NEW INSIGHTS AND CONTROVERSIES IN DIAGNOSIS AND TREATMENT OF ADULT GROWTH HORMONE DEFICIENCY, 2nd Edition

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PUBLISHED IN: Frontiers in Endocrinology

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-8325-2593-7
DOI 10.3389/978-2-8325-2593-7

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Publisher's note: This is a 2nd edition due to an article retraction.

Citation: Mancini, A., Persani, L., Arosio, M., Kreitschmann-Andermahr, I., eds. (2023). New Insights and Controversies in Diagnosis and Treatment of Adult Growth Hormone Deficiency, 2nd Edition. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-2593-7

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frontiers in Endocrinology

EDITORIAL
published: 10 January 2022
doi: 10.3389/fendo.2021.819527

Editorial: New Insights and Controversies in Diagnosis and Treatment of Adult Growth Hormone Deficiency

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Keywords: growth hormone, hypopituarism, growth hormon therapy, growth hormone deficiency (GHD), growth hormone deficiency (childhood onset)

Editorial on the Research Topic

New Insights and Controversies in Diagnosis and Treatment of Adult Growth Hormone Deficiency

OPEN ACCESS

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *21 November 2021*
Accepted: *25 November 2021*
Published: *10 January 2022*

Citation:
Mancini A, Arosio M, Kreitschmann-Andermahr I and Persani L (2022) Editorial: New Insights and Controversies in Diagnosis and Treatment of Adult Growth Hormone Deficiency. Front. Endocrinol. 12:819527. doi: 10.3389/fendo.2021.819527

Growth hormone (GH) is a hormone whose biochemical actions go far beyond of what its name implies. Surely it is the main regulator of growth in childhood until puberty, however its metabolic functions are central even in adult age.

The history of medical use of GH now spans several decades. The growth-promoting action of extracts of anterior pituitary was already known when human GH was isolated in 1944. In the 1960s, further metabolic activities were discovered, strengthening the notion that it would be beneficial to replace the hormone in case of deficiency. GH, extracted from human cadavers at the time, was employed to improve longitudinal growth in hypopituitary children with severe growth retardation for nearly 30 years, despite the difficulties and costs associated with delivering this medication. When, in 1985, the association between cadaveric GH replacement and fatal, slow-viral Creutzfeldt-Jakob disease was uncovered, an important era in endocrinology seemed to have come to a sudden halt. Yet, recombinant technology, which made the synthesis of biosynthetic recombinant human GH (rhGH) possible since 1981 and was used first in GH-deficient children and then in adults, revolutionized this field and the life of these patients.

GH is produced by the pituitary gland in the somatotropic cells, which differentiate from mammosomatotroph lines, which explains the chemical analogy between GH and prolactin. It is the product of different GH genes present in chromosome 17: two GH genes, GH1 (or GH-N) and GH2 (or variant, GH-V), and three placental genes (also known as placental lactogens). GH-N is mostly expressed in the pituitary gland and, therefore, codes the circulating GH forms (22kDa and 20kDa), however it is also expressed in other tissues where GH can act as an autocrine and/or paracrine factor. Only about half of the GH is free in the circulation, the other half is tied up to a binding protein (GHBP). GHBP can be expressed in two different isoforms, of which the most abundant high-affinity one corresponds to the extracellular domain of GH liver receptor, and it is generated by

tumor necrosis factor (TNFα)-converting enzyme (a metalloproteinase known as TACE), which represents another level of fine control of GH activity.

Its secretion is regulated in the hypothalamus in two ways: by means of a stimulatory hormone (growth hormone releasing hormone, GHRH) and an inhibitory one (somatostatin). Its actions are mediated by so-called somatomedins, produced in the liver and the periphery of the body. Its best known representative is the insulin-like growth factor-I (IGF-I). IGF-I is involved in a negative feedback loop with GH itself and is transported by a class of binding proteins (IGF-BP). Among these (six overall) the major binding protein in serum is IGF-BP3, that is controlled by GH and has been claimed as a possible marker of GH secretion, especially in the syndrome characterized by prolonged excessive GH secretion, acromegaly, and in pediatric GHD. IGF-I and IGF-BP3 are present in circulation in a 150 kDa ternary complex also including the acid-labile subunit (ALS) whose hepatic synthesis is also under the direct control of GH. The ternary complex significantly lengthens the half-life of IGF-I and constitutes its major deposit. IGF-BP5 is important for bone tissue formation and also forms ternary complex with IGF-I and ALS. On the contrary IGF-BP1 forms only small 50 kDa binary complexes and has been linked to metabolic status; insulin is its main regulator.

Twenty years ago, the classical view of the GH-IGF-I axis has been revaluated: the somatomedin hypothesis has been replaced by a more complex concept which takes into account GH action on different tissues, where it can induce local IGF-I synthesis. The major importance of the local autocrine/paracrine versus endocrine IGF-I actions on growth has been shown in models of mice knock-out for IGF-I genes in different tissues. All the pleiotropic effects of the axis are due to the coordinated action of GH (sometimes with a local production, as in bone, endothelium, heart or gonads) and IGF-I.

Furthermore, the model of a selective regulation of this axis at the hypothalamic level is a simplification and represents only a small part of a larger, more complex mechanism involving also peripheral organs. One of the most important modulator of GH secretion is ghrelin (also known as GH-releasing peptide or GHRP), produced by cells of the gastrointestinal tract, a powerful orexigenic peptide, physiologically released in the fasting state. Ghrelin action in turn is modulated by other factors, such as liver expressed antimicrobial peptide (LEAP)-2. Another newly discovered player is Klotho, a protein with antiaging properties in mice. In humans, Klotho is mainly expressed in the kidney, but also in the endothelium, where it induces nitric oxide (NO) production. Klotho is a regulator of GH secretion, as shown both in animals and humans, inhibiting negative IGF-I feedback on GH release; its levels are low in GH deficient subjects, however, the complex relationship with the GH axis is still to be clarified. The discover of ghrelin, LEAP-2 and Klotho, has thrown light on connections of peripheral organs and the pituitary.

Other regulatory factors are age-dependent, acting only in specific periods of life. One of them is growth differentiation factor (GDF)-15, directly produced by cardiomyocytes and related to growth disorders in children affected by cardiomyopathies; it inhibits the stimulation of IGF-I expression in the liver by GH, and therefore, contributes to the coordination of growth and cardiac function.

Normal aging is accompanied by a gradual decline in GH and IGF-1 levels. At 70, the secretion of GH in 24 hours is equal to 1/3 of that of a young adult (somatopause). The somatotropic axis influences the various epochs of life differently. While IGF-I excess could be dangerous for the mature man for the risk of favoring cardiovascular and oncological diseases, as observed in acromegaly, the rise of GH alone associated with reduced IGF-1/insulin activity observed in fasting and calorie restriction could be the key to explain the increased longevity of this condition.

The function of GH-IGF-I axis is strictly linked to nutritional status. Both GH secretion and effects are strongly regulated, directly or indirectly, by glucose, lipid and protein metabolism. Obesity is a condition of low GH secretory state, as demonstrated by blunted response to dynamic tests. Lower secretion in obese patients is the result of several factors, including the excess of circulating free fatty acids. Whatever the cause, in turn, it could contribute to the progressive metabolic alterations and co-morbidities, from dyslipidaemia to sarcopenia and to cardiovascular deterioration observed in obese patients.

The syndrome of adult GH deficiency (aGHD) has still poorly defined contours: it affects several organs and systems. It is currently considered uncommon, due to the non-specific and nuanced clinical presentation. Due to GH episodic secretion and short half-life, only provocative tests can play a diagnostic role. Organic causes (pituitary masses, iatrogenic after pituitary surgery or radiation therapy, trauma) are the most common. Idiopathic aGHD is a rare condition, even if its prevalence is probably underestimated, as highlighted in different cohorts in literature.

The example of obesity suggests that functional hyposecretion is a matter of fact and it is unknown if it can be assimilated to a real GHD at tissue levels. Circulating IGF-I concentrations are indeed normal in most obese subjects and GH sensitivity is increased probably due to hyperinsulinemia. Similarly, partial GH deficiency, with less clinical implications, yet significant, for instance at cardiovascular level, has been described.

Current guidelines on GHD diagnosis and treatment have been elaborated, however many questions are still debated. New tests for diagnosis have been recently proposed; moreover, non-conventional indications for diagnosis and treatment deserve further investigations. Controlled trials on beneficial effects on morbidity and mortality are still lacking and new formulations of GH are under investigation. Several questions are related to the age of affected patients (from transition age to ageing) and no clear indicators on how long therapy should be continued, are available. Other concerns are related to a possible pro-oncogenic effect, especially in patients who develop the deficiency because of a cancer or its treatment. The interrelations with other pituitary axes need further clarification since isolate GHD and multiple pituitary deficiencies may have a different clinical spectrum. Considering all these aspects, the aim of this Research Topic is to foster deeper insight in all questions related to aGHD, from molecular pathways involved in the pathophysiology to diagnostic tools and replacement therapy. Main advances in the papers, published in the Research Topic, are summarized and commented in this editorial.

GROWTH HORMONE REGULATION

Devesa present a very interesting review on new insights about GH regulation, focused on three circulating hormones involved in the modulation of GH secretion, which are ghrelin, klotho and nesfatins, all basically acting at pituitary levels. Interestingly, the same hormones play a role in energy metabolism, and, in the case of klotho, also in mineral metabolism (which explains why the kidney is the main site of production). A detailed description of ghrelin synthesis, activation (in an acylated form), signalling and effects is presented. Interestingly, this activation is performed by an enzyme (GOAT) by attachment of a fatty acid, underlining the link of GH stimulation with nutritional fuels. Ghrelin colocalizes with GHRH neurons and therefore a hypothalamic mechanism is also likely. Circulating klotho is part of a transmembrane protein and is capable of stimulating GH release and antagonizing the inhibitory feedback on GH exerted by IGF-1. Some data are reported in humans, with reduced plasma levels of klotho in children and adults with GH deficiency, restored by replacement therapy. Finally, the role of nesfatins is far from being understood. Undoubtfully they exert a regulatory role in feeding behaviour, inhibiting food intake. Concerning GH physiology, they seem to be inhibitor of GH production by a downregulation of the cAMP/PKA/CREB signalling. They also can counteract the positive effect of ghrelin on GH synthesis and secretion.

GH DEFICIENCY: AETIOLOGY AND CLINICAL MANAGEMENT

Many questions arise about the transition period, which is very important from a metabolic point of view. Obviously, the role of GH in determining bone maturation is a main issue, but body composition and not secondarily sexual maturation are also affect by the hormone. Spaziani et al. review aspects, which are of fundamental importance in clinical management of childhood onset GHD. In the transition period, GH replacement appears critical for the achievement of an adequate peak bone mass; however, equally clear is the primary role of rhGH on body composition and metabolic profile and, probably, in the achievement of a complete gonadal and sexual maturation. The most relevant issue in the transition period is the high rate of spontaneous recovery of GH function after the achievement of final height. A percentage between 25% and 100% of subjects with previous GHD diagnosis during childhood display an adequate response when undergoing re-testing, thus raising several questions on a) the opportunity to treat children with partial GHD, b) when performing the re-testing; c) which is the most adequate test and relative cutoff for the confirmation of GHD. In Table 1 of their orginal publication, Spaziani et al. provide the list of the available GH provocative tests and related cutoffs.

Traumatic brain injury (TBI) ranges among the those aetiologies of hypopituitarism and, thus, aGHD, which are inbetween recognized as classical causes, as reviewed by Gasco et al. in his overview on post-traumatic hypopituitarism. First described in 1918, it was, for a long time, considered a rare sequel of trauma. However, the situation has radically changed in the last two decade and the prevalence of TBI is, nowadays, estimated between 27.5 and 32%. GH, together with gonadotropins, seems to be the most frequent hormone involved. The physiopatologic mechanisms underlying this condition are comprehensively described, according to their division into two distinct periods of time: the primary brain injury at the time of trauma, with direct damage of neural structures and hypoadrenalism as the main life-threatening hormonal deficit; and the secondary one, which is based on different mechanisms, such as excitotoxicity (mainly related to glutamate), secondary ischemia (considering the peculiarity of pituitary vascularization) and inflammatory response; the latter also including autoimmune mechanisms and, possibly, a genetic vulnerability. The diagnosis is based on the same tests used in other aetiologies of GHD, but with some peculiarities (for instance the risk of seizures after ITT) and the lack of hypothalamic derangement sensitivity when using GHRH plus arginine. Glucagon in this case seems to be the gold standard and 6-12 months after trauma appears to be the ideal period to perform GH test. The rationale for beneficial effects are based on pleiotropic actions of GH-IGF-1 axis on neurogenesis and neurorepair. Anabolic GH functions could also be a key point for the recovery. However, clinical studies are still heterogeneous, so that conclusive data are still far to be obtained. The authors underline that postTBI GHD, compared with GHD secondary to non-functioning pituitary adenomas, seems to exhibit a less severe biochemical picture, but worse quality of life (QoL) scores. The QoL improvement after GH replacement therapy seems to have a principal effect and was shown to be maintained for a long period, up to eight years.

The group of Giavoli provides and overview on the management of GHD patients with multiple pituitary deficiencies (MPHD). A condition of untreated GHD masking concomitant pituitary deficiencies, mainly central hypothyroidism and hypoadrenalism, is now a consolidated concept. Therefore, thyroid and adrenal functions should be soon re-tested after the introduction of rhGH replacement. In their manuscript, Profka et al. give information on the possible contexts in which GHD may develop and examining the proposed mechanisms at the basis of interactions between the GH/IGF-I system and other axes. A relevant part of the manuscript is dedicated to the sexual dimorphism of GH-IGF1 function and on the possible role of rhGH in the induction of fertility in different clinical conditions in both sexes.

EFFECTS OF GH REPLACEMENT THERAPY

GHD and Cardiovascular Risk

The issue of GHD, insulin resistance and cardiovascular risk is the topic of two articles in this Research Topic. In the first, van

Bunderen et al. present data from a clinical trial in which they investigated the effects of GH dose titration to low-normal or high-normal levels of IGF-I on (micro)vascular function, insulin resistance and body composition in order to explore the mechanisms underlying the U-shaped relation of IGF-I levels with cardiovascular disease. Based on the knowledge, that epidemiological data give evidence for a bidirectional link between serum IGF-I concentrations and cardiovascular disease with an increased cardiovascular risk (CVR) in states of aGHD but also in acromegaly, they investigated 30 patients with aGHD on GH replacement, titrated to low vs. high-normal IGF-I levels. They found that an increase of GH dose with subsequent high-normal IGF-I levels led to a reduction in waist circumference, but also to a significant increase in insulin resistance. Also, neurogenic and endothelial vasomotion domains were affected by a change in GH dose, paralleling, in part, the changes in waist circumference. They concluded from their results that higher IGF-I levels may be beneficial for body composition but seem to be detrimental in terms of insulin resistance. While van Bunderens results must be considered preliminary and do not allow, at present, to provide clear dosing strategies, they open up avenues for further research in this important field.

In the second article, Ren et al. introduce a further potential player into the intricate relationship between GHD, CVR and insulin resistance. Based on the knowledge that patients with aGHD have elevated levels of circulating inflammatory factors, accompanied by increased levels of oxidative stress and endothelial dysfunction, they explored levels of mesencephalic astrocyte-derived neurotrophic factor (MANF) in aGDH patients and normal controls. MANF is a secreted stress-response protein with selective protective effects on dopamine neurons and immune modulatory properties, which serves as a regulator of metabolic homeostasis. 101 aGHD patients and 100 matched healthy controls were included in the analysis. The authors found that circulating MANF content of aGHD patients was significantly lower than in the controls and that, moreover, MANF levels were linearly correlated with homeostasis model assessement)-insulin resistance (HOMA-IR) in the aGHD population. Those patients with MANF at the lowest concentration tertile, had a significantly higher disease odds ratio, Framingham risk socre and 10-year-risk of atherosclerotic cardiovascular disease than the hightest concentration tertile. In sum, the authors were able to show that MANF is strongly associated with insulin resistance and abnormal lipid metabolism under aGHD conditions. Thus, in the future, MANF may play a role in aGHD diagnosis and even provide therapeutic potential for later cardiovascular disease.

GHD in elderly is a particular topic which is addressed by two papers in the Research Topic. Ricci Bitti et al. performed a minireview about the peculiarity of clinical presentation, diagnosis and outcomes of aGHD patients in this period of life. Due to the similarity of the ageing process and GHD symptoms, the diagnosis of aGHD in older patients is particularly complex, since no clear adjustments for diagnostic cut-offs in GH dynamic test are available. There is agreement to start therapy at low doses and up-titrate according to clinical response, including IGF-I levels, which should be maintained between -1/-2 and +1/+2 DS for age, monitoring of metabolic parameters and of side effects, which could be more harmful in elderly people. Few randomized and controlled studies have been reported, which are still inconclusive due to the number and heterogeneity of patients; moreover, no data are available about efficacy and long-term therapy in patients above 80 years. Greater attention should be placed on cardiovascular morbidity and mortality and on cognitive function. Despite no clear evidence is reported on increase of muscle strength, there is sufficient suggestion that GH may reduce its age-related decline. However, in the authors' opinion, the main goal of GH replacement in the elderly should be the improvement of QoL, in turn related to frailty and the risk of loss of independence, typical of the ageing; they underline the importance of personalized treatment and careful follow-up.

The other is a single-centre observational study (Scarano et al.) which gives an important experience, selecting a group of GHD patients treated for 7 years, comparing the effects of therapy in groups divided according to age (10 elderly and 29 adult-onset GHD); they were recruited by a large cohort of 196 hypopituitary patients, with an inclusion criterion of this therapy period; a comparison with age-matched control group is also presented. According to concepts above described, the mean GH dose was lower in the elderly group, but with the same aim to maintain IGF-levels in the normal range for the specific age. The study shows that the effects on body composition are more evident in AGHD (reduction of waist and hip circumferences and waist-hip ratio) than in EGHD (that showed only reduction in hip circumference): similarly lipid profile was improved more in AGHD (decreased in total and LDL-cholesterol and triglycerides and increase in HDL-cholesterol) than in EGHD (only triglycerides significantly decreased). An increase in morning glycemia was observed only in AGHD, but without modification of HbA1c. EGHD showed, as expected, higher systolic blood pressure, which however did not significantly change after treatment. Interestingly the prevalence of diabetes mellitus did not differ from that of general population; a risk of develop it could be related to impaired glucose homeostasis in obese GH adults. On the contrary, the authors showed a higher prevalence of dyslipidaemia in adult controls than AGHD. The prevalence of Metabolic Syndrome is increased in AGHD during treatment, due to the increase in glucose levels, BMI and systolic blood pressure in this long-term study. In agreement with other study, the main conclusion was the beneficial effect on body composition and lipidic pattern, less pronounced, but present, also in elderly GHD people.

In this context, Chen et al. performed a meta-analysis to evaluate the efficacy and safety of weekly long-acting growth hormone (LAGH) replacement therapy, a new frontier for GHD, compared to daily growth hormone in children with short stature. This analysis reveals that LAGH has no significant difference compared to daily growth hormone in children with short stature on several clinical parameters (height velocity, final height SDS, bone age, IGF1-SDS, as well as on incidence of

adverse events). This is of significance in medical practice due to the various nuisances of daily injection in adherence to treatment, for example, as the authors well defined in the introduction. One possible counfounding factor in this analysis is the availability of six different LAGH formulations, each one tested in a small number of patients so far. Despite the limited number of children treated with LAGH, the meta-analysis would indicate that both short- and long-acting rhGH formulations can be used without major consequences on children's growth or on their side effects.

NEW THERAPEUTIC ISSUES

The challenges about GH treatment in adults concern the entire lifespan (from transition to aging). The metabolic role indicates that it is not simply a growth hormone; nevertheless it is not a antiaging therapy. A precise definition of GH deficiency in different clinical situations is mandatory before starting treatment. Another open question which could be of interest in clinical management regards the objective evaluation of patients' adherence.

Few studies have investigated the adherence to GH therapy in the adult GHD population and the psychological reasons that influence it. In children a review showed that up to 71% of the young patients were non adherent to their GH medication. The group of CJ Strasburger and I Kreitschmann-Andermahr, on behalf of the German PATRO Board, studied this important and overlooked aspect in depth using for the first time in GHD a methodology already well validated in other chronic diseases. Using specific questionnaires they analyzed three major psychological domains, that is: strategy of coping with their chronic disease, beliefs about medications and quality of life and related them to adherence to GH therapy. Their series consists of 107 patients (53% M, mean age 50 years) with severe GHD in almost all cases from organic causes, followed up in 5 German referral centers and in stable current therapy with rhGH. The AA note that the majority of the patients had high rhGH specific adherence scores and are strongly convicted of their need for GH medication. In addition the AA find that active coping is the most common adaptation strategy, and the one that most correlates with adherence to therapy; that most patients judge the benefits of rhGH greater than the potential negative effects, with only 4 patients whose fear for side effects outweighs the perceived benefits.

Of particular interest is the evaluation of QoL in these patients: in fact, if there are many studies that have shown severe QoL impairment in untreated GH-deficient patients in respect to the general population, very few have evaluated it in the course of replacement GH therapy. Well, the physical QoL remains reduced by more than 1 SD in 13% and by more than 2 SD in approximately 7% of these patients, and, surprisingly, mental QoL in 12% and 25%, respectively. This shows a severe mental impairment, not related to age, in a large proportion of the investigated patients, which is mainly due to a reduced vitality and a bad perception of the one's general health status. Noteworthy, the adherence to therapy is negatively correlated to the mental QoL, conversely, a lower physical QoL, as observed in the oldest patients, correlates with higher adherence to therapy.

Although the study did not include a control group of untreated patients, as the questionnaire used (SF-36) is the same, some comparisons can be made with historical series of untreated GHD patients showing overall better QoL of patients with hypopituitarism on replacement GH therapy. The important observation remains that those patients with impaired mental QoL often demonstrate a depressive coping and also have a lower adherence to therapy, as if they are less able to translate their belief in the usefulness of therapy with GH into action and these will be the patients clinicians need to recognize and to focus their efforts on in the future.

Yuen et al. provide an excellent overview on the present situation of long-acting GH (LAGH) analogs, the development of which has been prompted by issues of patients' non-adherence to the presently approved daily recombinant human GH (rhGH) preparations. LAGH analogs that allow for a decreased injection frequency may offer increased patient acceptance, tolerability and therapeutic flexibility. However, the authors also point out that there may be pitfalls associated with these LAGH analogs, among them an unphysiological GH profile and different molecular structures that might pose clinical problems in terms of dose initiation, therapeutic monitoring, incidence and duration of side-effects and long-term safety. Moreover, the technology used to prolong GH action may cause fluctuations of peak and trough serum GH and IGF-I levels and variations in therapeutic efficacy. Non-inferiority to daily rhGH has already been proven for some LAGH analogs, not only in terms of increased growth velocity but also improved body composition in children and adults. With two LAGH analogs marketed in Asia, one recently approved in the United States, one more approved but not marketed in Europe along with several others proceding through various stages of clinical development, there seem to be exciting new treatment opportunities for pediatric and adult GH indications at the horizon. However, the authors caution that long-term surveillance of safety and efficacy of LAGH analogs are needed to establish their worth in clinical practice.

CONCLUDING REMARKS

GHD syndrome has still poor defined features and many unsolved question. Due to new discovered function of GH, the search for clinical/biochemical parameters, which could be useful in risk prediction, is yet to be expanded.

The possible role of GH in other diseases, such as osteoporosis, infertility, cardiac failure and many more, could represent a "non-conventional" indication to perform dynamic GH tests unveiling masked and underestimated GHD. Therefore, it has also the aim to sensitize physicians, who are not familiar with this Research Topic, to extend their cultural interest and clinical practice in GH physiopathology.

AUTHOR CONTRIBUTIONS

AM, MA, IK-A, and LP wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

Partially supported by the Ricerca Corrente Funds of Istituto Auxologico Italiano (IPOFICTUS; code: 05C303_2013).

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Endocrinology

ORIGINAL RESEARCH
published: 09 February 2021
doi: 10.3389/fendo.2020.619173

Titrating Growth Hormone Dose to High-Normal IGF-1 Levels Has Beneficial Effects on Body Fat Distribution and Microcirculatory Function Despite Causing Insulin Resistance

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *19 October 2020*
Accepted: *18 December 2020*
Published: *09 February 2021*

Citation:
van Bunderen CC, Meijer RI, Lips P, Kramer MH, Serné EH and Drent ML (2021) Titrating Growth Hormone Dose to High-Normal IGF-1 Levels Has Beneficial Effects on Body Fat Distribution and Microcirculatory Function Despite Causing Insulin Resistance. Front. Endocrinol. 11:619173. doi: 10.3389/fendo.2020.619173

To clarify the mechanism underlying the described U-shaped relation of both low and high levels of IGF-1 with cardiovascular disease this study explores the effect of decreasing and increasing growth hormone dose in GH deficient adults on (micro)vascular function, body composition and insulin resistance. In this randomized clinical trial, thirty-two subjects receiving GH therapy with an IGF-1 concentration between –1 and 1 SD score (SDS) for at least one year were randomized to receive either a decrease (IGF-1 target level of –2 to –1 SDS) or an increase of their daily GH dose (IGF-1 target level of 1 to 2 SDS) for a period of 24 weeks. Microvascular endothelium (in)dependent vasodilatation and vasomotion, vascular stiffness by pulse wave analysis, and HOMA-IR were measured. At the end of the study 30 subjects (65.6% men, mean age 46.6 (SD 9.9) years) were analyzed. There was a favorable effect of increasing the IGF-1 level on waist circumference compared to decreasing the IGF-1 level (p=0.05), but a detrimental effect on insulin resistance (p=0.03). Decreasing IGF-1 level significantly lowered the endothelial domain of vasomotion (p=0.03), whereas increasing IGF-1 level increased the contribution of the neurogenic domain (p=0.05). This change was related to the favorable change in waist circumference. In conclusion, increasing IGF-1 levels was beneficial for body composition but detrimental with respect to insulin resistance. The contribution of the neurogenic vasomotion domain increased in parallel, and could be explained by the favorable change in waist circumference.

Clinical Trial Registration: ClinicalTrials.gov, identifier NCT01877512.

Keywords: growth hormone, insulin-like growth factor-1, growth hormone deficiency in adults, insulin resistance, vasomotion, vascular endothelium, growth hormone treatment

INTRODUCTION

Epidemiological evidence for a bidirectional link between serum IGF-1 concentrations and cardiovascular disease (CVD) has been repeatedly demonstrated. On the one hand, growth hormone (GH) deficient adults are characterized by an adverse lipid profile and altered body composition with increased fat mass which may put them on an increased risk for cardiovascular disease (1, 2). Moreover, even low-normal IGF-1 levels have been associated with the development of ischemic heart disease and stroke in the general population (3–5). On the other hand, high levels of IGF-1 such as observed in acromegaly are also associated with an adverse cardiovascular risk profile and a higher prevalence of CVD (6). These data suggest a U-shaped relationship between IGF-1 concentrations and CVD, which was corroborated by the finding of a U-shaped relationship with cardiovascular mortality in a Dutch cohort of healthy older people (7). Presently, it is unclear whether such a U-shaped association also exists in GH deficient adults treated with GH. Many studies demonstrate favorable effects of GH replacement therapy in adults with GH deficiency (8, 9), and of normalization of GH and IGF-1 levels in acromegaly (10), on cardiovascular risk factors, but the presented data suggest that there may be an optimal target level of IGF-1. In addition, the underlying mechanisms of this U-shaped relationship remain unresolved. On the one hand, IGF-1 is postulated to protect against (micro) vascular endothelial dysfunction, atherosclerotic plaque development, and ischemic myocardial damage (11). Interestingly, cultured endothelial cells, isolated microvessels, as well as the capillaries of perfused hearts, all possess distinct surface binding sites for both IGF-1 and insulin (12). Capillary density has been shown to be lower in untreated GH deficient patients than in control subjects, which increased to a level that was not different from that in control subjects after GH treatment normalized plasma IGF-1 (13). On the other hand, high levels of IGF-1 such as observed in acromegaly are associated with profound insulin resistance (6), which may offset the beneficial (micro)vascular effects of IGF-1. Insulin resistance itself has been linked to a lower capillary density and a change in vasomotion, the rhythmic change in vascular diameter, which is thought to influence capillary perfusion (14).

In order to elucidate possible mechanisms underlying the U-shaped relationship of IGF-1 with CVD, the aim of the present study is to explore the effects of titrating GH dose to low-normal or high-normal levels of IGF-1 for 24 weeks in GH deficient adults on (micro)vascular function, body composition and insulin resistance.

MATERIALS AND METHODS

Study Design

This study presents data from a randomized, open-label, clinical trial conducted at one university hospital (VU University Medical Center, Amsterdam, The Netherlands) comparing decreasing and increasing GH dose for 24 weeks. The study investigates the efficacy and safety measures of GH replacement therapy before and after reaching low-normal and high-normal IGF-1 target levels (15). At entry subjects were receiving GH treatment according to general clinical practice (daily subcutaneous somatropin injections using automated pen systems manufactured by Pfizer Inc., Novo Nordisk Inc., and Eli Lilly and Co.). Subjects were selected based on having an IGF-1 concentration between −1 and 1 SDS (adjusted for age and gender) during GH replacement therapy. Randomization was done by a computer-generated random sequence and was stratified by gender. Subjects were randomized to receive either a decrease of their regular dose of GH treatment (IGF-1 target level of −2 to −1 SDS) (low dose=LD group), or an increase of their regular dose (IGF-1 target level of 1 to 2 SDS) (high dose=HD group), for 24 weeks. After 4 weeks the GH dose was adjusted when the target level of IGF-1 was not reached. At visit one (baseline) and visit two (after 24 weeks) blood samples were drawn and measurements performed to assess micro- and macrovascular function.

Patients

The study group consisted of 32 adult patients with documented severe GH deficiency and more than one year of GH treatment, with an IGF-1 level between −1 and 1 SD score (SDS) for at least six months. Other pituitary hormone deficiencies had to be substituted when indicated and be stable for at least six months and during follow up. Severe GH deficiency was diagnosed prior to the study and defined according to the consensus guidelines of the GH Research Society for the diagnosis and treatment of adults with GH deficiency (16). In the Netherlands, the approval of GH treatment (and reimbursement of costs by the health insurer) was judged by an independent board of endocrinologists wanting to see two abnormal GH stimulation tests (mostly used: insulin-tolerance test and GHRH-arginin test) or one abnormal test or low IGF-1 in combination with panhypopituitarism or profound congenital GH deficiency. Patients were not eligible if they had a recent or current malignancy, craniopharyngioma as cause of hypopituitarism, were (planning to become) pregnant, or had a cardiovascular event within the last year before recruitment. Patients with prior Cushing's disease or acromegaly were not excluded since an earlier study did not demonstrate significant interaction with the effect of GH treatment on cardiovascular mortality in GH deficient adults in The Netherlands (17). Patients were included after oral and written informed consent. The study protocol was approved by the Ethics Committee of the VU University Medical Center, Amsterdam. The study was performed according to Good Clinical Practice and the Declaration of Helsinki. This study is registered with ClinicalTrials.gov, number NCT01877512.

Laboratory Investigations

Laboratory investigations included total IGF-1, and insulin and glucose to calculate insulin resistance by HOMA-IR. Blood samples were drawn after an overnight fast prior to every visit. Total IGF-1 was measured by a non-competitive (sandwich) chemiluminescence immunoassay (Liaison, DiaSorin S.p.A., Italy). The inter-assay coefficient of variation (CV) was 7.4%. Insulin was measured by an immunometric assay, Luminescence (Advia Centaur, Siemens Medical Solutions Diagnostics, USA). The inter-assay coefficient of variation (CV) was 8%.

Microvascular Function

Endothelial function was assessed by microvascular measurements of the skin blood flow including endothelium (in)dependent vasodilatation and vasomotion. Endothelium-(in)dependent vasodilation of finger skin microcirculation was evaluated by measuring skin blood flow in perfusion units (PU) by a laser Doppler system (Periflux 4000, Perimed, Stockholm, Sweden) in combination with iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP), respectively, as described previously (18). All measurements were performed in the fasting state, in the sitting position with the investigated hand at heart level in a temperature-controlled room. Skin temperature was registered continuously and was above 28°C at the start of all microvascular measurements. ACh (1% Miochol; Bournonville Pharma, Braine d'Alleud, Belgium) was delivered to the skin on the middle phalanx of the third finger using an anodal current, consisting of seven doses (0.1 mA for 20 s) with a 60 s interval between each dose. SNP (0.01%, Nipride; Roche, Woerden, The Netherlands) was delivered on the middle phalanx of the second finger using a cathodal current, consisting of seven doses (0.2 mA for 20 s) with a 90 s interval between each dose. In order to perform vasomotion analyses skin blood flow was measured during 30 min with a laser Doppler probe positioned at the dorsal side of the wrist of the arm. A bandpass filter with cut-off frequencies at 20 Hz and 20 kHz, and a time constant of 0.2 s, was selected. Wavelet analysis of the signals with a minimum of 30 min (with a sampling frequency of 32 Hz resulting in approximately 58.000 data points) in length was conducted to assess the frequency spectrum between 0.01 and 1.6 Hz. Wavelet analysis was performed using the wavelet toolbox in Matlab (7.8.0.347; The Mathworks, Inc., Natick, MA, USA), as described earlier (19). Scales are chosen for a resulting frequency range from 0.01 to 1.6 Hz which can be divided in five frequency intervals as described by Stefanovska et al. (20). The first three lower frequencies are locally generated; 0.01–0.02 Hz as endothelial activity, 0.02–0.06 Hz as neurogenic activity, and 0.06–0.15 Hz as myogenic response of the vascular smooth muscle cells (VSMC). The higher frequencies originate upstream and are: 0.15–0.4 Hz as respiratory function and 0.4–1.6 Hz as heart beat frequency. To eliminate edge effects, the first and last 2,000 samples were removed from the resulting wavelet transform. The relative amplitude was calculated for each of the five frequency bands by dividing the average amplitude within a band by the average amplitude of the entire spectrum. This normalization takes into account the variation in the signal strength between subjects and/or within subjects during an intervention (21).

Macrovascular Hemodynamics and Vascular Stiffness

Blood pressure and heart rate were measured automated by Dinamap (PRO 100 V2), with a proper sized cuff, after 3 min of rest, three times with at least 1 min in between, where the two last measurements were averaged. Vascular stiffness was assessed by Pulse Wave Analysis, determining pulse wave velocity (PWV) and augmentation index (AIx) by a validated noninvasive automated device. The Sphygmocor Pulse Wave Velocity system uses applanation tonometry in conjunction with a three-lead ECG to take sequential measurements at two arterial sites. The timing of the onset of systole of the pressure waves were compared with the timing of the corresponding R waves on the ECG recording, with the same delay calculated by the software. PWV was calculated as the ratio of the distance traveled and the foot-to-foot time delay between pulse waves and expressed in meters per second. A high fidelity peripheral artery blood pressure waveform at the radial artery is used to calculate the AIx. The cardiac index (l/min/m^2) at rest was determined by a non-invasive continuous hemodynamic monitoring system (Nexfin monitoring system, BMEYE B.V., Amsterdam, The Netherlands).

Statistical Analyses

Categorical data were expressed as number (percentage) and continuous data as mean (SD), or as median (interquartile range (IQR)) for skewed variables. Parametric or non-parametric tests were used when appropriate. General Linear Model (GLM) for repeated measures was used for between-group differences for change over time. Skewed variables were transformed when needed. Adjustments for baseline value were conducted to account for regression to the mean for the different outcome measures. Moreover, at baseline the LD and HD groups differed with respect to childhood onset (CO) and adult onset (AO) GH deficiency, and this variable was therefore added as covariate to the final GLMs. Subsequently, linear regression analyses were performed to investigate whether the association of change in IGF-1 SDS with some domains of the vasomotion analysis remained when adjusting for relevant covariates. Data were examined by use of Pearson's correlation. Two sided *P* values of 0.05 or less were considered significant. Statistical analyses were performed by the statistical software package IBM SPSS statistics 20.0 (SPSS Inc., Chicago, IL).

RESULTS

Between May 31, 2013, and April 11, 2014, we enrolled 32 patients. One subject withdrew after start of the study because of personal reasons. Another subject was excluded from the analyses due to the inability to reach the proper IGF-1 target level. The final analyses were conducted with 15 subjects in each group. **Table 1** shows the baseline characteristics of the groups. There were more subjects with CO GH deficiency in the LD group and consequently fewer patients with a history of pituitary surgery. This corresponds with the underlying diagnosis of GH deficiency being 50% congenital in the LD group (compared to 19% in the HD group) and 50% (treatment of) pituitary tumor in the HD group (compared to 25% in the LD group). Of all 13 pituitary tumors, six concerned a non-secreting adenoma, five a prolactinoma, and two an ACTH producing adenoma.

The median daily dose of GH was decreased from 0.25 (IQR 0.35) to 0.10 (IQR 0.15) mg/day ($p<0.001$) in the LD group and increased from 0.25 (IQR 0.30) to 0.50 (IQR 0.60) mg/day ($p<0.001$) in the HD group. The IGF-1 concentration

TABLE 1 | Baseline characteristics of the low dose group in which the IGF-1 target level was between −2 and −1 standard deviation score (SDS), and the high dose group in which the IGF-1 target level was between 1 and 2 SDS.

	Low Dose		High Dose		*P value*
No. of patients	16		16		
Age, year	47.4	(10.8)	46.4	(9.3)	*0.80*
Sex, no. of females (%)	6	(37.5)	5	(31.2)	*0.71*
Onset of GHD, CO (%)	10	(62.5)	3	(18.8)	*0.01*
Cranial radiotherapy (%)	1	(6.2)	2	(12.5)	*1.00*
Pituitary surgery (%)	2	(12.5)	8	(50.0)	*0.02*
Isolated GHD (%)	4	(25)	4	(25)	*1.00*
TSH deficiency (%)	8	(50)	11	(68.8)	*0.28*
LH/FSH deficiency (%)	10	(62.5)	7	(43.8)	*0.29*
ACTH deficiency (%)	10	(62.5)	10	(62.5)	*1.00*
ADH deficiency (%)	0		4	(25)	*0.10*
Cardiovascular disease (%)	3	(18.8)	4	(25)	*1.00*
Diabetes mellitus (%)	3	(18.8)	0		*0.23*
Smoking (%)	4	(25)	2	(12.5)	*0.65*
GH dose, mg/day[a]	0.23	(0.36)	0.28	(0.30)	*0.93*
Duration GH treatment, year[a]	15.1	(17.9)	12.6	(12.7)	*0.09*

Values are mean (SD) unless stated otherwise.
[a]Median (IQR).
GHD, growth hormone deficiency; CO, childhood onset; GH, growth hormone.

decreased from 21.40 (SD 4.87) at baseline to 12.43 (SD 2.25) nmol/L (p<0.001) in the LD group after 24 weeks, and increased from 18.53 (SD 2.77) to 28.13 (SD 5.15) nmol/L (p<0.001) in the HD group (**Figure 1** shows the IGF-1 levels in SDS adjusted for age and gender).

The effects of increasing or decreasing IGF-1 levels on body composition, macrovascular hemodynamics and vascular stiffness and insulin resistance are presented in **Table 2**. In parallel to the favorable effect of increasing the IGF-1 level on waist circumference compared to decreasing the IGF-1 level (p=0.05), there was a significant difference in the effect on insulin resistance (p=0.03). Increasing IGF-1 by augmenting the GH dose in the HD group significantly increased insulin resistance compared to baseline (1.12 vs. 0.79, p=0.01), whereas no significant change was detected during decreased levels of IGF-1 (0.74 vs. 0.80, p=0.24). With respect to microvascular function, no (difference in) effect on endothelial-dependent, nor endothelial-independent, vasodilatation was found. Decreasing IGF-1 level significantly lowered the endothelial domain of vasomotion (p=0.03). Increasing IGF-1 level increased the contribution of the neurogenic domain (p=0.05) (**Figure 2**).

Correlation analyses (**Figure 3**) demonstrated that the change in waist circumference was inversely correlated with the change in the neurogenic vasomotion domain (r −0.39, p<0.05), but not with change in IGF-1 SDS or HOMA-IR. In addition, the change in IGF-1 SDS was positively correlated with the change in the endothelial vasomotion domain (r 0.38, p<0.05), but not with changes in waist circumference or HOMA-IR. Subsequently, these associations and possible confounders were explored in the regression models presented in **Table 3**.

DISCUSSION

This exploratory study on the possible mechanisms linking IGF-1 and CVD investigated the effect on (micro)vascular function, body composition and insulin resistance of changing the IGF-1 concentration to low- or high-normal levels during GH treatment in GH deficient adults. The most clear finding was that increasing the GH dose to a high-normal IGF-1 level led to a significant increase in insulin resistance, but a reduction in waist circumference. Moreover, although the overall effect on (micro) vascular function was limited, both the neurogenic and

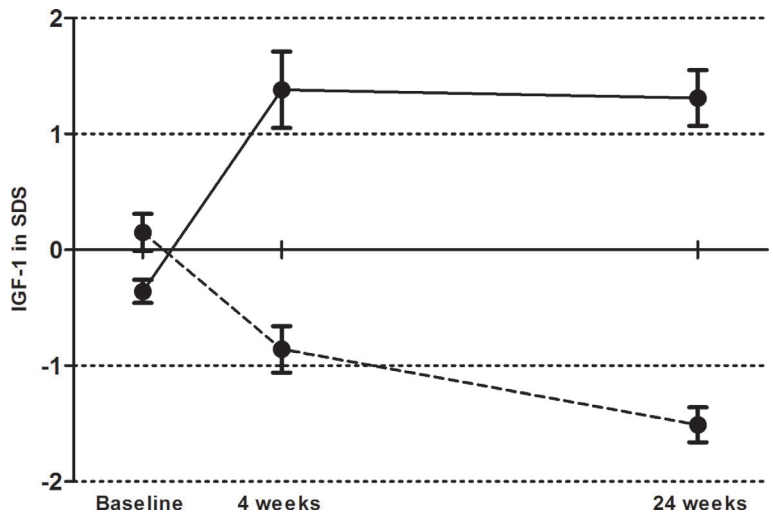

FIGURE 1 | Mean serum total IGF-1 level in SD score (± SEM) at baseline, after four weeks of growth hormone (GH) treatment and at end of follow up in the low dose (dashed line) and high dose (solid line) group.

TABLE 2 | The effect of increasing or decreasing IGF-1 level on measurements of body composition, insulin resistance, and macrovascular hemodynamics and vascular stiffness.

	Low Dose				High Dose				*P value for between group difference*[a]
	Baseline		Follow up		Baseline		Follow up		
BMI, kg/m^2	28.2	(9.8)	28.2	(9.4)	28.8	(4.2)	28.4	(3.3)	*0.47*
Waist circumference, cm [b]	97	(24)	99	(23)	105	(11)	102	(8)	*0.05*
Insulin resistance (HOMA-IR)[c]	0.80	(1.24)	0.74	(1.48)	0.79	(0.57)	1.12	(0.88)*	*0.03*
Fasting glucose, mmol/L	4.3	(0.7)	4.5	(1.2)	4.3	(0.9)	4.7	(0.6)	*0.54*
Insulin, pmol/L [c]	43	(77)	40	(86)	42	(34)	62	(48)*	*0.01*
Total cholesterol, mmol/L	5.02	(1.01)	4.86	(0.75)	5.15	(0.98)	4.99	(1.02)	*0.90*
HDL cholesterol, mmol/L	1.51	(0.53)	1.54	(0.51)	1.47	(0.40)	1.50	(0.43)	*0.63*
LDL cholesterol, mmol/L	3.04	(0.88)	2.86	(0.56)	3.10	(0.94)	2.92	(0.79)	*0.93*
Triglycerides, mmol/L	1.03	(0.38)	1.03	(0.40)	1.27	(0.59)	1.33	(0.45)	*0.65*
Free fatty acid, mmol/L	0.42	(0.18)	0.42	(0.19)	0.45	(0.22)	0.51	(0.22)	*0.58*
Systolic blood pressure, mmHg	129	(18)	127	(12)	126	(14)	124	(16)	*0.71*
Diastolic blood pressure, mmHg	80	(8)	78	(8)	77	(9)	74	(10)	*0.42*
Heart rate, beats/min	68	(8)	63	(8)*	59	(8)	61	(9)	*0.54*
Cardiac index, liter/min/m^2	3.0	(0.5)	3.0	(0.7)	2.7	(0.5)	2.9	(0.4)	*0.36*
Pulse Wave Velocity, m/s	7.2	(1.0)	7.2	(0.7)	7.1	(1.2)	7.1	(1.5)	*0.76*
Augmentation index	24	(18)	25	(17)	20	(15)	17	(11)	*0.17*

Values are mean (SD) unless stated otherwise.
*[a]The effect in both groups (delta) are compared and adjusted for baseline value and onset of GHD, [b]Waist circumference reference value men <102 cm, women <88 cm, [c]Median (IQR), *P value < 0.05 for change from baseline.*

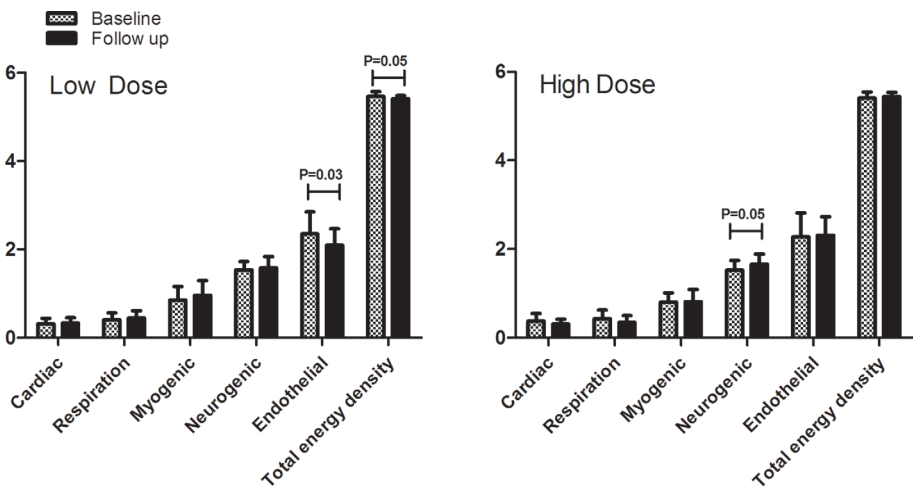

FIGURE 2 | The contribution of different frequency intervals to microvascular vasomotion (expressed in energy density) by laser Doppler of skin blood flow in both treatment groups before and after 24 weeks. The frequency intervals are: 0.4–1.6 Hz = cardiac function, 0.15–0.4 Hz = respiratory function, 0.06–0.15 Hz = myogenic response of the vascular smooth muscle cells, 0.02–0.06 Hz = neurogenic activity, 0.01–0.02 Hz = endothelial activity.

endothelial vasomotion domain were affected by a change in the GH dose. Interestingly, insulin resistance and (central) obesity have been demonstrated to be associated with a decreased activity of the neurogenic and endothelial vasomotion domain (22). In the present study, however, the changes in insulin resistance and the vasomotion domains were disconcordant, i.e. insulin resistance became worse, whereas the contribution of the neurogenic and endothelial vasomotion domains increased after increasing the GH dose to a high-normal IGF-1 level. The changes in vasomotion seem, in part, to parallel the changes in waist circumference.

Vasomotion is the rhythmic change in vascular diameter which is thought to influence capillary density and capillary exchange of substances between blood and tissues (23). As already mentioned IGF and insulin receptors can be detected on the microvascular endothelium, and therefore IGF and insulin should in theory be able to influence microvascular vasomotion. Indeed, insulin has been shown to alter arteriolar vasomotion with a resultant increase in the capillary exchange surface (14, 24, 25). Systemic hyperinsulinemia affects vasomotion by increasing neurogenic and endothelial activity in skin, and the change in the neurogenic vasomotion domain is

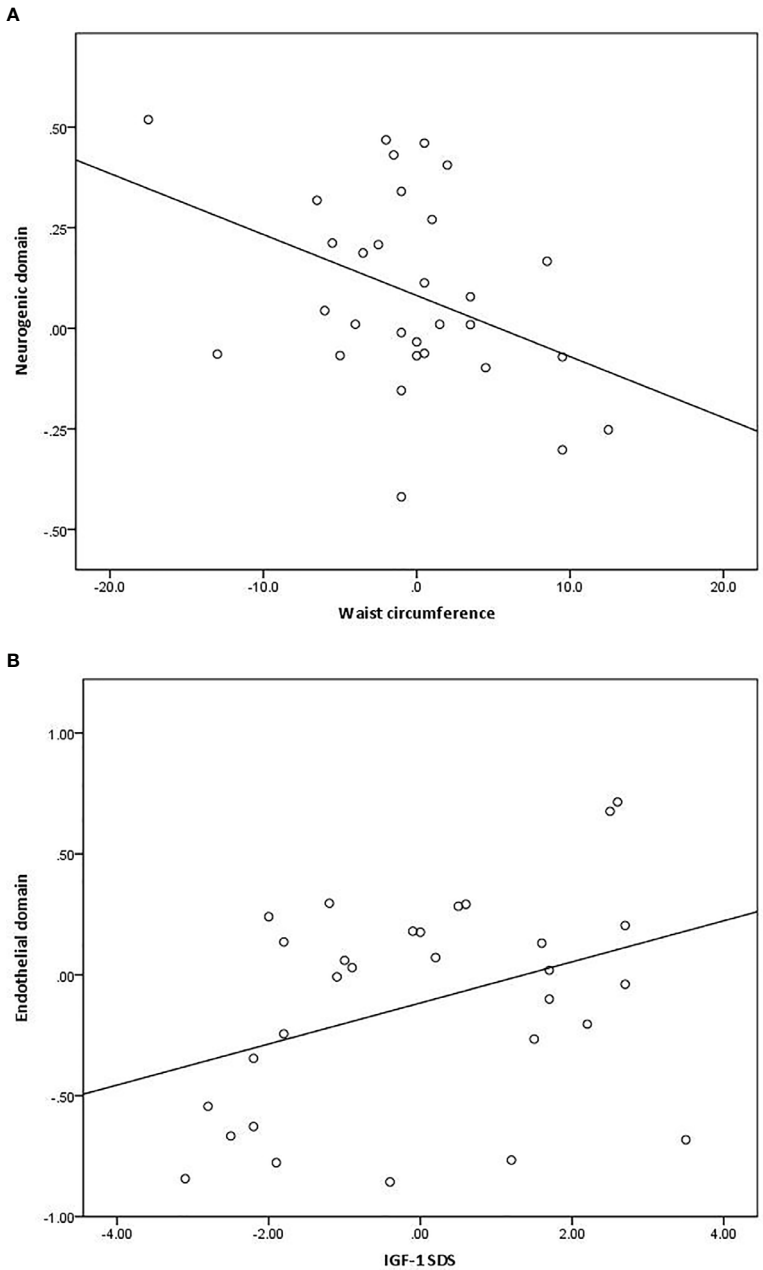

FIGURE 3 | Associations between change in the activity of the neurogenic vasomotion domain and waist circumference [**(A)**; r –0.39, p<0.05] and the change in the activity endothelial vasomotion domain and IGF-1 standard deviation score (SDS) [**(B)**; r 0.38, p<0.05].

directly associated with the increase in capillary density during hyperinsulinemia (14, 25). Moreover, in obese, insulin-resistant subjects, the contribution of the neurogenic and endothelial vasomotion domains is impaired (22). Data on the effects of GH on vasomotion are presently lacking, but the finding that GH replacement therapy is able to increase capillary density in a similar fashion as insulin, suggests that IGF-1 may influence vasomotion. Indeed, in the present study, increasing IGF-1 level leading to significantly more insulin resistance but a lower waist circumference, resulted in more neurogenic activity, whereas decreasing IGF-1 levels resulted in less endothelial activity in the vasomotion analysis. However, increasing IGF-1 levels did result in a reduction in waist circumference, which could have had a favorable effect on the microcirculation. These results are in line with previous studies investigating microvascular vasomotion. De Jongh et al. found that the contribution of the

TABLE 3 | Regression analysis for change in IGF-1 standard deviation score (SDS) and the change in neurogenic and endothelial vasomotion domains and adjusted for change in waist circumference and/or insulin resistance.

	Neurogenic domain			Endothelial domain		
	B	95% CI	P value	B	95% CI	P value
Model 1						
IGF-1 SDS	0.032	(–0.014–0.078)	0.16	0.085	(0.004–0.166)	0.04
Model 2						
IGF-1 SDS	0.015	(–0.033–0.064)	0.52	0.073	(–0.017–0.162)	0.11
Waist circumference	-0.013	(–0.029–0.002)	0.09	–0.009	(–0.037–0.019)	0.50
Model 3						
IGF-1 SDS	0.019	(–0.032–0.071)	0.45	0.078	(–0.014–0.171)	0.10
Insulin resistance	0.112	(–0.087–0.311)	0.26	0.058	(–0.299–0.416)	0.74
Model 4						
IGF-1 SDS	0.001	(–0.053–0.054)	0.97	0.065	(–0.036–0.166)	0.20
Waist circumference	–0.014	(–0.029–0.002)	0.08	–0.010	(–0.038–0.019)	0.50
Insulin resistance	0.119	(–0.073–0.310)	0.21	0.063	(–0.299–0.425)	0.72

frequency spectrum of the neurogenic activity to vasomotion was lower in obese compared to lean women (22). De Boer et al. demonstrated an inverse association of body mass index and trunk fat with the neurogenic vasomotion domain in a different cohort (21). However, these studies were both cross-sectional. This study now demonstrates that by changing waist circumference (by changing IGF-1 level) the neurogenic domain of vasomotion is affected, which strengthens this finding. Next to the change in neurogenic activity, decrease in IGF-1 level in our study led to a decreased contribution of the endothelial activity to the vasomotion, which seemed to be independent of change in waist circumference or insulin resistance. Consequently, this appears to be a direct effect of low IGF-1, perhaps due to the decreased formation of NO (26). Studies on the effect of IGF-1 on endothelial function are scarce. Endothelial cells have high-affinity IGF-1 binding sites and IGF-1 stimulates NO formation by endothelial cells and VSMCs (27). Christ et al. demonstrated in patients with GH deficiency that GH treatment had a beneficial effect on endothelial function (measured by using venous occlusion plethysmography before and after infusion of ACh and of SNP) mediated by endothelium-dependent NO production and/or increase in sensitivity of VSMC to NO (28). One might have expected also an improvement of endothelial function and arterial stiffness in the present study due to the effect of raising NO formation by increasing IGF-1 levels. The lack of effect could be due to sample size and limited follow up time in which the IGF-1 levels were adjusted in a relative small width. Rossi et al. (23) also suggest that skin vasomotion investigation is a more sensitive evaluation of microvascular endothelial function than skin blood flow measurements, explaining that in our study the effect on endothelial function was first demonstrated in the vasomotion measurements after change of GH dose.

This is one of the first studies to explore possible mechanisms for the association of IGF-1 levels within the normal range with cardiovascular risk factors in GH treated GH deficient adults. As mentioned above, a limitation of the study is the overlapping effects of both higher GH doses and higher IGF-1 level which could have influenced the results, for instance with respect to dose-dependent effect of GH on insulin resistance (29). Another factor to take into account when interpreting the results is the total number of statistical tests performed in a relatively small sample. Some of the findings could have been due to chance alone. However, most changes were in the expected direction and a larger sample size or prolonged duration of the intervention with proper adjustments for multiple testing could be expected to demonstrate similar results.

In conclusion, in this exploratory study to elucidate possible mechanism underlying the U-shaped relationship of IGF-1 with CVD, we demonstrated that higher IGF-1 levels are beneficial for body composition but seem to be detrimental with respect to insulin resistance. The contribution of the neurogenic vasomotion domain increased in parallel, and could be explained by the favorable change in waist circumference. It remains to be seen whether the effects on the neurogenic vasomotion domain are indeed beneficial for capillary perfusion and cardiovascular homeostasis, and therefore can be considered a measure of optimal IGF-1 levels. Since the really long-term effect of high-normal IGF-1 target levels during GH treatment remain to be investigated, and also the known suggested association with tumor progression, no clear recommendation on dosing strategy can be given at this moment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the VU University Medical Center, Amsterdam. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CV and MD were responsible for design, execution, analysis of the study, and writing of the manuscript. RM, PL, MK, and ES thoroughly assisted in the design of the study, analysis and writing of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

CV is supported by an AGIKO grant of The Netherlands Organisation for Health Research and Development (ZonMw) (grant number: 92003591). This work was partly supported by an investigator-initiated grant from Pfizer bv.

ACKNOWLEDGMENTS

We direct special gratitude to all participating patients, and to Ingrid Knufman-van Ravenzwaay, research nurse at the clinical research unit Internal Medicine of the VU University Medical Center, for her assistance.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

MINI REVIEW
published: 24 February 2021
doi: 10.3389/fendo.2021.634288

Clinical, Diagnostic, and Therapeutic Aspects of Growth Hormone Deficiency During the Transition Period: Review of the Literature

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *27 November 2020*
Accepted: *11 January 2021*
Published: *24 February 2021*

Citation:
Spaziani M, Tarantino C, Tahani N, Gianfrilli D, Sbardella E, Isidori AM, Lenzi A and Radicioni AF (2021) Clinical, Diagnostic, and Therapeutic Aspects of Growth Hormone Deficiency During the Transition Period: Review of the Literature. Front. Endocrinol. 12:634288. doi: 10.3389/fendo.2021.634288

The role of growth hormone (GH) during childhood and adulthood is well established. Once final stature is reached, GH continues to act during the transition, the period between adolescence and adulthood in which most somatic and psychological development is obtained. The achievement of peak bone mass represents the most relevant aspect of GH action during the transition period; however, equally clear is its influence on body composition and metabolic profile and, probably, in the achievement of a complete gonadal and sexual maturation. Despite this, there are still some aspects that often make clinical practice difficult and uncertain, in particular in evaluating a possible persistence of GH deficiency once final stature has been reached. It is also essential to identify which subjects should undergo re-testing and, possibly, replacement therapy, and the definition of unambiguous criteria for therapeutic success. Moreover, even during the transition phase, the relationship between GH substitution therapy and cancer survival is of considerable interest. In view of the above, the aim of this paper is to clarify these relevant issues through a detailed analysis of the literature, with particular attention to the clinical, diagnostic and therapeutic aspects.

Keywords: transition period, bone, body composition, metabolism, gonad function, growth hormone deficiency, cancer survivors

INTRODUCTION

The importance and effectiveness of somatotropic hormone replacement therapy (rh-GH) in children with low stature and/or linear growth decline associated with growth hormone deficiency (GHD) is well established. In childhood, there is a diagnostic procedural standard and therapeutic success can be easily evaluated based on the increase in the linear growth rate and the repositioning in the family height target. Nevertheless, there are not the same certainties during the transition period, both in diagnostic and therapeutic terms, even though it has been widely debated in the last 2 decades (1).

The transition phase begins with the achievement of final height and ends with the achievement of peak bone mass. The mean age of the transition onset differs according to sex and the achievement of Prader's pubertal stage 5 or final adult height. According to Prader, in pubertal stage 5, the mean age is 14.7 ± 2.2 years in boys and 14.0 ± 2.4 years in girls; when considering the final height, the mean age is 16.8 ± 2.2 years in boys and 15.2 ± 2.0 years in girls, and in both cases with a growth velocity <1.5–2 cm/year (2). The end of the transition period usually corresponds with peak bone mass, which occurs at an average age of 23.1 years in males and 19.9 years in females (3). Other Authors define the end of the transition by referring to the sleep chronotype, A percentage of between 25 and 100% of subjects, which occurs at a mean age of 20.9 years for males or 19.5 years for females (4).

The aim of this review is to address the main questions and doubts related to transition, focusing on the diagnostic, clinical and therapeutic tools.

TABLE 1 | List of the different available GH provocative tests, whit their respective cut-off values.

Test	BMI	Cut-off	References
GHRH + Arginine test	BMI <25 kg/m2	<11 μg/L	(8, 10)
	BMI 25–30 kg/m2	<8 μg/L	(8, 10)
	BMI >30 kg/m2	<4 μg/L	(8, 10)
		<19 μg/L	(11)
Insulin Tolerance Test (ITT)		<5 μg/L	(10)
		<6,1 μg/L	(12)
		<5,6 μg/L	(13)
Glucagon test	BMI <25 kg	<3 μg/L	(10, 14)
	BMI 25–30 kg/m2 (high pretest probability)	<3 μg/L	(10, 14)
	BMI 25–30 kg/m2 (low pretest probability)	<1 μg/L	(10, 14)
	BMI >30 kg/m2	<1 μg/L	(10, 14)
Arginine test		<0.4 μg/L	(11)

GHD DIAGNOSIS IN TRANSITION: THE IMPORTANCE OF RE-TESTING

A percentage of between 25% and 100% of subjects with previous childhood-onset GHD (CO-GHD) display a normalization of GH secretion when undergoing re-testing (5).

It is usually recommended 1–3 months between the end of the childhood rh-GH and the time of re-testing (6). This period should not exceed 6 months (5, 7). Of note, not all subjects need to be re-tested: patients with more than two pituitary hormone deficits; isolated GHD associated with an identified mutation and or a specific pituitary/hypothalamic structural defect, except for an ectopic posterior pituitary; the presence of a transcription-factor mutation (6, 8). The positive predictive value for GHD in adulthood is 96% in the presence of three or more pituitary hormone deficiencies and even 99% in the case of four hormone deficits (9). Re-testing should be carried out in the following cases: idiopathic isolated GHD with or without a small pituitary/ectopic posterior pituitary; GHD associated with only one other pituitary hormone deficiency; patients previously treated with radiation therapy.

Table 1 provides a systematic list of the different available provocative tests, with their respective cut-off values. The gold standard is the Insulin Tolerance Test (ITT) (10–12). In the case of contraindications, such as seizures or confirmed cerebrovascular and cardiovascular risk factors, an alternative is the Glucagon test (10, 14). Another tool used for the diagnosis of GHD is the Arginine test, but it has been shown not to be very effective (11). Finally, the GHRH+Arginine test is not recommended in case of idiopathic GHD, because it may result in a false-positive response (8, 10). In addition, a 2005 study showed that this test should not be used for the diagnosis of GHD in those adults treated with cranial irradiation (CRT) for childhood acute lymphoblastic leukemia, as it could result in a false-positive response (15). For this test, the Italian Medical Agency set the cut-off value to 19 μg/L (16). A recent study investigated the diagnostic accuracy of the GHRH+Arginine test concluding that it should provide specific cut-off points depending on the pathological condition, such as congenital GHD, isolated GHD, multiple pituitary hormone defects and cancer survivors (17).

The possibility of anticipating the time of re-testing, to avoid a hypothetical over-treatment, has been recently investigated by Penta and colleagues (18). The Authors claimed that it would be useful to re-test patients with idiopathic and isolated GHD before reaching final stature or Tanner stage 5, in order to avoid over-treatment.

A special mention should be made of IGF-1: a normal or high IGF1 value alone cannot rule out the diagnosis of GHD (19), as well as a low value alone is not sufficient to make the diagnosis (17, 20). Moreover, to date the chemiluminescence immunoassay should be the preferred method as it is considered the most appropriate and accurate (21).

THE ROLE OF GH DURING TRANSITION: EFFECTS ON BONE, BODY COMPOSITION, METABOLISM, AND GONADAL FUNCTION

There are numerous studies on the effect and importance of GH during the transition phase. Most of them focus on the influence on bone, metabolism and body composition. In this section, we will also mention the impact of GH on fertility.

Bone

GH and its mediator IGF-1 have a cumulative effect of raising bone turnover and mass, leading to an increase in endochondral ossification and linear growth.

Although most studies in the literature agree on the role of GH in improving bone quality, there is no unequivocal opinion

in this regard. Starting with Randomized Controlled Studies (RCTs), Carroll and Drake respectively showed an increase in total body BMC (TB-BMC) of about 6% and lumbar spine BMC (LS-BMC) of about 5% in treated subjects compared to untreated (22, 23). In a 2-year RCT, an increase of 5% in the total body-bone mineral density (TB-BMD) was found in subjects treated with rh-GH, compared to 1.3% of untreated patients (24). In another 2-year RCT, subjects were randomized either to an adult dose of rh-GH or to a paediatric dose or to a placebo, with an increase of TB-BMD, respectively of 3.3%, 5%, and 1.3% (25). By contrast, another multicentre RCT study, in which 18 GHD subjects were randomized to rh-GH and 15 to placebo, no substantial increase in BMD between the two groups was demonstrated (26). The same findings were obtained by Boot and Hyldstrup (27, 28).

A 2014 cross-sectional study compared 18 subjects with previous CO-GHD diagnosis, who were further subdivided into two sub-groups of nine patients, each based on the persistence or not of GHD after re-testing, with 18 healthy subjects. They showed a reduction in TB-BMD and LS-BMD both in GHD and in non-GHD subjects compared to controls, demonstrating that the previous administration of rh-GH was not sufficient to determine a normal BMD (29).

Of note, CO-GHD patients tend to have smaller bones than control subjects; this may lead to underestimation of Areal BMD assessed by dual energy X-ray absorptiometry (DXA) scan (30, 31) and contribute to discrepancies in the interpretation of results among different investigations. Therefore, it should be considered that those patients with CO-GHD, not treated with rh-GH during childhood, have a significant reduction of the Areal BMD, but a normal Volumetric BMD.

Some studies also assessed the impact of GH on the micro-architectural characteristics of bone. A significant increase in both BMD and in cortical thickness in subjects undergoing rh-GH has been found by Hyldstrup et al (28)., whereas another study found a significant reduction in cortical bone area and thickness in untreated CO-GHD adults compared with AO-GHD (32). Of note, there are no studies that unequivocally demonstrate an increased risk of fractures in GHD subjects during the transition (33, 34).

It should be noted that changes related to eating habits, physical activity and socioeconomic status of patients might occur during rh-GH and they can influence the outcome of bone, metabolic, cardiac and body composition parameters. This makes it more difficult to understand the weight of rh-GH alone in all these changes. Moreover, other pituitary deficiencies and their treatments could play a role in bone metabolism, as the prevalence of fractures is markedly increased in patients with multiple pituitary deficiencies (35).

Body Composition

Some studies highlighted that a long period off rh-GH induces a consistent increase in the percentage of fat mass (FM) and trunk fat, with a decrease in lean mass (LM) (36–38). During the transition phase, several papers argued that in patients with a GHD persistence and that do not resume rh-GH, there was a reduction in LM and an increase in FM compared to control subjects or those who resumed rh-GH. The LM decreased by about 8%, compared to an average increase of about 15% in FM (22, 39, 40). The resumption of rh-GH, showed a clear improvement, with an increase in LM of about 14% and a decrease in FM of about 7% after 2 years of therapy (24, 41). In contrast, Mauras et al. found no improvement in body composition after 2 years of treatment (26).

Another paper investigated early body composition changes after the rh-GH break in a population with persistent GHD, finding a significant increase in FM especially in those with multiple pituitary deficits, and a significant reduction in cross-sectional muscle area Z-score (42).

GH exerts considerable effects on skeletal muscle, inducing a global effect of muscle growth through the stimulation of free-fatty acids and amino-acid uptake, and the increase of protein synthesis. Rh-GH should not be used during the transition with the only aim of muscle growth, even though several studies have shown clear lower muscle strength in confirmed GHD subjects compared to sufficient or healthy controls (43). A recent study assessed how 12 weeks of resistance exercise alone could improve muscle strength during the transition (44). Interesting is the interconnection between GH, leptin and ghrelin. Leptin, that reduces the sense of appetite, is primarily secreted by white adipose tissue, and its concentration correlates positively with total FM. Ghrelin has an opposite effect and is mainly secreted by P/D1 cells lining the fundus of the stomach and epsilon cells in the pancreas. Ghrelin represents a powerful stimulator to GH secretion, as it is a natural ligand for the GH-secretagogue receptor (45). In a study by Roemmler and colleagues, it was shown that rh-GH was able to reduce the total FM and consequently the leptin levels, albeit there was a no significant increase of ghrelin (46). This connection between GH, ghrelin and leptin paves the way for the possible role of GH in appetite regulation (47).

Metabolic Aspects and Cardiovascular Risk

Many studies report a worsening of the lipid pattern after discontinuation of the rh-GH (41, 42, 48), although others do not show any substantial difference between patients who resume therapy and subjects who do not (22, 24, 26).

As a general statement we can say that the later treatment begins in childhood and the longer the period off rh-GH lasts during the transition, the worse the lipid profile becomes, with higher total cholesterol and triglyceride levels (49), suggesting that discontinuation of rh-GH during the transition is associated with a pro-atherogenic lipid profile.

Some studies also demonstrated the influence of GH on post-prandial lipid values. Lanes and co-Authors, in 2004, showed an increase in post-prandial triglyceride values (4 h after a high-fat meal) and peripheral inflammatory and fibrinolytic markers in

untreated compared to treated subjects (50). In addition, higher levels of basal triglycerides and overlapping values of post-prandial triglycerides compared to an age-matched control group have been found in a population of GHD subjects. This metabolic situation remained unchanged after 4 months of rh-GH (51).

Regarding the effects on glucose metabolism, there are no unequivocal results. While some papers reported an improvement in insulin sensitivity following therapy discontinuation after reaching final stature (52), other studies came to opposite conclusions. One study, in particular, reported a significant increase in insulin resistance following a period of 6 months off rh-GH, which tended to disappear 6 months after the resumption of therapy (53). Some papers highlighted a possible gender-specific difference in carbohydrate metabolism and body composition. Specifically, in a 2004 metanalysis, it is reported that males are more sensitive to the effect of rh-GH on insulin sensitivity (54). Regarding body composition, women seem to be less sensitive than men, and require higher rh-GH doses to achieve the same benefits (55).

There is currently insufficient data to determine whether rh-GH may induce an increased risk of type 2 diabetes mellitus in the future.

The effects of rh-GH on the heart were evaluated in an echocardiographic study, which compared 21 previously treated patients with 21 age- and sex-matched healthy controls. The Authors found that in the 21 studied patients both the heart height and size were lower than in the controls, despite long-term rh-GH during childhood. Then, eight of the 21 patients were subjected again to rh-GH for 15 months during the transition period and they showed a significant increase in left ventricular mass and an improvement in endothelial function within the first 6 months of restarting rh-GH (56). Finally, a 2003 study reported an increase of intima-media thickness (IMT) in subjects with previous CO-GHD, compared to both adult-onset GHD patients and to controls (57); however, this finding was not confirmed in two further studies (53, 58).. An interesting study, carried out in 20 GH-naive Brazilian adults, due to a homozygous mutation in GHRH receptor gene, demonstrated a significant improvement in their lipid metabolic profile after 6 months of rh-GH, while a progressive increase in the number of atherosclerotic carotid plaques was still noted. Moreover, a relevant increase in both cardiac structural parameters (left ventricular mass index, posterior wall, and septum thickness) and carotid IMT was found after 6–12 months of rh-GH suspension (59).

In relation to vascular reactivity, a study conducted in 10 GHD-treated adolescents, 12 GHD untreated adolescents and 14 controls, noted a lower flow-mediated endothelium-dependent increase in the diameter of the brachial artery during hyperemia in untreated subjects. In addition, the hyperemia-induced blood flow increase was higher in treated patients than in controls and in untreated GHD adolescents. The presence of such vascular abnormalities, together with the increased epicardial adipose tissue thickness, lead to an increased cardiovascular risk in non-treated subjects, even though it is reversible after rh-GH (60). Abnormal vascular reactivity in young GHD adults was also confirmed in a previous study, which demonstrated a reduction of the brachial artery vasodilation induced by specific vasodilators (acetylcholine and sodium nitroprusside) in seven childhood-onset GHD patients. However, these vascular abnormalities were not confirmed in subjects with CO-GHD who had received adequate rh-GH, confirming that GHD was a trigger (61).

Finally, rh-GH can also influence blood pressure. Most of the studies argue that rh-GH induces a reduction in diastolic blood pressure and no change in systolic blood pressure, through an increase in nitric oxide formation, stimulation of the renin-aldosterone system and decrease in intima-media thickness (62). To date, there is only a study in which rh-GH was clearly associated with hypertension (63).

GH and Gonadal Function

The effect of rh-GH on gonadal function has been widely investigated, since it influences the hypothalamic-pituitary-gonadal axis, facilitating the release of GnRH and consequently of gonadotropins (64).

In 1994 our group described the effect of the rh-GH on the improvement of spermatogenesis in 10 infertile patients presenting with idiopathic severe oligozoospermia, normogonadotropinemia, or moderate hypergonadotropinemia, with low IGF-1 values or values within the lower limit of the normal range. Short-term rh-GH led to an improvement in both sperm concentration and motility in 50% of subjects (65). In a subsequent paper, we ruled out the possibility of prolonged rh-GH use negatively affecting testicular development or function (66).

In women, IGF-1 plays a role in the proliferation and differentiation of granulosa cells and stimulates steroidogenesis in large follicles and theca cells. Furthermore, a recent study showed that GH, along with IGF-1, interacts with local ovarian factors, such as VEGF-A and FGF-2, thus being a necessary actor in the ovarian angiogenesis (67).

IMPACT ON QUALITY OF LIFE

The impact of rh-GH on the quality of life (QoL) of GHD patients during the transition period seems less clear than in adulthood.

However, Abs and co-authors showed a positive relationship between stature gain after rh-GH in childhood and the improved QoL during the transition, while a negative relationship between the duration of rh-GH discontinuation and QoL was reported (34). Another paper evaluated through a survey the impact of rh-GH on QoL at baseline and after 1 and 2 years of rh-GH, finding that body shape and sexual arousal were significantly lower after rh-GH suspension, thus negatively affecting the QoL (68). In contrast to Mauras' paper (26), several studies agreed that rh-GH is effective in improving QoL, with a significant positive change in health-related aspects (68, 69).

Finally, the effect of therapy resumption was studied in another couple of studies, which showed a worsening of the QoL after 1 year of rh-GH suspension, counterbalanced following 6 months of rh-GH resumption (70, 71).

WHAT IS THE RIGHT THERAPEUTIC DOSAGE DURING THE TRANSITION?

During the transition, the tendency is to start with an intermediate dose between 0.22 and 0.30 mg/kg/week as in childhood and 0.01–0.1 mg/kg/week as in adulthood (72).

In case the therapy was suspended after reaching final height, it should be resumed at a dosage of 0.21 mg/kg/week, and then titrate based on age, IGF-1 values, clinical response and the possible appearance of adverse effects (73). However, based on our clinical practice, we recommend starting with the lowest therapeutic dose and then adjust dosage according to IGF-1 levels, clinical response and absence (even minimal) of adverse effects. **Table 2** summarises the possible main side effects associated with rh-GH.

In women receiving oral oestrogen replacement therapy, higher doses of rh-GH are typically required, as oral oestrogens seem to attenuate the metabolic actions of GH on its liver receptor, lowering IGF-1 secretion. It would therefore be preferable to use transdermal oestrogens, in order to avoid/attenuate the effect on the liver (74).

Regarding the production of thyroid hormones, rh-GH can cause a slight reduction in FT4 and TSH levels, and an increase in FT3 and could therefore unmask central hypothyroidism (75). Therefore, before starting rh-GH and during treatment, thyroid function should be monitored closely, particularly in the first 6 months.

At the adrenal level, GH can reduce the activity of the enzyme 11βHSD1, resulting in reduced conversion of cortisone to cortisol. Thus, an assessment of the HPA axis function (through the evaluation of basal AM and PM cortisol levels and, when needed, after stimulation, e.g., ACTH test or ITT) should be made before and after starting rh-GH, as GHD could mask the presence of a hidden central hypoadrenalism (76).

Rh-GH, even during the transition, must be carefully monitored to avoid the onset of possible adverse events. Every 6 months, it is advisable to carry out a haematochemical screening, with the evaluation of IGF-1, serum glucose, HbA1c and lipid profile, and a clinical evaluation based essentially on the measurement of weight and waist circumference and blood pressure. Every year a possible improvement in bone mineral density through a DXA scan, and of the intima media thickness by ultrasound examination should be evaluated, as well as any positive changes in QoL (12).

TABLE 2 | Main side effects associated with rh-GH.

Side effects
Headache
Glucose disorders
Insulin resistance
Idiopathic intracranial hypertension
Increased intraocular pressure
Arthralgia

GH TREATMENT IN CANCER SURVIVORS DURING TRANSITION

GHD is the most frequent endocrine disorder in childhood cancer survivors, especially in those with a history of pathology (and treatments) of the hypothalamic-pituitary region (20). In this respect, there is no unequivocal data on the risk of recurrence, as several studies indicate a very low or no-risk (77–80), whereas others provide no certain conclusions (81). The same risk of recurrence in GH-treated cancer survivors compared to non-treated subjects was found in two different studies (82, 83).

Others found no increased risk of cancer recurrence in a rh-GH treated paediatric population (84) and in patients with previous brain cancer (85, 86). As to the risk of developing a secondary neoplasm, a recent meta-analysis indicated that rh-GH seems not increase this risk (87). However, opposite results were found in other relevant studies, such as CCSS, GeNeSIS, and HypoCCS, in which the percentage of second neoplasms was 3.8% in GeNeSIS and 6% in HypoCCS (77, 88).

The Endocrine Society suggests starting rh-GH 1 year after stopping cancer therapy, when there is no more evidence of cancer disease. In the case of chronic or not totally eradicable oncological diseases, the choice whether to start rh-GH or not should be tailored according to the characteristics of the tumor and the patient, after a proper discussion with the oncologist (20).

A very controversial topic is the choice of the therapeutic rh-GH dose. However, it should be appropriate to use the minimum effective dose so as to decrease symptomatology. The most widely used and free of side effects therapy is the daily administration of 0.21 mg/kg/week, adjusted so as to reach normal IGF-1 levels (89).

To summarise, papers published so far show that somatotropin is indicated in those patients who have GHD and it appears safe in terms of tumor recurrence. Of course, caution is extremely important in the follow-up of GHD patients during the transition period, but it would be inappropriate to deprive them of therapy that is central to treating this deficiency.

CONCLUSIONS

Transition is a period in life in which the maturation of the organism is completed. During the transition, GH plays a relevant role on bone maturation, metabolism and body composition. GHD increases cardiovascular risk and impacts fertility negatively. For these reasons, GH is considered extremely important for a good quality of life. There are still uncertainties about when and how to re-test to confirm or not a persistent GHD in patients previously treated with somatotropin, and there is still no total agreement regarding the therapeutic dosage. The link between rh-GH and tumor recurrence in cancer survivors is not

totally clear. However, most of the studies report no evidence of increased recurrence risk in these patients during the transition.

Further studies with a longer duration of rh-GH are needed, in order to assess with more accuracy, the effects of rh-GH during the transition, and eliminate those uncertainties that are still present on this fascinating but often too feared hormone.

AUTHOR CONTRIBUTIONS

All the authors have made a substantial, direct and intellectual contribution to the work. MS revised it critically. All authors approved the work for publication. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 24 February 2021
doi: 10.3389/fendo.2021.637209

Usefulness and Potential Pitfalls of Long-Acting Growth Hormone Analogs

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Daily recombinant human GH (rhGH) is currently approved for use in children and adults with GH deficiency (GHD) in many countries with relatively few side-effects. Nevertheless, daily injections can be painful and distressing for some patients, often resulting in non-adherence and reduction of treatment outcomes. This has prompted the development of numerous long-acting GH (LAGH) analogs that allow for decreased injection frequency, ranging from weekly, bi-weekly to monthly. These LAGH analogs are attractive as they may theoretically offer increased patient acceptance, tolerability, and therapeutic flexibility. Conversely, there may also be pitfalls to these LAGH analogs, including an unphysiological GH profile and differing molecular structures that pose potential clinical issues in terms of dose initiation, therapeutic monitoring, incidence and duration of side-effects, and long-term safety. Furthermore, fluctuations of peak and trough serum GH and IGF-I levels and variations in therapeutic efficacy may depend on the technology used to prolong GH action. Previous studies of some LAGH analogs have demonstrated non-inferiority compared to daily rhGH in terms of increased growth velocity and improved body composition in children and adults with GHD, respectively, with no significant unanticipated adverse events. Currently, two LAGH analogs are marketed in Asia, one recently approved in the United States, another previously approved but not marketed in Europe, and several others proceeding through various stages of clinical development. Nevertheless, several practical questions still remain, including possible differences in dose initiation between naïve and switch-over patients, methodology of dose adjustment/s, timing of measuring serum IGF-I levels, safety, durability of efficacy and cost-effectiveness. Long-term surveillance of safety and efficacy of LAGH analogs are needed to answer these important questions.

Keywords: long-acting growth hormone, treatment adherence, growth hormone deficiency, growth hormone replacement, adults, children

OPEN ACCESS

Edited by:
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Reviewed by:
Martin Bidlingmaier, Ludwig Maximilian University of Munich, Germany
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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *03 December 2020*
Accepted: *11 January 2021*
Published: *24 February 2021*

Citation:
Yuen KCJ, Miller BS, Boguszewski CL and Hoffman AR (2021) Usefulness and Potential Pitfalls of Long-Acting Growth Hormone Analogs. Front. Endocrinol. 12:637209. doi: 10.3389/fendo.2021.637209

INTRODUCTION

The long-term safety and efficacy of daily recombinant human growth hormone (rhGH) therapy in children with GH deficiency (GHD) are well-studied (1–3). However, maintaining maximal treatment adherence with daily rhGH injections is challenging, not only for children, but also for caregivers and for adults with GHD because of device limitations, pain at injection sites, inconvenience of daily injections, lack of perceived immediate benefits, insurance barriers, and costs (4, 5), leading to frequent dose omissions and treatment cessation (6). Thus, it has been hypothesized that a LAGH analog with a lower injection frequency might help mitigate treatment non-adherence, and thereby potentially improve treatment outcomes.

To this end, many pharmaceutical companies have spent a significant amount of money developing LAGH analogs using a several different yet novel technologies to prolong GH action that may allow for weekly (7–18), bi-weekly (19–21), or monthly administration (22, 23). However, there are theoretical reasons to suspect that LAGH analogs might be inferior to daily rhGH administration. The physiologic secretory pattern of GH occurs in an episodic and pulsatile pattern, with several peaks throughout the day and an increased number in the second half of the night during sleep. Concerns that elevated and non-pulsatile GH exposure may downregulate or desensitize GH receptor signaling were unfounded when a study by Laursen et al. (24) demonstrated that subjects who received continuous subcutaneous infusions of GH over 6 months maintained their serum IGF-I levels within the normal range and did not develop any signs or symptoms of acromegaly. In 1999, the first LAGH analog (Nutropin Depot) was approved and marketed in the United States, but later withdrawn due to manufacturing issues and inferior efficacy reported during post-marketing follow-up (21). The latter problems may be related to increased pain at the injection sites compared to daily rhGH injections. After this experience, the lesson learnt from this experience was that the success of developing future LAGH analogs should not only take into consideration of convenience and ease of injection administration, but also non-inferiority in therapeutic efficacy and a side-effect profile comparable to that of daily rhGH therapy.

TREATMENT OF GHD IN CHILDREN AND ADULTS: WHERE WE WERE AND WHERE WE ARE NOW

Treatment with rhGH in children with GHD has been well-established for over 35 years in inducing linear growth and attaining adult height appropriate for genetic potential (2). In early studies involving children with GHD, these patients were receiving rhGH that were administered intramuscularly three days a week because this dosage regimen was based on several clinical observations of its effects on growth response (25). The concept of administering subcutaneous rhGH injections daily was first proposed in 1983 by Kastrup et al. (26), and this treatment regimen was found to be efficacious in inducing linear growth and less inconvenient to children. By contrast, current recommendations are weight-based or body surface-based dosing at the start of treatment followed by individualized dosing in accordance with clinical response, with higher rhGH doses preferred for those with greater severity of GHD, and subsequent dose adjustments made according to growth response (1, 2). Serum IGF-I levels are used to monitor for adherence, efficacy and safety (27); if these levels exceed the upper limit of the age-appropriate reference range, then reductions of the rhGH dose should be considered for safety reasons (1, 2). In some cases where treatment response has been inadequate, re-evaluation of other etiologies of short stature and non-adherence are recommended (27).

The first studies assessing the effects of rhGH replacement in adults with GHD was performed in 1989 (28, 29). These studies demonstrated improvements in body composition, exercise capacity, muscle strength, bone remodeling, and dyslipidemia. This prompted a flurry of publications in the literature between 1989 and 1999 in adults with GHD, and the results from many of these studies corroborated with the observations from the two initial pivotal trials in 1989 (28, 29). Further dose-finding clinical studies in various age groups were then performed (30–32) and interestingly, these studies found that adults are inherently more sensitive to the effects of rhGH than children in terms of serum IGF-I generation and rate of side-effects (33), and that males are more responsive to rhGH therapy than females (34, 35). These and other data have resulted in the approval for rhGH replacement in children and adults with GHD by the United States Food and Drug Administration (FDA) and European Medicines Agency in 1985 and 1996, respectively, and translated into several published consensus guidelines for the management of children (2) and adults with GHD (36, 37). Subsequent studies in adults with GHD performed since 1999 have further corroborated the positive effects of rhGH therapy on quality of life, exercise capacity and bone mineral density (38). However, whether rhGH replacement can normalize or decrease the mortality rates in these patients remains debatable. Treatment-related side-effects, mainly related to fluid retention and impaired glucose tolerance, are dose-dependent and related to increased GH sensitivity associated with aging, and are often reversible upon dose reductions or treatment cessation. Caution and close monitoring are recommended with an emphasis on lower rhGH dosing at treatment initiation and maintenance to avoid over-treatment, especially in older, obese, and glucose-intolerant patients as they are generally more susceptible to side-effects. More recent consensus guidelines have emphasized the importance of individualized rhGH dosing in the treatment of adults with GHD, with the primary objective of achieving normalization of age-appropriate serum IGF-I levels (38).

LONG-ACTING GH ANALOGS: USEFULNESS, MECHANISM/S AND CURRENT STATUS

The main indication for the development of LAGH analogs in children and adults with GHD is to improve patient adherence

and to ease the burden of chronic daily injections. While many early LAGH analogs were not shown to be effective or practical (39), two LAGH analogs (Eutropin Plus and Jintrolong) are currently being marketed in Asia, one (Somapacitan) was recently approved in the United States, one (Eutropin Plus) was previously approved in Europe but never marketed, and several more close to being considered for regulatory approval in the United States and Europe (**Table 1**). The methodology for creating LAGH analogs can be classified into two broad groups: formulations that create a subcutaneous depot from which native or modified GH is slowly released into the circulation, and formulations that permit rapid absorption from the delivery site in the subcutaneous tissue and delayed removal from the circulation. Other new development methods being studied include reversible complexes to stabilize the GH molecule, fabrication of sustained release preparations that utilize various matrices to bind to the GH molecule, and structural modifications of the GH molecule itself.

In 1979, after the publication by Lippe et al. (71) using a depot GH preparation in gelatin solution, the next generation of LAGH analogs to be developed was a native rhGH that was micronized, zinc-stabilized and encapsulated in microspheres (Nutropin Depot). Several other LAGH analogs have since been developed and additional studies performed to assess longitudinal growth velocity in children and changes in body composition in adults as primary endpoints (16, 19, 21, 22, 72).

TABLE 1 | Overview of the development history of LAGH analogs.

Company	LAGH analog	Modification to GH molecule	Frequency of administration	Current status	Research data
Depot Formulation		**Depot Chemical**			
Altus Pharmaceuticals	ALTU-238	Long-extended release formulation using protein crystallization technology (22 kDa) (39)	7 days	Althea acquired assets in 2010	No further studies planned
Critical Pharmaceuticals	CP016	Supercritical carbon dioxide, formed when CO_2 exceeds its thermodynamic critical point, used to create the depot (22 kDa) (39)	14 days	Company under liquidation	Evidence of ongoing studies at other corporations
Genentech	Nutropin Depot®	Encapulsated in biocompatible, biodegradable, polylactide-coglycolide polymer microsphere (22 kDa) (40)	14 days	Removed from market (39)	
LG Life Sciences, Ltd	Eutropin Plus™(LB03002)	Microparticles containing GH incorporated into sodium hyaluronate and dispersed in an oil base of medium-chain triglycerides (22 kDa)	7 days	Marketed in Korea for childhood GHD; approved in Europe but not marketed in the EU	Phase 3 trial in CGHD suggest non-inferiority (41), safety data from a Korean registry database in children with growth disorders (42), Phase 2 trial in children with ISS demonstrated non-inferiority and well-tolerated (43)
PEGylated Formulations		**PEGylation prolongs *in vivo* mean residence time of GH, through slowing absorption and protection from proteolysis**			
Ambrx	ARX201	30-kDa PEG added to unnatural amino acid incorporated into GH (52 kDa)	7 days	No longer being developed (39) due to PEGylated-containing vacuoles in the epithelial cells of the choroid plexus in monkeys (44)	
Bolder BioTechnology	BBT-031	Site-specific PEGylated GH analog (not available)	7 days (planned)	Preclinical studies (45)	
GeneScience Pharmaceuticals Co, Ltd	Jintrolong®	40-kDa PEG attached to GH (62 kDa)	7 days (13, 16)	Marketed in China for CGHD	Phase 3 studies show good IGF-I profile, Phase 4 studies now ongoing
Novo Nordisk	NNC126-0083	43-kDa PEG residue attached to glutamine 141 (65 kDa)	7 days	Unsatisfactory IGF-I profile peak and duration (46)	No longer being developed as of 2011
Pfizer	PHA-794428	Branched 40 kD PEG on N-terminus of GH (62 kDa)	7 days	High rate of lipoatrophy at injection site (47)	No longer being developed as of 2009

(Continued)

TABLE 1 | Continued

Company	LAGH analog	Modification to GH molecule	Frequency of administration	Current status	Research data
Pro-Drug formulation		**Mechanism of conversion to active drug**			
Ascendis	TransCon GH® (ACP-001)	Unmodified rhGH transiently bound to a PEG carrier molecule *via* a self-cleaving linker that is dependent upon pH and temperature (22 kDa)	7 days (8, 12, 14, 18, 48)	Phase 2 studies in CGHD and AGHD showed comparable GH and IGF-I profile to daily GH dosing Phase 3 studies in CGHD showed positive growth response (49)	Completed Phase 3 study in CGHD and data submitted to FDA and EMA Phase 3 study in AGHD currently planned
Non-covalent albumin binding GH compound(s)		**Albumin binding**			
Novo Nordisk A/S	Sogroya® (NNC0195-0092)	Single-point mutation in GH, with albumin binding moiety attached (non-covalent albumin-binding properties) (50, 51) (23 kDa)	7 days (52)	Phase 2 studies in CGHD showed comparable IGF-I profile to daily GH dosing (53) Phase 3 studies in AGHD well tolerated (54–56) Approved by the FDA in August 2020 for use in AGHD but not marketed yet	Phase 3 studies in CGHD, Phase 2 studies in SGA
GH Fusion Proteins		**Protein fused with GH**			
Ahngook Pharmaceutical Co, Ltd	AG-B1512	Recombinant GH genetically fused to a polypeptide linker and an anti-human serum albumin Fab antibody (~72 kDa)	14 or 28 days (57)	Preclinical studies show IGF-I level elevation sustained for 20 days	Ongoing research
Alteogen	ALT-P1	rhGH fused with NexP™, recombinant a1-antitrypsin (~74 kDa) (58)	unknown	Stopped Phase 2 study in CGHD (59)	
Asterion	ProFuse™ GH	GH binding protein (~82 kDa) (60)	1 month (planned)	Preclinical studies to provide intravascular stores of inactive GH	
Genexine and Handok	GX-H9	rhGH fused to hybrid non-cytolytic immunoglobulin Fc portions of IgD and IgG4 (100 kDa) (61)	7-14 days (62)	Phase 2 studies in AGHD completed (63) Phase 2 studies in CGHD showed reassuring height changes	Phase 3 studies in CGHD with twice-monthly dosing ongoing
Hanmi Pharmaceutical Co	LAPS rhGH (HM10560A)	Homodimeric aglycosylated IgG4 Fc fragment (~51 kDa) (64)	7-14 days (64)	Phase 2 in AGHD show good tolerability	Phase 3 studies in AGHD (65)
JCR Pharmaceuticals	JR-142	Engineered hGH fused at C-terminus with modified human serum albumin at N-terminus (~88 kDa) (66)	7 days	Preclinical trials	Phase 1 study completed (67)
OPKO Health and Pfizer	Somatrogon (MOD-4023)	rhGH fused to three copies of carboxyl-terminal peptide (CTP) of hCG β-subunit (47.5 kDa)	7 days (11, 15)	Phase 2 studies in CGHD (68), Phase 3 studies in AGHD did not meet primary endpoint of truncal fat reduction (17) Phase 3 studies in CGHD showed non-inferior improvement in height velocity with good tolerability	Phase 3 study in CGHD completed (69), and extension studies now ongoing
Teva	Albutropin (TV-1106)	Human serum albumin fused to N-terminus of GH (88 kDa)	7 days (9, 10)	Studies in AGHD discontinued for unknown reason; presumed unfavorable benefit:risk profile	
Versartis	Somavaratan (VRS-317)	Fusion protein of rhGH and the pharmacologically inactive portion of long chains of natural hydrophilic amino acids (XTEN technology)	7, 14 or 28 days (22)	No longer being developed as of 2017 as the Phase 3 study did not meet its primary end-point for non-inferiority comparison against daily rhGH for height velocity in CGHD (22)	

AGHD, adults with GH deficiency; CGHD, children with GH deficiency; EMA, European Medicines Agency; EU; European Union; FDA, Food and Drug Administration; kDa, kilodalton; ISS, idiopathic short stature; PEG, poly(ethylene glycol); rhGH, recombinant human GH; SGS, small for gestational age. Table is modified from Miller BS, et al. (70).

Among them, Eutropin Plus, a depot formulation of rhGH, was approved in South Korea in 1992 and in Europe in 2013, but because it was not marketed in Europe for 3 years, its authorization in Europe lapsed. Jintrolong, a PEGylated GH analog, and Somapacitan, an analog of rhGH containing a fatty acid linker which binds reversibly to serum albumin, have been approved for use in children and adults in China and United States, respectively. In September 2017, Versartis, Inc., the

manufacturer of Somavaratan (VRS-317), a molecule with extra amino acids added to the head and the tail of GH, reported data from the VELOCITY Phase 3 clinical trial that the drug had failed to meet its primary end-point for non-inferiority when compared against daily rhGH (Genotropin) for height velocity in children with GHD (9.44 cm *vs* 10.70 *cm* for those receiving daily rhGH) (22). Based on these findings, the company subsequently made the decision to suspend its manufacture and all clinical trials, and withdrew its United States Investigational Drug Application and equivalent filings in other countries (73). Other LAGH analogs also met the same fate in being discontinued from manufacture for a multitude of reasons: ARX201 due to the development of PEGylated-containing vacuoles in the epithelial cells of the choroid plexus in monkeys (39, 74), NNC126-0083 related to unsatisfactory IGF-I profiles at the doses administered (39, 74), PHA-794428 due to high rates of injection-site lipoatrophy (mainly in women) (47), TV-1106 due to the development of potentially inactivating antibodies (10), and ALTU-238 because the manufacturer had run out of funds (74, 75). On the other hand, studies on the efficacy and safety of other LAGH analogs, such as GX-H9, show promise and are currently being evaluated in ongoing Phase 3 clinical trials (76).

In April 2020, TransCon GH (Lonapegsomatropin®, Ascendis Pharma A/S), a sustained-release inactive prodrug of unmodified GH transiently bound to an inert carrier molecule designed to release fully active GH over one week, was granted Orphan Drug Designation by the FDA, after previously receiving Orphan Designation for the treatment of GHD in Europe from the European Commission in October 2019 (https://www.globenewswire.com/news-release/2020/04/15/2016859/0/en/Ascendis-Pharma-A-S-Receives-Orphan-Drug-Designation-for-TransCon-hGH-as-Treatment-for-Growth-Hormone-Deficiency-in-the-United-States.html). In a Phase 1 randomized trial, 44 healthy subjects were treated with 4 different doses of weekly TransCon GH and 2 different doses of daily rhGH. These investigators discovered that TransCon GH was well-tolerated with no binding antibody formation and comparable levels of serum GH and IGF-I were obtained (12). Phase 2 trials in 37 adults with GHD and 53 previously untreated prepubertal children with GHD also revealed comparable safety and efficacy of TransCon GH when compared to daily rhGH (8, 14). In a recent Phase 3 heiGHt trial (NCT02781727) (18) involving 161 previously untreated prepubertal children with GHD that received either weekly TransCon GH or daily Genotropin, annualized height velocity after 1 year was greater with TransCon GH compared to Genotropin (11.2 *vs* 10.3 cm). The preliminary data of the Phase 3 fliGHt trial (NCT03305016) on children with GHD who switched over from daily rhGH injections to once-weekly TransCon injections were presented at the 2020 Endocrine Society Annual Meeting (ENDO 2020) (77). In this study, dose titrations of TransCon GH demonstrated a predictable serum IGF-I response and a similar side-effect profile to daily rhGH therapy. Ascendis Pharma A/S reports that an application to the FDA and European Medicines Agency has been filed based on these data and those from fliGHt and enliGHten (long-term extension) studies with a Prescription Drug User Fee Act date tentatively set for June 25, 2021 (78).

In June 2020, the data from a Phase 3 trial of Somatrogon hGH-CTP (MOD-4023, Pfizer/OPKO Biologics), a long-acting derivative of rhGH modified by the addition of three C-terminal peptide segments from human chorionic gonadotropin to allow for once-weekly delivery, in children with GHD (NCT02968004) were presented at ENDO 2020 (79). Previously untreated prepubertal children with GHD received either weekly Somatrogon hGH-CTP (0.66 mg/kg/wk) or once daily Genotropin (0.24 mg/kg/wk) for 12 months. The annualized height velocity annualized height velocity after 1 year on Somatrogon hGH-CTP therapy was higher and non-inferior compared to Genotropin (10.1 *vs* 9.8 cm). This trial also demonstrated that children receiving Somatrogon hGH-CTP reported good tolerability with lower treatment burden than Genotropin. Based on these data, Pfizer Inc. is expected to file for FDA approval in early 2021 (80).

In August 2020, once-weekly Somapacitan (Sogroya®, Novo Nordisk A/S, Denmark) was approved by the FDA for treatment of adult GHD (https://www.fda.gov/drugs/drug-safety-and-availability/fdaapproves-weekly-therapy-adult-growth-hormone-deficiency). Somapacitan is a long-acting human GH derivative to which a small noncovalent albumin-binding moiety is attached to facilitate reversible binding to endogenous albumin, delaying its elimination, and thereby extending its duration of action with little to no accumulation of the drug when administered once-weekly (52). In previous short-term clinical trials, Somapacitan was well-tolerated in healthy adults (81) and in adults and children with GHD (53, 54, 82), and provided similar safety and efficacy to daily rhGH in previously rhGH-treated adults with GHD (54, 55). In a Phase 3, 26-week randomized, controlled multi-center study of 92 adults with GHD treated with Somapacitan or daily Norditropin, Somapacitan was well-tolerated, IGF-I standard deviation scores remained in the therapeutic range, and patients preferred the weekly Somapacitan therapy (54). In another Phase 3 study (REAL 1 trial –NCT02229851) of 257 adults with GHD, Somapacitan treatment for 86 weeks demonstrated superiority to placebo in improving body composition and serum IGF-I levels (56), and was well-tolerated with patients preferring Somapacitan to daily rhGH injections for administration convenience. However, although recently approved by the FDA in the United States, Somapacitan will not be available for commercial use in the foreseeable future as the launch date is yet to be determined by its manufacturer. Conversely, the Phase 3 testing of Somapacitan (REAL 4 trial – NCT03811535) in children with GHD commenced in 2019 and is expected to conclude sometime in 2021 (83).

POTENTIAL PITFALLS OF LAGH ANALOGS

Questions have arisen regarding dosing (particularly whether there are any differences in dose initiation between GH-naïve and switch-over patients from rhGH daily injection and how to adjust dosing during therapy), safety monitoring, and whether

LAGH analogs would be as effective and safe compared to daily rhGH because of the differences in pharmacokinetics and pharmacodynamics, as they are not physiologic. Furthermore, because the therapeutic response to daily rhGH injections can be highly variable among patients and may be influenced by multiple factors (e.g., age, time of diagnosis of GHD, gender, body mass index, severity of GHD, quality of life, other pituitary hormone replacements, and GH receptor polymorphisms) (84), it is likely that similar variability to therapeutic responses will be observed with LAGH analogs as well.

It is also anticipated that LAGH analogs will share many, if not all, of the known side-effects of daily rhGH. However, because of the mechanism by which GH action is prolonged and the duration of prolongation, additional safety risks may be present. New safety concerns may include the formation of neutralizing anti-drug antibodies, and growth and metabolic effects related to the altered profile of serum GH and IGF-I levels during therapy. Furthermore, in those drugs where modifications of the GH molecule have been made, there may be a risk of anti-GH antibodies developing. Anti-GH antibodies formed against rhGH given as a daily injection have not been previously shown to be clinically relevant, except in individuals with GH gene deletions (85, 86). If neutralizing antibodies develop against a modified GH molecule, there is a possibility that the individual would not or only partially respond to the unmodified rhGH. As the methods of measuring anti-drug antibodies are not universally consistent, it is important to determine its clinical impact and long-term clinical relevance. Additionally, accurate and reliable anti-drug antibody assays for each LAGH available are required and be made available to clinicians, and when to test for these antibodies while on treatment. Furthermore, it remains unknown if the likelihood of developing anti-drug antibodies is increased if an individual is inevitably switched from one LAGH analog to another.

Another potential pitfall of LAGH analogs is the impact of prolonged elevated serum GH levels after an injection of a LAGH analog resulting in the relative lack of daily GH nocturnal peak and daytime trough profile, unlike the profile with daily rhGH injections at bedtime. This may cause long-term metabolic aberrations since GH is closely involved in the regulation of fat and glucose metabolism, and body composition (39, 87, 88). Furthermore, due to the low levels of GH prior to the next LAGH injection, the use of LAGH in infants and young children with hypoglycemia associated with severe GHD may put them at unnecessary risk. To date, clinical trials of LAGH have not included children less than 2.5 years of age. Growth hormone fusion proteins may have differing therapeutic efficacy profiles because access of the modified GH may be restricted to different key target tissues due to the large overall size of the protein.

The profile of the IGF-I response to each LAGH analog that differs from daily rhGH injections may present with some unique safety concerns. Early epidemiological studies have demonstrated associations of elevated and high normal serum IGF-I levels with increased risk of cancers (89). A specific serum IGF-I cut-off level has not been identified above which there is documented increase in the risk of any known side-effect of daily rhGH injections (1). Depending upon the bioavailability of the LAGH analogs and dose administered, peak serum IGF-I levels achieved with LAGH may need to be relatively higher in order to achieve therapeutic clinical efficacy, but whether there are negative implications of transient elevations of serum IGF-I levels remains to be elucidated. Better understanding of the pharmacokinetic and pharmacodynamic profiles of each individual LAGH analog is required to ascertain the optimal timing of serum IGF-I measurements for both safety and efficacy. Other methodologies of assessing serum IGF-I levels that do not need to take into account of the timing of serum IGF-I measurement in relation to the LAGH analog injections, such as calculating the IGF-I area under the curve, utilizing a mathematical formula and/or measuring other surrogate markers, may be considered but needs further studies to validate their accuracy and reliability. It is also important to avoid inducing supra-physiological IGF-I levels for too long in between LAGH analog injections (39), as this could increase the risk of iatrogenic acromegaly, neoplasia and glucose intolerance. In children with GHD, although monitoring of serum IGF-I levels is recommended, hard evidence supporting this practice or finding a "safe" upper limit to target serum IGF-I levels are lacking (1). Conversely, the question of when to measure serum IGF-I levels does not pose such an issue with daily rhGH injections because these levels stabilize several days after injection, so measurements of that hormone at any time during therapy can been used as a basis to guide dosing. As for LAGH analogs, serum IGF-I levels can rise and fall over several days and may differ in the degree of fluctuations between injections with different LAGH analogs. Therefore, it is still unclear if dosing adjustments of LAGH analogs should be adjusted based on the peak, nadir, or a mathematically calculated mean of several serum IGF-I measurements in between injections, and whether these factors differ between other LAGH analogs.

When new LAGH analogs become commercially available, their use in clinical practice will be determined by coverage through insurance programs or government health policies. In countries with a single payer program, the coverage of LAGH analogs will be assessed not only for safety and efficacy, but also for cost-effectiveness compared with daily rhGH injections. It is possible that insurance carriers and governmental health policies may decide against covering LAGH analogs simply for the sole purpose that LAGH analogs are "convenient" because of the lower frequency of administration, especially if the costs are higher than daily rhGH injections.

Finally, post-marketing surveillance registries are recommended to enable surveillance of LAGH analogs for efficacy, safety, tolerability, cost-effectiveness, and therapeutic durability. Since each individual LAGH analog is unique in its formulation and molecular structure, further studies are needed for each individual LAGH molecule to better understand its pharmacokinetic and pharmacodynamic properties. It would be even more beneficial to set up a combined registry of all LAGH analogs used for treatment of children and adults with GHD in an independent data repository supported by the manufacturers of these compounds. This would enable manufacturers to fulfil their obligatory safety reporting

requirements from governmental agencies, facilitate collaborative "real-world" studies, and increase the power of the studies. A global registry would also be an ideal platform to capture the data on the impact of patients being initiated or switched from daily rhGH to LAGH analogs and from one LAGH analog to another.

DISCUSSION

The major usefulness of LAGH analogs when compared with current rhGH formulations is that the former requires significantly lesser number of injections compared to the latter. However, given the unphysiologic profile of LAGH analogs, new safety concerns have been raised. Prolonged elevated GH levels might induce supra-physiologic serum IGF-I levels and induce iatrogenic acromegaly, neoplasia and glucose intolerance. Nevertheless, these concerns have reassuringly not been substantiated by any robust evidence in numerous published clinical trials thus far. Because each individual LAGH analog has its own unique pharmacokinetic and pharmacodynamic features, safety issues, dose titrations and therapeutic monitoring need to be individually addressed. Pitfalls of LAGH analogs include whether there are pathophysiological long-term implications of prolonged supra-physiologic elevations of serum GH and IGF-I levels, differences in tissue distribution and tissue sensitivity to modified GH molecules, development of anti-drug antibodies, and differences in the side-effect profile compared with daily rhGH injections. The cost-effectiveness of LAGH analogs *vs* daily rhGH injections is another key question that requires resolution. Perhaps the key overarching question is will LAGH analogs increase treatment adherence, and improve treatment efficacy and long-term outcomes without sacrificing patient safety? Though it seems plausible that this presumption might hold true in certain patient populations, this question to date has not been proven and needs to be prospectively tested further in well-designed clinical trials with the answer likely to be dependent on multiple external and individual factors. Clearly there is still much to be learned moving forward in the coming years, but for now, the available data seem to suggest that LAGH analogs are a useful addition to currently available daily rhGH injections, especially for patients who are not coping with the rigors of daily rhGH injections but yet are wanting to continue as they are obtaining clear benefits from this therapy. Finally, we recommend starting surveillance registries once LAGH analogs are approved and become commercially available so that data on efficacy, safety, tolerability, and cost-effectiveness can be collected in large numbers to improve our understanding of the effects of prolonged exposure to these analogs.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of Interest: KY is an investigator on research grants from Pfizer, Novo Nordisk, and OPKO Biologics, and has consulted for Pfizer, Novo Nordisk, Sandoz, and Ascendis. BM is an investigator on research grants from Alexion, Abbvie, Amgen, Ascendis, Novo Nordisk, OOKO Biologics, Protalix, Sangamo, Sanofi Genzyme, Tolmar, and Takeda and has consulted for Abbvie, Ascendis, BioMarin, Bluebird Bio, Novo Nordisk, Pfizer, Sandoz, Sanofi Genzyme, Tolmar, and Vertice. AH is supported by the Biomedical Research Service of the Department of Veterans Affairs and has consulted for Ascendis, GeneScience, Genexine, Novo Nordisk, Pfizer, and Versartis.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CORRECTION
published: 28 June 2021
doi: 10.3389/fendo.2021.705241

Corrigendum: Usefulness and Potential Pitfalls of Long-Acting Growth Hormone Analogues

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 04 May 2021
Accepted: 24 May 2021
Published: 28 June 2021

Citation:
Yuen KCJ, Miller BS, Boguszewski CL and Hoffman AR (2021) Corrigendum: Usefulness and Potential Pitfalls of Long-Acting Growth Hormone Analogues. Front. Endocrinol. 12:705241. doi: 10.3389/fendo.2021.705241

Keywords: long-acting growth hormone, treatment adherence, growth hormone deficiency, growth hormone replacement, adults, children

A Corrigendum on

Usefulness and Potential Pitfalls of Long-Acting Growth Hormone Analogs
by Yuen KCJ, Miller BS, Boguszewski CL, Hoffman AR. (2021). Front. Endocrinol. 12:637209. doi: 10.3389/fendo.2021.637209

ERROR IN TABLE 1, 5TH COLUMN, 19TH ROW

In the original article, there was a mistake in **Table 1** as published. In **Table 1**, 5th column, 19th row, the company "Alteogen" under the "Current Status" column was incorrectly stated that the company was "bankrupt in 2009". This statement is incorrect as the company remains a currently viable bio-tech company globally.

The authors apologize for this inadvertent error with the statement and a modified **Table 1** is provided below, where the statement "bankrupt in 2009" is now deleted. This new table does not change the scientific conclusions of the article in any way. The original article has been updated.

TABLE 1 | Overview of the development history of LAGH analogues.

Company	LAGH analog	Modification to GH molecule	Frequency of administration	Current status	Research data
Depot Formulation		**Depot Chemical**			
Altus Pharmaceuticals	ALTU-238	Long-extended release formulation using protein crystallization technology (22 kDa) (39)	7 days	Althea acquired assets in 2010	No further studies planned
Critical Pharmaceuticals	CP016	Supercritical carbon dioxide, formed when CO_2 exceeds its thermodynamic critical point, used to create the depot (22 kDa) (39)	14 days	Company under liquidation	Evidence of ongoing studies at other corporations
Genentech	Nutropin Depot®	Encapsulated in biocompatible, biodegradable, polylactide-coglycolide polymer microsphere (22 kDa) (40)	14 days	Removed from market (39)	
LG Life Sciences, Ltd	Eutropin Plus™ (LB03002)	Microparticles containing GH incorporated into sodium hyaluronate and dispersed in an oil base of medium-chain triglycerides (22 kDa)	7 days	Marketed in Korea for childhood GHD; approved in Europe but not marketed in the EU	Phase 3 trial in CGHD suggest non-inferiority (41), safety data from a Korean registry database in children with growth disorders (42), Phase 2 trial in children with ISS demonstrated non-inferiority and well-tolerated (43)
PEGylated Formulations		**PEGylation prolongs *in vivo* mean residence time of GH, through slowing absorption and protection from proteolysis**			
Ambrx	ARX201	30-kDa PEG added to unnatural amino acid incorporated into GH (52 kDa)	7 days	No longer being developed (39) due to PEGylated-containing vacuoles in the epithelial cells of the choroid plexus in monkeys (44)	
Bolder BioTechnology	BBT-031	Site-specific PEGylated GH analog (not available)	7 days (planned)	Preclinical studies (45)	
GeneScience Pharmaceuticals Co, Ltd	Jintrolong®	40-kDa PEG attached to GH (62 kDa)	7 days (13,16)	Marketed in China for CGHD	Phase 3 studies show good IGF-I profile, Phase 4 studies now ongoing
Novo Nordisk	NNC126-0083	43-kDa PEG residue attached to glutamine 141 (65 kDa)	7 days	Unsatisfactory IGF-I profile peak and duration (46)	No longer being developed as of 2011
Pfizer	PHA-794428	Branched 40 kD PEG on N-terminus of GH (62 kDa)	7 days	High rate of lipoatrophy at injection site (47)	No longer being developed as of 2009
Pro-Drug formulation		**Mechanism of conversion to active drug**			
Ascendis	TransCon GH® (ACP-001)	Unmodified rhGH transiently bound to a PEG carrier molecule *via* a self-cleaving linker that is dependent upon pH and temperature (22 kDa)	7 days (8, 12, 14, 18, 48)	Phase 2 studies in CGHD and AGHD showed comparable GH and IGF-I profile to daily GH dosing Phase 3 studies in CGHD showed positive growth response (49)	Completed Phase 3 study in CGHD and data submitted to FDA and EMA Phase 3 study in AGHD currently planned
Non-covalent albumin binding GH compound(s)		**Albumin binding**			
Novo Nordisk A/S	Sogroya® (NNC0195-0092)	Single-point mutation in GH, with albumin binding moiety attached (non-covalent albumin-binding properties) (50, 51) (23 kDa)	7 days (52)	Phase 2 studies in CGHD showed comparable IGF-I profile to daily GH dosing (53) Phase 3 studies in AGHD well tolerated (54–56) Approved by the FDA in August 2020 for use in AGHD but not marketed yet	Phase 3 studies in CGHD, Phase 2 studies in SGA
GH Fusion Proteins		**Protein fused with GH**			
Ahngook Pharmaceutical Co, Ltd	AG-B1512	Recombinant GH genetically fused to a polypeptide linker and an anti-human serum albumin Fab antibody (~72 kDa)	14 or 28 days (57)	Preclinical studies show IGF-I level elevation sustained for 20 days	Ongoing research

(Continued)

TABLE 1 | Continued

Company	LAGH analog	Modification to GH molecule	Frequency of administration	Current status	Research data
Alteogen	ALT-P1	rhGH fused with NexP™, recombinant a1-antitrypsin (~74 kDa) (58)	unknown	Stopped Phase 2 study in CGHD (59)	
Asterion	ProFuse™ GH	GH binding protein (~82 kDa) (60)	1 month (planned)	Preclinical studies to provide intravascular stores of inactive GH	
Genexine and Handok	GX-H9	rhGH fused to hybrid non-cytolytic immunoglobulin Fc portions of IgD and IgG4 (100 kDa) (61)	7-14 days (62)	Phase 2 studies in AGHD completed (63) Phase 2 studies in CGHD showed reassuring height changes	Phase 3 studies in CGHD with twice-monthly dosing ongoing
Hanmi Pharmaceutical Co	LAPS rhGH (HM10560A)	Homodimeric aglycosylated IgG4 Fc fragment (~51 kDa) (64)	7-14 days (64)	Phase 2 in AGHD show good tolerability	Phase 3 studies in AGHD (65)
JCR Pharmaceuticals	JR-142	Engineered hGH fused at C-terminus with modified human serum albumin at N-terminus (~88 kDa) (66)	7 days	Preclinical trials	Phase 1 study completed (67)
OPKO Health and Pfizer	Somatrogon (MOD-4023)	rhGH fused to three copies of carboxyl-terminal peptide (CTP) of hCG β-subunit (47.5 kDa)	7 days (11, 15)	Phase 2 studies in CGHD (68), Phase 3 studies in AGHD did not meet primary endpoint of truncal fat reduction (17) Phase 3 studies in CGHD showed non-inferior improvement in height velocity with good tolerability	Phase 3 study in CGHD completed (69), and extension studies now ongoing
Teva	Albutropin (TV-1106)	Human serum albumin fused to N-terminus of GH (88 kDa)	7 days (9,10)	Studies in AGHD discontinued for unknown reason; presumed unfavorable benefit:risk profile	
Versartis	Somavaratan (VRS-317)	Fusion protein of rhGH and the pharmacologically inactive portion of long chains of natural hydrophilic amino acids (XTEN technology)	7, 14 or 28 days (22)	No longer being developed as of 2017 as the Phase 3 study did not meet its primary end-point for non-inferiority comparison against daily rhGH for height velocity in CGHD (22)	

AGHD, adults with GH deficiency; CGHD, children with GH deficiency; EMA, European Medicines Agency; EU, European Union, FDA, Food and Drug Administration; kDa, kilodalton; ISS, idiopathic short stature; PEG, poly(ethylene glycol); rhGH, recombinant human GH; SGS, small for gestational age. Table is modified from Miller BS, et al. (70).

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 25 February 2021
doi: 10.3389/fendo.2021.635983

Impact of Long-Term Growth Hormone Replacement Therapy on Metabolic and Cardiovascular Parameters in Adult Growth Hormone Deficiency: Comparison Between Adult and Elderly Patients

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 30 November 2020
Accepted: 14 January 2021
Published: 25 February 2021

Citation:
Scarano E, Riccio E, Somma T, Arianna R, Romano F, Di Benedetto E, de Alteriis G, Colao A and Di Somma C (2021) Impact of Long-Term Growth Hormone Replacement Therapy on Metabolic and Cardiovascular Parameters in Adult Growth Hormone Deficiency: Comparison Between Adult and Elderly Patients. Front. Endocrinol. 12:635983. doi: 10.3389/fendo.2021.635983

Growth hormone deficiency (GHD) in adults is due to a reduced growth hormone (GH) secretion by the anterior pituitary gland which leads to a well-known syndrome characterized by decreased cognitive function and quality of life (QoL), decreased bone mineral density (BMD), increased central adiposity with a reduction in lean body mass, decreased exercise tolerance, hyperlipidemia and increased predisposition to atherogenesis. Considering some similar features between aging and GHD, it was thought that the relative GH insufficiency of the elderly person could make an important contribution to the fragility of elderly. GH stimulation tests are able to differentiate GHD in elderly patients (EGHD) from the physiological reduction of GH secretion that occurs with aging. Although there is no evidence that rhGH replacement therapy increases the risk of developing Diabetes Mellitus (DM), reducing insulin sensitivity and inducing cardiac hypertrophy, long-term monitoring is, however, also mandatory in terms of glucose metabolism and cardiovascular measurements. In our experience comparing the impact of seven years of rhGH treatment on metabolic and cardiovascular parameters in GHD patients divided in two groups [adult (AGHD) and elderly (EGHD) GHD patients], effects on body composition are evident especially in AGHD, but not in EGHD patients. The improvements in lipid profile were sustained in all groups of patients, and they had a lower prevalence of dyslipidemia than the general population. The effects on glucose metabolism were conflicting, but approximately unchanged. The risk of DM type 2 is, however, probably increased in obese GHD adults with impaired glucose homeostasis at baseline, but the prevalence of DM in GHD is like that of the general population. The increases in glucose levels, BMI, and SBP in GHD negatively affected the prevalence of Metabolic Syndrome (MS) in the long term, especially in AGHD patients. Our results are in

accordance to other long-term studies in which the effects on body composition and lipid profile are prominent.

Keywords: growth hormone (GH) treatment, elderly, metabolism, cardiovascular, hypopituitarism

INTRODUCTION

Growth hormone deficiency (GHD) in adults is due to a reduced growth hormone (GH) secretion by the anterior pituitary gland which leads to a well-known syndrome characterized by decreased cognitive function and quality of life (QoL), decreased bone mineral density (BMD), increased central adiposity with a reduction in lean body mass, decreased exercise tolerance, hyperlipidemia, and increased predisposition to atherogenesis (1–3). For this reason, twenty years ago GH replacement therapy started to be used also in adults with GHD and not only in children with impaired growth. In fact, in 1989 initial studies on rhGH treatment in adult hypopituitary patients were published, with positive effects on the main features of the above-mentioned syndrome (4–9). On these bases, GH treatment was approved for Adult GHD (AGHD) in Europe in 1995 and in the United States (US) in 1996 (10). On this concern, elderly patients are a peculiar task: in these patients, whom we assist at a physiological reduction of GH secretion due to the advancing age, it is difficult to establish the right clinical threshold to start rhGH replacement therapy (11, 12). The reduction of GH/insulin like growth factor I (IGF-I) activity is considered to be one of the causes of catabolic process of normal aging and can partly explain the age-related variations in the bone metabolism, muscle mass, cardiovascular system, immune system and well-being, although sex steroids and malnutrition have an important role too. Considering some similar features between aging and GHD, it was thought that the relative GH insufficiency of elderly people could make an important contribution to the fragility of the elderly (13). GH stimulation tests are able to differentiate GHD in elderly patients (EGHD) from the physiological reduction of GH secretion that occurs with aging (11). A small number of randomized controlled trials have studied the effects of rhGH therapy in EGHD with no univocal data and no long period of treatment (14–16).

On these bases, the aim of our study was to evaluate the impact of seven years of rhGH treatment on metabolic and cardiovascular parameters in EGHD and AGHD. In addition, we evaluated the prevalence of dyslipidemia, type II diabetes mellitus (DM), metabolic syndrome (MS) according to IDF (MS-IDF) and ATP III (MS-ATPIII) criteria and arterial hypertension in GHD patients, and we compare it to prevalence of these comorbidities in a group of age-matched no GHD hypopituitary patients and in a group of age-matched controls.

MATERIALS AND METHODS

The study was approved by the local ethics committee and complied with the Declaration of Helsinki, in line with the Guidelines for Good Clinical Practice. All patients provided written informed consent before entering the study, with respect to study participation, and confidentiality statement of data collection according to the Italian privacy policy.

Patients

In this study we evaluated 196 consecutive hypopituitary patients (125 GHD and 71 no GHD patients) followed at the outpatient clinic of the Department of Clinical Medicine and Surgery, Section of Endocrinology, "Federico II" University, Naples, Italy, from 1998 to 2010. We started recruiting patients in 1998 and ended in 2010, but we waited until 2017 to ensure that all patients had a 7-year follow up. From 2017 to nowadays we processed data and develop the manuscript.

The longest follow-up period that was available for our study population of adult patients was seven years. Seven years represent for GHD patients in treatment with rhGH a long-term follow-up. Therefore, to homogenize the sample the maximum follow-up period was set at seven years.

The following exclusion criteria were taken into account: 1) gpatients with rh-GH therapy discontinuation (more than 1 year); 2) patients who had missed visits more than three times. During the study period 34 patients died: myocardial infarction (n 6), stroke (n 4), breast cancer (n 2), pulmonary cancer (n 2), colon cancer (n 2), other cancers (n 3), old age (n 7), dementia (n 1), traumatic injury (n 2), and sudden death of unknown cause (n 5); 42 GHD patients discontinued therapy more than 1 year: diagnosis of cancer (n 11), tumor recurrence (n 7), poor compliance (n 16), lack of subjective improvement (n 8); 49 patients were lost to follow-up (**Figure 1**). So, we recruited 39 GHD patients (21 F, 18 M, mean age 48.38 ± 13.33 years, range 26–71 years) who achieved at least seven consecutive years of rhGH treatment and 32 no GHD hypopituitary patients (19 F, 13 M, mean age 48.84 ± 15.55years, range 27–75 years) who achieved at least seven consecutive years of follow-up (**Figure 1**).

All GHD patients had known pituitary disease or other anterior pituitary hormonal deficiencies. GHD was mainly caused by treatment for pituitary adenomas (20 patients: 17 treated with surgery and three treated with surgery and radiotherapy (RT)). In 10 patients GHD was due to primary empty sella or pituitary hypoplasia and in four patients GHD occurred after treatment for craniopharyngioma (two treated with surgery and two treated with surgery and RT). Indeed, one patient had GHD for other pituitary lesions (arachnoid cyst treated with surgery). Finally, in two patients GHD occurred after traumatic brain injury and in two patients for Sheehan's syndrome. (**Table 1**). Most GHD patients had multiple anterior pituitary hormonal deficiencies (**Table 2**). The diagnosis of GHD was based on a peak GH <9 μ/l after GHRH + Arginine (GHRH + ARG) test. All GHD patients were divided in two groups:

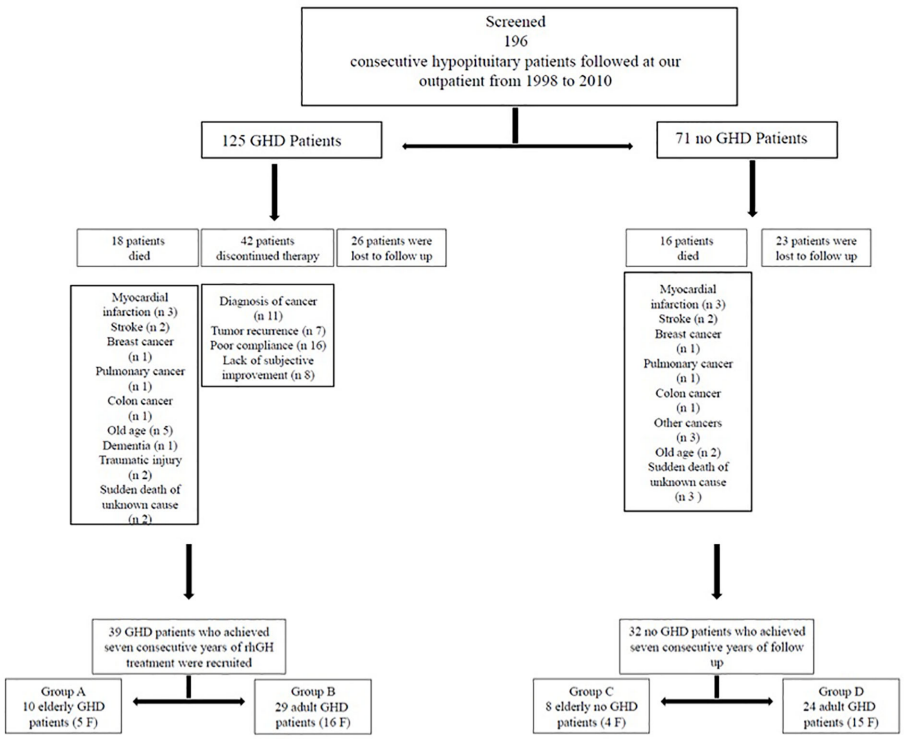

FIGURE 1 | GHD and no GHD patients' selection.

TABLE 1 | Causes of pituitary deficiency in the study population of 39 GHD patients.

Causes	N of Patients
Pituitary adenoma	20
Primary empty sella/Pituitary hypoplasia	10
Craniopharyngioma	4
Other pituitary lesions (Arachnoid Cyst)	1
Traumatic brain injury	2
Sheehan's Syndrome	2
Total	39

GHD, growth hormone deficiency.

TABLE 2 | Number of pituitary deficiencies in the study population of 39 GHD patients.

Type of deficiency	N of Patients
Isolated GHD	3
1 additional deficiency	6
2 additional deficiencies	8
3 additional deficiencies	16
4 additional deficiencies (+ Diabetes insipidus)	6

GHD, growth hormone deficiency.

group A, 10 EGHD patients (five F, five M, mean age 66.6 ± 2 years, range 65–71 years); group B, 29 AGHD patients (16 F, 13 M, mean age 42.1 ± 8.9 years, range 26–55 years) (**Figure 1**).

Causes of hypopituitarism in no GHD hypopituitary patients were: treatment for pituitary adenomas (17 patients: 15 treated with surgery and two treated with surgery and RT). In nine patients hypopituitarism was due to primary empty sella or pituitary hypoplasia, and in three patients hypopituitarism occurred after treatment for craniopharyngioma (two treated with surgery and one treated with surgery and RT). Indeed, one patient had GHD for other pituitary lesions (ependymoma treated with surgery and RT). Finally, in two patients GHD occurred after traumatic brain injury (**Table 3**). A number of anterior pituitary hormonal deficiencies are shown in **Table 4**. All no GHD hypopituitary patients were divided in two groups: group C, eight elderly no GHD hypopituitary patients (four F, four M, mean age 69.87 ± 3.31years, range 65–75 years); group D, 24 adult no GHD hypopituitary patients (15 F, nine M, mean age 41.83 ± 10.61 years, range 27–63 years) (**Figure 1**).

We recruited also 37 age-matched controls (21 F, 16 M, mean age 48.21 ± 16.29 years, range 26–72 years), which were divided in two groups: group E, nine elderly controls (five F, four M, mean age 67.77 ± 2.63 years, range 65–72 years); group F, 28 adult controls (16 F, 12 M, mean age 41.92 ± 13.53 years, range 26–64 years).

Parameters

All parameters were evaluated at baseline and after three months during the first year of therapy and every six months during the following seven years of therapy.

TABLE 3 | Causes of pituitary deficiency in the study population of 32 no GHD hypopituitary patients.

Causes	N of Patients
Pituitary adenoma	17
Primary empty sella/Pituitary hypoplasia	9
Craniopharyngioma	3
Other pituitary lesions (ependymoma)	1
Traumatic brain injury	2
Total	32

GHD, growth hormone deficiency.

TABLE 4 | Number of pituitary deficiencies in the study population of 32 no GHD hypopituitary patients.

Type of deficiency	N of Patients
1 deficiency	5
2 deficiencies	6
3 deficiencies	14
4 deficiencies (+ Diabetes insipidus)	7

GHD, growth hormone deficiency.

In each group of GHD patients we evaluated body height, body weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist–hip ratio (WHR), systolic blood pressure (SBP), and diastolic blood pressure (DBP). Body weight was measured in the morning to the nearest 0.1 kg, and body height was measured to the nearest 0.01 m while subject was wearing light indoor clothes without shoes. BMI was calculated as body weight (in kilograms) divided by squared height (in squared meters). WC was measured with a soft tape, midway between the lowest rib margin and the iliac crest, in the standing position. HC was measured over the widest part of the gluteal region, and then WHR was calculated.

BP was measured at the right arm with the patient in the sitting position. The average of three measurements using a mercury sphygmomanometer. Hypertension was diagnosed when SBP exceeded 140 mmHg, and DBP exceeded 90 mmHg.

The presence of MS was evaluated according to both the IDF and the ATPIII criteria. Thus, using the IDF criteria, the subject with abdominal obesity, defined as a WC >94 cm for men and WC >80 cm for woman, was considered to have MS, with at least two of the following factors: high values of TG value: >150 mg/dl or specific treatment for such dyslipidemia, reduced HDL-C values: <40 mg/dl in humans and <50 mg/dl in women or hypercholesterolemia, high BP >130/85 mm/Hg or hypertension treatment, high fasting blood glucose: >100 mg/dl or diagnosis of type 2 diabetes mellitus (17–19). Using the ATPIII criteria, subject was considered to have MS if subject had three of the following factors: abdominal obesity, defined as a WC >102 cm for men and WC >88 cm for women, high values of TG value: >150 mg/dl or specific treatment for such dyslipidemia, reduced HDL-C values: <40 mg/dl in men and <50 mg/dl in women or hypercholesterolemia, high BP >130/85 mm/Hg or hypertension treatment, high fasting blood glucose: >100 mg/dl or diagnosis of type 2 diabetes mellitus (20).

In each group of GHD patients we evaluated total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), triglycerides (TG), blood glucose, glycosylated hemoglobin (HbA1c), and IGF-I; Venous blood sampling was performed between 8 and 12 am. The blood samples were immediately centrifuged, and the sera were stored at a temperature of −80°C until they were analyzed. Serum levels of TC, HDL-C, and TG were determined by enzymatic method on fasting serum. LDL-C was calculated according to Friedewald's formula adjusted to SI units (21). Serum LDL-C was excluded in patients with serum TG >400 mg/dl. Blood glucose was measured by fasting on serum or plasma sample. Blood HbA1c was determined by HPLC method.

For the diagnosis of GHD, the GHRH + Arginine stimulation test was used, in agreement with studies in which adult patients with GHD with GH peak after ITT <3 μg/l have a GH response to GHRH + ARG <9 μg/l, while normal subjects have a GH response always >16.5 μg/l (22). Arginine (arginine hydrochloride, Damor, Naples, Italy and SALF®, Bergamo, Italy) was administered at a dose of 0.5 g/kg up to a maximum dose of 30 g by slow infusion from 0 to 30 min, while GHRH (Geref, Serono, Rome, Italy and GHRH Ferring, Milan, Italy) was injected at a dose of 1 μg/kg in intravenous bolus at time 0. Blood withdrawals were performed every 30 min from time 0 to 90 min.

Serum GH was measured by RIA using kits provided by Radim (Pomezia, Italy): the normal GH range was less than 5 ng/ml, and the sensitivity was 0.2 mg/L, and by CLIA using Liaison hGH kit of Diasorin: the hGH sensitivity is 0.052 μg/l; thus undetectable GH levels were arbitrarily considered 0.05 μg/l. The intraassay CVs were 4.4, 1.6, and 2.0% for the low, medium, and high points of the standard curve, respectively. The inter-assay CVs were 6.0, 7.7, and 6.8% for the low, medium, and high points of the standard curve. The hGH values were evaluated against the World Health Organization Second International Standard reference reagent 98/574, when possible. Plasma IGF-I was measured after ethanol extraction by immunoradiometric assay using kits provided by Diagnostic System Laboratories (Webster, TX): in our laboratory the normal IGF-I ranges in 20- to 30-, 31- to 40-, 41- to 50-, and over 50-year-old subjects were 110–502, 100–494, 100–303, and 78–258 ng/ml, respectively and by CLIA after automatized extraction using Liaison IGF-I kit of DiaSorin: The IGF-I sensitivity is <3 μg/l. The intra-assay CVs were 4.3, 3.0, and 3.3% for the low, medium, and high points of the standard curve, respectively. The inter-assay CVs were 4.4, 3.3, and 3.6% for the low, medium, and high points of the standard curve. The IGF-I values were evaluated against 1st WHO International Standard for Insulin-like Growth Factor-I NIBSC 02/254, when possible. In addition, an assessment of other pituitary axis hormones (TSH, FT3, FT4, FSH, LH, estradiol, testosterone, PRL, ACTH, cortisol, and urinary free cortisol) in order to determine the adequacy of substitution therapy, was periodically performed (at baseline, after three months, and every six months). Serum testosterone, estradiol, free forms of thyroid hormones, TSH, FSH, LH, PRL, and free urinary cortisol have been dosed with common kits on the market.

Treatment Regimens

Each GHD patient initially received a GH dose according to regimens suggested by the international guidelines at the time of diagnosis (Group A, mean dose: 1.5 ± 0.2 mg/week; group B,

mean dose: 2 ± 0.7 mg/week). During the following years of treatment, the GH dose was gradually titrated on the basis of IGF-I concentration (according to age/sex related values) (23). Dose titration and safety monitoring were performed every three months during the first year and every six months thereafter. When required, in GHD and no GHD hypopituitary group, patients received adequate and stable therapy with glucocorticoids, thyroid hormone, testosterone, estrogen, and desmopressin.

Statistical Analysis

Data were analyzed using SPSS Software for Windows, version 20.0 (SPSS, Inc., Cary, NC package) and were reported as Mean ± Standard Deviation (SD) or as percentages. For all variables, within-group differences were calculated using a repeated-measures ANOVA, followed by a *post hoc* analysis performed using Bonferroni or Student–Newman–Keuls tests where applicable. The t student test was used for intergroup/intragroup comparison. Then the prevalence of arterial hypertension, dyslipidemia, MS according to both IDF and ATPIII criteria and DM was calculated for evaluation of intragroup differences with the chi-square test. Significance was set at 5%.

RESULTS

GH Dose, Serum IGF-I, Body Composition, and Blood Pressure

Mean rhGH dose at baseline was 1.52 ± 0.26 mg/week in Group A and 2.02 ± 0.74 mg/week in group B. During follow-up rhGH dose was gradually titrated according IGF-I normal value for age and sex with a mean dose of 0.96 ± 0.46 mg/week in Group A and 3.07 ± 2.28/week in Group B after seven years' treatment. Serum IGF-I concentration was increased in the two groups with a mean value within the normal range (± 2 S.D.) during the follow-up. After seven years of therapy, WC and HC significantly decreased in Group B (p = 0.004 and p = 0.011 respectively). WHR significantly decreased in Group B (p = 0.021). In group A there was a significant reduction only of HC (p = 0.03) with no modification of WC and WHR. No differences in Group A and B were found in BP parameters (**Tables 5** and **6**). No differences in these parameters were observed between groups, except for SBP that was higher in Group A than in Group B (p <0.001) (**Table 7**).

Lipid Profile and Glucose Metabolism

TC significantly decreased in Group B (p = 0.006) after seven years of treatment. In Group B also HDL-C increased (p = 0.02), and LDL-C (p <0.001) significantly decreased at study end. Indeed, TG significantly decreased in Groups A and B (p = 0.048 and p = 0.047 respectively) (**Tables 5** and **6**). No differences were observed in other lipid parameters between groups at baseline and after treatment (**Table 7**).

Blood glucose increased in Group B (p = 0.023), while there was no difference in HbA1c after seven years of rhGH (**Tables 5** and **6**). No significant modifications of these parameters were found between groups at baseline and at study end (**Table 7**).

Prevalence of Dyslipidemia, Metabolic Syndrome, Diabetes Mellitus, and Hypertension

In Group B, an increased prevalence of hypertension (13.7 *vs* 90%) was observed (p = 0.031). No differences were observed on prevalence of hypertension in Group A and on prevalence of dyslipidemia, MS-IDF, MS-ATP, and DM in Groups A and B (**Table 8**). There was no difference in the prevalence of all these comorbidities in Groups C and D (**Table 9**). In Groups E and F there was a higher prevalence of dyslipidemia (p = 0.045 and p = 0.034 respectively) after 7 years of follow-up (**Table 10**).

We also compared the prevalence of dyslipidemia, MS, DM, and hypertension of all our GHD patients, according to age, to prevalence of these diseases in no GHD hypopituitary patients and in controls. There were no differences between GHD

TABLE 5 | Clinical features of EGHD (Group A) patients at baseline and after 7 years of rhGH treatment.

Parameters	Baseline	7 years	P
BW (kg)	74.18 ± 11.72	73.40 ± 12.39	NS
BMI (kg/m^2)	30.44 ± 3.87	30.21 ± 4.18	NS
WC (cm)	99.30 ± 6.01	98.50 ± 8.19	NS
HC (cm)	106.10 ± 3.66	102.70 ± 5.49	0.03
WHR	0.93 ± 0.02	0.95 ± 0.03	NS
SBP (mmHg)	139.20 ± 6.56	130.9 ± 13.56	NS
DBP (mmHg)	75.30 ± 11.77	79.60 ± 9.30	NS
TC (mg/dL)	219.50 ± 25.84	193.40 ± 42.91	NS
LDL-C (mg/dl)	125.96 ± 24.56	112.38 ± 19.55	NS
HDL-C (mg/dl)	52 ± 7.21	53.10 ± 11.14	NS
TG (mg/dl)	206.70 ± 96.18	139.5 ± 34.2	0.048
Fasting glucose (mg/dl)	87.80 ± 13.54	88.80 ± 6.21	NS
HbA1c (%)	5.69 ± 0.63	5.75 ± 0.59	NS
IGF-I (mg/dl)	75.64 ± 35.18	165.80 ± 78.00	0.002
GH dose (mg/week)	1.52 ± 0.26	0.96 ± 0.46	0.009

EGHD, elderly growth hormone deficiency; rhGH, recombinant human growth hormone; BW, body weight; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist to hip ratio, SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; HbA1c, glycated haemoglobin.

TABLE 6 | Clinical features of AGHD (Group B) patients at baseline and after 7 years of rhGH treatment.

Parameters	Baseline	7 years	P
BW (kg)	77.73 ± 16.92	79.01 ± 19.49	NS
BMI (kg/m^2)	29.24 ± 5.68	29.60 ± 6.00	NS
WC (cm)	105.72 ± 14.60	94.27 ± 14.11	0.004
HC (cm)	110.98 ± 10.19	104.43 ± 8.74	0.011
WHR	0.95 ± 0.08	0.90 ± 0.08	0.021
SBP (mmHg)	121.24 ± 14.19	123.00 ± 18.00	NS
DBP (mmHg)	76.10 ± 8.61	77.52 ± 10.4	NS
TC (mg/dl)	230.9 ± 41.35	200.18 ± 39.7	0.006
LDL-C (mg/dl)	149.47 ± 35.71	116.60 ± 21.06	<0.0001
HDL-C (mg/dl)	50.17 ± 15.57	60.01 ± 15.80	0.020
TG (mg/dl)	154.28 ± 92.06	116.62 ± 39.06	0.047
Fasting glucose (mg/dl)	84.79 ± 15.99	90.83 ± 21.84	0.023
HbA1c (%)	5.73 ± 0.65	5.61 ± 0.73	NS
IGF-I (mg/dl)	84.25 ± 41.57	178.94 ± 80.17	<0.001
GH dose (mg/week)	2.02 ± 0.74	3.07 ± 2.28	0.016

AGHD, adult growth hormone deficiency; rhGH, recombinant human growth hormone; BW, body weight; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist to hip ratio, SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; HbA1c, glycated haemoglobin.

TABLE 7 | Comparison between Group A (EGHD) and Group B (AGHD) patients at baseline and after 7 years of rhGH treatment.

Parameters	Baseline	7 years
	P Group A *vs* B	P Group A *vs* B
BW	NS	NS
BMI	NS	NS
WC	NS	NS
HC	NS	NS
WHR	NS	NS
SBP	<0.001[a]	NS
DBP	NS	NS
TC	NS	NS
LDL-C	NS	NS
HDL-C	NS	NS
TG	NS	NS
Fasting glucose	NS	NS
HbA1c	NS	NS
IGF-I	NS	NS
GH dose	NS	NS

[a]*A > B. EGHD, elderly growth hormone deficiency; AGHD, adult growth hormone deficiency; rhGH, recombinant human growth hormone; BW, body weight; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist to hip ratio, SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; HbA1c, glycated haemoglobin. NB, data of parameters of each group are shown in* ***Tables 5*** *and* ***6****.*

patients (Groups A and B) and no GHD hypopituitary patients (Groups C and D), but there was a higher prevalence of dyslipidemia in adult controls (Group F) than adult GHD (Group B) after 7 years (p = 0.011) (**Tables 11** and **12**).

DISCUSSION

This is a single-center observational study on the effects of long-term rhGH replacement on body composition and cardiovascular risk factors in GHD patients, dividing subjects in EGHD and AGHD patients according to the age of onset of GHD. As there are limited data in literature on the long-term effect of GH therapy in elderly patients, our paper implements and strengthens the knowledge on the long-term management of rhGH therapy, with particular regard to its efficacy and safety, in a subset of patients in whom GHD contributes to their fragility. In addition, although there are other similar studies with matched populations, these studies have shorter follow-up periods or, if long enough, to evaluate GHD adult patients without differences between adult and elderly people.

In our study 7 years of GH treatment improved body composition of AGHD patients. In fact, in our AGHD patients, WC, HC, and WHR decreased after GH therapy, and this is in line with other short-term studies (24, 25). Even if we did not perform a body composition analysis with DEXA or magnetic resonance, we used WHR as indicator of visceral fat mass (VFM) according to the World Health Organization (WHO) that states that abdominal obesity is defined as a WHR above 0.90 for M and above 0.85 for F (26). Indeed, WHR is reported to be of clinical utility in identifying patients with cardiovascular risk factors in an adult population (27–29). WC is also a parameter used as indicator of VFM (30), and in our AGHD patients WC was decreased. So, the reduction in WC and WHR in our AGHD patients should reflect an effect of GH to limit central body fat deposit that occurs with age (31) and suggest a reduction in VFM in our patient. This is in line with a previous study of Svensson et al. in which seven years of GH treatment reduced body fat (32). These changes are reported also in longer observational studies (≥10 years) (33, 34) where body fat was reduced when corrected for age related-increase in body fat in normal aging (35). Using Framingham model, the GH-induced difference in WHR with an improvement in body composition would represent a 3–4% decrease in the incidence of coronary heart disease over 10 years (36, 37).

In our EGHD patients there was only a reduction of HC, while WC and WHR were not modified by GH treatment, and this is in contrast with long term (10 years) follow-up results

TABLE 8 | The prevalence of metabolic co-morbidities at study entry and after 7 years of rhGH treatment in each group of GHD patients (elderly and adult patients).

Patients		Dyslipidemia n (%)	MS-IDF n (%)	MS-ATPIII n (%)	DM n (%)	Arterial hypertension n (%)
Group A	**Baseline**	7 (70)	7 (70)	6 (60)	1 (10)	9 (90)
(10 patients)	**7 years**	7 (70)	7 (70)	7 (70)	1 (10)	8 (80)
	p	NS	NS	NS	NS	NS
Group B	**Baseline**	15 (51.7)	7 (17.2)	3(10.3)	2 (6.89)	4 (13.7)
(29 patients)	**7 years**	16 (55.1)	6 (20.6)	4 (13.7)	3 (10.3)	8 (27.5)
	p	NS	NS	NS	NS	0.031

Group A, elderly growth hormone deficiency patients; Group B, adult growth hormone deficiency patients; rhGH, recombinant human growth hormone deficiency; MS-IDF, metabolic syndrome according IDF criteria; MS-ATPIII, metabolic syndrome according ATPIII criteria; DM, diabetes mellitus.

TABLE 9 | The prevalence of metabolic co-morbidities at study entry and after 7 years in each group of no GHD hypopituitary patients (elderly and adult patients).

Patients		Dyslipidemia n (%)	MS-IDF n (%)	MS-ATPIII n (%)	DM n (%)	Arterial hypertension n (%)
Group C	**Baseline**	4 (50)	2 (25)	2 (25)	2 (25)	4 (50)
(8 patients)	**7 years**	4 (50)	4 (50)	4 (50)	2 (25)	4 (50)
	p	NS	NS	NS	NS	NS
Group D	**Baseline**	11 (45.8)	8 (33.3)	6 (25)	2 (8.33)	8 (33.3)
(24 patients)	**7 years**	7 (29.1)	10 (41.6)	8 (33.3)	2 (8.33)	7 (29.1)
	p	NS	NS	NS	NS	NS

Group C, elderly no GHD hypopituitary patients; Group D, adult no GHD hypopituitary patients; MS-IDF, metabolic syndrome according IDF criteria; MS-ATPIII, metabolic syndrome according ATPIII criteria; DM, diabetes mellitus.

study (38) in which authors assessed a reduction in body fat (BF) (especially after 5 years of treatment). This difference probably reflects the use of a different technique (DEXA) which is a direct method of body composition measurement and the slightly younger mean age of the Swedish study's patients, who would be less affected than our patients by the increased central body fat that occurs with aging (35). Other studies about body composition and metabolic parameters in EGHD have a less treatment period (≤2 years) (16, 39–43).

Regarding lipid profile, in our AGHD patients TC and LDL-C were decreased and HDL-C was increased. This pattern did not change after exclusion of patients using lipid-lowering drugs and is due not only to improvements in body composition, but also to a direct effect of GH on lipid metabolism. In fact, GH increases the expression of LDL receptors (44), which increases the clearance of LDL-C as well as the hepatic uptake of partially de-lipidated VLDL particles (45). GH also enhances the available intrahepatic lipid substrate through its lipolytic action, stimulating very low-density lipoprotein (VLDL) apo B secretion (44). Other long-term studies showed an improvement in lipid profile after GH substitution (32–34, 46–50) with a reduction in TC and LDL-C and an increase in HDL-C. In the general population, the cardiovascular risk of hypercholesterolemic patients is reduced to 15% by the reduction in 10% cholesterol levels (51), so any additional effect of GH treatment next to conventional lipid-lowering drugs may be advantageous.

In contrast to our findings, the majority of the mentioned studies did not report a reduction in TG that occurred in our cohort, except for the observational study of Gotherstrom and colleagues (49) in which there was a reduction of TG after 5 years of GH replacement. This finding was not confirmed by the same Sweden study group after 10 (34) and 15 (33) years of therapy. GH activates the hormone-sensitive lipase, increases triglycerides hydrolysis in fatty acids and glycerol, induces lipolysis, and reduces the re-esterification of free fatty acids to triglycerides (52). These effects can explain our findings. EGHD patients showed no changes in lipid profile except for a reduction in TG; TC and LDL-C had a decreasing trend and HDL-C an increasing one even if not significant. Previous studies (39, 41–43, 53) reported in EGHD lipid changes similar to AGHD patients.

Despite favorable changes in lipid profile, the prevalence of dyslipidemia does not decrease after therapy in the two groups of our patients, but the reduction of LDL-C levels after treatment is of particular importance if we consider the correlation between LDL-C blood levels and cardiovascular risk (54–56). Comparing the prevalence of dyslipidemia of our patients, according to age, to prevalence of this comorbidity in no GHD hypopituitary patients and in controls, we found no differences between GHD patients and no GHD hypopituitary patients, but there was a higher prevalence of dyslipidemia in adult controls than adult GHD patients after 7 years. Probably, this is due to the fact that in the control group there was a significant increase of prevalence of dyslipidemia after 7 years of follow-up, both in elderly and adult.

About glucose metabolism, as demonstrated in previous studies (33, 34, 49, 57, 58) we observed in our AGHD cohort an increase in blood glucose, even if there was no modification in HbA1c after seven years of rhGH treatment. The increase in fasting blood glucose concentration could, at least to some extent, be an effect of the normal aging of patients. It was hypothesized by Gotherstrom and colleagues (49) that this increase in fasting blood glucose in the morning may be affected by the bedtime GH injections and does not reflect 24-h glucose homeostasis in GHD adults receiving GH replacement which is lower (59). This is in line with no modification of

TABLE 10 | The prevalence of metabolic co-morbidities at study entry and after 7 years in each group of controls (elderly and adult controls).

Patients		Dyslipidemia n (%)	MS-IDF n (%)	MS-ATPIII n (%)	DM n (%)	Arterial hypertension n (%)
Group E	**Baseline**	4 (44.4)	3 (33.3)	3 (33.3)	2 (22.2)	4 (44.4)
(9 patients)	**7 years**	8 (88.8)	4 (44.4)	3 (33.3)	2 (22.2)	5 (55.5)
	p	0.045	NS	NS	NS	NS
Group F	**Baseline**	17 (60.7)	8 (28.5)	9 (32.1)	4 (14.2)	4 (14.2)
(28 patients)	**7 years**	24 (85.7)	9 (32.1)	10 (35.7)	4 (14.2)	8 (28.5)
	p	0.034	NS	NS	NS	NS

Group E, elderly controls; Group F, adult controls; MS-IDF, metabolic syndrome according IDF criteria; MS-ATPIII, metabolic syndrome according ATPIII criteria; DM, diabetes mellitus.

TABLE 11 | Differences in prevalence of metabolic co-morbidities at study entry and after 7 years between the two groups of elderly patients (GHD and no GHD hypopituitary patients) and between elderly GHD patients and elderly controls.

	Patients	Dyslipidemia n (%)	MS-IDF n (%)	MS-ATPIII n (%)	DM n (%)	Arterial hypertension n (%)
Baseline	**A**	7 (70)	7 (70)	6 (60)	1 (10)	9 (90)
	C	4 (50)	2 (25)	2 (25)	2 (25)	4 (50)
	p	NS	NS	NS	NS	NS
7 years	**A**	7 (70)	7 (70)	7 (70)	1 (10)	8 (80)
	C	4 (50)	4 (50)	4 (50)	2 (25)	4 (50)
	p	NS	NS	NS	NS	NS
Baseline	**A**	7 (70)	7 (70)	6 (60)	1 (10)	9 (90)
	E	4 (44.4)	3 (33.3)	3 (33.3)	2 (22.2)	4 (44.4)
	p	NS	NS	NS	NS	NS
7 years	**A**	7 (70)	7 (70)	7 (70)	1 (10)	8 (80)
	E	8 (88.8)	4 (44.4)	3 (33.3)	2 (22.2)	5 (55.5)
	p	NS	NS	NS	NS	NS

Group A, elderly GHD patients; Group C, elderly no GHD hypopituitary patients; Group E, elderly controls; MS-IDF, metabolic syndrome according IDF criteria; MS-ATPIII, metabolic syndrome according ATPIII criteria; DM, diabetes mellitus.

TABLE 12 | Differences in prevalence of metabolic co-morbidities at study entry and after 7 years between the two groups of adult patients (GHD and no GHD hypopituitary patients) and between adult GHD patients and elderly controls.

	Patients	Dyslipidemia n (%)	MS-IDF n (%)	MS-ATPIII n (%)	DM n (%)	Arterial hypertension n (%)
Baseline	**B**	15 (51.7)	7 (17.2)	3(10.3)	2 (6.89)	4 (13.7)
	D	11 (45.8)	8 (33.3)	6 (25)	2 (8.33)	8 (33.3)
	p	NS	NS	NS	NS	NS
7 years	**B**	16 (55.1)	6 (20.6)	4 (13.7)	3 (10.3)	8 (27.5)
	D	7 (29.1)	10 (41.6)	8 (33.3)	2 (8.33)	7 (29.1)
	p	NS	NS	NS	NS	NS
Baseline	**B**	15 (51.7)	7 (17.2)	3(10.3)	2 (6.89)	4 (13.7)
	F	17 (60.7)	8 (28.5)	9 (32.1)	4 (14.2)	4 (14.2)
	p	NS	NS	NS	NS	NS
7 years	**B**	16 (55.1)	6 (20.6)	4 (13.7)	3 (10.3)	8 (27.5)
	F	24 (85.7)	9 (32.1)	10 (35.7)	4 (14.2)	8 (28.5)
	p	0.011	NS	NS	NS	NS

Group B, adult GHD patients; Group D, adult no GHD hypopituitary patients; Group F, adult controls; MS-IDF, metabolic syndrome according IDF criteria; MS-ATPIII, metabolic syndrome according ATPIII criteria; DM, diabetes mellitus.

HbA1c that is observed in our study and that is the expression of the mean blood glucose level during several weeks.

The insulin sensitivity impairs with increasing age in the general population (60, 61). It is known that during initial months of GH treatment, there is an initial deterioration of insulin sensitivity that could return to baseline values after several months of treatment, probably for the favorable effect of GH on body composition (49) and on the well-being and physical activity. In a study of Christopher and colleagues (62), authors found unchanged insulin sensitivity after 2 years of GH treatment compared with baseline. In another long-term study insulin sensitivity was determined using hyperinsulinemic, euglycemic clamp technique

in 11 GHD patients after 7 years of treatment (32). The authors reported an initial increase of blood glucose during the first year of treatment with an unchanged insulin sensitivity at study end even if there was a tendency to be higher than in controls. Since in our GHD patient we have no modification of HbA1c and since it is known that there is an inverse correlation between insulin sensitivity and TG (63), the reduction of TG observed in our study may imply an improvement in insulin sensitivity in our patients. RhGH therapy could therefore prevent the age-related insulin sensitivity decline in treated GHD patients.

Our EGHD patients showed no change in blood glucose and HbA1c levels. This is in line with results of another study (64) in which GH therapy did not affect glucose metabolism, although the follow-up is too short (1 years).

All groups of GHD patients of our study showed no modification of prevalence of DM at the end of follow-up, and this prevalence did not differ from the prevalence of DM in no GHD hypopituitary patients and in controls at study end.

All patients who had DM in our study were obese (BMI ≥30 kg/m^2). This is confirmed in other two studies based on international database (65, 66) where obesity and disturbed glucose metabolism at baseline increased risk of DM whose incidence was reported similar to the background population (66). In a more recent study using Patro database (67), the authors conclude that rhGH treatment did not increase the risk of DM and glucose impairments.

Concerning BP, contrasting with two long term studies (33, 57) that reported higher SBP after treatment, in our EGHD and AGHD patients we found no difference in SBP and DBP after 7 years' treatment, even if we observed an increase in prevalence of hypertension in AGHD.

Moreover, in our EGHD patients SBP was found higher than AGHD. These two findings may reflect the increase in BP with normal aging. Despite these results, we found no difference between the prevalence of hypertension in GHD patients and the prevalence of hypertension in no GHD hypopituitary patients and in controls.

Regarding the prevalence of MS-IDF and MS-ATPIII, there were no modifications in both GHD groups after GH treatment, even if there was a not significant trend to be higher at the end of the study according to the ATPIII criteria. Despite improvement of lipid spectrum, this increased MS-ATPIII prevalence probably is mainly due to an increase in BP and hyperglycemia which was reported respectively in EGHD and AGHD patients. Two studies have shown a persistently high MS prevalence after 3 and 5 years of rhGH treatment, respectively (50, 68). In the study of Claessen et al (48)., the authors found a further increase in MS prevalence to 57% after 10 years of rhGH substitution. However, in our study we found no differences if this prevalence is compared to the prevalence of MS in no GHD hypopituitary patients and in controls.

A limitation of our study is the lack of a control GHD group and thereby the lack of control for aging. Because the beneficial effects of rhGH therapy are well-established in the short term, it is unethical to deny GH therapy for long period to patients with GHD when no contraindications were assessed. Therefore, it is difficult if not impossible, to perform long-term randomized, controlled trials including GHD patients without rhGH treatment.

To reduce the effect of this limitation on our assessments, we compared our results with data of no GHD hypopituitary patients and healthy controls.

Another limitation is the relative small number of patients in the EGHD cohort, but it was difficult for us to recruit a large number of these patients; some of them stopped GH treatment before the established follow-up period for lack of subjective improvement or opted not to have GH therapy because they disliked injections or because they felt sufficiently well without additional treatment.

In conclusion, the present study showed the beneficial effects of long-term rhGH therapy in GHD patients on body composition and lipid profile as in line with other previous studies. These effects are more evident in AGHD than EGHD patients. The improvement in lipid profile is confirmed by finding that adult GHD patients have lower prevalence of dyslipidemia than controls. Findings on glucose metabolism are contrasting although the prevalence of DM type 2 is similar to controls. The increases in glucose levels, BMI, and SBP in GHD negatively affected the prevalence of the MS in the long term, especially in AGHD patients even if there is no difference of MS prevalence with control population.

Future larger studies are needed to investigate whether the increased mortality seen in hypopituitary GHD adults not receiving GH can be affected by GH replacement, especially in EGHD patients in which an analysis including cost-effectiveness and QoL is required.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Dipartimento di Scienze Biomediche Avanzate-Sezione di Medicina Legale-Università di Napoli "Federico II", Naples Italy. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

The authors' responsibilities were as follows: ES and CD were responsible for the concept of this paper and drafted the manuscript. ER, TS, RA, FR, ED, GD, and AC provided a critical review of the paper. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

SYSTEMATIC REVIEW
published: 11 March 2021
doi: 10.3389/fendo.2021.636403

The Complex World of Regulation of Pituitary Growth Hormone Secretion: The Role of Ghrelin, Klotho, and Nesfatins in It

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The classic concept of how pituitary GH is regulated by somatostatin and GHRH has changed in recent years, following the discovery of peripheral hormones involved in the regulation of energy homeostasis and mineral homeostasis. These hormones are ghrelin, nesfatins, and klotho. Ghrelin is an orexigenic hormone, released primarily by the gastric mucosa, although it is widely expressed in many different tissues, including the central nervous system and the pituitary. To be active, ghrelin must bind to an n-octanoyl group (n = 8, generally) on serine 3, forming acyl ghrelin which can then bind and activate a G-protein-coupled receptor leading to phospholipase C activation that induces the formation of inositol 1,4,5-triphosphate and diacylglycerol that produce an increase in cytosolic calcium that allows the release of GH. In addition to its direct action on somatotrophs, ghrelin co-localizes with GHRH in several neurons, facilitating its release by inhibiting somatostatin, and acts synergistically with GHRH stimulating the synthesis and secretion of pituitary GH. Gastric ghrelin production declines with age, as does GH. Klotho is an anti-aging agent, produced mainly in the kidneys, whose soluble circulating form directly induces GH secretion through the activation of ERK1/2 and inhibits the inhibitory effect that IGF-I exerts on GH. Children and adults with untreated GH-deficiency show reduced plasma levels of klotho, but treatment with GH restores them to normal values. Deletions or mutations of the *Klotho* gene affect GH production. Nesfatins 1 and 2 are satiety hormones, they inhibit food intake. They have been found in GH3 cell cultures where they significantly reduce the expression of *gh* mRNA and that of pituitary-specific positive transcription factor 1, consequently acting as inhibitors of GH production. This is a consequence of the down-regulation of the cAMP/PKA/CREB signaling pathway. Interestingly, nesfatins eliminate the strong positive effect that ghrelin has on GH synthesis and secretion. Throughout this review, we will attempt to broadly analyze the role of these hormones in the complex world of GH regulation, a world in which these hormones already play a very important role.

Keywords: growth hormone, IGF-I, GHRH, somatostatin, ghrelin, klotho, nesfatins

OPEN ACCESS

Edited by:
Antonio Mancini
Catholic University of the Sacred Heart, Rome, Italy

Reviewed by:
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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *01 December 2020*
Accepted: *12 February 2021*
Published: *11 March 2021*

Citation:
Devesa J (2021) The Complex World of Regulation of Pituitary Growth Hormone Secretion: The Role of Ghrelin, Klotho, and Nesfatins in It. Front. Endocrinol. 12:636403. doi: 10.3389/fendo.2021.636403

INTRODUCTION

Our group was the first to describe in humans the existence of an intrinsichypothalamic-somatotrophic rhythm, sexually dimorphic, that conditions the secretion of growth hormone (GH) (1). We also described for the first time that this rhythm depends mainly on the negative tonic effect of somatostatin (SS) on hypothalamic GHRH release and pituitary GH secretion (2). Several studies by our group showed that the control of SS and GHRH depends on the rate of delivery of hypothalamic norepinephrine (NA) to the SS and GHRH neurons (3–7). The role of catecholamines in the control of GH release is related to the classically known metabolic actions of GH as a counterregulatory (hyperglycemic-inducing), lipolytic, and anabolic hormone. This is the reason why most of the tests used to analyze deficient GH secretion are based on increasing the supply of NA to SS neurons, as occurs with the administration of insulin (ITT) to induce hypoglycemia and consequently the release of NA, or the administration of clonidine, an alpha 2 agonist, alone or followed by the administration of GHRH (8). Other classical tests, such as arginine administration, are known to inhibit SS secretion (9, 10), but the exact mechanism by which it occurs has not yet been established. However, in recent years, the complex world of GH secretion regulation has changed due to two main factors:

1. We currently know that GH is a pleiotropic hormone that, in addition to its metabolic and growth effects, has very important positive effects on practically all organs and tissues (11).
2. GH expression has been shown to exist at many extra-pituitary sites, including the nervous system, reproductive system, immune system, cardiovascular system, muscle tissue, dermal tissue, skeletal tissue, and even in the eyes (11–14), where the hormone exerts physiological or pathological auto/paracrine roles.

It seems logical, then, that both the knowledge of the multiple functions that GH plays in the body and the peripheral expression of the hormone have increased the knowledge of the complexity of GH regulation, far beyond the classical concept (2), with the discovery of new factors involved in neuroregulation and/or paracrine regulation of the expression of this hormone. In this review, we will analyze how three of these factors, ghrelin, klotho, and nesfatins, act on the expression and release of pituitary GH.

GHRELIN

In 1990 it was published that a synthetic hexapeptide called GH-releasing peptide (GHRP) could act as a potent GH secretagogue in normal humans (15). Furthermore, the effect of this peptide was independent from that of GHRH and it acted synergistically with this GH-releasing hormone discovered a few years earlier (16, 17). The discovery of synthetic GH secretagogues (GHS) led to investigate on how they could act on the secretion of this hormone. Thus, in 1996 a heterotrimeric receptor coupled to a GTP-binding protein was discovered; it was present in the pituitary and arcuate ventromedial and infundibular hypothalamus of several species, including humans (18). Detection of such a receptor implied that an unknown natural hormone had to exist. This hormone was identified and purified in the rat stomach in 1999 and was called ghrelin (19). Ghrelin is a 28 amino acid peptide hormone in which the third amino acid, normally serine, is modified by a fatty acid, a key modification for ghrelin activity (20). Therefore, GH secretion from the pituitary is regulated not only by the GHRH-SS interaction, but also by gastric ghrelin. The question should now be: why does a gastric hormone play a positive role in GH secretion? The answer to this question is given by the different actions that ghrelin performs in the body. Soon after its characterization, it was found that ghrelin is an orexigenic hormone that is present in the blood in times of fasting and reaches the central nervous system to which it transmits a hunger signal. This is the reason why patients with anorexia nervosa normally show increased plasma concentrations of ghrelin, whereas in obesity they are reduced (21). These facts also explain why GH secretion is increased in anorexia nervosa and reduced in obesity, and also why there is an age-related decrease in plasma ghrelin concentrations in the elderly, a stage of life in which GH secretion is practically absent and there is a decrease in appetite (21). Based on these apparently unrelated effects of ghrelin, the stimulation of hunger and the induction of GH secretion in the pituitary, it is feasible to think that ghrelin appeared in evolution to induce eating behavior and optimize the use of food digested by promoting the release of an anabolic hormone, as GH (22). These concepts are schematized in **Figure 1**.

There are two forms of ghrelin: acyl ghrelin (octanoylated form) and des-acyl ghrelin (non-octanoylated form). The former is the active form responsible for most of the physiological functions of this peptide, including the induction of pituitary GH secretion. Acyl ghrelin is produced by attaching an n-octanoyl group to serine at position 3 (23) (**Figure 2**).

As indicated above, the discovery of ghrelin occurred as a consequence of the interest in the search for a natural hormone capable of interacting with the receptor for synthetic secretagogues that induce GH secretion. This receptor is a G-protein-coupled receptor (GHSR-1a) expressed mainly in the pituitary and hypothalamus, and responsible for mediating the endocrine activities of acyl ghrelin (**Figure 2**). This receptor cannot be activated by des-acyl ghrelin (19, 24, 25), which despite being more abundant than acyl ghrelin lacks known endocrine activity (26), although more studies are needed to better understanding its actions.

Ghrelin Gene and Ghrelin Production Sites

The *Ghrelin* gene is made up of six exons and three introns, located on chromosome 3, at the 3p25-2 locus, although the first exon made up of 20 bp is a non-coding exon (exon 0 of 20 bp) (27). In humans, ghrelin is produced primarily in P/D1 cells (X/A-like cells in rats) and is distributed throughout the stomach

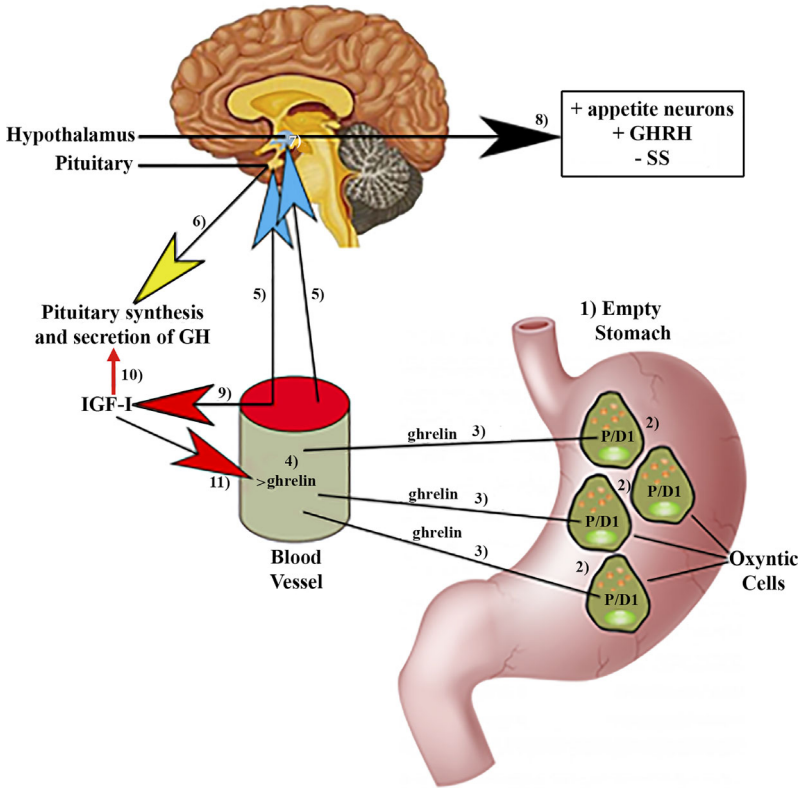

FIGURE 1 | Ghrelin production by the stomach and effects of ghrelin on pituitary GH synthesis and secretion. (1)In fasting situations, an empty stomach increases ghrelin production in specific P/DI cells (2) in humans located in oxyntic cells. Then, ghrelin is released into the circulation (3, 4) from where it reaches (5) the pituitary gland, inducing the synthesis and secretion of GH (6). Circulating ghrelin also reaches the hypothalamus (7), where it induces stimulation (8) of the appetite neurons, GHRH, and inhibits the release of SS. (9)Furthermore, circulating ghrelin inhibits the inhibitory effects of IGF-I on the synthesis and pituitary secretion of GH (10). In turn, IGF-I inhibits the increase in circulating ghrelin (11) produced in response to fasting. Blue arrows: stimulation; Red arrows: Inhibition; Yellow arrow: synthesis and secretion of GH; Black arrow: Hypothalamic effects of ghrelin; +, stimulation; –, inhibition.

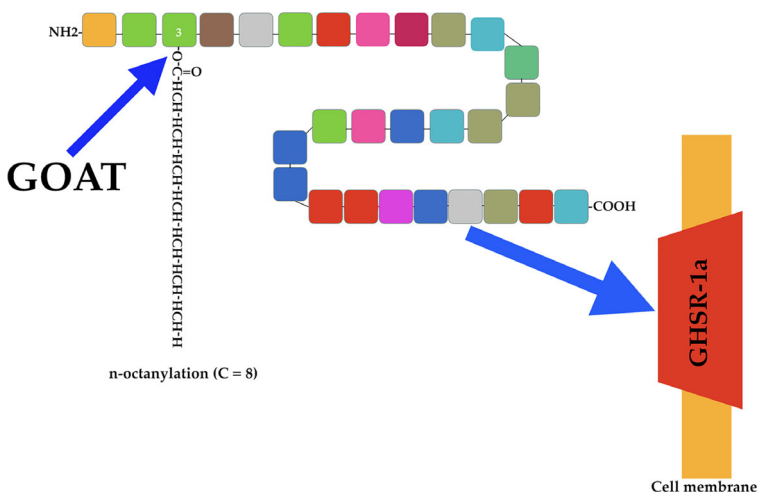

FIGURE 2 | Ghrelin acylation and ghrelin receptor. The endocrine active form of ghrelin is produced by attaching an n-octanoyl group (usually 8 C) to serine at position 3 of the ghrelin molecule (blue arrow). This is done by the enzyme ghrelin-O-acyl-transferase (GOAT). Active acyl ghrelin acts on a receptor (blue arrow) expressed mainly in the pituitary and hypothalamus. This receptor is a G-protein-coupled receptor (GHSR-1a), and cannot be activated by des-acyl ghrelin.

mucosa (28, 29); the greatest amount of ghrelin found in plasma comes from these cells (30, 31) (**Figure 1**).

However, despite the fact that after its discovery, ghrelin was considered a "hunger hormone" and a regulator of GH secretion in the pituitary, current data indicate that it is a pleiotropic hormone that exerts many different effects on the human body and, therefore, can be produced, as its receptor, in a wide variety of tissues and organs. Besides the stomach, ghrelin and its receptor are expressed in many different regions of the brain (32–34), pituitary (35, 36), kidneys (34), heart (37, 38), lung (34), ovaries (34), intestine (39), and pancreatic islets (40, 41). This indicates that the hormone exerts multiple actions, endocrine and/or auto/paracrine, in these tissues, although this is not the objective of this review.

Ghrelin Acylation and Secretion

As stated above, ghrelin requires acylation to interact with and activate its receptor. This acylation is performed by ghrelin O-acyl-transferase (GOAT), which links a fatty acid side chain (C8) to serine 3 of ghrelin (42, 43) (**Figure 2**). At this point is of interest to highlight that the lipids involved in this acylation are mainly those present in nutrition because the ghrelin-producing cells in the stomach are located within the oxyntic gastric glands, which allows direct access to the ingested lipids (44), mainly middle chain fatty acids, because they can be absorbed into the circulation without undergoing breakdown by lipases and bile acids (45).

The GOAT-ghrelin system appears to be a nutrient sensor to signal to the brain that calorie-rich foods are available, leading to optimization of nutrient partitioning and growth signals (46, 47).

Acyl ghrelin is deacylated by plasma esterases and then degraded by plasma proteases and excreted in the urine.

Knowledge about how gastric ghrelin secretion is regulated can be useful to know how this peptide acts on GH secretion in the pituitary.

Gastric ghrelin synthesis and secretion increase during fasting and decrease during feeding (48). This is the reason why chronic intake of high-calorie diets, prolonged ingestion of high fats, and obesity lead to a reduction in gastric ghrelin production and secretion (48, 49), while a low protein supply significantly increases plasma ghrelin (49) (**Figure 3**).

Interestingly, some studies have shown that ghrelin secretion increases in response to stimulation of the sympathetic nerves (50, 51) or by local infusion of adrenergic hormones in the stomach (52), while SS inhibits it (52).

Adrenergic hormones stimulate the release of gastric ghrelin by acting directly on the ß1 receptors of ghrelin-producing cells, especially rich in this type of adrenergic receptors (53). In the same study, the authors confirmed the role of these ß1 receptors in ghrelin production by administering the ß1 receptor blocker atenolol. This prevented the increase in plasma ghrelin that appears after fasting. Furthermore, when they depleted neuronal catecholamines with reserpine, they again observed that there was no ghrelin release after fasting. All this led these authors to propose that fasting acts on gastric ghrelin-secreting cells through the sympathetic nervous system (53). Of course, alpha-adrenergic antagonists also induce an increase in plasma ghrelin concentration (30), as does the administration of muscarinic agonists (54). Furthermore, excitation of the vagus nerve in the gastric mucosa directly stimulates ghrelin-producing cells (55).

As indicated in the *Introduction*, the autonomic nervous system plays a prominent role in the neuroregulation of GH (2), but also in the regulation of gastric ghrelin secretion, as we

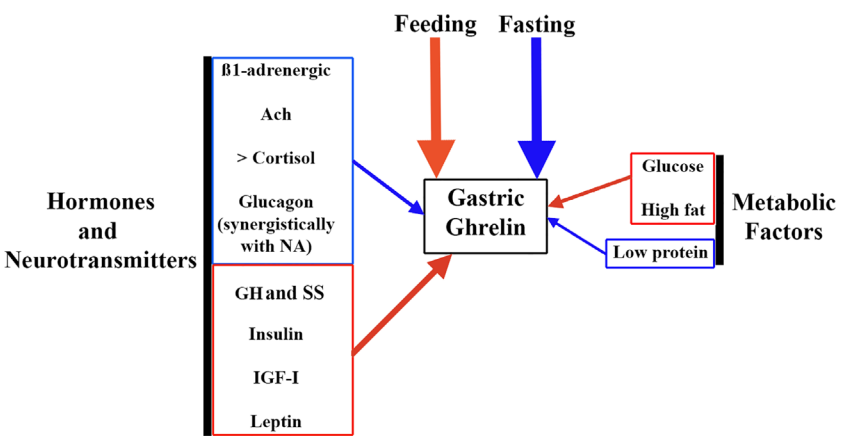

FIGURE 3 | Factors involved in the regulation of gastric ghrelin secretion. As ghrelin is an orexigenic hormone, the main factors that regulate its gastric secretion are fasting (stimulates it, blue arrow) and feeding (inhibits it, red arrow). But in addition, a series of factors such as hormones and neurotransmitters, and metabolic factors play an important role in this regulation. Among neurotransmitters, ß-1 adrenergic pathways and the cholinergic system (Ach) stimulate gastric ghrelin production (blue arrow). Among the hormones, increased plasma cortisol and glucagon levels also stimulate gastric ghrelin production, although in the case of glucagon its effect is most likely to depend on an action carried out synergistically with NA. In contrast, other hormones such as GH, SS, insulin, IGF-I and leptin inhibit gastric ghrelin production (red arrow). Among metabolic factors, glucose and high fat content inhibit gastric ghrelin production (red arrow), while low protein intake stimulates it (blue arrow).

have just seen. What then are the relationships between these two hormones?

In addition to the autonomic system, some hormones and metabolic factors contribute to modulate gastric ghrelin production. For example, GH exerts a negative feedback effect on ghrelin production and secretion, which makes sense (56). More complex is the relationship between insulin and gastric ghrelin. Insulin has been reported to affect ghrelin production and signaling (57), but the reverse is also true (58). The mammalian target of rapamycine (mTOR) is closely involved in metabolic changes in various tissues after postprandial insulin secretion (59), and plays a key role in insulin signaling (60). Components of the mTOR signaling pathway are expressed in endocrine cells of the gastric mucosa, and most of these ghrelin-producing cells show positivity when stained for these components of the mTOR signaling pathway (61). On the contrary, physiological levels of ghrelin impair the functions of pancreatic ß-cells, inhibiting insulin secretion of (62). Most likely, this inhibitory effect depends on the stimulation of pancreatic SS production (63). The intricate relationships between ghrelin and insulin, both peripherally and in the CNS, deserve further explanation, but this is beyond the scope of this review. In any case, it is clear that both hormones play an important role in balancing energy expenditure and metabolic homeostasis.

Another important hormone, such as cortisol, exerts a positive effect on gastric ghrelin secretion (64), which seems to depend directly on cortisol itself and not on CRH or ACTH, since although plasma ghrelin concentrations increase in response to stimulation by ACTH (induced by stress or after exogenous ACTH administration), when metyrapone (which blocks cortisol synthesis) was administered, ACTH increases but plasma ghrelin levels decrease (64). This and other studies by the same group indicate that the hypothalamic-pituitary-adrenal axis is only capable of increasing ghrelin secretion when plasma cortisol is elevated (64). Furthermore, this positive effect of cortisol seems to depend on its plasma levels.

In the case of glucagon, another hormone related to metabolism, mainly glucose homeostasis, this hormone has been shown to induce a significant decrease in ghrelin secretion, which seems not to depend on changes in glucose or insulin concentrations (65), nor in ghrelin-producing cells in the stomach (66), and is suppressed when there is a lesion in the hypothalamic-pituitary axis (65), suggesting that the inhibitory effect of glucagon on ghrelin release is exerted at the hypothalamic-pituitary level, perhaps inducing hypothalamic somatostatin release (67). However, another study indicates that glucagon may participate in the pre-prandial peak of ghrelin, because: a) the glucagon receptor exists in the endocrine cells of the gastric mucosa; b) ghrelin increases in rat plasma during glucagon perfusion; c) glucagon can stimulate ghrelin gene transcription. These led to the claim that ghrelin can be directly regulated by glucagon which acts synergistically with NA (68).

Leptin is another hormone involved in controlling ghrelin release, which is logical given its effects, different from ghrelin, on appetite control. Leptin significantly reduces plasma ghrelin levels and decreases food intake. The weight-reducing effects of leptin are exerted by its direct central effects on the hypothalamus and by its inhibitory actions on gastric release and central actions of ghrelin (69).

Since plasma levels of IGF-I are important mediators of most of the peripheral actions of GH, it is reasonable to assume that there must be important relationships between IGF-I and gastric ghrelin secretion. Data obtained from a large cohort of middle-aged subjects indicate that plasma IGF-I concentration is a significant determinant of plasma ghrelin concentration, with a negative correlation between them (70). A similar negative correlation had previously been found in children and adolescents (71–73). It is important to note that a large amount of circulating IGF-I binds to the transporter protein IGFBP3, so when analyzing the relationships between IGF-I and ghrelin, only free IGF-I, which is the bioactive form, should be considered. On this basis, the highest concentration of ghrelin was observed in GH-deficient children in whom there was low bioavailability of IGF-I (74). That is, low plasma levels of IGF-I induce the synthesis and secretion of ghrelin, while in turn ghrelin decreases plasma levels of IGF-I.

Although many other hormones can help regulate gastric ghrelin synthesis and secretion, their role is not as relevant as that of the hormones just described.

With regard to metabolic factors, it is well known that plasma ghrelin concentrations decrease in normal subjects after oral or intravenous glucose administration (75). The effect of these factors on gastric ghrelin secretion is schematized in **Figure 3** and **Table 1**.

To finish this subsection on how gastric ghrelin synthesis and secretion is regulated, it seems essential to describe a recent study in which it is reported that there are specific cellular mechanisms for the detection of nutrients in the mouse stomach; these are chemosensors expressed primarily in a region-specific way in gastric and stomach ghrelin cells, and their function is to

TABLE 1 | Main factors involved in the regulation of the gastric secretion of ghrelin.

Factors	Inductors	Inhibitors
Hormones and neurotransmitters	ß1-adrenergic stimulation	GH and Somatostatin
	Alpha-adrenergic antagonism	
	Ach	Insulin
	> Plasma cortisol	Plasma IGF-I
	Glucagon	Leptin
Food	Fasting	Feeding
Metabolic Factors	Low protein intake	Glucose, High fat

Different factors can act on the gastric secretion of ghrelin, either stimulating it or inhibiting its secretion. >, Increased.

modulate gastrointestinal responses to food intake, for example by inhibiting ghrelin secretion (76).

Ghrelin and Pituitary GH Secretion

Ghrelin stimulates GH release in the pituitary by acting at two levels: 1) directly on the pituitary somatotrophs and 2) antagonizing the hypothalamic and pituitary effects of SS and inducing GHRH secretion. Indirectly, a third mechanism of action could be considered since ghrelin decreases plasma levels of IGF-I, consequently inhibiting the negative effect of IGF-I on GH secretion exerted directly on the somatotrophs, and indirectly because IGF-I induces hypothalamic secretion of SS (2) (**Figure 4A**).

The first clear evidence that in addition to GHRH and SS, another factor had to be involved in the regulation of GH secretion came from several studies in humans in which it was shown that the nocturnal increase in GH was not inhibited by the infusion of octreotide, an analog of SS (77, 78). The last of these studies proposed that ghrelin could be responsible for the diurnal rhythm of GH secretion (78). This confirms other studies in which it was shown that patients with inactive GHRH receptor, due to mutations, still had rhythmic GH secretion, suggesting that another factor, in addition to GHRH, was acting on pituitary GH secretion (79).

There is a possibility that ghrelin interacts with GHRH at the hypothalamic level, since it has been shown that transgenic rats that have a decreased expression of the ghrelin receptor GHSR-1a in the arcuate nucleus of the hypothalamus, where GHRH is produced, show a decrease of GHRH in the neurons that produce it (80). Administration of a ghrelin receptor antagonist leads to a decrease in the amplitude of GH pulses in rodents (81). Consistent with this finding, in humans, a non-sense mutation affecting ghrelin receptor activity is associated with short-stature (82). This possibility of a hypothalamic ghrelin-GHRH interaction appears to be reinforced by the finding of ghrelin in the hypothalamic arcuate nucleus from where it increases GHRH release and antagonizes the inhibitory effects of SS (83) (**Figure 4A**).

The complicated relationships between ghrelin, GHRH, and SS in controlling episodic GH release were extensively analyzed in an elegant study conducted in male rats in 2003 (84). In that study, intravenous administration of ghrelin during a physiological GH peak was shown to induce a marked increase in plasma GH, suggesting that ghrelin acted synergistically with GHRH; this required the integrity of a functional GHRH system, because the immunoneutralization of GHRH led to a virtual absence of ghrelin-induced GH secretion; however, when ghrelin was administered during a physiological trough period the GH response was clearly attenuated, although a recovery in its secretion was observed 15 min after ghrelin administration. Immunoneutralization of SS reversed the early blunted response to ghrelin in the trough periods, the GH response being similar to that observed when ghrelin was administered during episodes of peak GH. This indicates that ghrelin is a functional SS antagonist. Interestingly, when ghrelin was administered *via* intracerebroventricular during a trough

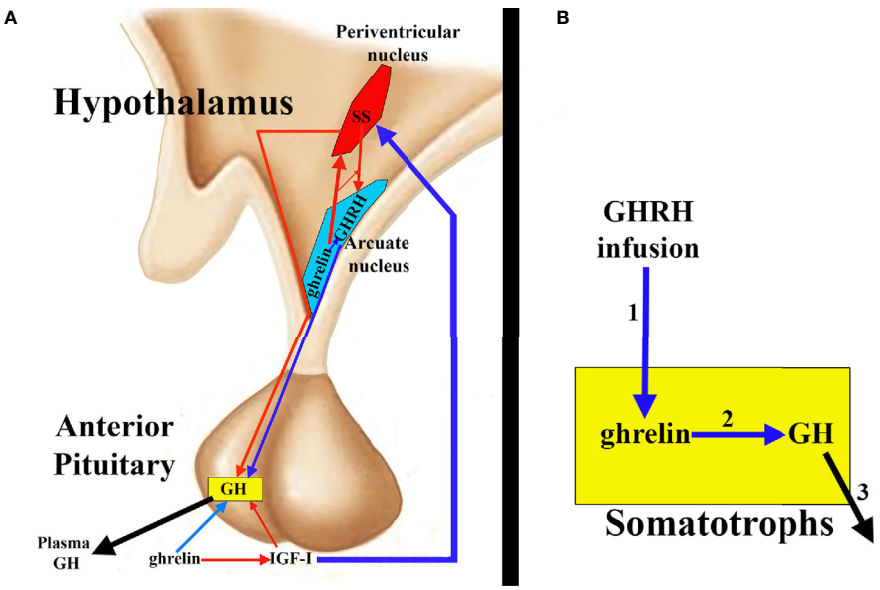

FIGURE 4 | Ghrelin and pituitary GH secretion. **(A)** Ghrelin directly stimulates pituitary GH secretion (blue arrow), but indirectly it also contributes to this secretion, since ghrelin inhibits IGF-I (red arrow) and, therefore, the inhibitory effect of this peptide on GH; furthermore, the inhibition of IGF-I impedes the stimulatory effect of this hormone on the hypothalamic release of SS (blue arrow), which allows the release of GHRH to the portal circulation (blue arrow) with its consequent positive effect on the synthesis and secretion of GH. Furthermore, it appears that ghrelin co-localizes with GHRH in the hypothalamic arcuate nucleus, inducing its release, directly or inhibiting the release of SS from the periventricular nucleus (red arrow), thus inhibiting the inhibitory effect that SS exerts on the release of GHRH and on the synthesis and secretion of pituitary GH. **(B)** ghrelin and its receptor are expressed in the pituitary, where they could play an auto/paracrine role in the regulation of GH release. In fact, GHRH infusion (1) increases pituitary ghrelin mRNA levels that could induce GH stimulation (2) and release (3).

period, no increase in plasma GH was observed, indicating that SS may behave as a functional ghrelin antagonist that acts centrally (in GHRH neurons) and in the pituitary gland (84).

As noted above, ghrelin and its receptor are also expressed in the pituitary (35, 36), thus the possibility exists that pituitary ghrelin plays an auto/paracrine role in the regulation of GH release. GHRH infusion increases pituitary ghrelin mRNA levels, suggesting that GHRH may be a regulator of pituitary ghrelin production (85) (**Figure 4B**). In situations where GHRH expression increases (GH deficiency due to GH gene mutations, hypothyroidism, etc.), pituitary ghrelin expression also increases; conversely, when hypothalamic GHRH expression decreases (GH replacement therapies, glucocorticoid treatments, hyperthyroidism, etc.), pituitary ghrelin expression also decreases. All of this suggests that pituitary ghrelin is dependent on an adequate supply of GHRH to the pituitary gland. GHRH stimulation of pituitary cell cultures in the presence of a specific ghrelin receptor inhibitor significantly decreased the GH response to GHRH challenge, although this effect was not observed in the absence of GHRH stimulation. These results suggest that pituitary ghrelin may act physiologically on GH secretion, improving or optimizing the response of somatotrophs to GHRH. **Table 2** summarizes these effects of ghrelin on pituitary GH secretion.

TABLE 2 | Ghrelin and pituitary GH secretion.

Level	Effect
Anterior Pituitary	Direct effect on GH secretion by inhibiting SS action
Arcuate nucleus	Increases GHRH releaseAntagonizes the inhibitory
Periventricular nucleus	effects of SS on GHRH release
Anterior Pituitary	Inhibits the inhibitory effect of IGF-I on GH secretion
Anterior Pituitary	Acts synergistically with GHRH in inducing GH secretion
Anterior Pituitary	Auto/paracrine induction of GH secretion dependent on GHRH supply.
Anterior Pituitary	> Transcription Na+ channels > NOS/NO + PLC > > Ca2+

Effects of ghrelin on GH secretion and levels at which it acts. >, Increases. +, Stimulates.

Regarding the signaling pathways involved in the release of GH, GHRH binds to a G-protein-coupled receptor (GPCR) that once activated induces the activation of adenylyl cyclase that generates the conversion of ATP to cAMP; cAMP induces a conformational change in protein kinase A (PKA) regulatory subunits that allows phosphorylation of serine or threonine residues in proteins that are then activated (83). Ghrelin also acts through a GPCR but in this case the activation of this receptor leads to the stimulation of the activity of phospholipase C (PLC) that induces the formation of inositol 1,4,5-triphosphate (IP3), and diacylglycerol (DAG) (**Figure 5**); both IP3 and DAG induce an increase in cytosolic calcium that allows the release of GH (83). Furthermore, *in vitro* studies demonstrated that ghrelin requires activation of the NOS/NO pathway and its subsequent guanylate cyclase (GC)/cGMP signal transduction pathway to induce GH release from the pituitary (86). A more recent study demonstrated that GH release from cultured bovine somatotrophs during chronic ghrelin treatment is associated with a significant increase in Na^+ macroscopic current, the blockade of which with tetrodotoxin (TTX) nullifies GH release induced by ghrelin (87). In this study, it was also observed that chronic treatment with ghrelin produced an upregulation of GH transcription levels, as well as that of two isoforms of Na^+ channels sensitive to TTX expressed in somatotrophs, such as NaV1.1 and NaV1.2, indicating that ghrelin also regulates the expression of the Na^+ channel gene in these pituitary cells (87) (**Figure 5**). Of interest here is the recent description that AMP-activated protein kinase (AMPK), a hypothalamic enzymatic complex involved in the hypothalamic control of energy and metabolic homeostasis, and activated by ghrelin, participates in the control of GH secretion, as its blocking or its functional impairment inhibits ghrelin- or

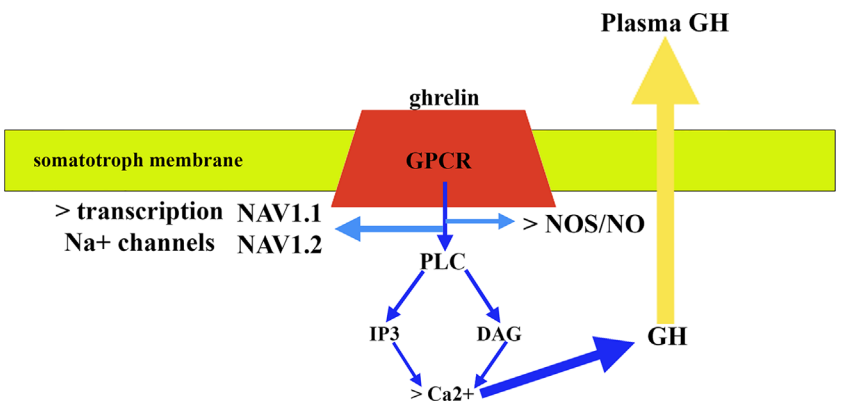

FIGURE 5 | Mechanism of action of ghrelin in pituitary somatotrophs. After binding to a G-protein-coupled receptor (GPR) and activating it, the signaling pathways involve stimulation of phospholipase C (PLC) activity. This activation leads to the formation of inositol 1,4,5 triphosphate (IP3) and diacylglycerol (DAG) (blue arrows). Both IP3 and DAG induce an increase in cytosolic calcium that allows GH release (yellow arrow), although activation of NOS/NO pathway is also required. In addition, the effect of ghrelin includes the activation of the transcription of the Na+ channel gene that leads to the expression of two isoforms of Na+: NAV1.1 and NAV1.2.

GHRH-induced GH secretion, most likely by increasing SS tone (88).

The great importance of ghrelin in the regulation of GH could perhaps be deduced from the data obtained in a study carried out in six healthy young male volunteers in which GH secretion was analyzed every 15 min (−30 to +210 min) in response to 1) iv administration of acyl ghrelin (1 μg/kg); 2) iv infusion of salbutamol (SLB; 0.06 μg/kg/min); 3) acyl ghrelin + SB; 4) saline infusion. While SB led to a significant inhibition of spontaneous GH secretion that remained abolished for up to 75 min after SB withdrawal, acyl ghrelin led to a marked increase in plasma GH levels that was unaffected by SB (89). Since SB is a ß-adrenergic agonist and ß-adrenergic agonists increase hypothalamic SS secretion and inhibit hypothalamic GHRH release (2), these data suggest that acyl ghrelin is refractory to the inhibitory effect of SS. Perhaps this is the mechanism by which gastric ghrelin acts at the pituitary level on the release of GH.

It is of interest now to analyze what happens to the secretion of ghrelin and GH in the elderly. It is well known that aging is associated with a decrease in GH secretion from the second decade of life (90). A similar age-related decline in circulating ghrelin levels has been reported (21, 91). However, the pituitary ghrelin receptor does not decline with aging, at least in mice (92), and the GH response to ghrelin is still seen in the elderly, although there is an age-related decline (93). This is an interesting study topic because, as described, senescence is associated with a decreased appetite (21). What is the reason why the secretion of an orexigenic hormone, such as ghrelin, and also that of an anabolic hormone, such as GH, is lost with aging?

Summary

In summary ghrelin is a very complex hormone, because in addition to its two main actions: orexigenic and strong inducer of GH release in the pituitary, ghrelin exerts many different effects on practically the entire human body. The main source of ghrelin is the stomach, from where this hormone is released in response to different nervous, hormonal, and metabolic stimuli. These are related to the main action of ghrelin as a "hunger hormone," which acts on the central nervous system to stimulate food intake. Additionally, ghrelin induces the secretion of pituitary GH, an anabolic hormone.

Ghrelin acts after being acylated by ghrelin O-acyl-transferase (GOAT), which binds a fatty acid side chain (C8) to serine 3 of ghrelin, forming acyl ghrelin, the active form, which acts on its receptor, a G-protein-coupled receptor (GHSR-1a).

The effects of acyl ghrelin on pituitary GH secretion occur both centrally and directly on pituitary somatotrophs. At the central level, ghrelin is expressed in many neurons that produce GHRH, which most likely facilitates the release of GHRH by antagonizing the inhibitory effect of somatostatin on this secretion. Furthermore, ghrelin exerts a synergistic effect with GHRH in the induction of GH secretion. At the pituitary level, acyl ghrelin has a direct effect on GH secretion; this effect probably depends on gastric ghrelin. Ghrelin expression has been detected in pituitary somatotrophs, which may suggest a paracrine effect of this peptide on GH secretion. Another effect of ghrelin on GH secretion comes from its inhibitory effects on plasma IGF-I. Since IGF-I inhibits GH secretion directly in somatotrophs and indirectly by activating hypothalamic somatostatin release, this action of ghrelin is another factor that positively contributes to GH secretion in the pituitary gland.

Finally, gastric ghrelin secretion decreases with age, as occurs with GH; however, the pituitary ghrelin receptor does not experience this decrease, and a GH response to ghrelin is still seen in the elderly.

KLOTHO

Like ghrelin, the klotho transmembrane protein performs many different functions in the human body, among them it plays a very important role in controlling GH secretion. Klotho was first identified in 1997 as an anti-aging agent (94), since an impairment in its genetic expression, in mice, leads to a syndrome that mimics human aging: short lifespan, infertility, arteriosclerosis, skin atrophy, osteoporosis, and emphysema. Klotho-deficient mice (*kl/kl*) develop normally up to 3 weeks of age, but from this age they begin to show a severe aging phenotype, including growth retardation (94). Klotho was initially thought to be expressed in the distal tubules of the kidney and the choroid plexus of the brain, but was later shown in many different tissues, including the gonads and the pituitary gland. This may explain not only its role in the control of GH secretion, but also how it acts positively in pathological processes, such as arteriosclerosis, and many physiological processes in healthy humans (22). We now know that klotho is a circulating hormone that can be found in body fluids, including blood and cerebrospinal fluid (95), and also in many territories where klotho is not expressed.

The extracellular region of klotho contains two homologous domains, KL1 and KL2, which can be shed from the cell surface (96). Klotho cleavage occurs at a site directly above the plasma membrane (α-cut) or between the KL1 and KL2 domain (ß-cut), resulting in soluble full-length klotho or KL1 and KL2 fragments (97) (**Figure 6**). These different cleavages are carried out by proteases, including a disintegrin and metalloproteinase (ADAM10 and ADAM 17), mainly responsible for α-cut cleavage in kidney cells (97). After its release from the cell membrane, circulating soluble klotho exerts its biological effects on many different organs and tissues. There is no known receptor for soluble klotho, rather there is a coreceptor formed by shed klotho, fibroblast growth factor receptor (FGFR) and FGF23, indicating that shed klotho is an enzyme-dependent active scaffold protein (98). Thus, klotho is an essential cofactor for the binding of FGF23 to its receptor.

Klotho Regulation and GH Secretion

To date, it has not been clearly established how klotho secretion is regulated, although the fact that the kidneys are the main source of klotho suggests that hormones and factors involved in mineral homeostasis play a role in this regulation. This is the case with adiponectin, a hormone that reduces renal secretion of

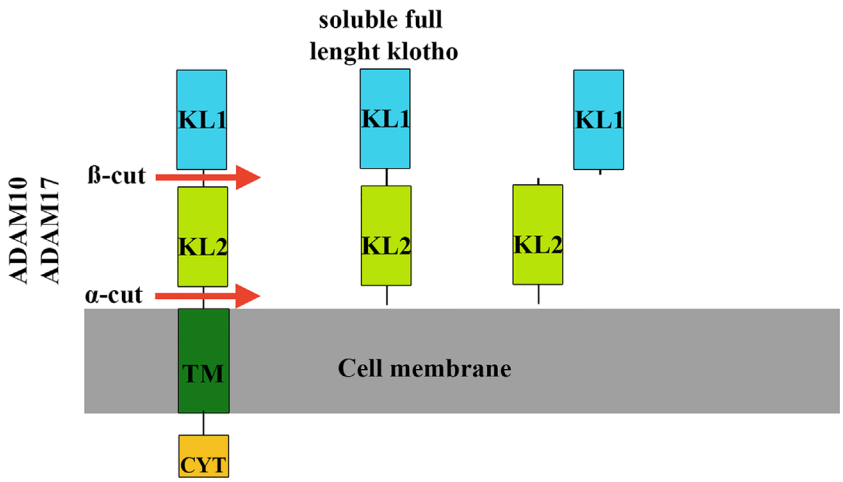

FIGURE 6 | Klotho cleavage mechanisms. Klotho cleavage occurs in the extracellular domain of the klotho protein and is carried out by proteases (ADAM10 and ADAM17). In kidney cells, cleavage at a site directly above the cell membrane (α-cut) results in full-length circulating soluble klotho, which can act in many different organs and tissues. The other type of cleavage (ß-cut) occurs between the KL1 and KL2 domains that give rise to the KL2 and KL1 fragments. TM, membrane portion of klotho. CYT, intracellular portion of klotho.

klotho (99). Interestingly, adiponectin sensitizes insulin and insulin stimulates the cleavage and release of the extracellular domain of klotho (96). Similar to insulin, IGF-I appears to stimulate klotho secretion (22, 100), whereas klotho inhibits insulin/IGF-I signaling (101) (**Figure 7**), and activation of both hormone receptors (101). Interestingly, in mice, intraperitoneal injections of klotho for 4 weeks produced a significant increase in the levels of liver mRNA of IGF-I and its carrier protein IGFBP3 (102), which seems to contradict the inhibition that klotho exerts on the inhibitory effect of IGF-I in the pituitary secretion of GH. This and other studies indicate that klotho is a positive regulator of GH release. Perhaps this is the reason why *kl/kl* mice have hypotrophic somatotrophs and a reduced number of secretory granules (94).

The mechanisms by which klotho induces GH secretion involve the activation of the ERK1/2 pathway (**Figure 8A**), also demonstrated in GH3 cells (102); in these cultured cells cotreatment of klotho and bFGF further increased ERK1/2

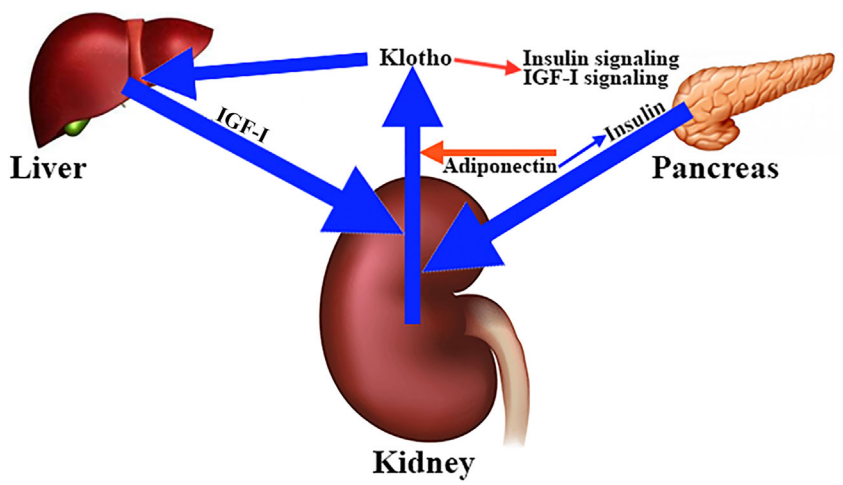

FIGURE 7 | Main factors involved in the regulation of klotho secretion in the kidney. The kidneys are the main source of circulating klotho. Different hormones and factors involved in mineral homeostasis contribute to the regulation of klotho secretion. For example, adiponectin decreases renal klotho secretion (red arrow), while insulin stimulates cleavage and release of full-length soluble klotho (blue arrow), an effect that is sensitized by adiponectin (blue arrow). The same effect on klotho secretion is carried out by IGF-I (blue arrow), whose hepatic production appears to be stimulated by klotho (blue arrow). This is curious because klotho inhibits the insulin and IGF-I signaling pathways (red arrow).

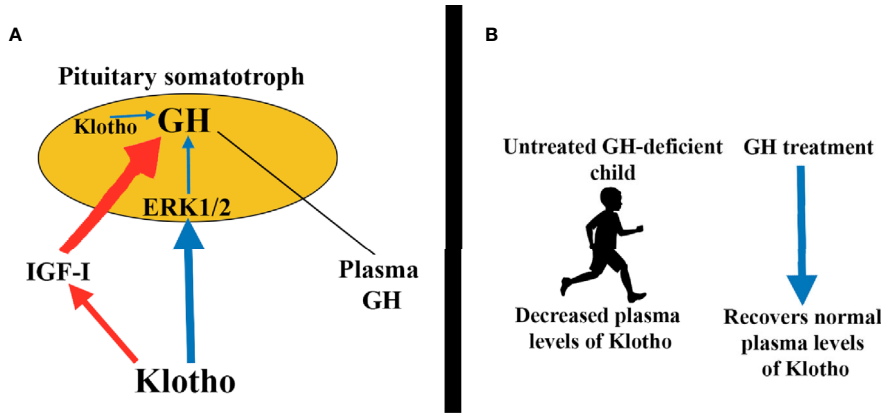

FIGURE 8 | Klotho induces pituitary GH secretion. **(A)** Circulating klotho directly stimulates GH secretion by activating the ERK1/2 pathway (blue arrows). Indirectly, klotho also promotes GH secretion by inhibiting IGF-I and consequently its inhibitory effects on GH release (red arrows). Additionally, klotho is expressed in somatotrophs, perhaps to modulate auto/paracrine GH production (blue arrow). **(B)** Interestingly, plasma klotho levels are decreased in untreated GH-deficient children, but GH replacement therapy brings klotho to normal values.

phosphorylation, while inhibition of ERK1/2 activation leads to abolition of klotho-induced GH release in normal pituitaries (102). Klotho plasma levels are decreased in untreated GH-deficient children and adults, but increased during GH treatment (100) (**Figure 8B**). This was associated with an increase in plasma IGF-I levels dependent on the activation of Akt-mTOR pathway (100). However, in the case of klotho genetic deletions (*kl/kl* in mice) GH administration cannot lead to normal growth in these mice (103).

In acromegaly, increased levels of circulating klotho have been reported, returning to normal values shortly after surgery (104). This is most likely due to the fact that the elevated IGF-I levels that exist in this pathology lead to increased klotho secretion which, in turn, further increases GH secretion. Another possibility, not investigated, is that since klotho is also produced in the somatotrophs, perhaps to modulate auto/paracrine GH production, increased klotho in acromegaly could be a consequence of increased GH secretion; that is, klotho would be released from the pathological pituitary accompanying GH secretion.

Interestingly, a study in patients with pituitary adenomas showed that there was expression of klotho in both GH-secreting and non-GH-producing adenomas; this expression of klotho, proven by immunohistochemistry, was higher in non-GH-secreting adenomas, suggesting that non-GH-secreting pituitary cells are capable of producing klotho.

Summary

Klotho is an anti-aging agent that is expressed primarily in the kidneys and the cerebral choroid plexus, but also in many other different tissues and organs, such as the gonads and the pituitary gland. Although it is a transmembrane protein, it can be found in the circulation, at the expense of proteolytic cleavage of its extracellular region. The soluble form of klotho thus generated is then capable of exerting multiple effects related to the mineral homeostasis of the organism, but also with physiological functions in different organs and tissues. Interestingly, there is no known receptor for klotho, rather there is a coreceptor formed by shed klotho, FGFR, and FGF23, which indicates that shed klotho is an enzyme-dependent active scaffold protein; therefore, klotho is a key cofactor for the binding of FGF23 to its receptor.

Among the multiple effects of klotho, we have to comment in relation to this review, the effect of klotho on pituitary GH secretion. Klotho induces GH secretion at the expense of ERK1/2 phosphorylation. Children and adults with untreated GH-deficiency show reduced plasma levels of klotho, but GH replacement therapies restore these low levels of klotho to normal values (**Figure 8B**). This does not occur when klotho is absent due to genetic mutations or deletions (*kl/kl* in mice, for example). In these situations, GH replacement therapy cannot lead to normal growth.

Insulin and IGF-I appear to stimulate the secretion of klotho, whereas klotho inhibits insulin/IGF-I signaling and the activation of both hormone receptors.

Klotho is expressed in pituitary adenomas and its expression is higher in non-GH-secreting adenomas than in GH-secreting adenomas, suggesting that non-GH-secreting pituitary cells are capable of producing klotho.

In summary, klotho is part of the complex world of regulating pituitary GH secretion, acting positively on it. The relationships between GH and klotho are summarized in **Table 3**.

TABLE 3 | Effects of klotho on GH secretion and *vice versa*.

Level	Effect
Anterior Pituitary	+ Phosphorylation of ERK ½ Paracrine induction of GH secretion Inhibits the inhibitory effect of IGF-I
Plasma levels of klotho	Decreased in untreated GH-deficient patients GH administration recovers normal values of klotho

+, *Stimulates.*

NESFATINS

In 2006, a study described nesfatin-1 as a hypothalamic and brainstem peptide whose expression decreased during fasting, suggesting a role for this peptide in energy balance (105). Other studies using RT-PCR demonstrated that nesfatin-1 was expressed in various areas of the brain involved in metabolic regulation and eating behavior (105–108). Nesfatin-1 is also expressed in the adipose tissue and has been found in serum (108). The wide distribution of nesfatin-1 in the CNS indicates that this peptide also exerts endocrine and autonomic effects on energy expenditure (109); for example, nesfatin-1 has been found to be co-localized with neuroendocrine hormones, including GHRH or somatostatin, among others (109). Interestingly, nesfatin-1 has also been found to be produced in ghrelin-producing cells of the stomach (31), where it may be involved in the des-acyl ghrelin-induced inhibition of peripherally administered orexigenic ghrelin in free-fed rats (110).

In 2019, two DNA and calcium-binding peptides called nucleobindins (NUCB1 and NUCB2) were reported to be involved in many physiological processes as multifunctional regulators of cell biology, including activation of G protein signaling (111). These NUCBs can give rise to smaller peptides called nesfatin-1 (NESF) and nesfatin-1-like peptide (NLP) that share a 76.6% amino acid sequence identity with NESF (112). Although the full function of these peptides is not well understood, they suppress food intake and contribute to modulating energy homeostasis (106, 113–115), and they also produce endocrine effects, such as stimulating insulin secretion (112, 116) or regulating gonadal function (117, 118). From these data, it is feasible to assume that nesfatins are pleiotropic hormones that act through G-protein-coupled receptors (GPCR) (107, 119).

Nesfatins Inhibit GH Synthesis and Secretion

In a very recent study, both NESF and NLP have been shown to inhibit GH synthesis and its ghrelin-induced release in mammalian somatotrophs (120). This study was carried out in cultured cell lines (GH3 and RC-4B/C) in which the authors demonstrated that both *nucb1* and *nucb2* mRNA expression existed, as well as their corresponding NUCB1 and NUCB2 proteins. NLP was found to be mainly located in the cytoplasm, while the distribution of NESF was more diffuse and was also found in the nucleus. Both NESF and NLP bind to the membrane of GH3 cells, suggesting the possibility of a GPCR-mediated action of NESF and NLP in these cells.

The expression of *gh* mRNA was down-regulated in these cells when incubated in the presence of low and high, but not medium, concentrations of these nesfatins. The same occurred with the expression of the pituitary-specific positive transcription factor 1 (*pit-1*), although in this case a significant down-regulation was only observed for NESF at 1 and 24 h of incubation, while NLP only led to a significant down-regulation of *pit-1* at 24 h. Consequently, GH protein levels decreased by an amount of approximately 31% at low and high concentrations of NESF after 1 and 6 h of incubation, while the decrease observed when incubating with NLP was slightly less (27%) in the same time periods.

Interestingly, the significant increase observed in *gh* and *pit-1* expression when GH3 cells were incubated in the presence of ghrelin was abrogated when these cells were pre-incubated or co-incubated with NESF or NLP. The signaling pathway responsible for these effects appears to be cAMP/PKA/CREB, which in this study was shown to be negatively regulated by both NESF and NLP (**Figure 9**).

Taken together, these results indicate that in mammalian somatotrophs, nesfatins play an inhibitory role on GH synthesis and secretion, although their physiological significance, for example in humans, has not yet been established.

Summary

Although little is known about the role that nesfatins play in the human body, their wide distribution and the fact that they are circulating pleiotropic hormones indicates that they have to contribute to the modulation of many different physiological processes. For example, they have to act as counteracting hormones that inhibit the orexigenic effects of ghrelin, acting as signals that suppress food intake and modulate energy homeostasis. They also appear to act as neurohormones that modulate the function of many endocrine glands, such as the pancreas, gonads, and pituitary, acting through G-protein-coupled receptors.

In relation to its effects on the pituitary synthesis of GH and its secretion, the action of nesfatins is inhibitory. They reduce

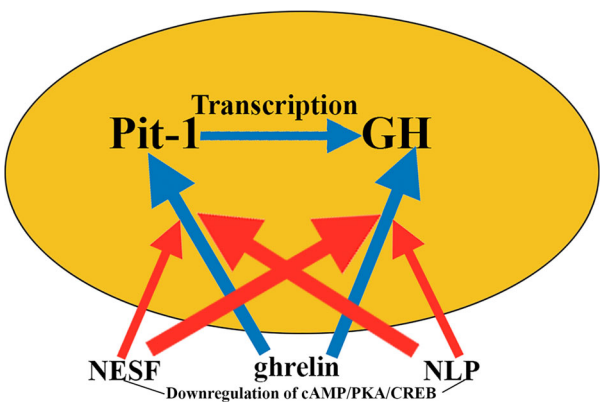

FIGURE 9 | Nesfatins and GH secretion. Nesfatins are involved in suppressing food intake, although they also have many different endocrine effects. Therefore, it is logical that in GH cells cultured with ghrelin, which induces the expression of pit-1 and GH (blue arrows), the addition of nesfatins to the medium blocks, if not totally to a great extent, these effects of ghrelin on pit-1 and GH (red arrows), although its effects in intensity and time vary depending on the nesfatin used. The mechanism by which nesfatins induce these effects appears to be the downregulation of cAMP/PKA/CREB. NESF, nesfatin-1; NLP, nesfatin-1-like peptide.

the expression of *pit-1*, and consequently that of the *gh* gene, by negatively regulating the cAMP/PKA/CREB signaling pathway and also block the stimulating effects of ghrelin on GH secretion in the pituitary. Given that these inhibitory effects have been demonstrated in cultured GH-producing cells, they appear to be paracrine and independent of any action on hypothalamic somatostatin release, although it would be interesting to analyze whether nesfatins play a role in hypothalamic control of the synthesis and secretion of pituitary GH exerted by SS-GHRH, or on the neurotransmitters involved in this hypothalamic regulation of GH. The fact that nesfatin-1 co-localizes with neuroendocrine hormones, such as GHRH or somatostatin, may support this possibility.

CONCLUSIONS

Throughout this review, we have analyzed the effects of three peripheral hormonal factors, ghrelin, klotho, and nesfatins, on pituitary GH synthesis and secretion. Adding these factors to the world of GH regulation radically changes the classic concept of how pituitary GH is regulated by somatostatin and GHRH, and these neurohormones by hypothalamic adrenergic pathways (2). These three factors act basically at the pituitary level, although one of them, ghrelin, also performs its GH-secreting action facilitating the release of hypothalamic GHRH, perhaps inhibiting the hypothalamic release of somatostatin.

Interestingly, two of these factors, ghrelin and nesfatins, are involved in the regulation of energy homeostasis, although with opposite actions since, while ghrelin could be considered the hunger hormone, stimulating appetite, nesfatins would be the hormones of satiety, inhibiting orexigenic action of ghrelin. This may justify the fact that these hormones are involved in the control of the synthesis and secretion of GH, a hormone with important metabolic actions: hyperglycemic, lipolytic, and anabolic, which is stimulated by ghrelin and inhibited by nesfatins. Changes in the metabolic needs of the body at each moment of the day and throughout life would justify the secretion and actions of each of these hormones to optimize energy homeostasis at the expense of GH, among other factors. Interestingly, gastric ghrelin secretion decreases with age, as occurs with GH, while the pituitary ghrelin receptor remains present in the pituitary. It is very important that ghrelin has to be acylated by ghrelin O-acyl-transferase (GOAT), which binds a fatty acid side chain (C8) to serine 3 of ghrelin, forming acyl ghrelin, the active form that acts on its receptor, a G-protein-coupled receptor (GHSR-1a).

The other factor, klotho, is an anti-aging agent, the soluble circulating form of which directly induces GH secretion by activating ERK1/2 and inhibits the inhibitory effect that IGF-I exerts on GH. Klotho is also involved in the regulation of mineral homeostasis. Perhaps this is why the kidneys are the main source of this peptide. Klotho expression has also been found in the pituitary, where it perhaps induces a paracrine effect on GH synthesis and secretion. There are very important relationships between klotho and GH; untreated children and adults with GH-deficiency show reduced plasma levels of klotho, but GH treatment restores them to normal values (121). Deletions or mutations of the *klotho* gene affect GH production, but GH treatment cannot induce a normal growth in mice displaying these deletions (*kl/kl* mice).

TABLE 4 | Growth Hormone transcription factors.

FACTORS	Inducers	Inhibitors
Pit-1	+	
Ghrelin	+	
NESF and NLP		+
Klotho	?, +?	

+, *Induces.*

In summary, these three factors must be added to the world of GH control. All of them act after being released from the peripheral level, directly on the pituitary gland (not yet proven for nesfatins) but they can also act on the GHRH-SS system and even paracrine in the pituitary. The broad spectrum of actions that these factors play in the body, along with their actions on GH, reinforces the idea that GH is more than just a growth hormone (11). The role of ghrelin and nesfatins in pituitary GH transcription is summarized in **Table 4**, along with the major GHRH-induced GH transcription factor Pit-1.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

This review has been funded by Foundation Foltra, Teo, Spain, grant number Foltra 2020-06.

ACKNOWLEDGMENTS

I acknowledge Diego de Souza for his valuable collaboration in ordering and formatting the References.

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Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 15 March 2021
doi: 10.3389/fendo.2021.634415

Traumatic Brain Injury as Frequent Cause of Hypopituitarism and Growth Hormone Deficiency: Epidemiology, Diagnosis, and Treatment

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Traumatic brain injury (TBI)-related hypopituitarism has been recognized as a clinical entity for more than a century, with the first case being reported in 1918. However, during the 20th century hypopituitarism was considered only a rare sequela of TBI. Since 2000 several studies strongly suggest that TBI-mediated pituitary hormones deficiency may be more frequent than previously thought. Growth hormone deficiency (GHD) is the most common abnormality, followed by hypogonadism, hypothyroidism, hypocortisolism, and diabetes insipidus. The pathophysiological mechanisms underlying pituitary damage in TBI patients include a primary injury that may lead to the direct trauma of the hypothalamus or pituitary gland; on the other hand, secondary injuries are mainly related to an interplay of a complex and ongoing cascade of specific molecular/biochemical events. The available data describe the importance of GHD after TBI and its influence in promoting neurocognitive and behavioral deficits. The poor outcomes that are seen with long standing GHD in post TBI patients could be improved by GH treatment, but to date literature data on the possible beneficial effects of GH replacement therapy in post-TBI GHD patients are currently scarce and fragmented. More studies are needed to further characterize this clinical syndrome with the purpose of establishing appropriate standards of care. The purpose of this review is to summarize the current state of knowledge about post-traumatic GH deficiency.

Keywords: traumatic brain injury, hypopituitarism, growth hormone deficiency, pituitary, brain damage

OPEN ACCESS

Edited by:
Antonio Mancini, Catholic University of the Sacred Heart, Rome, Italy

Reviewed by:
Maria Chiara Zatelli, University of Ferrara, Italy
Andrzej Lewinski, Medical University of Lodz, Poland
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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *27 November 2020*
Accepted: *16 February 2021*
Published: *15 March 2021*

Citation:
Gasco V, Cambria V, Bioletto F, Ghigo E and Grottoli S (2021) Traumatic Brain Injury as Frequent Cause of Hypopituitarism and Growth Hormone Deficiency: Epidemiology, Diagnosis, and Treatment. Front. Endocrinol. 12:634415. doi: 10.3389/fendo.2021.634415

INTRODUCTION

Traumatic brain injury (TBI) is one of the leading causes of disability and mortality affecting many people each year and resulting in a serious burden of devastating health consequences (1–3). The most common mechanism for TBI are falls, especially in older adults and very young children; motor vehicle accidents are instead the most frequent cause of TBI among young adults (1). TBI may lead to permanent or transient pituitary insufficiency (4, 5). The clinical picture presents a very large spectrum determined by the kind, number and severity of hormonal deficiency and could go

from mild and non-specific complaints to life-threatening conditions. The reported prevalence of hypopituitarism is quite variable among the available studies (6–9). The pathophysiologic mechanisms underlying pituitary damage in TBI patients include a primary injury that may lead to direct trauma to the hypothalamus or pituitary gland, or to compressive effect from surrounding structures; secondary injuries, on the other hand, are mainly related to an interplay of a complex and ongoing cascade of specific molecular/biochemical events. The diagnosis of pituitary dysfunction is very challenging both due to the common occurrence of TBI, the subtle character of clinical manifestations, the variable course of the disease, as well as the lack of proper diagnostic algorithms. Growth hormone deficiency (GHD) is the most common abnormality, followed by hypogonadism, hypothyroidism, hypocortisolism, and diabetes insipidus (8, 9). The purpose of this review is to summarize the current state of knowledge about post-traumatic hypopituitarism, and especially about post-traumatic GHD. However, well-designed studies are needed to further investigate the pathophysiology, epidemiology, and timing of pituitary dysfunction after a TBI with the purpose of establishing appropriate standards of care.

EPIDEMIOLOGY OF TBI AND TBI-RELATED HYPOPITUITARISM

Epidemiology of TBI

TBI is one of the leading causes of disability and mortality in western countries. It can be estimated that in the United States, every year there are around 2.5 million visits at the emergency department, 280,000 hospitalizations and 50,000 deaths related to TBI (1).

The highest rates of TBI are observed in older adults (≥75 years; 2232 per 100,000 population), followed by very young children (0 to 4 years; 1591 per 100,000) and young adults (15 to 24 years; 1081 per 100,000). There is a sex prevalence, with males showing a higher risk than females (959 per 100,000 vs 811 per 100,000). Falls are the most common mechanism for TBI, especially in older adults and very young children; motor vehicle accidents are instead the most frequent cause of TBI among young adults (1).

After the acute phase, TBI-survivors are often forced to deal with relevant and persistent long-term sequelae, with significant neurological and functional impairment. The prevalence of TBI-related long-term disability in the United States is estimated to affect between 3 and 6 million patients, i.e., 1% to 2% of the population (2, 3).

Apart from the individual sequelae, TBI clearly determines significant economic implications for society, related both to direct expenses for medical care and to indirect costs caused by injury-related work loss and disability (10). The former can be as high as 80,000 US dollars per person in the first year after trauma (11). The latter is more difficult to estimate, but it is likely to account for more than 80% of the total economic burden of TBI (10).

Epidemiology of TBI-Related Hypopituitarism

TBI-related hypopituitarism has been recognized as a clinical entity for more than a century, with the first case being reported in 1918 (12). However, during the 20th century it was considered only a rare sequela of TBI.

Most likely, hypopituitarism was under-recognized for such a long time for its generally subtle and nonspecific clinical features that also share a significant overlap with many of the somatic, psychiatric and neurological symptoms directly related to TBI. As a consequence, only patients with the frankest clinical pictures were probably identified as having a TBI-related hypopituitarism, leaving unrecognised the vast majority of TBI patients with some degree of pituitary deficits. This underestimation may probably have affected patient life expectancy and quality, as it happens to every patient living with unrecognised and untreated hypopituitarism (13).

The awareness of the critical relevance of hypopituitarism in TBI patients radically changed during the last two decades. The first two cornerstone studies that solidly proved that TBI-related hypopituitarism was a far more common sequela of head trauma than previously thought have been published in 2000 and 2001 (4, 5). Since then, several other studies of the endocrine function in patients after TBI have been published (6, 7, 14–40).

The reported prevalence of hypopituitarism is quite variable among the available studies, ranging from 1% (6) to 76% (7). However, this relevant heterogeneity should not surprise because the existing studies widely differ in many aspects, such as study design, age of patients, severity of trauma, time point of endocrinological evaluation and testing protocols for the diagnosis of the deficiency of the various pituitary axes.

Given these premises, an overall summary of the available evidence can be found in two major meta-analyses (8, 9). Pooled data show that the proportion of patients with some kind of pituitary disfunction can be estimated to be approximately 27.5-32.0% (8, 9). In most individuals only a single pituitary axis is affected (19.8-25.3%) (8, 9), while involvement of multiple pituitary axes is far less frequent (6.7-7.7%) (8, 9).

Furthermore, it has been consistently proved that not all pituitary axes are equally susceptible to TBI-mediated damage. The most sensitive ones appear to be GH and FSH/LH (12.4-22.1% and 10.2-12.5%, respectively) (8, 9); on the other hand, deficits of ACTH and TSH axes appear to be significantly less frequent (8.2-9.9% and 4.1-6.2%, respectively) (8, 9).

PATHOPHYSIOLOGY OF TBI-RELATED HYPOPITUITARISM

Overview on the Pathophysiology of TBI

TBI is a heterogeneous disease. There are many ways to categorize the patients, both in terms of clinical severity and pathophysiological mechanism of injury.

Clinical severity is usually assessed by specific severity scores; the most commonly used is the Glasgow Coma Scale (GCS), which evaluates three neurological domains (eye opening, best

verbal response, best motor response) and classifies TBI as mild (GCS 13-15), moderate (GCS 9-12) or severe (GCS ≤ 8) (41).

The pathophysiology of TBI is usually summarized into two separate categories: primary and secondary brain injury (42).

Primary brain injury occurs at the time of trauma, as a consequence of external mechanical forces transferred to intracranial content. The pathologic sequelae of primary brain injury include shearing of white matter tracts (also known as diffuse axonal injury), focal cerebral contusion/hemorrhages, and focal extra-axial hematomas/hemorrhages (i.e. epidural hematomas, subdural hematomas, subarachnoid hemorrhage and intraventricular hemorrhage) (42, 43).

Following this primary injury, extensive and lasting damage is sustained through a complex and ongoing cascade of events referred to as secondary brain injury. Pathogenesis is driven by complex, interacting mechanisms that include, among others, neurotransmitter-mediated excitotoxicity, secondary ischemia (from vasospasm or other secondary vascular injuries, such as focal microvascular occlusion), and inflammatory responses. As a final consequence, these mechanisms of injury lead to neuronal cell death, cerebral edema and increased intracranial pressure, which can further exacerbate brain damage (42, 44, 45).

Pathophysiology of TBI-Related Hypopituitarism

From a general point of view, the pathophysiological mechanisms underlying pituitary damage in TBI-patients are broadly similar to those described for TBI itself.

Primary injury may lead to direct trauma of the hypothalamus or pituitary gland, or to compressive effect from surrounding structures (42, 43); moreover, especially in case of skull base fracture, primary injury may determine pituitary stalk transection (46) (**Figure 1**).

On the other hand, secondary injuries are mainly related to an interplay of a complex and ongoing cascade of specific molecular/biochemical events (42, 44, 45) (**Figure 1**).

As already discussed, one of the three major mechanisms for secondary brain injury after head trauma is represented by excitotoxicity. Excitotoxicity is caused by the abnormal levels of excitatory neurotransmitters (mostly glutamate) that are uncontrollably released in patients with TBI. In fact, at high concentrations, these neurotransmitters act as excitotoxins, operating on ion channels and thus altering cell wall permeability with an unregulated electrolyte shift between intra- and extracellular spaces (47).

Another mechanism for secondary brain damage after TBI is represented by ischemia. Overall, the same pathophysiological events affecting brain are likely to underlie the pituitary-specific ischemic insult as well. However, some distinctive points related to the peculiar vascularization of the hypothalamic-pituitary area are still worth to be discussed. As known, the anterior pituitary receives its blood supply from the hypothalamic-hypophyseal portal circulation (48–50), which likely poses the gland to a greater risk of ischemic harm (**Figure 2**). In particular, long hypophyseal portal vessels substantially represent the only source of vascularization of the lateral portion and of pars tuberalis (mostly populated by GH, PRL and FSH/LH secreting cells) (52) (**Figure 3**). Instead, the antero-medial portion and the central wedge (mostly populated by TSH and ACTH secreting cells) (52) (**Figure 3**) receive a mixed supply by both long and short hypophyseal portal vessels (48–50) (**Figure 2**). Therefore, the ischemic susceptibility hypothesis may be one of the most plausible explanation for the differential frequency of pituitary axes involvement after TBI. In fact, the most vulnerable axes (GH and FSH/LH) are those whose blood supply only relies on long hypophyseal portal vessels, that are by themselves more prone to vascular damage; instead, the most resilient ones (ACTH and TSH) are those whose blood supply is guaranteed both by long and short hypophyseal portal vessels (48–50, 52) (**Figures 2**, **3**).

The third and last major mechanism implied in the pathophysiology of secondary pituitary injury after trauma is inflammation. Part of the inflammation mechanisms affecting

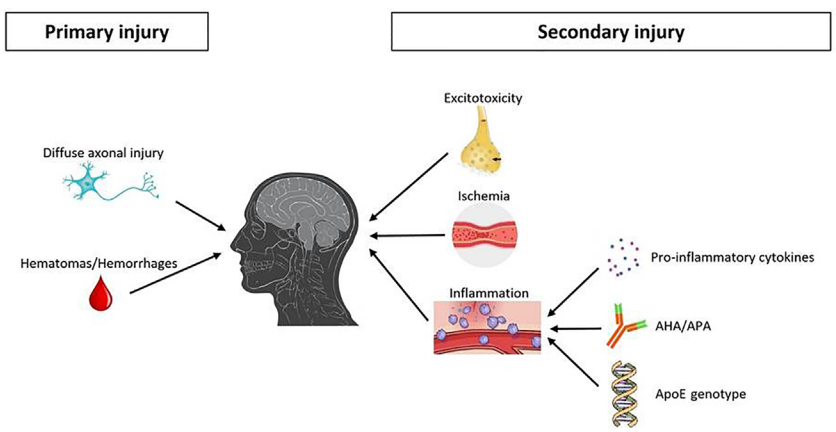

FIGURE 1 | Main pathophysiological mechanisms underlying pituitary damage after TBI. TBI, traumatic brain injury; AHA, anti-hypothalamus antibodies; APA, anti-pituitary antibodies.

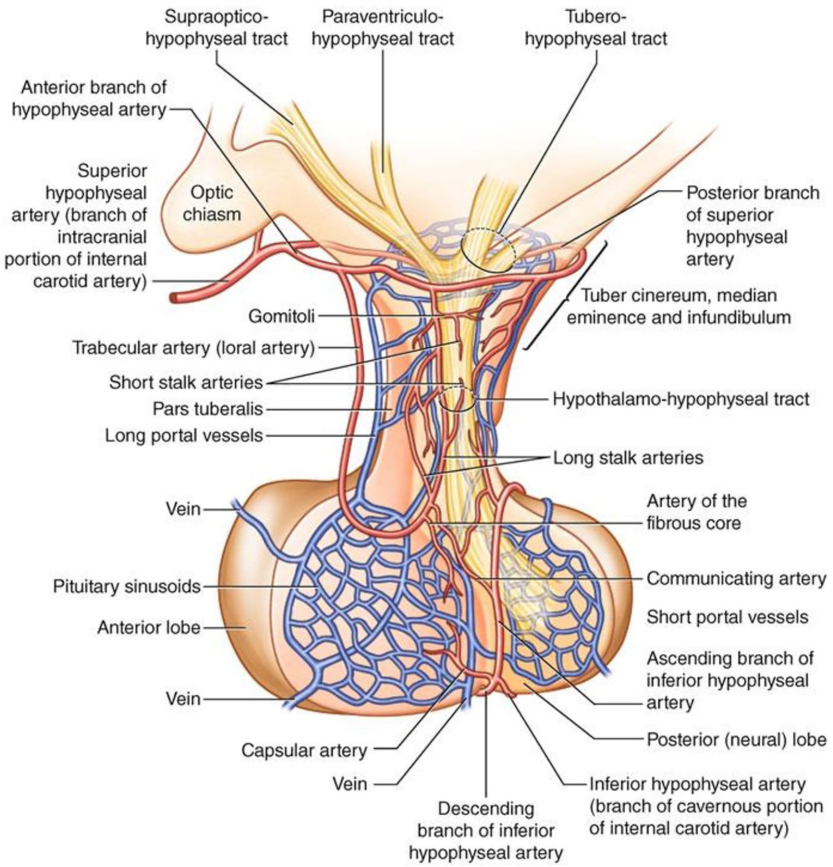

FIGURE 2 | Anatomy and vascularization of the hypothalamus and pituitary gland [reproduced with permission from (51)].

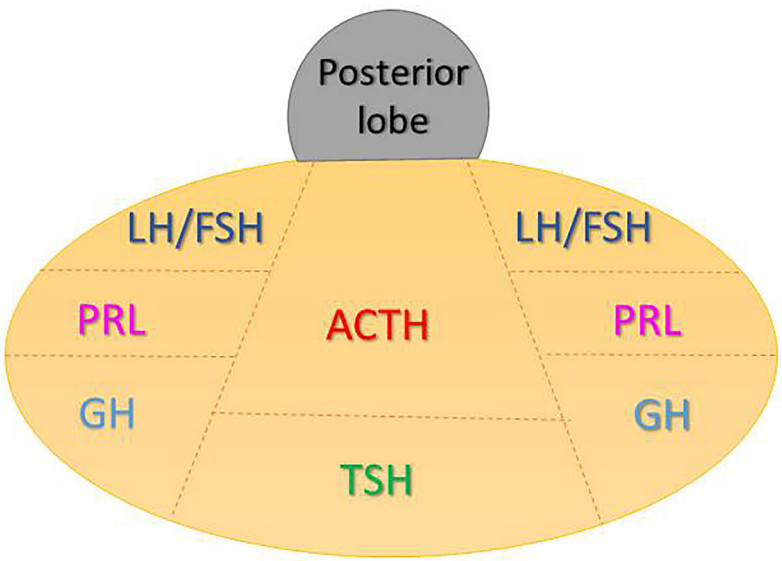

FIGURE 3 | Distribution of anterior pituitary cell subtypes.

the pituitary gland after TBI are probably shared with the general inflammation mechanisms known to affect the brain parenchyma as a whole, with an uncontrolled and self-sustaining release of pro-inflammatory cytokines such as interleukin 1 (IL-1) and tumor necrosis factor α (TNF-α) (53).

In addition, a pituitary-specific inflammatory mechanism may be related to autoimmunity and, more specifically, to the presence of anti-hypothalamus antibodies (AHA) and/or anti-pituitary antibodies (APA). The positivity to these antibodies was demonstrated to be more frequent in patients with previous TBI (ranging from 44% to 60% depending on the study) than in matched healthy controls (0%) (54, 55). A similar result, even if less pronounced, was found in a cohort of subjects who underwent chronic repetitive head trauma due to amateur boxing activity, in which a higher positivity of AHA (21%) and APA (23%) was found compared to matched controls (0%) (56). Moreover, among patients with previous TBI, various authors have demonstrated a statistically significant correlation between the presence of AHA and/or APA positivity and that of post-traumatic hypopituitarism, with an odds-ratio (OR) ranging from 2.2 to 8.5, depending on the study (54–56). Looking at the whole body of these results, it is therefore reasonable to hypothesize that autoimmunity may play a role in the pathogenesis of TBI-induced hypopituitarism. The genesis of AHA and/or APA production is likely related to hypothalamus and/or pituitary exposure and release of antigens that would have otherwise remained unexposed. Despite the aforementioned association, the available evidence is not clearly sufficient to establish whether AHA/APA positivity may actually play an active pathophysiological role in the propagation/perpetuation of TBI-related pituitary damage, or may instead represent only an epiphenomenon of hypothalamic/pituitary antigen exposure due to necrotic post-TBI alterations of these areas.

Finally, another potential pituitary-specific inflammatory modulation mechanism may be related to individual intrinsic genetic susceptibility. In the general context of traumatic brain injury, ApoE polymorphisms have been widely shown to be associated with various clinical outcomes after TBI, such as the onset of seizures (57), coma duration (58), and subsequent neurobehavioral recovery (59). In fact, ApoE is a key protein in enhancing lipid transport and metabolism within the nervous system and has a role in neuronal repair and maintenance. In the specific context of post-traumatic hypopituitarism, a study from Tanriverdi et al. confirmed the possible role of ApoE polymorphism on neuroendocrinological outcomes; in particular, this study demonstrated a likely protective role of ApoE3/E3 genotype against the development of post-traumatic pituitary disfunction, with an estimated OR of 0.29 (60).

Evidence From Histopathological Studies

Most histopathological studies in patients with TBI-related hypopituitarism have been published several decades ago, as autoptic case series. These studies showed that the most frequent pathological alterations of pituitary gland after TBI were anterior lobe ischemic necrosis, posterior lobe haemorrhage and pituitary stalk haemorrhage, each occurring in approximately 14-42% of patients (61–64). A normal pituitary could be found in a variable proportion of 14-74% of patients, depending on the study (61–64).

More recently, another study by Salehi et al. (65) provided a further insight into histopathological changes of the pituitary gland after TBI. The overall results were not in contrast with previous case series, but a more accurate analysis relating pathological findings to the timing of death after trauma has to be discussed. In fact, the authors showed that no abnormalities of the pituitary gland could be found in patients who died immediately after trauma, both at macroscopic and at microscopic level. On the other hand, several degrees of pituitary haemorrhage and/or ischemic necrosis could be found in 43% of patients who survived between 3 h and 7 days after trauma. Moreover, the extent of histopathological alterations in those patients was significantly related to the time elapsed between injury and death (< 10% of adenohypophysis involved in patients who died within 1 day, > 50% in patients who died between 1 and 7 days) (65).

In conclusion, the whole body of histopathological evidences supports the hypothesis that post-TBI neuroendocrine damage could be mostly related to pituitary vascular vulnerability. However, despite the interest of these results, it must be pointed out that they are based on autoptic studies looking at a very specific and selected subset of TBI patients, i.e. those dying in the acute phase after a severe TBI. This undoubtedly represents an important selection bias, as this category of patients accounts for just a small minority of all patients suffering from TBI. Moreover, this is a category of TBI patients in which correlation with hormonal outcomes is neither feasible nor relevant. Precise pituitary histopathological correlations in long-term TBI survivors are thus lacking, but further research in this field are clearly conditioned by the constitutional limit to conduct this kind of evaluation *in vivo*.

Evidence From Imaging Studies

Several studies have been published about the possible identification of microstructural abnormalities of the pituitary gland by imaging techniques in patients with TBI-related hypopituitarism. Also in this subset of patients the imaging modality of choice for the evaluation of the pituitary gland was mostly MRI.

No single features were found to predict with high accuracy the presence or the absence of hypopituitarism in patients experiencing TBI. Therefore, in clinical practice the role of imaging in the prediction of post-traumatic hypopituitarism is limited. However, the available evidence is still of significant interest, as it provides information on the possible pathophysiological mechanisms through which post-traumatic hypopituitarism develops.

In this regard, the role of imaging is surely less accurate than that of pathological studies, considering that the deduced evidences about the underlying pathophysiological mechanisms can be only indirect. On the other hand, the clear advantage is that these evidences may be obtained for all patients with TBI, regardless of trauma severity and mortality.

In the acute phase, Maiya et al. (66) showed a significant enlargement of pituitary gland dimensions after TBI compared

to healthy controls. In particular, this enlargement was evident not only in those patients with focal changes of the pituitary gland (haemorrhagic foci, heterogeneous signal intensities, macroscopical swelling) but also in those without specific focal pituitary abnormality. This is consistent with a pathophysiological hypothesis of an underlying pituitary edematous change, which is likely to be predominant in the first days after TBI. However, in this study no data about the subsequent pituitary functional outcomes were available, neither in the short nor in the long term. Therefore, it is not possible – based on these data – to explore the potential correlation of pituitary dimension in the acute phase and the subsequent development of pituitary deficits.

In the subacute phase, Zheng et al. (67) showed a reduction in apparent diffusion coefficient (ADC) in diffusion-weighted imaging (DWI) of pituitary gland in patients with previous TBI compared to healthy controls. DWI is based on the capacity of MRI to demonstrate the random, brownian diffusion movement of water. It is widely recognized as one of the best imaging modalities of cerebral ischemia, in which failure of the energy-dependent Na-K-ATPase determines a translocation of water from the interstitial to the intracellular space, and therefore a reduction of its diffusion capacity (68). This diffusion restriction determines a coherent change in signal intensity on DWI and on the derived ADC map, in which ischemic areas appear characteristically hypointense (69, 70). The results obtained by Zheng et al. thus confirm the probable role of ischemia in pituitary microstructural change in the first few weeks after TBI. Interestingly, even if ADC reduction with respect to healthy controls was found to be significant in the whole cohort of TBI patients, this alteration resulted to be more prominent in those which subsequently developed pituitary deficits at the hormonal follow-up; this is a further proof of the plausible relation between the severity of microstructural ischemic abnormalities in the short-term and the final functional outcome in the long-term.

In the chronic phase, Schneider et al. (71) showed that, after TBI, pituitary abnormalities at imaging were more frequent in patients with some degree of hypopituitarism (80%) than in those without pituitary hormone deficits (29%). The most common finding, in particular, was pituitary volume reduction, up to the degree of empty sella in some patients. The pathophysiological process underlying these changes is plausibly related to the long-term sequelae of pituitary tissue necrotization and subsequent fibrosis, which likely contribute to irreversible tissue loss.

The evidence of a pituitary volume reduction in the chronic phase finds further confirmation in a study by Tanriverdi et al. (72), in which the authors examined pituitary function and pituitary volume in a cohort of amateur boxers. As it is known, boxers are exposed during their career to mild chronic repetitive head traumas, which may determine pathophysiological consequences similar to classical TBI in the long term. In this study, 18% of boxers had some degree of pituitary dysfunction, and the presence of one or more hormonal deficits was significantly correlated with a lower pituitary volume at MRI.

TBI-RELATED HYPOPITUITARISM: CLINICAL PRESENTATION

Post-traumatic hypopituitarism (PTHP) can present with variable and non-specific clinical features, which may overlap with those resulting from the injury. The consequence is a delay in diagnosis, with a higher morbidity and mortality (39).

The clinical picture presents a very large spectrum determined by the kind, number and severity of hormonal deficiencies and could go from mild and non-specific complaints to life-threatening conditions (73).

Traditionally, an acute and a chronic phase can be distinguished, but pituitary dysfunction during the acute phase does not necessarily lead to long-term hypopituitarism. In a systematic review, the analysis of prospective studies showed that some of the early abnormalities are transient with complete recovery, while hypopituitarism can evolve over time and become detectable only later (8).

The first 2 weeks after trauma are considered the acute stage.

The most common hormonal changes in this phase are represented by gonadotropin and GH deficiency, but in the clinical presentation they are not the most evident.

In fact, the most fearsome endocrinological complication of the acute stage is adrenal insufficiency. Hyponatremia, hypoglycemia, hypotension, fatigue, mental confusion are some of its typical features. Patients affected by hypocortisolism require a higher dose of vasopressors and have a higher mortality rate; therefore hormone replacement therapy is crucial (74, 75).

Impaired vasopressin secretion (Syndrome of Inappropriate Antidiuretic Hormone Hypersecretion - SIADH or Central Diabetes Insipidus - CDI) could also be life-threatening, contributing to the hydro-electrolytic imbalance of the acute phase (76).

Hypothyroidism can be also reported, due to the adaptive response after trauma and the use of steroids. It contributes to the clinical picture of marked asthenia, lethargy and confusion, not always easy to discriminate in the acute stage.

GHD is considered a common alteration of the acute phase, reflecting the adaptive response after the traumatic event.

Hyperprolactinemia could be reported as a consequence of pituitary stalk compression or as a physiological reaction to stress. Together with hypogonadism, hyperprolactinemia may lead to the menstrual alteration and the sexual dysfunction of the post-TBI period.

Some of these abnormalities are transient: recovery from hypoadrenalism is described in 50% of patients, from CDI in up to 90% (77). Hypothyroidism and SIADH often resolve in 3-12 months (40).

The chronic phase starts at 3 months after TBI. The clinical features could be very variable and not specific, depending on the different axes involved. Lethargy or insomnia, fatigue, reduced attention, difficulty concentrating, memory impairment, anxiety, depression, irritability and diminished libido are often described (78).

Also in this phase GHD and hypogonadism are the most frequent anomalies, hypocortisolism and central hypothyroidism

are relatively rare and CDI could persist in a small percentage of patients (79).

These patients experience metabolic abnormalities, neurocognitive impairment, and a decreased quality of life (34, 80).

Metabolic Alterations

In patients with PTHP, altered function of hypothalamic nuclei and pituitary disfunction itself determine an adverse metabolic profile. Glycemic disorders, dyslipidemia, weight gain with abdominal fat distribution, changes in body composition and reduced bone mineral density determine the higher morbidity and mortality of these patients (78).

Different studies demonstrated that PTHP patients have higher BMI, increased LDL cholesterol and total cholesterol (19), altered glucose levels and insulin resistence (34).

Decreased thyroid function reduces the basal metabolic rate and hypogonadism affects bone and muscle mass, beyond the effect on libido and reproduction. It is however GHD that plays the major role, affecting glycemic and lipidic profile, increasing BMI and waist circumference, reducing bone mineral density and leading also to anemia (78).

GHD can affect also the rehabilitation: patients with post-TBI GHD seem to have lower aerobic capacity, a measure of physical resistance, which may delay or inhibit the recovery process (81).

Neurocognitive Alterations

Recently, more attention was paid to neuropsychiatric symptoms which are due both to PTHP and brain injury itself. Cognitive and affective impairment may be severe, prejudicing patients' social skills. In fact, attention, memory, executive functions and language can be involved.

Symptoms of PTHP can overlap with cognitive, sleep, mood, and anxiety disorders due to Post-Traumatic Stress Disorder (PTSD). Differential diagnosis can be challenging, but essential for the therapeutic implications.

Hypothyroidism is associated with neurocognitive function: low speed of information processing and deficits in short-term memory are the most described (73).

Hypogonadism is also associated with cognitive dysfunction: patients with lower testosterone levels after TBI seem to have an increased risk for Alzheimer's disease (82).

Also hypoadrenalism results in mood disorders, decreased memory, and frank psychosis in the chronic phase, in addition to the classical picture of fatigue, weakness, and impaired response to stress (78).

Although every hypothalamic-pituitary axis could be involved in cognitive functions, the role of GH and the effects of its deficiency are the most known and frequently observed (83).

In fact, somatotropic axis has a role in microtubular regeneration, dendritic growth and regrowth, regulation of the use of glucose in the brain and, probably, an action on hippocampal area. So, GHD may lead to impaired neuronal, somatic, and dendritic growth, affecting memory and cognitive function too (78, 84).

Several studies reported that patients with post-traumatic GHD have more severe cognitive impairment, in particular deficit in attention and memory, as well as emotional problems, than those with normal GH values (33, 85, 86).

Patients with post-TBI GHD have a higher risk to achieve poor cognition outcomes than those with an intact somatotropic axis after trauma (87). Park et al. showed that patients with GHD after TBI have decreased cerebral glucose metabolism in specific cortical areas involved in intellectual function, executive function, and working memory (88).

In contrast to all these studies, Pavlovic et al. did not find significant differences between patients with or without GHD after brain injury (89).

Quality of Life (QoL)

Another important feature is QoL: patients with post-TBI GHD are more likely to be depressed and with a poorer quality of life than those with GHD due to other causes. In particular, domains of physical health, energy and fatigue, emotional well-being, pain, and general health seem to be affected (85).

Also perceived poor QoL would negatively impact on rehabilitation after TBI.

TBI-RELATED HYPOPITUITARISM: DIAGNOSIS

PTHP, and in particular GHD, are often underdiagnosed: patients with post-TBI GHD seem to be diagnosed on average two and a half years later after the primary onset of disease when compared to those with Non-Functioning Pituitary Adenoma (NFPA) (87).

PTHP diagnosis is not different from hypopituitarism due to other causes. However, the time and type of hormonal assessment in TBI patients is controversial.

Who to Test

Despite the severity of trauma itself, hypopituitarism can develop in patients post-TBI, but testing all of them is not feasible because of the great amount of human and economic resources needed. A rational approach is to evaluate life expectancy, avoiding to test patients with poor prognosis who cannot benefit of hormonal replacement therapy. Conversely, patients with mild TBI could develop hypopituitarism, but not all of them achieve medical attention (90) and testing is not considered cost-effective (87).

Epidemiology could be useful to establish who to screen: in fact, PTHP frequency is better established in patients with moderate or severe TBI based on GCS score. Furthermore, other risk factors include age, intracranial hemorrhage, focal cortical contusion, seizures and skull base fracture.

In patients symptomatic for acute hypopituitarism (i.e. electrolyte unbalance and/or acute adrenal insufficiency) an endocrinological evaluation is mandatory.

Patients with mild TBI who need hospitalization (more than 24 h), a neurosurgical intervention, monitoring in an Intensive Care Unit, or those who present anatomical changes on CT scan, are considered complicated and screening is also recommended (87). Conversely, patients with a mild uncomplicated TBI should

be screened for hypopituitarism only if the clinical suspect is present (91).

Patients who required hospitalization for at least 24 h, those with radiological abnormalities on CT scan, and those who presents signs and symptoms of PTHP should be screened at three months and one year post-TBI. It is possible to perform hormonal screening even further, if symptoms persist (78).

Biochemical Evaluation: Time and Kind

Pituitary function evaluation could be challenging in the acute phase post-TBI. In fact, in this stage patients have hormonal changes as part of the stress response and acute adaptive response to injury. Hormonal levels are also affected by medications and surgery. Pituitary function during this time could be variable (78).

While some of the alterations are natural consequences of the trauma itself, others are life-threatening and require an immediate hormonal replacement therapy. So, basal hormonal evaluation should be performed in any patient with TBI who has been hospitalized and presents signs and symptoms suggesting adrenal insufficiency (92). For the diagnosis, a morning serum cortisol should be measured. Usually, a level beyond 15 μg/dL indicates a proper function, values between 3 and 15 μg/dL require further investigation using a stimulation test, while a concentration lower than 3 μg/dL allows a diagnosis of hypoadrenalism (93). In the post-TBI context, these cut-points are not considered applicable. In fact, plasma cortisol concentrations rise in parallel to the severity of acute illness, so Hannon et al. proposed that critically sick patients should have plasma cortisol levels >300 nmol/L (=10.875 μg/dL), suggesting the need of hormonal replacement therapy for lower values (74). Also the evaluation of cortisol reserve with a dynamic test seems to be not reliable in TBI patients, at least in the acute phase. In fact, Endocrine Society Clinical Practice Guidelines suggest performing an ACTH stimulation test (Synacthen test) when required for secondary adrenal deficiency diagnosis (93), preferring the low-dose (1 μg) version to reduce false negative results due to a supraphysiological stimulus (94, 95). However, in the setting of the acute phase of TBI a Synacthen test is inappropriate because of the acute nature of ACTH deficiency, that would not have time to lead to secondary adrenal insufficiency. Therefore, patients with TBI would have a normal response also to the low-dose ACTH stimulation test (74). Even an extremely accurate test as Insulin Tolerance Test (ITT) is not feasible for safety concerns in patients with TBI in the acute phase.

First post-acute phase evaluation should be scheduled 3-6 months after TBI. Thyroid and gonadal axes are frequently involved, so they should be evaluated. TSH and fT4 are dosed to confirm or exclude hypothyroidism, while gonadic evaluation is different between males and females. In men with suspected hypogonadism LH, FSH, testosterone and PRL levels should be dosed. In women of reproductive age with menstrual irregularities is recommended to measure PRL, LH, FSH and estradiol (E2), while in postmenopausal women gonadotropins reduction may be sufficient for the diagnosis (78, 83, 93). The adrenal axis should be re-evaluated measuring morning serum cortisol and, eventually, using an appropriate stimulation test.

To assess for CDI in patients with polyuria, serum and urine osmolality should be dosed simultaneously: urine osmolality/plasma osmolality ratio should be ≥2, after excluding glycosuria (78).

Somatotropic axis evaluation is recommended at least six months after TBI because of the possible spontaneous recovery in the post-acute phase (87). Some Authors proposed to postpone the evaluation until 1 year after TBI in adults, while children may require an earlier assessment (83). The diagnosis of GHD could not be based only on IGF-I levels; even if in patients with low IGF-I levels and multihormonal deficiency GHD diagnosis does not require further tests (96), in all other conditions different dynamic tests are available to confirm the suspected somatotropic deficiency with a high accuracy (96, 97). The choice of a test over another is usually based on patient's characteristics, availability of secretagogues and center experience (87). ITT is considered the gold standard and it evaluates both hypothalamic and pituitary integrity, but it can be used only after excluding contraindications (i.e. seizure, cardiac disease) (8, 90). Up to 22% of patients who had TBI develop seizure disorders, so ITT is often considered not safe in this setting (98).

The administration of GH Releasing Hormone (GHRH) plus Arginine (Arg) or GHRH plus GH Releasing Peptide 6 (GHRP-6) provides a strong stimulus to GH secretion and are considered safe, so they could be used as a dynamic test for GHD evaluation. However, they are not useful for GHD of hypothalamic origin and both GHRH and GHRP-6 are unavailable in many Countries (96). Glucagon Stimulation Test (GST) is not usually the first choice for GHD diagnosis. In fact, it could be labored, requiring a monitoring of at least 3 h, and it could lead to delayed hypoglycaemia (96). The diagnostic accuracy could be lower in patients with glucose intolerance (97).

For all GHRH+Arg, GHRH+GHRP-6 and GST different BMI-related cut-off points are available (99–101). Also for ITT new BMI-related cut-points have been recently proposed, allowing to avoid false positive results due to obesity (102). However, they need further validation.

Lately, Macimorelin test has been proposed for the diagnosis of adult GHD (97), but data about its use in post-TBI patients are lacking until now.

An overview of the main stimulation tests for GHD diagnosis is provided in **Table 1**.

TBI-RELATED GHD: MANAGEMENT AND OUTCOMES

Rationale for Treatment of Post-Traumatic GHD

GH is expressed not only at pituitary level but also in many other organs and tissues, including the central nervous system (CNS) (103) where it plays an important role in the regulation of cell

TABLE 1 | Main characteristics of GH stimulation tests.

Test	ITT	GHRH+Arg	GHRH+GHRP-6	GST	Macimorelin
Drug and dose administered	Human Regular Insulin 0.1-0.15 UI/kg iv.	GHRH 1-44 1 μg/kg iv + Arginine HCl 0.5 g/kg (max 30 g) infusion.	GHRH 1-44 1 μg/kg iv + GHRP-6 1 μg/kg iv	Glucagon 1-1.5 mg im.	Macimorelin 0.5 mg/kg in 1 ml/kg of water oa.
Sampling and measurements	GH and glucose at times 0′-30′-45′-60′-90′	GH at times 30′-45′-60′	GH at times 0′-15′-30′	GH and glucose at times 0′-30′-60′-90′-120′-150′-180′-210′-240′	GH at times 30′-45′-60′-90′
GH cut-points (μg/L)	- <5 (partial) or <3 (severe). - ≤3.5 if BMI <25 kg/m^2; (*) - ≤1.3 if BMI 25-30 kg/m^2; (*) - ≤2.2 if BMI >30 kg/m^2. (*)	- ≤11.5 if BMI <25 kg/m^2; - ≤8 if BMI 25-30 kg/m^2; - ≤4.2 if BMI >30 kg/m^2.	- <10 if BMI ≤35 kg/m^2; - <5 if BMI >35 kg/m^2.	- <3 if BMI <25 kg/m^2; - <1 if BMI ≥25 kg/m^2.	≤2.8
Side Effects	- Severe hypoglycaemia; - Late hypoglycaemia.	Flushing, nausea, smell and taste disorders	Flushing	Delayed hypoglycaemia, nausea, vomiting	Dysgeusia
Contraindications	Pregnancy, older age, history of seizure, history of CAD.	Chronic renal failure.	None	Severe fasting hyperglycaemia.	Use of drug that prolong QT.
Pros	- Possible simultaneous assessment of HPA function; - Evaluation of both hypothalamic and pituitary integrity.	- Strong selective stimulus; - Safe test.	- Strong selective stimulus; - Safe test.	Evaluation of both hypothalamic and pituitary integrity	- Oral administration; - High tolerability.
Cons	Symptomatic hypoglycaemia (<40 mg/dl) not always achieved in diabetic patients and obese.	- Not useful for GHD of hypothalamic origin; - GHRH not commercially available in every Country.	- Not useful for GHD of hypothalamic origin; - GHRH and GHRP-6 not commercially available in every Country.	- Long time needed; - Labored test; - Lower accuracy in patients with glucose intolerance.	Expensive
Notes	Gold standard for GHD diagnosis; (*) Need further validation of BMI-dependent cut points.	///	///	Not frequently the first choice for GHD diagnosis	Safety and diagnostic performance not available for patients <18 and >65 years.

ITT, Insulin Tolerance Test; GHRH, Growth Hormone-Releasing Hormone; Arg, Arginine; GST, Glucagon Stimulation Test; iv, intravenous; im, intramuscular; oa, oral administration; GHD, Growth Hormone Deficiency; BMI, Body Mass Index; CAD, Coronary Artery Disease; HPA, Hypothalamus-Pituitary-Adrenal.

proliferation and survival (104–107). It is well known that GH plays a role in brain repair, and several preclinical and clinical studies have demonstrated the positive effects of GH treatment on neurogenesis in both animals and humans (108, 109). Moreover, the whole GH system [GH, GH receptor (GHR) and IGF-I] is acutely and strongly upregulated after brain injury (110, 111), and specifically associated with stressed neurons and glia (112–114). However, to date, the role exerted by GH at CNS level and, in particular, its possible contribution to the recovery of neurologic injuries remains poorly understood.

GH exerts its beneficial effects on neural repair through different mechanisms that include regulation of the proliferation, survival, differentiation and migration of both neural progenitors and newly formed neurons.

Both *in vitro* and *in vivo* studies support the ability of GH to promote the proliferation of neural precursor. GH treatment promotes proliferation of both human fetal (115) and adult mice neural stem cells (NSCs) (106, 116). It has been demonstrated that peripheral administration of GH is able to induce cell proliferation in the brain of both normal (117) and hypophysectomized (107) adult rats (117); moreover, peripheral administration of GH seems to increase the proliferative response of hippocampal progenitors to kainate-induced injury (118). Despite the main role of GH on the proliferation of neural precursors, it has been suggested that GH may actually have a more prominent effect in the regulation of survival, differentiation, or even migration of newly formed neurons (113). In agreement with the control exerted by GH on cell survival, GH prevents the apoptotic death of both mature neurons (119–121) and primary neurospheres derived from embryonic mouse NSCs (122). On the other hand, an increased apoptotic death of NSCs is observed during the treatment with a GHR antagonist (123). Lastly, GHD has been shown to impair the survival of newborn neurons in the subgranular zone of adult rat dentate gyrus (124), while elevated GH levels within the hippocampus reduce apoptosis (125). All these neuroprotective effects would explain some of the acute consequences of GH therapy. Several studies support a role for GH in enhancing neuronal precursors differentiation (126, 127), while a possible effect of GH on neuronal precursors migration is less clearly established.

Finally, it cannot be ruled out that GH promotes neurogenesis and neurorepair, at least in part, through indirect mechanisms including both the synthesis and the release of IGF-I, epidermal growth factor (EGF) or erythropoietin (EPO) or changes in neurotransmitter turnover.

IGF-I is the main mediator of GH action and it is essential for CNS development (128, 129). IGF-I stimulates the proliferation of neuronal precursors and the survival and differentiation of both neurons and oligodendrocytes (130). As a result, brain growth is increased by IGF-I overexpression and reduced as a consequence of decreased IGF-I levels.

In this context, it must be pointed out that IGF-I has been shown to be a crucial modulator of CNS activity, including higher functions like cognition, and to modulate genes involved in microvascular structure and performance, and synaptic plasticity (131, 132).

On the other side, EGF has been demonstrated to be a powerful mitogen capable of inducing neurogenesis both in basal studies and after experimental injuries (133, 134). Since it has been shown that GH is able to induce the expression of both EGF and its receptor (EGFR) and to mediate EGFR activation (135, 136), it cannot be excluded that the action performed by GH at CNS level is mediated by these interactions.

EPO and its receptor (EPOR) are other factors involved in neurogenesis. Both EPO and EPOR have been identified in numerous areas of the CNS during development, and they are expressed in several neuronal cells like neurons, astrocytes, oligodendrocytes, microglia and cerebral endothelial cells, where EPO activates anti-apoptotic, anti-oxidant and anti-inflammatory signals and stimulates angiogenesis and neurogenesis. It is therefore not surprising that EPO can determine a strong protective effect on neuronal tissue in experimental models of stroke, cerebral hemorrhage, traumatic brain injury, and neuroinflammatory and neurodegenerative diseases (137).

Moreover, it has been shown that the blockade of EPOR in the CNS leads to impairments in neural cell proliferation and survival during embryonic development, and in post-stroke neurogenesis in adult brain, further confirming EPO's role in neurogenesis (138, 139). Finally, both *in vivo* and *in vitro* studies show that EPO is able to increase oligodendrogenesis and remyelination after stroke (140). Since GH induces EPO release from kidneys (141), it can be assumed that GH promotes neurogenesis and neurorepair, at least in part, through indirect mechanisms including EPO mediated effects.

Peripherally, GH is an anabolic hormone that promotes growth in skeletal and soft tissues (142) through the expression of GHR at many levels including liver, muscle, bone, and adipose tissue (143). GH plays an important role in metabolism stimulating lipolysis, reducing hepatic triglyceride secretion, activating the nitric oxide system (and so reducing vascular tone), increasing cardiac performance and exercise capacity, and promoting longitudinal skeletal growth (142). In patients with traditional causes of hypopituitarism such as pituitary tumors, GHD is associated with changes in body composition, worrisome metabolic dysfunction, reduced bone density, a significant decrease in QoL, increased cardiovascular risk, and impaired cardiac function (33, 144). GH replacement therapy has been shown to be partially beneficial in adults with GHD (145). However, the evidence of the benefit from GH replacement therapy in post-traumatic GHD is scant: more robust data are available about improving cognition and QoL, but not about all the other parameters.

Literature data on the possible beneficial effects of recombinant human GH (rhGH) therapy in patients with GHD post-TBI are currently scarce and fragmented. The studies available so far are few (14, 146–153), carried out on small series (147–150, 152–154), extremely variable with respect to the type of patients considered (severity of TBI, age and sex of the subjects, presence or absence of other associated hormonal deficits, time elapsed since TBI), the type of control group (subjects with GHD from another cause, subjects with GHD from TBI, subjects with previous TBI but without GHD), the way in which the condition of GHD is defined (different stimulation tests, some of which not even recognized for GHD diagnosis by the international guidelines), the rhGH dose which in some cases appears supraphysiological (148, 150), the outcomes considered and the tools used for their detection. Furthermore, the studies in general have a short duration (3-12 months) and the drop-out is often high (153). All these factors make the results of every single study difficult to compare and the results of every individual experience on a broader case series are scarcely generalizable.

An overview of the main studies investigating GH replacement therapy in adult post-TBI GHD patients is provided in **Table 2**.

Evidence for Treatment of Post-Traumatic GHD: Cognition

Several studies reported that patients with post-traumatic GHD have a severe cognitive impairment mainly characterized by deficit in attention and memory, as well as emotional problems, and poor verbal learning (7, 33, 85, 86). GHD also seems to be associated with poor mental health outcomes. The cognition and quality of life problems experienced by patients with GHD may be explained by the reduced expression of GH activity in specific CNS areas involved in memory, learning, and emotions, like the hippocampus and the limbic system (156–158). Park et al. showed that patients with GHD after TBI have decreased cerebral glucose metabolism in specific cortical areas involved in intellectual function, executive function, and working memory (88).

It has been demonstrated that in TBI patients the GH response to stimulation test was negatively correlated to paranoid ideation and somatization (33). GHD patients have a moderate to large impairment in each of the cognitive domains assessed when compared to the matched controls (159) and patients with post-TBI GHD have a higher risk to achieve poor cognition outcomes than those with an intact somatotropic axis after trauma (87). In contrast to all these studies, Pavlovic et al. did not find significant differences between patients with or without GHD after brain injury (89). It is well known that GH replacement therapy in patients with GHD from non-traumatic causes is of benefit (96, 160). A meta-analysis conducted on patients with GHD from any cause showed a moderate improvement in cognitive performance during treatment with rhGH; the improvement concerned mainly memory and attention domains (159).

TABLE 2 | Studies investigating GH replacement therapy in adult post-TBI GHD patients.

Authors	Study design	GHD post TBI patients (N)	Control group (N)	Age of treated patients	GHD testing	rhGH dose[§]	Duration of rhGH treatment	Time elapsed from TBI	Parameters analyzed
Kreitschmann-Andermahr I. et al. (146)	Retrospective database analysis	84	84 GHD patients due to NFPA	Patients: 36.7 ± 10.8 yrs Controls: 37.3 ± 10.3 yrs *p = NS*	ITT (31 TBI +39 NFPA); GHRH +Arg (4 TBI+8 NFPA); GHRH (16 TBI +18 NFPA); Arg (49 TBI +31 NFPA)	TBI: 0.41 ± 0.3 mg/day NFPA: 0.36 ± 0.2 mg/day	12 months*	CO-GHD: 21.0 ± 8.1 yrs AO-GHD: 6.2 ± 9.4 yrs *p = 0.000*	BMI; WHR; IGF-I SDS; GH-dose; fasting lipid profile; QoL-AGHDA
High W.M. et al. (14)	Open, prospective, randomized study	12 (5 GHD +7 GHI[#])	11 TBI (3 GHD+8 GHI)	Patients: 36.1 ± 10 yrs Controls: 39.1 ± 8.5 yrs *p = NS*	GST	Patients: 0.6 mg/day or uptitrated to achieve an IGF-I level in the upper half of the normal range Controls: *placebo*	12 months	Patients: 1.8-33.9 yrs Controls: 1.9-13.8 yrs *p < 0.058*	Muscle biopsy; VO_2; muscle strength; LBM; FM; language; visual/spatial functioning; upper extremity motor functioning; information processing efficiency; working memory/attention; learning and memory; executive functioning; intellectual functioning; emotional functioning
Maric N.P. et al0 (147)	Open, prospective study	4	2 GHD post TBI	39.3 ± 11 yrs	GHRH +GHRP-6	M: 0.3 mg/day; F: 0.4 mg/day	6 months	≥ 3 yrs	Psychiatric assessment: Zung Depression Inventory and SCL-90-R. Neuropsychological examination: MMSE, RAVLT, RCF, TMT, BNT, WCST
Reimunde P. et al. (148)	Open, prospective, placebo-controlled study	11	8 TBI without GHD	Patients: 53.4 ± 17.4 yrs Controls: 47.1 ± 14.6 yrs	GHRH +Arg	Patients: 1 mg/5 days/week Controls: *placebo* (All subjects received daily cognitive rehabilitation)	3 months	Patients: 44.6 ± 35.6 yrs Controls: 46.6 ± 28.8 yrs	Neuropsychological test battery (WAIS)
Moreau O. K. et al. (149)	Open, prospective, controlled study	23	27 TBI (15 without GHD + 9 with partial GHD° + 3 GHD who refused rhGH)	Patients: 37.9 ± 11.7 yrs Controls: 37.1 ± 12.4 yrs *p = NS*	GHRH +Arg ITT	0.2-0.6 mg/day	12 months	Patients: 7.8 ± 6.6 yrs Controls: 5.5 ± 6.2 yrs	BMI; Health-related QoL (QOLBI); RCF; TAP; NRS-R; pADL; iADL
Devesa J. et al. (150)	Open, prospective study	5	8 TBI without GHD	Patients: 27.4 ± 4.8 yrs Controls: 26.3 ± 12.9 yrs	GHRH +Arg	Patients: 1.0 ± 0.0 mg/5 days/ week resting 15 days every 2 months Controls: 0.94 ± 0.1 mg/5 days/ week resting 15 days every 2 months (All subjects received daily rehabilitation according to the specific individual needs)	Patients: 11.2 ± 1.6 months Controls: 8.9 ± 2.2 months	Patients: 44.1 ± 34.3 months Controls: 66.8 ± 47.2 months	Cognitive assessment (WAIS, MMSE); motor assessment (FAC, Tinetti); swallowing function (FOAMS); visual function; functional assessment (MBI); IGF-I
Gardner C. J. et al. (151)	Retrospective database analysis	161	1268 GHD patients due to NFPA	Patients: 42.6 yrs [40.8; 44.5 yrs]	IGF-I GHRH +Arg Arg	Patients: 0.37 [0.35; 0.40] mg/ day Controls:	12 months	Not available	BMI; WHR; IGF-I SDS; LBM; FM; BP; glucose metabolism; GH-dose; fasting lipid profile;, QoL-AGHDA

(Continued)

TABLE 2 | Continued

Authors	Study design	GHD post TBI patients (N)	Control group (N)	Age of treated patients	GHD testing	rhGH dose[§]	Duration of rhGH treatment	Time elapsed from TBI	Parameters analyzed
				Controls: 53.2 yrs [52.5; 53.8 yrs] p < 0.0001	GST GHRH ITT Other	0.33 [0.32; 0.34] mg/day p = 0.006			
Leonhardt M. et al. (152)	Open, prospective study	4 patients with isolated post-BI GHD (only 1 post TBI)	6 TBI patients without hormonal deficiency	Patients: 49.0 ± 9.8 yrs Controls: 49.5 ± 13.6 yrs	GHRH +Arg	0.2-0.5 mg/day	6 months	Patients: 25-1024 days Control: 41-2566 days	QoL (SF-12; EQ-5D; QoLBI; BDI; PSQI); Cognition (VLMT; test from the psychological TAP 2.3 test battery of attention); BMI; Abdominal fat distribution
Dubiel R. et al. (153)	Randomized, prospective, placebo-controlled study	31^	32 TBI^	Patients: 32.2 ± 15.2 yrs Controls: 30.1 ± 13.7 yrs p = NS	Arg^	Patients: 0.4 mg/day up- or down-titrated to achieve an IGF-I level in the upper quintile of the range for age and body weight, to a maximum dose of 1 mg/day Controls: *placebo*	12 months▪	Patients: 65.7 ± 30.4 days Controls: 62.5 ± 41.4 days *p = NS*	Glucose metabolism; fasting lipid profile; free T4; IGF-I; AEs; GOS-E; DRS; FIM; QoL (SWLS and SF-36); neuropsychological battery

AEs, adverse event; Arg, arginine: AO-GHD, adulthood onset GHD; BDI, Beck Depression Inventory; BI, brain injury (TBI, aneurysmal subarachoid hemorrhage, ischaemic stroke); BMI, body mass index; BNT, Boston Naming Test; CO-GHD, childhood onset GHD; DRS, Disability Rating Scale; EQ-5D, EuroQoL; F, females; FAC, Functional Ambulatory Category; FIM, Functional Independence Measure; FM, fat mass; FOAMS, Functional Outcome Assessment Measure of Swallowing; GHD, Growth hormone deficiency; GHI, Growth hormone insufficiency; GHRH, Growth Hormone Releasing Hormone; GHRH+Arg, Growth Hormone Releasing Hormone+arginine; GHRH+GHRP-6, Growth Hormone Releasing Hormone +Growth Hormone Releasing Peptide-6; GOS-E, Glasgow Outcome Scale-Extended; GST, glucagon test; iADL, independence in instrumental activities of daily living; IGF-I SDS, IGF-I standard deviation score; ITT, insulin tolerance test; LBM, lean body mass; M, males; MBI, Modified Barthel Index; MMSE, Mini Mental State Examination; NFPA, non-functioning pituitary adenoma; NRS-R, Neurobehavioral Rating Scale-Revised; pADL, independence in personal activities of daily living; PSQI, Pittsburgh Sleep Quality Index; QoL, quality of life; QoL-AGHDA, Quality of Life-Assessment of Growth Hormone Deficiency in Adults; QOLBI, Quality of Life after Brain Injury; RAVLT, Rey Auditory-Verbal Learning Test; RCF, Rey-Osterrieth Complex Figure Test; rhGH, recombinant human GH; SCL-90-R, Symptom-check-list; SF-12, 12-Item Short Form Health Survey; SF-36, Short-Form 36; SWLS, Satisfaction with Life Scale; TAP, Test for Attentional Performance; TBI, traumatic brain injury; Tinetti, balance and gait tests; TMT, Trail Making Test; VLMT, Verbal Learning and Memory Test; VO_2, peak oxygen consumption; WAIS, Wechsler Adults Intelligence Scale; WCST, Wisconsin Card Sorting Test; WHR, waist-hip ratio; yrs, years.

**Data available only from 61 out of 84 TBI patients.*

[#]GHI defined as a GH response to GST greater than 3 ng/ml but less than 8 ng/ml.

[§]After titration period.

°partial GHD defined as GH peak > 3 ng/ml but < 10 ng/ml to ITT or > 4.0 to 15.6 ng/ml (depending on the patient's age and BMI) but < the percentile threshold values from ref. (155) in the GHRH+Arg test.

^Not clear if all patients and controls were GHD subjects: only 24 out of 63 subjects underwent GH stimulation test.

▪Only 16 out of 31 patients and 18 out of 32 controls completed 12 months follow-up.

A beneficial effect of GH replacement therapy on cognition has also been reported in post-TBI GHD patients (14, 148, 150). However, till now, there is not sufficient evidence to support GH treatment with the aim of improving cognition in post-TBI GHD patients.

Recovery during a thorough rehabilitation program after TBI may be positively influenced by normal GH secretion as suggested by Bondanelli et al. who showed that GH peak during GHRH + ARG test was an independent predictor of positive outcomes suggesting that GH replacement therapy may be considered in post-traumatic GHD patients (161). The improvement in cognitive function is more significant and appears earlier than that in motor function; this observation supports the usefulness of GH replacement therapy in the rehabilitation process of these kind of disabilities (159, 162–164). In general, the improvement during GH therapy appears to be more marked in patients with worse cognitive impairment at baseline (149) and it has also been shown that discontinuation of GH therapy is accompanied by a worsening of cognitive performance (147).

Although to date the exact mechanism underlying these effects is not well understood, it cannot be excluded that it could be based on GH stimulating effect on neurogenesis in CNS areas related to recent memory like the hippocampal dentate gyrus. However, stimulation of neurogenesis does not appear to be the mechanism by which GH therapy improves other cognitive processes such as attention and concentration. In this case it has been hypothesized that the effect of GH replacement therapy is mediated by the action of the hormone

on some neurotransmitter pathways and this might explain the early responses observed after starting a treatment with rhGH (116, 118, 148, 150).

Evidence for Treatment of Post-Traumatic GHD: Metabolic and Cardiovascular Risk Factors

Regardless of pathogenesis, GHD is associated with adverse effects on body composition, alterations in glucose and lipids metabolism, reduced physical and cardiovascular performance (165); all these alterations are thought to be the cause of the incresed cardiovascular mortality observed in hypopituitaric patients (166–168). A systematic review showed a small but significant improvement on lean and fat body mass, low-density lipoprotein-(LDL) and total cholesterol, and diastolic blood pressure in GHD adults during GH replacemente therapy (169). In contrast, plasma glucose and insulin levels were increased during GH therapy (169). However, improvement in hard outcomes, such as reductions in cardiovascular events and mortality, has yet to be demonstrated (96, 170).

Several studies argue metabolic disorders in patients with post-traumatic GHD. In patients with post-TBI hypopituitarism, mainly GHD, high LDL and total cholesterol levels, waist circumference, and total fat mass have been showed (19). Treatment of these patients has shown variable results. In one observational study, weight or waist to hip ratio did not change in post-traumatic GHD patients treated with rhGH for a year (146), while in another study there was an improvement in blood pressure, total and LDL cholesterol after 1-year treatment in patients with GHD post TBI (151). A case study of two patients with GHD secondary to sport-related TBI showed some improvement in lipid profile and body composition after a 6-month treatment with rhGH (171).

Patients with TBI have been found to have a reduced aerobic capacity, a well-established measure of physical endurance and fatigue resistance, which may further delay or hinder the rehabilitative process (81). In particular, patients with TBI and a normal GH axis show suboptimal aerobic capacity and those with GHD performed even worse (81). Although GH treatment seems to improve skeletal muscle mass in patients with GHD from non-traumatic causes, data available are not so convincing in post-traumatic GHD patients and the improvement, if any, seems to concern only male patients with TBI and GHD (172). A case study of one patient showed an improvement in muscle force production, body composition, and aerobic capacity after treatment with rhGH for 12 months (173).

Evidence for Treatment of Post-Traumatic GHD: Bone Health

A higher risk of osteopenia, osteoporosis, and vertebral fractures is observed in hypopituitaric patients adequately replaced with glucocorticoids and thyroid hormones (174) suggesting a fundamental role of both GH and gonadotropins secretion disturbances in the pathogenesis of the reduced bone health observed in these patients. Apparently, in GHD patients there is no difference related to gender and age. GH replacement therapy increases bone mineral density (BMD) (175, 176) and seems to mitigate the increased fracture risk observed in GHD patients (174), but specific data for skeletal outcomes in TBI induced GHD are lacking.

Evidence for Treatment of Post-Traumatic GHD: QoL

Regardless of the cause, GH is characterized by a compromised QoL (177, 178). Patients with post-traumatic GHD are more likely to be depressed and with a poorer QoL than those with GHD due to other causes (85, 160). Interestingly, patients with post-TBI GHD, when compared to those with GHD secondary to NFPA, seemed to have a less severe GHD at biochemical level, but worse QoL scores (151). In particular, domains of physical health, energy and fatigue, emotional well-being, pain, and general health seem to be affected (85). This perceived poor QoL would negatively impact on rehabilitation after TBI. In general, the treatment of GHD of any cause seems to improve QoL as measured by the QoL-AGHDA (Quality of Life Assessment of Growth Hormone Deficiency in Adults) score and other evaluation tools (179). The QoL improvement is higher in post-traumatic GHD patients than in patients with GHD due to NFPA, especially in the domains of socialization, self-confidence, and tenseness (151). The observed improvement was maintained for a long time, up to eight years, and always based on continuation of treatment.

CONCLUSIONS

TBI is one of the leading causes of disability and mortality affecting many people each year and resulting in a serious burden of devastating health consequences. TBI may lead to transient or permanent pituitary insufficiency. After the initial primary injury, secondary mechanisms that involve an interplay of ischemia, inflammation, and cytotoxicity seem to result in hypopituitarism that causes adverse changes in body composition, worrisome metabolic dysfunction, decreased bone density, and a significant reduced QoL. GHD, the most common pituitary hormone deficiency after TBI, is associated with adverse sequelae, which may impair recovery and rehabilitation. The poor outcomes that are seen with long standing GHD in post TBI patients could be improved by treatment with rhGH, but literature data on the possible beneficial effects of GH replacement therapy in post-TBI GHD patients are currently scarce and fragmented. More studies are needed to further characterize the post-TBI GHD syndrome with the purpose of establishing appropriate standards of care.

AUTHOR CONTRIBUTIONS

VG, VC, and FB performed a literature search, wrote the first draft, and designed tables and figures. EG and SG supervised the work and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 24 May 2021
doi: 10.3389/fendo.2021.680964

Adults' Adherence to Growth Hormone Replacement in Relation to Medication-Related Beliefs, Coping and Quality of Life - An Exploratory Analysis

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Introduction: Little is known about psychological reasons associated with adherence to growth hormone (GH) replacement therapy (GHRx) in adults. As in other chronic diseases, medication-related beliefs, coping strategies and disease impact on quality of life (QoL) might play an important role. We thus explored these psychological factors in relation to adherence in patients with GH deficiency (GHD) in order to find leverage points for the improvement of adherence.

Patients and Methods: Cross-sectional analysis including 107 adult GHD patients on GHRx who completed self-assessment inventories on health-related QoL (Short-Form SF-36), coping style (Freiburg questionnaire on coping with illness, FKV-LIS) and medication beliefs (Beliefs about Medicine questionnaire, BMQ). Results were correlated to general and GH-specific adherence to medication.

Results: In the BMQ, 92.5% of the patients (n=99) reported a strong belief in the need for their medication, which correlated significantly with general adherence (r_s = 0.325). Active coping was significantly related to general (r_s = 0.307) and GH-specific adherence (r_s = 0.226). Better mental QoL (r_s = 0.210) but worse physical QoL (r_s = -0.198; all $p < 0.05$) were related to higher GH-specific adherence. Older age was associated with a higher degree of active coping, a higher belief in the necessity of medication and worse physical QoL.

OPEN ACCESS

Edited by:
Lucio Vilar, Federal University of Pernambuco, Brazil

Reviewed by:
Lara Porto, Regional Hospital of Taguatinga, Brazil
Luiz Augusto Casulari, University of Brasilia, Brazil

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *15 March 2021*
Accepted: *23 April 2021*
Published: *24 May 2021*

Citation:
Siegel S, Unger N, Streetz-van der Werf C, Karges W, Schilbach K, Schröder B, Szybowicz J, Sauerwald J, Zopf K, Grzywotz A, Bidlingmaier M, Kirstein C, Sommer H, Strasburger CJ and Kreitschmann-Andermahr I (2021) Adults' Adherence to Growth Hormone Replacement in Relation to Medication-Related Beliefs, Coping and Quality of Life - An Exploratory Analysis. Front. Endocrinol. 12:680964. doi: 10.3389/fendo.2021.680964

Conclusion: We provide preliminary data that most GHD patients on GHRx are strongly convinced of their need for medication and that adherence to GHRx is influenced by coping strategies and QoL. Patients with impaired psychological QoL are less able to translate their convictions into good adherence, a phenomenon to be addressed in future research.

Keywords: adult growth hormone deficiency, adherence, quality of life, coping, beliefs about medications

INTRODUCTION

Growth hormone (GH) deficiency in adults (aGHD) is associated with an adverse cardiovascular risk and plasma lipid profile, abnormal body composition, reduced bone mass, and impaired quality of life (QoL) (1, 2). These health-related alterations are believed to contribute to the excess morbidity and premature mortality observed in untreated aGHD patients compared to the normal population (3). Growth hormone replacement therapy (GHRx) in adults with recombinant GH, available since the 1990s, is able to ameliorate these negative effects (4–6), however, at the price of long-term treatment, currently involving daily subcutaneous injections. Different from GHRx in children in whom changes in longitudinal growth easily reflect therapy response, the benefits of GHRx in adults may not be as easily perceived. Treatment success in both patient groups is dependent on patients' continuous therapeutic adherence, i.e. their willingness and ability to follow the recommended injection regime (7).

A number of pediatric studies demonstrate that adherence to GH treatment is suboptimal, with some degree of non-adherence in up to 71% of all pediatric patients and their families as reported in a recent review (8). Preliminary data of Amereller et al. and our group indicate that adherence of aGHD patients to GHRx is higher than in children and adolescents (9, 10). Nevertheless, in clinical practice, a number of aGHD patients decide to refuse recombinant human (rh)GH treatment or to discontinue rhGH therapy over time. Poor therapy adherence as well as discontinuation or refusal of GH treatment may result in reduced QoL and economic burden of those patients (11). Little is known, however, about psychological reasons associated with adherence or non-adherence to GHRx in adults. It can be hypothesized that, as in other chronic diseases, coping strategies, patients' beliefs about their medication and impact of the disease on the patients' quality of life might play an important role. Since a better understanding of the psychological influencing factors could provide leverage points for the improvement of adherence, it was the aim of the current analysis to further characterize the beliefs and attitudes leading to adherence to GHRx in patients with GHD. We, therefore, analyzed three major psychological domains outlined below, known to be associated with adherence to medication in other chronic diseases.

Coping

Coping with a chronic illness includes all of a patient's cognitive and behavioral efforts to deal with the stress resulting from the disease (12). It is related to adherence to long-term therapies, e.g. hemodialysis in patients with renal disease (13) and medical treatment of type-2 diabetes (14). It has been shown that strategies of coping with chronic diseases differ significantly between patients of different age (15).

Beliefs About Medications

Beliefs about medications include the patients' attitudes towards medicines in general and specifically their own prescribed medicaments (16). It has been hypothesized that patients with chronic diseases weigh their beliefs about the necessity of their medication against their concerns about potential adverse effects. Horne and Weimann found this cost-benefit balance to be related to adherence across several chronic illness groups (asthma, renal disease, cardiac disease and oncological diseases) (17).

Quality of Life

Adherence can also be influenced by the perceived severity of the disease and its impact on day-to-day life (18). In line with this observation, it has been proposed, that health related QoL (hrQOL) and adherence influence each other (19). Patients might decide for the intake of medications based on the perceived impact of the disease on hrQOL and continually reassess hrQOL over the course of the treatment. This reassessment might influence future decisions on adherence, which again might impact hrQOL. Both hrQOL and change in hrQOL over the course of the treatment have been found to be associated with adherence (20, 21).

MATERIAL AND METHODS

Study Design

This cross-sectional study was part of an extensive research project on adult GHD and carried out in one large German neurosurgical and four large endocrinological university referral centers during a two-year recruitment period. Adult patients (age between 21 – 80 years) with biochemically proven severe GHD were included. Severe GHD had to be diagnosed either by means of a GH stimulation test performed according to local standards with local cut-offs or by insulin-like growth factor-I (IGF-I) levels more than two standard deviation scores (SDS) below normal in the presence of proven deficiency of three or more other pituitary hormone deficiencies (22). Patients with known active psychotic illnesses and known insufficient fluency of the German language were excluded from participation. A detailed study description and a description of the entire sample of investigated patients have been published previously (10).

For the present research question, a battery of psychological self-rating inventories covering the three domains outlined in the introduction, thought to impact on adherence, was analyzed in the large subgroup of patients on rhGH replacement (n = 107) at the time of the study. The questionnaire results were related to the patients' adherence to medication in general and to adherence to GHRx, the results of which have been reported previously (10).

The study protocol was approved by the local ethic committees of all participating centers with the lead vote provided by the Ethics Committee of the University of Duisburg-Essen. Patients were included if the signed consent form was returned with the filled-in questionnaires.

Sample Description

The present analysis includes 107 patients with severe GHD on rhGH treatment. 57 (53.3%) patients were male and 50 (46.7%) were female. The mean age of the study group was 49.9 ± 14.0 years at the time of the study. The etiology of GHD included pituitary adenoma (n = 47), craniopharyngioma (n =11), other tumors of the sellar and suprasellar region (n = 7), congenital (pan) hypopituitarism (n = 9, either genetic or due to hypothalamo-pituitary developmental lesions), empty sella syndrome (n = 6), idiopathic GHD (n = 6), hypophysitis (n = 5), cystic lesions of the pituitary (n = 4), mixed etiologies (such as trauma, Sheehan's syndrome, sarcoidosis; n = 7). Data on GHD etiology was missing in n = 5 patients. 70 patients (66.7%) had undergone neurosurgery at any time during the disease and 22 patients (21.0%) had received radiation therapy of the pituitary region.

Next to severe somatotropic insufficiency, 85.0% of the study patients suffered from additional gonadotropic insufficiency (n = 91, 79 of them on replacement therapy) and 77.6% had thyrotropic insufficiency (n = 83, all of them substituted). In 73.6% (n = 78 patients) corticotropic insufficiency had been diagnosed. All but one of these patients required regular hydrocortisone replacement. 23.8% (n = 25) of the study patients also suffered from diabetes insipidus which necessitated antidiuretic hormone replacement in all cases. Diabetes mellitus was present in 8.2% (n = 7), hypertension in 38.8% (n = 33) and coronary heart disease in 2.4% of the patients (n = 2). 20.6% of the patients had started rhGH therapy during childhood (n = 21), while 79.4% had started therapy during adulthood (n = 81). Two (1.9%) of all patients did not have a school leaving qualification, 30.2% (n = 32) had a basic school qualification (*Hauptschulabschluss*), 23.6% (n = 25) had a comprehensive school qualification (*Realschulabschluss*), whereas 44.3% of the patients had a high educational level (*Fachabitur* = university of applied sciences entrance qualification, or higher, n = 47). Sixty-six (62.3%) of the patients were working full-time or part-time at the time of the study.

Questionnaires

Freiburg Questionnaire on Coping With Illness (Freiburger Fragebogen zur Krankheitsverarbeitung; FKV-LIS)

Coping strategies were assessed with the German-language FKV-LIS questionnaire. The FKV-LIS allows the differentiation of a broad spectrum of coping strategies and is widely applied in German-speaking countries to assess illness coping in different disease entities. This 35-item questionnaire is divided into five subscales (*"depressive coping"*, *"active, problem-oriented coping"*; *"distraction and self-affirmation"*, *"religiousness and search for meaning"* and *"trivialization and wishful thinking"*). It assesses coping strategies for dealing with a disease on cognitive, emotional and behavioral levels. While coping strategies cannot be universally categorized as adaptive or maladaptive, *"active and problem-oriented coping"* as well as *"distraction and self-affirmation"* can usually be viewed as more beneficial than *"depressive coping"* and "*trivialization and wishful thinking"*. Answers are based on 5-point Likert scales, with higher scores indicating a higher intensity of coping in a particular domain. Since normative values for coping cannot be established, the raw scores are used for analysis (23). For better readability, we will refer to the scales in the following by abbreviated names, namely "depressive coping", "active coping", "distraction", "religiousness" and "wishful thinking".

Beliefs About Medicine Questionnaire (BMQ)

The BMQ questions cognitive and emotional representations of medication. It has two parts, the BMQ specific and the BMQ General, both of which were used in the present study. The BMQ-General comprises two 4-item factors assessing beliefs that medicines are harmful, addictive poisons which should not be taken continuously (*General Harm*) and that medicines are overprescribed by doctors (*General Overuse*). The BMQ-Specific comprises two 5-item factors assessing beliefs about the necessity of prescribed medication (*Specific Necessity*) and concerns about prescribed medication based on beliefs about the danger of dependency, long-term toxicity and the disruptive effects of medication (*Specific Concerns*) (24). For each of the scales the average score can be calculated. For easier interpretation the results can be dichotomized, with scores above the scale midpoint signifying a strong belief and scores below the scale midpoint signifying a weak belief. To assess the balance between perceived benefits and costs of the prescribed medication, the difference between *Specific Necessity* and *Specific Concerns* can be calculated (17). The resulting score ranges from -20 to 20, with negative values indicating that perceived concerns exceed benefits and positive scores indicating that perceived benefits exceed concerns. In this study, the German translation of the BMQ by U. Opitz was used (25).

Short Form-36 (SF-36)

The SF-36 is a generic quality of life outcome measure, consisting of 36 items, which assess health-related QoL of patients during either a one-week (acute) or a four-week (standard) recall period (26). The four-week recall version was used in the present study. The questionnaire has eight domains: *vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning*, and *mental health*. The answers can be combined to form two global measures, addressing physical (physical component summary score, SF-36 PCS) and mental (mental component summary score, SF-36 MCS) QoL. The raw values are transformed to allow comparison to the general population reference values. The transformed subscales are scaled from 0 to 100; the transformed

summary scores have a mean of 50 and a standard deviation (SD) of 10. Higher scores indicate a better QoL.

General Adherence Score and GH-Specific Adherence Score

These two questionnaires, developed by the authors have been described in detail previously (10). In brief, the general adherence questionnaire is focused on patients´ adherence with regard to medication in general whereas the GH-specific adherence score focusses on GHRx injections. For both questionnaires, answers could be given on a four-point Likert scale with values ranging between 0 and 3 which were summed up to a general or specific adherence score between 0 and 18, with higher values indicating a higher adherence.

Data Analysis

Database was generated by Microsoft Access 2010 (Microsoft Office 2010, Microsoft, Redmond/USA). Statistical analyses were conducted using IBM SPSS Statistics 22 (Statistical Package of the Social Sciences, SPSS Inc., Armonk/USA). Descriptive statistics of interval-scaled data were expressed as mean and standard deviations (SD). Categorical data were expressed as absolute frequencies and valid percent (n, %) which means that 100% are the number of available data per parameter out of the 107 included patients. A visual screening of the histogram revealed a severe skewness of the rhGH-specific adherence score and the general adherence score. Therefore, correlation analyses including these scores used the non-parametric Spearman's Rho coefficient (r_s). All other correlations were calculated by Pearsons' correlation coefficient (r) and point-biserial correlation (r_{pb}) for dichotomous variables. Where applicable, a p-value of ≤ 0.05 was regarded as statistically significant.

RESULTS

Results From the Questionnaires

Coping

As indicated by the average score in the FKV-LIS, patients used the strategy of active coping most frequently (3.04 ± 0.86). Distraction (2.82 ± 0.82) and religiousness (2.62 ± 0.75) were less commonly employed. The average scores for depressive coping (2.09 ± 0.81) and wishful thinking (2.09 ± 0.94) were lowest, suggesting that patients used these two coping strategies least.

Beliefs About Medicine

In the BMQ, 92.5% of the patients (n = 99) reached a Specific-Necessity Score above the scale midpoint. This documents a strong belief in the need for medication in this patient group. 22.4% of the patients (n = 24) had strong concerns about potential negative effects of their medication. The average Necessity-Concerns Difference was 10.21 ± 5.19, indicating that most patients judged the benefit of their prescribed medication highly, exceeding their concerns about potential negative effects. Only in 4 patients (3.7%), the Necessity-Concerns Difference was negative, meaning that their concerns outweighed the perceived benefit of their medication. The general assumption that medications are overprescribed by doctors was strongly prevalent in 35.5% of the patients (n = 38). Only 5.6% of the patients (n=6) reached a General Harm Score above the scale midpoint, which indicates that the belief that medicines are generally harmful or poisonous was rare.

Quality of Life

Physical QoL in the SF-36 (PCS) was reduced to 1 SD below normal in 13.3% (n = 14) and to 2 SD below normal in 6.7% (n = 7) of the patients. In 66.7% (n = 70) physical QoL was normal and 13.3% (n = 14) reported a physical QoL of at least 1 SD above average. This even distribution of values led to a mean PCS of 47.97 ± 10.31 only slightly below the scale midpoint of 50. Mental QoL (MCS) was 1 SD below average in 12.4% (n = 13) and 2 SD below average in 24.8% (n = 26) of the patients. 60.0% (n = 63) reported normal mental QoL and 2.9% (n=3) reported above average mental QoL. Although the mean MCS was 45.43 ± 11.94 and, thus, also close to the scale midpoint, the large percentage of 24.8% of patients with a mean mental QoL 2 SD below average indicates that mental QoL was severely impaired in a large proportion of the investigated patients. Of the individual subscales, *vitality* (50.1 ± 20.84) and *general health perceptions* (57.17 ± 23.10) received the lowest scores.

Adherence

The mean general adherence score (16.17 ± 1.7) and the mean rhGH-specific adherence score (15.82 ± 2.01; as reported already in (10) were almost equally high, indicating good adherence to medication in general as well as GHRx.

Table 1 gives the results of all questionnaires used in the present study.

Correlations Between Questionnaire Scores and Adherence

Coping

The only coping strategy significantly related to adherence was active coping. It correlated positively with the General Adherence and GH-specific Adherence Scores of our self-developed scale. This indicates that the more patients relied on active coping, the better was their adherence to medication in general and to GHRx. All other coping strategies were unrelated to General Adherence and GH-specific Adherence.

Beliefs About Medicine

Figure 1 illustrates the limited variability of the BMQ *Specific-Necessity* scores and general and GH-specific adherence scores, indicating strong beliefs and high adherence to medication and GHRx in the great majority of the investigated patients. The subscale *Specific-Necessity* thus only correlated moderately with General Adherence, but not with GH-specific adherence. For the same reason, the Necessity-Concerns Difference, which measures the balance between perceived benefits and costs of the prescribed medication, was not correlated with adherence (also see **Figure 1**).

The *General Harm* subscale of the BMQ, was negatively correlated to GH-specific adherence. Thus, patients who had the opinion that medicines are addictive or poisonous and

TABLE 1 | Descriptive results of the questionnaires.

	N	M	SD	Min	Max
Adherence					
General Adherence	107	16.17	1.70	9.00	18.00
rhGH-specific Adherence	107	15.82	2.01	9.00	18.00
FKV-LIS[#]					
Depressive coping	105	2.09	0.81	1.00	4.60
Active coping	105	3.04	0.86	1.00	4.80
Distraction	105	2.82	0.82	1.00	4.40
Religiousness	105	2.62	0.75	1.00	4.20
Wishful thinking	104	2.09	0.94	1.00	5.00
BMQ					
Specific Necessity	107	4.36	0.73	1.80	5.00
Specific Concerns	107	2.32	0.85	1.00	4.60
Necessity-Concerns Difference	107	10.21	5.19	-3.00	20.00
General Overuse	107	2.75	0.91	1.00	5.00
General Harm	107	1.79	0.75	1.00	4.00
SF-36					
Physical functioning	107	81.84	21.99	5.00	100.00
Physical role functioning	107	67.52	39.04	0.00	100.00
Bodily pain	107	76.17	26.62	22.00	100.00
General health perception	107	57.17	23.10	5.00	100.00
Vitality	107	50.51	20.84	5.00	95.00
Social functioning	107	77.45	24.91	12.50	100.00
Emotional role functioning	107	69.81	40.51	0.00	100.00
Mental well-being	107	67.73	19.68	0.00	100.00
Physical Component Score	106	47.97	10.31	16.51	68.54
Mental Component Score	106	45.43	11.94	11.86	60.63

[#]*Please note that abbreviated scale names for the FKV-LIS are reported here for better readability. For the full scale names please refer to the Methods section.*

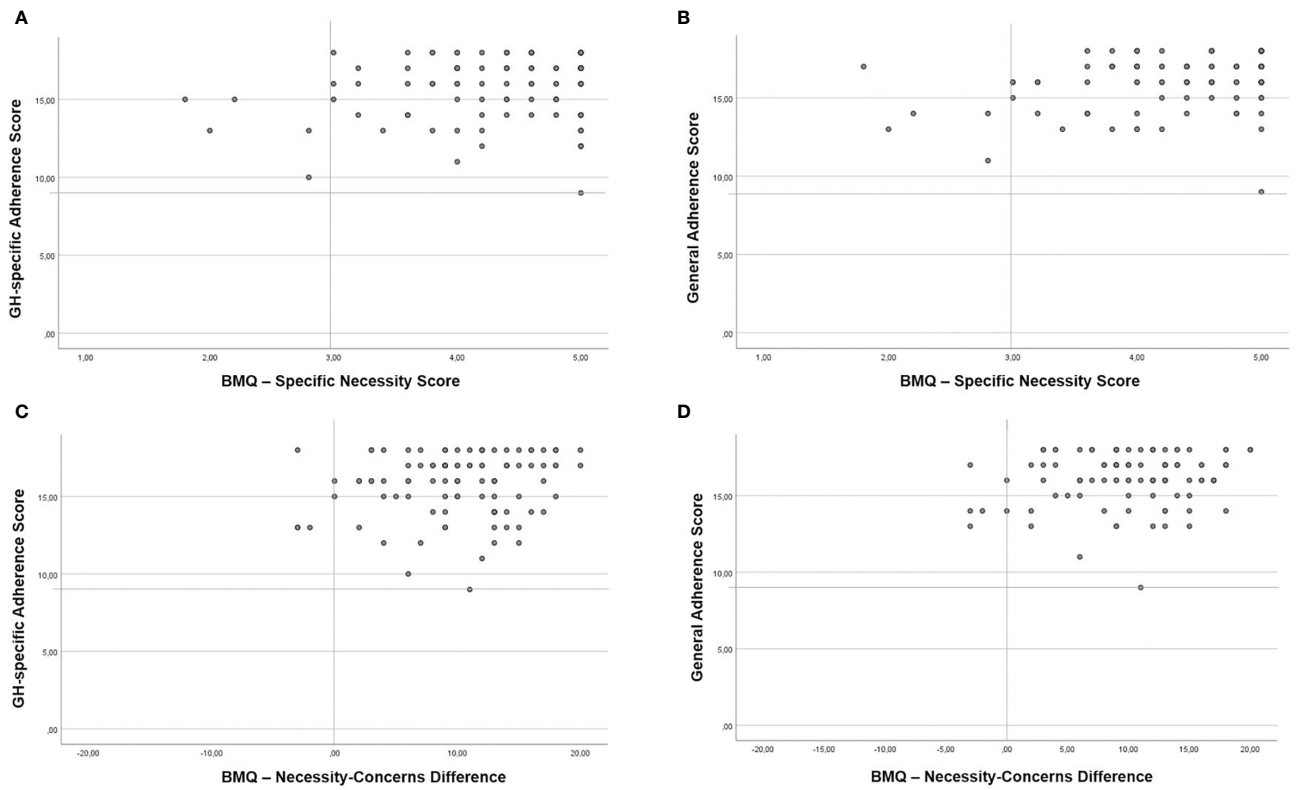

FIGURE 1 | BMQ Specific Necessity Scores in relation to GH-specific Adherence **(A)** and General Adherence **(B)** and BMQ Necessity-Concerns Difference in relation to GH-specific Adherence **(C)** and General Adherence **(D)**.

should not be taken continuously were more likely to be inadherent.

Quality of Life

General adherence was significantly negatively related to mental QoL as measured by the SF-36 MCS. Thus, the worse the mental QoL was, the lower was the General Adherence Score. The SF-36 subscales Vitality and Mental well-being correlated positively with General Adherence, as well. GH-specific Adherence was also significantly correlated with SF-36 PCS and MCS. Interestingly, better mental QoL but worse physical QoL were related to higher adherence (cf. **Figure 2**). None of the SF-36 subscales yielded significant correlations with GH-specific adherence.

Table 2 gives the nonparametric correlations between the adherence scores and the questionnaire results.

Correlations Between Coping and Quality of Life

The SF-36 MCS was negatively correlated with depressive coping ($r = -0.705$, $p < 0.001$), wishful thinking ($r = -0.459$, $p < 0.001$) and religiousness ($r = -0.257$, $p = 0.008$). Patients, who engaged more frequently in these coping strategies, thus, had a lower psychological QoL. The SF-36 PCS was only correlated to religiousness ($r = -0.262$, $p = 0.007$), indicating that the physical QoL was worse in those patients who stated to rely on religiousness to cope with their illness.

Associations With Age

Older age was significantly related to presence of comorbidities ($r_{pb} = 0.221$, $p = 0.022$) and number of additional hormone deficiencies ($r = 0.236$, $p = 0.015$). Accordingly, age correlated

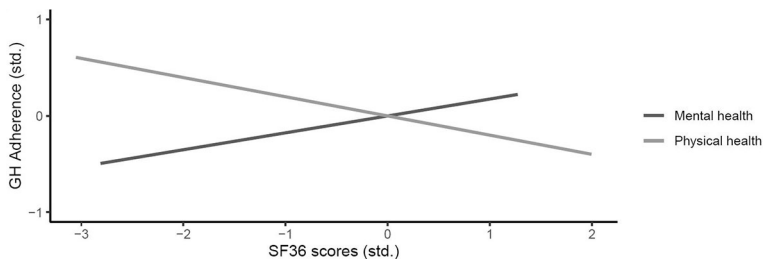

FIGURE 2 | Correlation between GH-specific adherence and the SF-36 sum scores (z-standardized).

TABLE 2 | Nonparametric correlations between general and rhGH-specific adherence and the results from the psychometric questionnaires.

	N	General Adherence		Adherence GH	
		r_s	p	r_s	p
Adherence					
rhGH specific Adherence	107	**0.564*** **	< 0.001	–	–
FKV-LIS[#]					
Depressive coping	105	-0.128	0.193	-0.132	0.179
Active coping	105	**0.307** **	0.001	**0.226***	0.020
Distraction	105	0.188	0.054	0.182	0.063
Religiousness	105	0.046	0.640	-0.015	0.877
Wishful thinking	104	-0.083	0.405	0.003	0.974
BMQ					
Specific Necessity	107	**0.325** **	0.001	0.145	0.135
Specific Concerns	107	0.108	0.268	-0.046	0.639
Necessity-Concerns Difference	107	0.142	0.145	0.104	0.286
General Overuse	107	0.004	0.969	0.036	0.716
General Harm	107	-0.177	0.068	**-0.193***	0.046
SF-36					
Physical functioning	107	-0.019	0.848	-0.074	0.451
Physical role functioning	107	-0.068	0.488	-0.110	0.260
Bodily pain	107	0.005	0.957	-0.079	0.417
General health perception	107	-0.080	0.414	-0.101	0.299
Vitality	107	**0.225***	0.020	0.173	0.074
Social functioning	107	0.156	0.108	0.132	0.176
Emotional role functioning	106	0.144	0.141	0.044	0.656
Mental well-being	107	**0.252** **	0.009	0.137	0.159
Physical Component Score	106	-0.170	0.082	**-0.198***	0.042
Mental Component Score	106	**0.255** **	0.008	**0.210***	0.030

[#]*Please note that abbreviated scale names for the FKV-LIS are reported here for better readability. For the full scale names please refer to the Methods section.*p< 0.05, **p< 0.01, ***p < 0.001.*

negatively with the SF-36 PCS (r = -0.228, p = 0.019), signifying a lower physical QoL in older patients. Since the standardized SF-36 sum scores takes gender and age specific norm values into account, the reported significant correlation between age and lower PCS scores in the present study thus exceeds the normal physical decline of older patients in a healthy population. The SF-36 MCS tended to be higher than in younger patients, albeit not significantly so (r = 0.168, p = 0.086). Older age was associated with a higher degree of active coping (r = 0.227, p = 0.020) and distraction (r = 0.195, p = 0.047), but not with religiousness. Older patients also stated to believe in the necessity of their medication more often than younger patients (BMQ Specific Necessity, r = 0.206, p = 0.033). On the other hand, the general belief that medications were overprescribed was more frequently shared by older patients (r = 0.235, p = 0.015). Their specific concerns and belief in the general harm of medications did not differ from younger patients, neither did their Necessity-Concerns Difference (all r<0.170, ns.).

DISCUSSION

Characterization of the Study Group

In the present study we investigated for the first time the association between psychological factors and adherence to medication in general and to GH replacement in adult patients with hypopituitarism including GHD. We found our investigated patients to rely on adaptive coping strategies such as active coping and self-distraction most often and to be strongly convinced of the necessity of their medication. Physical QoL of the entire sample was evenly distributed around the normative mean. However, older patients reported a significantly worse physical QoL than the younger ones, exceeding the normal decline of physical QoL in the general population, which might be explained by the high prevalence of comorbidities and additional hormone deficiencies in this subgroup. Mental QoL, on the other hand, was severely impaired with 2 SD below average in almost a quarter of our study population and unrelated to age. While a number of studies demonstrate that adults with untreated GHD have impaired QoL as compared to the general population (2, 27), there are only scarce data on the QoL of patients on stable, long-term GHRx, stemming from only one investigation published in 2003 (28). Despite being on stable GHRx for three years and adequate replacement of other hormones, multiple aspects of QoL remained impaired in comparison to age- and sex-matched controls in the then investigated participants. However, despite the relatively high percentage of patients with impaired mental well-being in our group, a comparison of the individual subscales of the SF-36 between the historic cohort mentioned above [view table 3 in their original publication (28)] and the present one indicates a now overall better QoL of patients with hypopituitarism on GHRx, which is perhaps related to more routine and confidence with hormonal replacement regimes evolving over time.

Influence of Psychological Factors and Age on Adherence

Of the psychological constructs investigated, we found the coping strategy of active coping to be most associated with adherence to GHRx and general adherence, followed by the negative influence of poor mental QoL on both adherence scales. Worse physical QoL was significantly associated with a better adherence to GHRx and displayed an insignificant trend for a better adherence to medication in general. Expectedly, the subscale of the BMQ exploring the belief that medications are addictive poisons was associated with significantly worse adherence to GHRx and, again, showed an insignificant trend to worse adherence to medication in general. Older patients were significantly more likely to use active coping and were more strongly convinced of the need for their medication, despite their more frequent belief that medications are generally overprescribed.

The association of better adherence and predominant use of beneficial coping strategies such as active coping in our study did not come unexpected and fits well into the literature on adherence in other chronic diseases such as rheumatoid arthritis and diabetes mellitus (29, 30). The result now also provides a psychological explanation for our previous finding that adherence to GHRx is age-dependent (10) in that older patients in our subanalysis succeeded in coping more effectively with their illness than the younger ones. Moreover, their strong conviction of the necessity of medication might be fueled by their disproportionally poor physical QoL, resulting in a better adherence to GHRx. One could have expected the same to apply to the relationship between mental QoL and adherence, in the sense that more severely impaired mental QoL would be a trigger for higher adherence in order to ameliorate this impairment. In the present study, however, we found the opposite effect - despite a high belief in the necessity for the taken medication, not only in older patients but the entire study group. How can this apparent contradiction be resolved? Acknowledging the high concordance between depression and impaired mental QoL in general, as well as the association between impaired mental QoL and depressive coping in the investigated study group, we assume a high prevalence of depression in our patients and propose the following explanation: Strong evidence suggests, that depression is associated with a poorer adherence to treatment in many physical diseases (31). It has further been proposed, that this relation is mediated by psychological processes, as, for example, proposed in the theory of planned behavior (TPB) (32). In short, this model states that favorable behavioral intentions (i.e., the intent to adhere to GHRx) can only then be translated into actual behavior (i.e., good adherence), if the individual has control over his/her behavioral engagement. Since depression has consistently been related to negative illness beliefs, helplessness and lack of perceived illness control [for an overview see (33)], it may be concluded that patients with severely impaired mental QoL are less able to realize their intent to adhere to GHRx and other medications because of a lack of behavioral control over their illness and adequate coping strategies. A similar relationship has

already been shown in breast cancer survivors (34), strengthening our hypothesis.

Strengths, Limitations and Implications for Further Research

To our knowledge, the present study is the first to explore psychological influencing factors of adherence to GHRx in adults with GHD. The large dataset of this cohort provided us the opportunity for a stable statistical analysis, taking into account three major psychological domains associated with adherence to medication in other diseases. However, the study was exploratory in nature. While it provides first indications that coping strategies, beliefs about medication and QoL are associated with adherence to GHRx, the results await further confirmation using a study design with independent hierarchical regression analysis, in which the order of entry is assigned by the researcher. Also, we did not specifically investigate depression as a comorbidity and its potential impact on adherence to GHRx. Last, but not least, we acknowledge a potential response bias in that we cannot rule out that predominantly highly motivated and adherent patients participated in this study, who might not constitute a representative sample of the general group of patients on GHRx. However, this is a problem shared by all studies relying on self-reporting measures and cannot be avoided in patient-reported outcome research.

Our results open avenues for further research, especially pertaining to the theory of planned behavior and its relationship to depression. Future studies should specifically address behavioral control and the intention to adhere to GHRx, especially in younger adults and patients in the transition period who might not have learned to cope effectively with chronic illness yet. For clinical practice, it would be interesting to learn if GHRx with long-acting GH preparations could facilitate adherence in those patients with poor psychological quality of life and lack of effective coping strategies. Moreover, the influence of interventions targeted at improving coping strategies in patients on GHRx on adherence needs to be investigated.

CONCLUSION

These pilot study data indicate that most GHD patients on GHRx are strongly convinced of the need for their medication and that adherence to GHRx is influenced by coping strategies and QoL. Patients with impaired mental QoL are less able to translate their convictions into good adherence. We believe this phenomenon to be caused by a lack of behavioral illness control and negative coping strategies, a hypothesis to be investigated in future research. Since younger patients coped less effectively and had a poorer adherence to GHRx, interventions to improve adherence should be developed for and investigated especially in this patient group.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Medical Faculty of the University of Duisburg-Essen. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SS, NU, CS-W, WK, KS, MB, CS and IK-A contributed to the conception of the study. BS, JSz, JSa, KZ and AG collected the data, SS, IK-A and CK conducted the statistical analysis. All authors took part in the interpretation of the data. SS, CS and IK-A wrote the first draft of the paper. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: MB, CS and IK-A are members of the German PATRO Board and, as such, have received consulting and speaker fees as well as travel support from Hexal AG. WK has received honoraria from NovoNordisk and Novartis. CS-W has received speakers' fees and/or travel grants from NovoNordisk and Pfizer. SS and AG have received travel grants from Hexal AG.

The study was supported by an independent-investigator initiated grant from Hexal Germany. The funder had no role in the study design, data collection, data analysis or decision to publish. However, the expertise of HS, who is an employee of Hexal with a long-standing scientific background in human growth hormone deficiency, was appreciated for the interpretation of the data and manuscript revision. She, therefore, is a co-author of the manuscript.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

MINI REVIEW
published: 15 June 2021
doi: 10.3389/fendo.2021.680579

GH Replacement in the Elderly: Is It Worth It?

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Growth hormone (GH), once the age of linear growth is completed, continues to play a fundamental role for the human body. In adulthood, GH contributes to regulate muscle, cardiovascular and bone metabolism. The same happens in old age, although there is less data on the effect of GH in the elderly. Regardless the age of onset, a reduced quality of life (QoL), an increased cardiovascular risk and an accelerated age-related decline in physical strength have been demonstrated in the elderly with GH deficiency (EGHD). In adults with GH deficiency (AGHD), recent studies suggest a role of GH replacement therapy (GHrt) in improving lean/fat mass ratio, blood pressure, lipid profile, bone metabolism and QoL. Despite these recent studies, there is still a lack of randomized controlled trials proving these positive effects in EGHD. Moreover, the lack of a long-term positive outcome on mortality, and the cost of GHrt could often impact on treatment decision-making and lead to postpone or avoid the prescription. The aim of this mini-review is to summarize the available data on GHrt in EGHD, in order to highlight its weaknesses and strengths and to provide directions to clinicians that will help in the management of this specific set of patients.

Keywords: growth hormone, growth hormone deficiency (GDH), growth hormone replacement therapy, IGF-1, elderly, GHD diagnosis

OPEN ACCESS

Edited by:
Antonio Mancini, Catholic University of the Sacred Heart, Rome, Italy

Reviewed by:
Marija Pfeifer, University of Ljubljana, Slovenia
Moises Mercado, Mexican Social Security Institute (IMSS), Mexico

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *14 March 2021*
Accepted: *02 June 2021*
Published: *15 June 2021*

Citation:
Ricci Bitti S, Franco M, Albertelli M, Gatto F, Vera L, Ferone D and Boschetti M (2021) GH Replacement in the Elderly: Is It Worth It? Front. Endocrinol. 12:680579. doi: 10.3389/fendo.2021.680579

INTRODUCTION

The role of growth hormone (GH), which performs its most important functions through its peripheral mediator, the insulin-like growth factor (IGF-1), is certainly of primary importance in growth. In adult life, the pituitary production of GH physiologically decreases and its circulating levels are progressively reduced; however, the integrity of the GH/IGF-1 axis continues to ensure the maintenance of homeostasis of many organs and systems (1). Adult GH deficiency (AGHD) is a specific condition, diagnosed when GH levels in adults are pathologically reduced (2). It could be the continuation of a childhood-onset GH deficiency or begin in adulthood. AGHD commonly results from pituitary tumours, from the treatments of these disorders or traumatic brain injury (3–5). Apart from the physiological age-related decrease in GH and IGF-1 during life span, elderly patients may suffer from GHD (EGHD) (6). To date, several studies have shown that AGHD patients present physical deficiencies such as an increased cardiovascular risk and bone fragility, unfavourable fat/lean mass ratio, reduced muscle strength (2, 7–9), as well as psychological deficiencies, such as impaired quality of life (QoL) and social alienation (10, 11). It has been proved that GHrt results in

stable improvement in these alterations (9, 12–14) and that it is safe in both the short and long term (3, 15, 16). The majority of scientific societies do not share a specific opinion on the identification of EGHD cases for which treatment is required, as there are no precise criteria for deciding whether to start therapy or when and if a previously initiated GHrt should be discontinued (17) once old age is reached (6, 16–18).

The aim of this review is to critically analyse the available peer reviewed papers on EGHD and the disease management in the elderly population. The definition of elderly population has undoubtedly changed over the years. Nonetheless, the majority of the analysed studies identify over-65 patients as elderly.

DIAGNOSIS OF GHD IN THE ELDERLY: WHEN TO SUSPECT IT AND HOW TO CARRY IT OUT

The diagnosis of GHD in adults and in the elderly must be achieved through the use of standard stimulating tests, except for those patients in whom GHD arises from a non-modifiable structural brain defect, already producing partial hypopituitarism (coexistence of minimum 3 pituitary axes deficit) and low serum IGF-1 (< -2.0 SDS). The need to rely on a provocative test is based on the evidence that the simple measurement of the IGF-1 levels do not distinguish between normal and GHD subjects; in fact, a low IGF-1 is a reliable diagnostic indicator of GHD in the presence of hypopituitarism, but a normal IGF-1 does not rule out GHD (17–19). Current guidelines recommend to firstly determine the probability of an impaired pituitary function and to use further diagnostic investigation in those patients with other pituitary deficient axes especially if clinical signs such as dyslipidaemia, central obesity and loss of muscle mass are present. One or two positive stimulus tests are required to formulate the diagnosis of GHD, depending on the pre-test diagnostic suspicion (16, 18). It is recommended that the decision to carry out the diagnostic protocol for EGHD be corroborated by a strong suspicion to avoid false positive results, given the higher incidence of side effects of the GHrt in old age (11). At the same time, the symptoms and signs of GHD are often non-specific (asthenia, fatigue, reduced muscle strength, increased visceral fat, dyslipidaemia, osteoporosis). Therefore, especially in the elderly, formulating a clinical suspicion can be difficult and the diagnosis could be misrecognized. We can infer that establishing an accurate diagnosis of GHD in the elderly is challenging and even more so if considering the variability in response and interpretation of stimulus tests available (19, 20). In fact, these tests lack age-adjusted cut-offs, despite the well-known physiological decrease of GH and IGF-1 with age (21, 22).

The latest guidelines do not provide suggestions on the most appropriate test to diagnose EGHD and no dedicated studies have been performed to define it. Moreover, two of the most widely used tests for the diagnosis of GHD - the insulin tolerance test (ITT) and the glucagon stimulation test (GST) - are generally avoided in the elderly, due to their potential detrimental effect on patients with multiple comorbidities (23, 24). The GHrh plus arginine test seems to have the best accuracy/safety ratio in the elderly. Indeed, the side effects of this test are negligible, with the only limitation of unequal availability. The GH cut-off point after GHrh plus arginine test to determine AGHD is different, depending on the country and the effect of BMI is not always considered (25). In Italy it is established at 9 μg/l in the normal-weight and at 4.2 μg/l in obese people (BMI > 30 kg/m^2) (26). In the United States, where GHrh was withdrawn from the market in 2008, the better test for EGHD diagnosis seems to be Macimorelin, which has excellent tolerability and minimal side effects (27), despite several pharmacological interferences (28).

RECOMBINANT GH REPLACEMENT THERAPY (GHRT) IN THE ELDERLY: WHEN, HOW AND WHY

The effects of GHrt in AGHD have been widely studied and an improvement in most of the metabolic and psychological abnormalities associated with this condition has been recorded (10). Recent studies have suggested that most beneficial effects of GHrt basically last over the long term (29–32). In EGHD, as well as in AGHD, GHrt should be individually tailored and it is recommended that therapy is started at low doses and up titrated according to the clinical response, side effects, and IGF-1 levels. Periodic monitoring of both benefits and adverse events must be ensured in order to appropriately titrate the dose of therapy. Side effects consist primarily on fluid retention and increase in insulin resistance, typically seen at the beginning of therapy and/or after the increase of the dose. Adverse events are more common in the elderly and, generally, disappear with dose reduction or end of therapy (18, 28, 32).

As in AGHD, the goals of treatment in EGHD are an adequate clinical response, the achievement of IGF-1 levels within the normal range for age and the minimization of side effects (25, 33–35). Based on our clinical experience with EGHD, the treatment goal should be to maintain IGF-1 between -1 and +1 SD, in accordance with the findings of a study by Van Bunderen et al., specifically aimed at comparing different target IGF-1 therapeutics (36). However, clinical practice is not uniform (25, 37, 38) and the therapeutic goal is not univocal: for example, American guidelines suggest a wider range (IGF-1 between -2 and + 2 SD) (16).

In EGHD, initial doses of GHrt of 0.1 mg/day are recommended (34).

Toogood et al. conducted a dose-finding study to identify the minimum effective dose in EGHD, concluding that the majority of patients maintains an IGF-1 adequate level on a dose of 0.33 mg/day (39). In our clinical practice an average dose of 0.2 mg/day is generally sufficient to maintain IGF-1 between the normal range. Standard follow-up interval in treated EGHD is initially 1 or 2 months; the up-titration of GHrt dose is carried out with increments of 0.1 to 0.2 mg/day, based on the clinical response, IGF-1 levels, occurrence of side effects and individual considerations. In AGHD, once the maintenance dose is achieved, follow-up can be deferred to approximately 6 to 12 months. In EGHD, shorter follow-up and smaller dose

increments are recommended, especially for those patients with other comorbidities such as diabetes mellitus (40). The parameters to be evaluated during treatment are circulating IGF-1, fasting glucose, glycosylated haemoglobin levels, lipid profile, BMI, waist circumference and waist-to-hip ratio.

It is known that GHrt influence thyroid, glucocorticoid and sex hormone requirements; hence, these hormones should be closely monitored during follow-up (16, 34).

Contraindications to GHrt in EGHD are the same ones identified for AGHD: active neoplasia and active proliferative or severe non-proliferative diabetic retinopathy. GHrt should be initiated with caution in pre-existing type II diabetic patients or with a strong family history of cancer (15, 16, 18) (**Table 1**).

To date, only few randomized placebo controlled trials have assessed the effects of GHrt in EGHD and there are no data on the efficacy and long-term safety in patients above 80 years of age (41). Given the benefits of GHrt in AGHD, and considering the similarity between some signs and symptoms of GHD and of aging, GHrt has been proposed in the past as anti-aging agent in healthy elderly subjects. However, the use of GHrt in this setting appears marginal and its benefits are offset by troublesome side effects (42).

CARDIOVASCULAR EFFECTS

The effects of GHrt on the cardiovascular system in the elderly are the most studied to date. In EGHD patients, attention to cardiovascular risk should be a priority, given the demonstrated increase in cardiovascular morbidity and mortality from this cause in elderly patients and the supposed, although not yet unequivocally defined, increase in cardiovascular risk related to GHD itself (7, 14, 31, 43–45). Some studies show an improvement in HDL/LDL ratio after GHrt of up to 20% (11, 46, 47). In a study comparing treated EGHD and AGHD it was shown that, despite a lower dose of GHrt was used in EGHD, this group surprisingly displayed a more pronounced reduction in waist-to-hip ratio and LDL cholesterol levels (14). In 31 EGHD (age range 60-79 years) Elgzyri et al. demonstrated that GHrt leads to a transient increase in heart rate, an improvement in the resistance to a maximal exercise and an improved LDL/HDL ratio (44). These data suggest the importance to bring IGF-1 levels to the physiological threshold in order to reduce the cardiovascular risk in elderly patients with GHD. There were no clear consistent effects of GHrt on arterial blood pressure, as confirmed by many studies, especially in registries (46). Moreover, the reduction in waist-to-hip ratio after GHrt in EGHD appears controversial (11, 47–51). Considering the few existing studies and their heterogeneity in terms of patients, enrolment criteria and study design, it still remains difficult to establish the power of benefits in terms of clinical implications, such as reduced cardiovascular morbidity and mortality (41) (**Figure 1**).

EFFECTS ON COGNITIVE FUNCTION

A higher incidence of mental disorders, more pronounced mental distress and cognitive dysfunctions are also symptoms of AGHD (10). However, the positive effect of GHrt, in terms of cognitive function, remains doubtful. In healthy elderly subjects, it has been shown that some cognitive aspects correlate inversely with IGF-1 levels (52, 53) and that regional cerebral blood flow, during the performance of memory tasks, increases more in healthy elderly with high circulating IGF-1 than in a group with "low" IGF-1 levels (54).

Sathiavageeswaran et al. in 2007 carried out the first double blind, randomized, placebo-controlled study to establish the effects of GH on cognition in EGHD. It has been found that certain aspects of cognitive function improved over the years in the GHrt group, while the placebo group deteriorated even further. There were no effects in patients without cognitive impairment at baseline. Therefore, the results of this study provide a basis for further investigations in this setting of patients (55).

EFFECTS ON MUSCLE STRENGTH

Reduced levels of GH and IGF-1 are known to correlate with the grade of impairment of muscle strength (**Figure 1**). Indeed, a

TABLE 1 | A brief report about clinical suspicion, diagnosis, GH replacement therapy (GHrt) dose and titrating and follow up in elderly patients with GHD (EGHD).

GROWTH HORMONE DEFICIENCY IN ELDERLY		
Clinical suspicion	**Symptoms and signs:** Asthenia, fatigue, reduced muscle strength, increased visceral fat, dyslipidaemia, osteoporosis	**Hormonal assessment:** IGF-I SDS < -2Other deficient pituitary axes
Diagnosis	**- GHrh + Arginine test-** Macimorelin (if GHrh + Arginine is not available);	**Cut-off GH peak (GHrh + Arginine test):** BMI 25-30 kg/m^2= <9.0 µg/l BMI >30 kg/m^2= <4.2 µg/l
Dosage	**Starting dose:** 0,1 mg/die – 0,2 mg/dieincreasing dose 0.1 mg every 1-3 months to achieve IGF-1 level in the normal range for age (we suggest -1<SDS<1)	Daily subcutaneous injection
Follow up	Serum IGF-1, fasting glucose, glycosylated haemoglobin, lipid profile, other pituitary axes, BMI, W/H ratio, waist circumference	Every 1-3 months until the maintenance dose is achieved, then every 6 months
Absolute Contraindications	Active neoplasia, diabetic retinopathy	
Caution	Diabetes Mellitus, family history of cancer	

W/H, waist to hip.

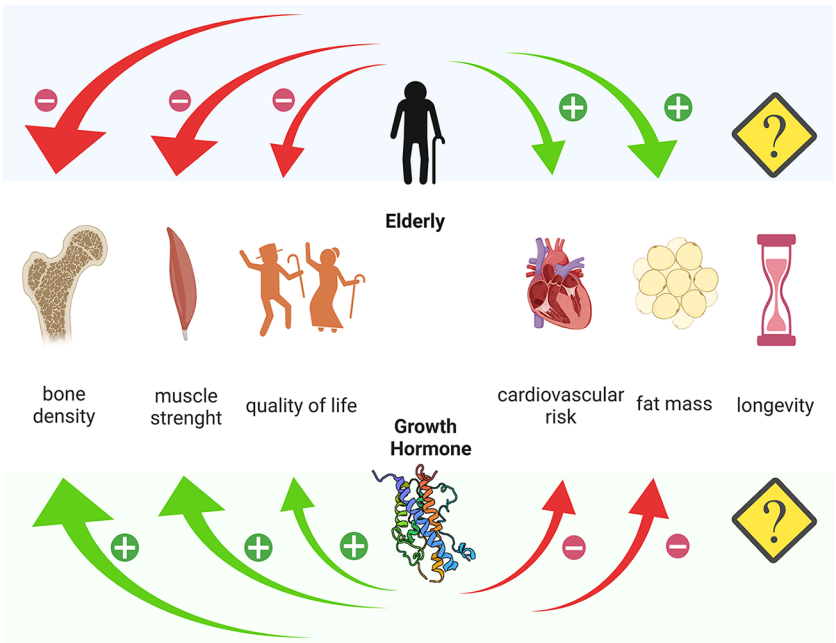

FIGURE 1 | Different action of age and Growth Hormone on bone density, muscle strength, quality of life, cardiovascular risk and fat mass. Created with Biorender.com.

recent Chinese cross-sectional study of more than 3200 healthy elderly patients demonstrated how IGF-1 levels negatively correlate with the incidence of sarcopenia in both sexes (56). In AGDH, GHrt significantly improves muscle strength over the years (57). In EGHD, the correlation between GHD and muscle strength is still controversial, due to a very limited number of studies and assessed subjects. Based on the results of a prospective open-label study evaluating the effect of 10 years of GHrt on muscle strength in 24 EGHD (61-74 years), it seems that GHrt does not directly increase muscle strength, but it may reduce its age-related decline (58). Prospective studies in larger populations might be necessary.

EFFECTS ON BODY COMPOSITION AND BONE METABOLISM

It is known that in AGHD, the imbalance between fat and lean masses is in favour of the former, (**Figure 1**) and there are some data suggesting an improvement in fat/lean mass ratio after GHrt. In EGHD, the data on this subject are still scarce. Toogood et al. demonstrated that EGHD show significant differences in body fat distribution but the response to treatment was not assessed (50). Moreover, more than one study, demonstrate a positive effect of GHrt on body composition (14, 39, 49), and the data extracted from the large KIMS database also support this evidence (51).

In AGHD, GHrt induces a progressive increase in bone mass and density (29), especially after 5–6 years of treatment (59), but results considerably differ according to age, gender, duration and schedule of treatment, including the dose (60, 61). According to the few studies available, GHrt in EGHD seems to improve bone metabolism (49, 62); however, given the lack of long-term prospective controlled studies, there is no clear evidence of a direct impact on the risk of fracture. This is a crucial point, because sarcopenia is an important risk factor for falls and fractures. Therefore, the maintenance of muscle strength (58) might have beneficial effects on reduction of falls and, indirectly, it could also reduce the fracture risk. We believe that targeted studies are needed to prove this assumption.

EFFECTS ON QUALITY OF LIFE (QOL)

Reduced QoL in AGHD patients is one of the most consolidated evidence in the literature. GHrt improves QoL in AGHD patients, particularly by increasing energy and stabilizing emotionality (3, 30). Many studies have shown that the improvement in QoL seems more proportional to the degree of the baseline evaluation rather than the changes in IGF-1 levels (28). The assessment of QoL was proposed as a part of the clinical management in GHD patients, complementary to the measurement of surrogate biological markers or other clinical end points. In fact, in the NICE guidance the QoL score questionnaire is mandatory to decide whether to continue the GHrt (63) or not.

In a large recent study (64) including GHD patients older than 50 years old, 4 years of GHrt resulted in beneficial effects in terms of QoL, but no relevant differences were found in GHrt response between early or late initiation of treatment. Li Voon

Chong et al. were among the first groups to study QoL in EGHD. They demonstrated that those patients had reduced energy, hypo-mobility, and lack of fulfilment in personal life, became socially isolated and suffered from mental fatigue (65). Remarkable improvements in QoL have been described after 6 months of GHrt and this has long been considered the goal of this therapy in patients with GHD (51). QoL should always be measured using validated questionnaires, such as the QoL-AGHDA (66) and we believe that in EGHD patients, an assessment of QoL, in addition to other clinical parameters, may be a valid determinant of whether to initiate replacement therapy or not.

DISCUSSION

We can confirm that GHrt in EGHD can contribute to the restoration of a physiological state of health, without inducing significant adverse effects, in particular when the treatment is properly and individually titrated. The main, though few, evidences concern improvements in QoL, cardiovascular risk factors and metabolic features; however, targeted studies on this population are strongly recommended to confirm these results, to adequately test the effectiveness and safety of GHrt in old age and to optimize diagnostic aspects (i.e. to determine peak GH cut-offs stratified by age). It must also be considered that fat mass increases in the elderly and, therefore, correcting the cut-off for weight alone is not always reliable.

We believe it would be important to select suitable patients for treatment, taking into account their health status, comorbidities, life expectancy and on-going medications (67), as well as adherence to chronic treatment (i.e., cognitive status, presence of a caregiver, *etc.*). There are currently several on-going studies with long-acting recombinant GH preparations (68), which use a variety of technologies to prolong the action of GH by deferring administration over time (e.g., weekly). This is intended to improve patient compliance, especially among the elderly, who often take a high number of medications (28).

The evidence available to date suggests, in patients with hypopituitarism, GHD contributes to excess mortality and GHrt contributes to bring mortality rates to those of normal subjects, but a true relationship between mortality reduction and GHrt has not been conclusively established with long-term prospective controlled trials (69). However, it seems unlikely that such studies could be easily conducted, especially in EGHD (11, 49, 69).

We can conclude by stating that the main goal of GHrt in the elderly patient is to improve QoL, prolong independence, and avoid frailty (69, 70). The age-related decline in GH-IGF-1 levels does not justify GHrt supplementation, but patients with established GHD should be considered for treatment, regardless of age, but rather taking into account general conditions, comorbidities, and life expectancy, analysing each case individually (34, 37, 38). We add that considering the costs of GHrt and the mainly long-term effects, the cost/benefit ratio must always be carefully evaluated (38), especially in a population with a reduced life expectancy (71).

In our opinion, the main goal of GHrt in EGHD should be a significant improvement in QoL, which can only be achieved through the development of personalized treatment and careful follow-up. To achieve this, it is necessary to expand studies of GHrt in the elderly population.

AUTHOR CONTRIBUTIONS

SR and MB conceived and wrote the manuscript. MF, MA, LV and FG realized the bibliography research. DF supervised the draft. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 16 June 2021
doi: 10.3389/fendo.2021.671126

Correlation of Significantly Decreased Serum Circulating Mesencephalic Astrocyte-Derived Neurotrophic Factor Level With an Increased Risk of Future Cardiovascular Disease in Adult Patients With Growth Hormone Deficiency

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Objective: Adult growth hormone deficiency (AGHD) is a rare chronic inflammatory disease caused by damage to the pituitary gland and is accompanied by disorders of multiple metabolic pathways. By examining the correlation between the serum mesencephalic astrocyte-derived neurotrophic factor (MANF) levels of AGHD patients and those of normal controls, we hope to elucidate the close relationship among MANF, lipid metabolism and insulin resistance in AGHD and discuss the potential therapeutic value of MANF.

Methods: This study included 101 AGHD patients and 100 healthy subjects matched for sex, age, height, and weight. Anthropometric parameters and biochemical indicators such as body mass index, waist circumference, hip circumference, serum MANF level, blood lipids and insulin level were measured. The above patients were also divided into several subgroups for correlation analysis based on indicators such as insulin resistance and BMI.

Results: The serum circulating MANF content of AGHD patients was significantly lower than that of the normal control group (5.235 (0.507-17.62) ng/ml (n=101) *vs.* 10.30 (1.84-16.65) ng/ml (n=100); p<0.0001), and circulating MANF levels were linearly correlated with HOMA-IR in the AGHD population (R=0.481, P=0.0041). When MANF was at pathological concentrations (lower than the mean circulating MANF of normal controls), the lowest concentration tertile (OR=21.429 p<0.0001) had a significantly higher disease odds ratio, Framingham risk score and 10-year risk of atherosclerotic cardiovascular disease than the highest concentration tertile.

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *23 February 2021*
Accepted: *01 June 2021*
Published: *16 June 2021*

Citation:
Ren Z, Wang Y, Chen Q, Long J, Zhang R, Wu X, Qian W, Chen Y, Liu D and Ren W (2021) Correlation of Significantly Decreased Serum Circulating Mesencephalic Astrocyte-Derived Neurotrophic Factor Level With an Increased Risk of Future Cardiovascular Disease in Adult Patients With Growth Hormone Deficiency. Front. Endocrinol. 12:671126. doi: 10.3389/fendo.2021.671126

Conclusions: MANF has a significant correlation with insulin resistance in the AGHD state. There is a strong correlation with abnormal glucose and lipid metabolism in the obese AGHD population. MANF is also a good assessment factor for the risk of cardiovascular disease in AGHD patients and has excellent therapeutic potential.

Keywords: adult growth hormone deficiency, mesencephalic astrocyte-derived neurotrophic factor, insulin resistance, lipid metabolism, cardiovascular risk

INTRODUCTION

Growth hormone is a polypeptide secreted by somatotroph cells of the anterior pituitary gland that is released in pulses mainly at night, and it can participate in anabolism and promote the growth and development of the body (1, 2). Acquired adult growth hormone deficiency (AGHD) is a special endocrine condition in which the growth hormone secretion ability of somatotroph cells is inhibited due to structural damage to the pituitary gland in adults, ultimately resulting in lower than normal absolute levels of growth hormone. Due to the presence and widespread expression of growth hormone receptors in various tissues and organs of the body, AGHD is characterized by abnormal body composition, imbalanced energy metabolism, decreased exercise capacity, impaired heart and kidney function, and even impaired psychological health (3). It has been shown that AGHD, similar to metabolic syndrome, is strongly associated with a chronic inflammatory state and that serum levels of lipocalin-2 (LCN2) are significantly elevated in patients with AGHD compared to the healthy population (4). Numerous studies suggest that disruption of the balance of the growth hormone/insulin-like growth factor 1 axis can induce the development of various heart diseases. Patients with AGHD have elevated levels of circulating inflammatory factors, accompanied by increased levels of oxidative stress and endothelial dysfunction (5). A body of evidence suggests that patients with AGHD have a higher risk of developing cardiovascular disease.

Mesencephalic astrocyte-derived neurotrophic factor (MANF) was first discovered by Canadian scientists in 2003 as a secreted protein with selective protective effects on dopamine neurons (6). However, in recent years, researchers have increasingly turned their attention to the association of MANF with metabolic diseases. In addition to neuronal tissues, MANF protein and mRNA are also widely expressed in metabolically active nonneuronal tissues and organs such as the testis, thyroid, and adrenal gland (7). It is also significant for neuroendocrine organs such as the thalamus and pituitary gland. MANF-deficient mice have a smaller anterior pituitary gland size than wild-type mice, thus reducing the number of cells producing growth hormone and prolactin due to the reduced pituitary gland size. This eventually leads to dysregulation of pituitary hormone expression and increased endoplasmic reticulum (ER) stress and apoptosis (8). Although MANF does not share any protein sequence homology with typical neurotrophic factors, MANF can exert the same extracellular effects as typical neurotrophic factors in regulating the cellular cascade. Interestingly, MANF can also act intracellularly as a reactive protein to ER stress and participate in the unfolded protein response (9). It is well known that to balance the harmful effects of ERs, the body initiates the unfolded protein response (UPR), which stimulates the secretion of MANF to reduce the accumulation of misfolded proteins and restore the normal function of the endoplasmic reticulum (10).

Pituitary cells are more susceptible to endoplasmic reticulum stress due to their high secretion of growth hormone and various prohormones (11). Increasing evidence also suggests a strong link between MANF and pituitary cells. The MANF-/- mouse model exhibited severe growth defects compared to normal controls. The size of the pituitary gland was significantly smaller, and ER stress and apoptosis were significantly increased in the pituitary gland (8). MANF has been shown to play an important therapeutic role in a variety of endoplasmic reticulum stress-related diseases. MANF has shown promising protective effects in neurodegenerative diseases, diabetes, and ischemic diseases of the heart and brain, and it can even modulate inflammatory factor expression to suppress chronic inflammatory diseases (12).

A growing body of evidence suggests that MANF appears to have highly valuable therapeutic potential for AGHD, a specific chronic metabolic endoplasmic reticulum stress-related disorder. Whether MANF can be a novel therapeutic target for AGHD needs to be supported by various lines of evidence. The purpose of this research was to investigate whether circulating MANF is associated with newly diagnosed AGHD and to clarify the association from a clinical point of view. No relevant research about serum MANF levels in healthy subjects and patients with AGHD has been reported thus far.

METHODS

Subjects

We recruited 101 patients (68 females, 33 males; mean age of 45.87 ± 14.45 years; range 20-76) with newly diagnosed AGHD from January 2017 to October 2020 in the Department of Endocrinology of our hospital. AGHD was diagnosed according to the insulin tolerance test (ITT), which is recommended by The American Endocrine Society as the gold standard, with a growth hormone (GH) peak <5.0 μg/L. None of the patients had been treated with GH prior to diagnosis, and all the patients were evaluated for thyroid, gonadal, and adrenal function and received stable hormone replacement therapy for more than 6 months according to the function of their respective endocrine glands to ensure the stability of these glands and

related hormone levels. A total of 100 healthy subjects (66 females, 34 males; mean age of 44.71 ± 10.47 years; range 26-77) were recruited as controls for our study. The abovementioned healthy volunteers were roughly matched with AGHD patients in terms of age, sex, height, and weight. All subjects participating in this study were informed of the experimental method and purpose and provided informed consent signed by themselves. All experimental protocols conformed to the Declaration of Helsinki and were approved by the Ethics Committee of our hospital.

Inclusion Criteria

The inclusion criteria were as follows: 1. the subjects in the experimental group met the diagnostic requirements of ITT (GH peak value < 5.0 μg/L); 2. the subjects did not have diabetes or chronic diabetic complications; 3. the subjects were not treated with drug regimens to interfere with glucose lipid metabolism; 4. the subjects did not have hypertension or cardiovascular disease; 5. the subjects did not have a mental illness or malignant tumors; 6. the subjects did not have severe or chronic kidney or liver disease; and 7. the subjects did not have acute symptoms of infection.

Experimental Group Design

In this study, 101 patients with AGHD were divided into the following three subgroups according to different BMIs, pathogenic factors and degrees of insulin resistance to investigate the possible correlation between MANF and different factors. We first divided the 101 patients with AGHD into an overweight group (BMI>25.0 (n=34)) and a nonobese group (BMI<25.0 (n=67)) based on the World Health Organization (WHO) proposed criteria that a body mass index (BMI) over 25 is considered overweight (13). The above patients were then regrouped according to the most common cause of AGHD into a postoperative pituitary tumor group (craniopharyngioma×3; Rathke cleft cyst×2; nonfunctional pituitary adenoma ×22, n=27), an idiopathic AGHD group (menstrual disorders of unknown origin×6; primary amenorrhea×2; insidious onset×52, n=60) and a Sheehan's syndrome group (adrenocorticotropic hormone combined with thyroid hormone deficiency×10; sex hormone combined with adrenocorticotropic hormone deficiency×4, n=14) (14). However, since all the patients with Sheehan's syndrome were female, which may lead to gender bias in the statistical results, this study excluded all male patients in the etiological classification for subsequent comparison (postoperative pituitary tumors group (nonfunctional pituitary adenoma×19), n=19; idiopathic AGHD group (menstrual disorders of unknown origin×6; primary amenorrhea×2; insidious onset×27), n=35; Sheehan's syndrome group (adrenocorticotropic hormone combined with thyroid hormone deficiency×10; sex hormone combined with adrenocorticotropic hormone deficiency×4), n=14). Finally, 91 patients with AGHD (10 of whom did not undergo fasting insulin testing) were divided into an insulin-resistant group (HOMA-IR > 2.71, n=34) and a noninsulin-resistant group (HOMA-IR < 2.71, n=57) according to the homeostasis model assessment proposed by the University of Oxford (15). The possible differences and associations of MANF in the above subgroups were explored separately.

Anthropometric Parameters and Biochemical Indexes

All the participants completed a comprehensive clinical questionnaire detailing their physical measurements, including basic information on physical examination, type of nongrowth hormone replacement medication, and medication dosage. After 12 hours of fasting, all the participants had elbow venous blood collected for the evaluation of relevant biochemical parameters. Fresh serum was selected for analysis of the fasting glucose, fasting insulin (Fins), glycated hemoglobin (HbA1c), insulin-like factor-1 (IGF-1), insulin-like factor binding protein-3 (IGFBP3), circulating lipids, transaminases, and high-sensitivity C-reactive protein (hsCRP) levels. The remaining serum was promptly frozen at -80°C for the later measurement of circulating MANF levels. Plasma glucose was measured using the glucose oxidase method. Lipid metabolic spectra were measured by a biochemical autoanalyzer (CX-7 Biochemical Autoanalyzer; Beckman, Brea, CA, USA). The serum insulin, GH, and IGF1 levels were detected by chemiluminescence immunoassay (Immulite1000).

Ten-year risk scores for atherosclerotic cardiovascular disease (ASCVD) and Framingham risk scores were determined for all the participants to assess the risk level for developing cardiovascular disease over the next ten years (16, 17).

The formulas involved in this research were as follows: this assay adopts the homeostatic model to assess the insulin resistance index using the following formula: HOMA-IR = fasting insulin (mU/L) × [fasting plasma glucose (mmol/L)/22.5]. β cell function was calculated as follows: HOMA-β = [20 × fasting insulin (mU/L)]/[fasting plasma glucose (mmol/L)-3.5]. The lipid accumulation product, LAP, was calculated as follows: LAP (male) =[waist circumference, WC (cm) -65]×triglycerides, TG (mmol/L); LAP (female) =[WC (cm) -58]×TG (mmol/L). The visceral adiposity index was calculated as follows, VAI: VAI (Male) = WC (cm)/(39.68+1.88×BMI)×TG (mmol/L)/1.03×1.31/HDL (mmol/L); VAI (Female) = WC (cm)/(39.68+1.89×BMI)×TG (mmol/L)/0.81×1.52/HDL (mmol/L). The quantitative insulin sensitivity index was calculated as follows: QUICKI =1/[log(fasting insulin) + log (fasting glucose)].

Measurements of Serum MANF

Serum circulating MANF levels were determined by human MANF ELISA kits (ab215417, Abcam, USA). The ELISA kit has an optimal measurement range of 0.25 ng/ml - 16 ng/ml and a sensitivity of 30 pg/ml. This study used the same batch of kits to avoid differences between batches that could affect the accuracy of the experiment.

Statistical Analysis

All analyses in this study were performed with the Statistical Package for Social Sciences version 26 (SPSS Inc., Chicago, IL, USA). In this experiment, we performed Kolmogorov–Smirnov tests on all test data from participants to determine their normal distribution. Continuous variables are expressed as the mean ± standard deviation (SD), and skewed distribution data are expressed as medians with interquartile ranges. Demographic and laboratory characteristics were compared between the AGHD population and

the normal control group. The Wilcoxon rank-sum test was used for comparison of skewed distribution data, and the independent samples t-test was used for normal variables. Among the subgroups divided by BMI and homeostatic model, we used Spearman's correlation analysis to test the correlation between the MANF level and other demographic and laboratory characteristics. In multiple linear regression, we tested multicollinearity for all independent variables, and if the VIF value exceeded 5, the variables were considered to be multicollinear. After dividing all subjects by MANF concentration tertile, we used the Kruskal-Wallis H test to examine differences in Framingham risk scores and 10-year risk scores for ASCVD between groups. Binary logistic regression analysis was used to calculate the odds ratio of AGHD at different serum MANF concentrations. All reported confidence interval (CI) values were calculated at the 95% level. Categorical variables are expressed as absolute and relative (%) values or proportions. In all analyses, a P value <0.05 was considered statistically significant.

RESULTS

Clinical Characteristics of the Study Subjects

All the AGHD and control subjects were matched for height, weight, age, and sex (P>0.05). **Table 1** details the comparison and differences in demographic data and laboratory parameters between the AGHD patients and healthy controls. In the AGHD group, the waist circumference and the levels of TC, TG, HDL, LDL, hsCRP, LAP, VAI, ALT and AST were significantly different from those in the control group. In the AGHD population, the circulating lipid levels were significantly increased, whereas the HDL level was significantly lower than that in the control group. The VAI, LAP, and other lipid metabolism parameters were significantly higher than those in the control participants.

Serum MANF Levels in AGHD Were Significantly Lower Than Those in the Control Population

Circulating serum MANF levels were significantly lower in the AGHD subjects than in the healthy controls (5.235 (0.507-17.62) ng/ml (n=101) *vs.* 10.30 (1.84-16.65) ng/ml (n=100); p<0.0001), as shown in **Figure 1**.

Circulating MANF Levels in Overweight AGHD Patients Were Significantly Higher Than Those in the Nonobese Group

Since AGHD is closely related to lipid metabolism, 101 patients with AGHD were grouped according to the WHO definition of BMI=25.0 as the limit of overweight, were divided into two subgroups with BMI>25.0 (n=34) and BMI<25.0 (n=67) and were subjected to comparative statistical analysis. As shown in **Figure 2**, serum circulating MANF levels in AGHD patients with BMI <25.0 were significantly lower than those in subjects with BMI >25 (4.67 (0.51-17.61) ng/ml *vs.* 6.92 (0.55-17.62) ng/ml;

TABLE 1 | Main clinical characteristics in AGHD and controls.

Variables	Control (n=100)	AGHD (n=101)	P value
Age (y)	45.5(35-52)	46.00(35-56)	0.703
Height (cm)	161.14±7.19	160.09±8.43	0.329
Weight (kg)	58.621±9.84	61.59±13.52	0.411
BMI	23.71±4.01	23.03±3.46	0.501
Gender (M/F)	34/66	34/67	0.842
Waist circumference (cm)	78.08±9.11	85.48±10.94	<0.0001*
SBP (mmHg)	116.91±14.97	119.76±16.99	0.485
DBP (mmHg)	74.00(66.25-80.00)	75.00(66.25-83.00)	0.366
FPG (mmol/L)	5.1(4.90-5.40)	5.2(4.75-5.60)	0.945
TC (mmol/L)	4.44(3.87-4.75)	4.81(4.15-5.85)	<0.0001*
TG (mmol/L)	1.337±0.67	2.187±1.53	<0.0001*
HDL (mmol/L)	1.395(1.19-1.60)	1.18(0.93-1.66)	0.013*
LDL (mmol/L)	2.70(2.19-3.09)	3.09(2.30-3.60)	0.004*
hsCRP (mg/L)	0.51(0.15-0.91)	1.68(0.66-3.76)	<0.0001*
LAP	22.00(8.82-35.04)	47.12(26.79-79.66)	<0.0001*
VAI	1.525(0.91-2.47)	2.65(1.51-4.16)	<0.0001*
AST (u/L)	17.00(14.50-21.00)	22.00(18.00-30.25)	0.004*
ALT (u/L)	15.00(11.50-21.50)	21.00(14.00-30.00)	<0.0001*
ALB (g/L)	45.371±2.31	44.157±4.43	0.102
Cr (μmol/L)	65.50(57.00-77.00)	72.00(52.25-83.75)	0.059
UA (μmol/L)	297.50(259.00-375.50)	295.00(235.00-370.00)	0.524
HOMA-IR	2.11(1.22-3.57)	1.53(0.75-2.03)	<0.0001*
HOMA-β	110.40(65.56-177.78)	58.43(38.42-107.53)	<0.0001*

SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, Triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LAP, lipid accumulation product; VAI, visceral adiposity index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Cr, creatinine; UA, uric acid.

*The data are presented as the mean±standard deviation or medians with interquartile ranges. *The p values for the comparisons between the two groups were two-tailed and considered significant at p < 0.05.*

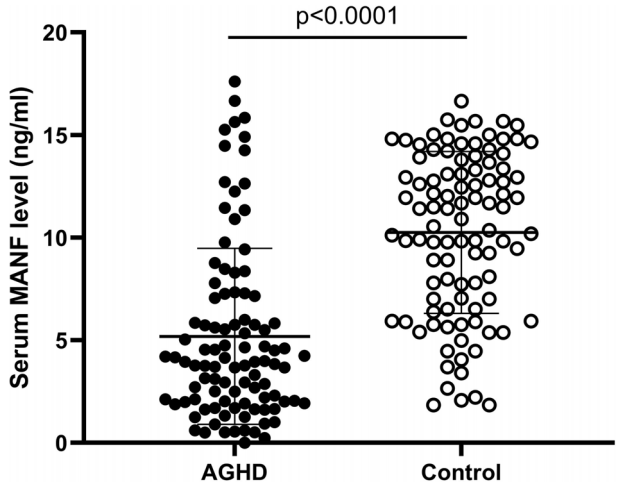

FIGURE 1 | Serum circulating MANF levels in AGHD patients and control populations.

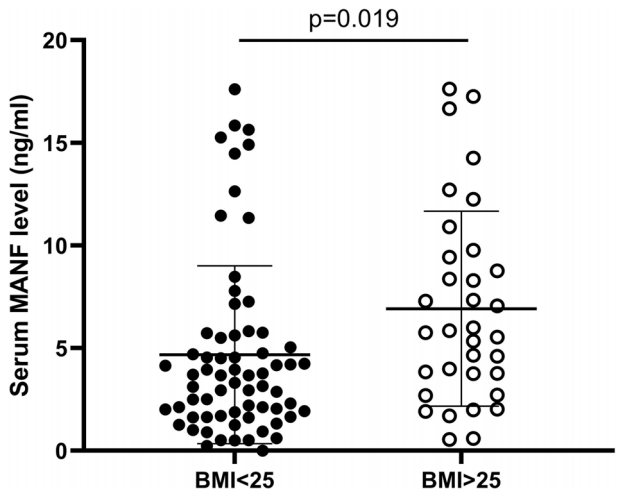

FIGURE 2 | Serum circulating MANF levels in two AGHD subgroups of patients according to BMI=25.

p=0.019). The Mann-Whitney U test suggested significant differences between the two subgroups of subjects in the Framingham risk scores, 10-year risk scores for ASCVD, Fins, HOMA-IR, and QUICK ($p<0.05$). All of the above data in the obese group were higher than those in the nonobese group. Next, we examined the relationship between serum MANF levels and other various parameters in patients in the BMI>25.0 group using Pearson's correlation analysis. The results showed that serum MANF was positively correlated with the levels of TC (r=0.477** p=0.004), TG (r=0.415* p=0.015), LDL (r=0.391* p=0.022), and LAP (r=0.434* p=0.01). Multiple linear regression revealed *an independent correlation between the TC and LDL levels and the level of serum circulating MANF (***Figure 3***).*

Correlation of Circulating MANF Level and Metabolism in AGHD Patients Under Different Pathogenic Factors

Due to the diverse etiologies of AGHD, this study aimed to further investigate the association between serum circulating MANF factors and the metabolic axis in AGHD patients with different etiological factors. We categorized 101 patients with AGHD into the following three groups according to different etiologic factors: the postoperative group, idiopathic AGHD group, and Sheehan's syndrome group, The results of the Kruskal-Wallis H test suggested that there were differences in the TC, TG, LDL, VAI, GH, and AST levels among the three groups of subjects, and thus, further comparisons were made between groups. Significant differences were found in the TC ($p<0.001$) and LDL ($p<0.001$) levels between the postoperative and idiopathic groups after Bonferroni correction (significantly higher in the postoperative group than in the remaining two groups). For comparison of the anthropometric indexes, we adjusted the sex of the patients in the postoperative and idiopathic groups and finally selected all female subjects in both groups for subsequent comparisons (group 1 n=19; group 2 n=35; group 3 n=14). In terms of height (although not reaching statistical significance, a trend can be seen, p=0.052), weight, BMI, and IGF1 level, the subjects in Sheehan's group exhibited significantly lower values than those in the other two groups. The postoperative group exhibited significantly higher values than the other two groups in terms of the TC and LDL levels, the two lipid metabolism indexes (**Table 2**). The analysis revealed that the level of MANF was correlated with different parameters in the three subgroups. In the idiopathic AGHD group, Spearman's correlation analysis showed that the level of MANF was positively correlated with Fins, HbA1C, VAI, QUICK, weight, BMI, waist circumference, LAP, and ALB. In the postoperative group, the level of MANF was positively correlated with DBP and UA. In conclusion, however, the level of MANF was more closely associated with lipid metabolism in AGHD.

Circulating MANF Levels in AGHD Subjects in the Insulin-Resistant and Noninsulin-Resistant Groups

To investigate the relationship between circulating MANF levels and insulin resistance in the AGHD population, the circulating serum MANF levels in both groups of subjects are shown in **Figure 4**. In the insulin-resistant group, circulating MANF levels were significantly higher than those in the noninsulin-resistant group (3.86 (0.236-12.64) ng/ml (n=56) *vs.* 6.94 (0.604-17.62) ng/ml (n=34); p=0.0006). Spearman correlation analysis showed that there was a significant positive correlation between the level of MANF and Fins (r=0.306** p=0.005), HOMA-IR (r=0.288** p=0.009), HOMA-β (r=0.324** p=0.003), HbA1c (r=0.31* p=0.032), and waist circumference (r=0.331** p=0.002) in 91 subjects. To verify the existence of an independent linear correlation between MANF and insulin resistance, multiple linear regression was performed. In this experiment, *MANF level was found to have an independent influence on HOMA-IR after consecutively adding the remaining influencing factors, as shown in* **Figure 5**.

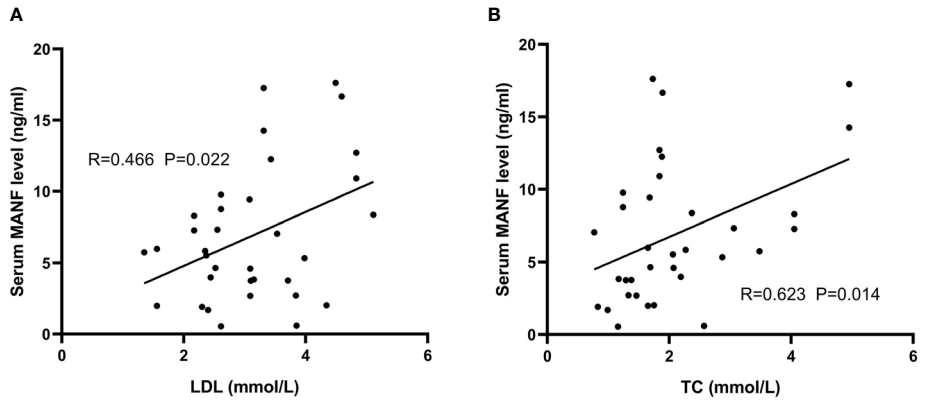

FIGURE 3 | Associations between lipid metabolism indexes and circulating MANF in overweight AGHD patients. Scatter plots and correlation coefficients. Scatter plot and correlation coefficient (R) between LDL **(A)** and TC **(B)** and cycle MANF. P values were considered significant at $p < 0.05$.

TABLE 2 | Comparison of selected parameters between the three groups of subjects after gender correction.

Pathogenic		Height,cm	Weight,kg	BMI	IGF1	TC,mmol/L	LDL,mmol/L
G1 (Postoperative, n=19)		160.28±6.31	58.72±8.51	22.31±3.36	78.85(34.40-124.75)	5.75(4.61-6.22)	3.56(2.89-3.73)
G2 (idiopathic AGHD, n=35)		156.68±6.94	57.5±11.18	23.98±4.24	121.5(83.60-221.75)	4.47(3.96-4,84)	2.42(2.02-3.14)
G3 (Sheehan's syndrome, n=14)		155.00±6.47	51.55±6.94	21.48±2.30	48.25(28.63-129.25)	4.74(4.00-5.22)	2.95(2.13-3.56)
Overall Comparison							
Intergroup comparison		0.052	0.030*	0.047*	0.002*	0.001*	0.012*
G1 VS G3	P value		0.073	0.798	1.000	0.067	0.077
G2 VS G3			0.038*	0.047*	0.025*	1.000	1.000
G1 VS G2			1.000	0.537	0.092	0.001*	0.001*

*G1 means the postoperative group; G2 means the idiopathic AGHD group; G3 means the Sheehan's syndrome group. The data are presented as the mean±standard deviation or medians with interquartile ranges *The p values for the comparisons between the two groups were two-tailed and considered significant at $p < 0.05$.*

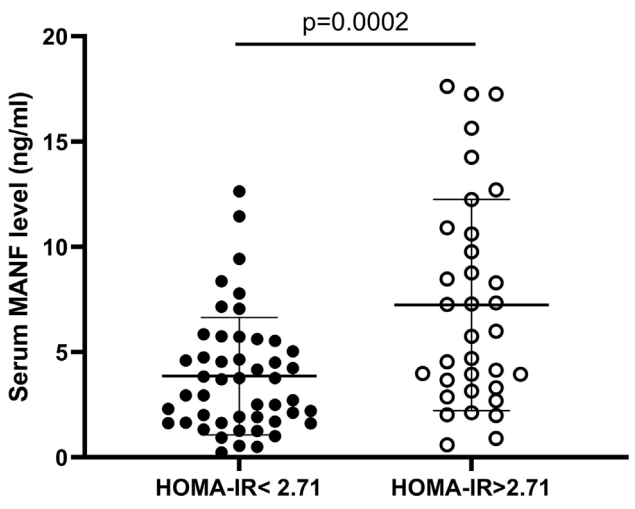

FIGURE 4 | Serum circulating MANF levels in two AGHD subgroups of patients according to homeostasis model assessment.

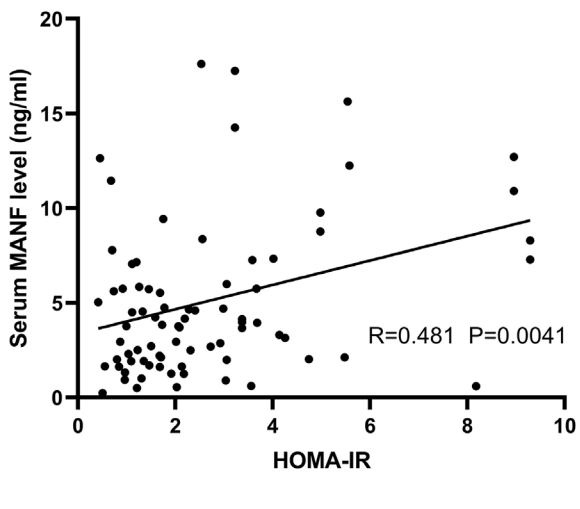

FIGURE 5 | Scatter plot and correlation coefficient (R) between HOMA-IR and circulating MANF levels in the AGHD population. P values were considered significant at $p < 0.05$.

Different Circulating MANF Concentrations and Cardiovascular Risk Assessments

We divided all the subjects into three subgroups based on the tertile of the circulating MANF concentration (group 1: <4.17 ng/ml; group 2: 4.17-10.36 ng/ml; group 3: >10.36 ng/ml). The high MANF concentration group showed lower Framingham risk scores than group 1 ($p<0.0001$). In terms of circulating lipid levels, group 3 also exhibited lower TC, TG, and LDL levels and higher HDL levels than group 1 and group 2 (however, due to sample size limitations, statistical significance was not reached). Binary logistic regression showed that the odds ratio for AGHD in group 1 was 20.429 times higher (OR=20.429 $p<0.0001$) than the ratio in group 3. The odds ratio for AGHD in group 2 was 2.869 times (OR=2.869 P=0.012) higher than in group 3. The model was adjusted for potential confounders such as height, age, sex and weight.

DISCUSSION

Growth hormone is involved in many metabolic pathways such as sugar, lipid, and protein pathways, but its main metabolic function is to promote lipolysis (18). Adult growth hormone deficiency is caused by structural injury to the pituitary gland or tumors in the pituitary region that leads to a sharp decrease in the amount of growth hormone in the body, which triggers structural damage to the heart, skeleton and other organs and is accompanied by endocrine metabolic disorders. An imbalance in the GH/IGF1 axis leads to an excessive accumulation of lipids, which increases the risk of cardiovascular disease. High levels of LDL as well as low levels of HDL are known to be high-risk factors for the development of cardiovascular disease (19). In this study, we found that patients with AGHD have significantly higher blood lipid levels than normal controls, which corroborates previous studies stating that AGHD patients are at a higher risk of developing cardiovascular disease (20). Interestingly, circulating serum levels of a class of endoplasmic reticulum stress-associated secreted proteins, such as MANF, were also significantly reduced in the AGHD population. To the best of our knowledge, this study is the first to link the level of MANF with AGHD. It also investigates the correlation of MANF levels with lipids, glucose metabolism and potential therapeutic value in the AGHD population.

In recent years, increasing evidence has shown that MANF has unique advantages in relation to endocrine metabolic pathways and stress protection in the endoplasmic reticulum (21). In animal models, $MANF^{-/-}$ mice show abnormal pituitary hormone secretion accompanied by a state of insulin resistance and growth hormone deficiency, while the mice exhibit severe growth retardation and endocrine gland atrophy. The endocrine organs of MANF+/+ mice are more active and express greater levels of hormone secretion (8). However, the expression of MANF is inconsistent in human disease, and studies have shown that circulating serum MANF levels are significantly elevated in patients with primary diabetes and early diabetes with abnormal glucose tolerance (22). In another PCOS-related study, it was noted that the circulating serum MANF level of the PCOS population was significantly lower than that of the control group (23). In our study, a trend toward significantly lower circulating MANF levels was found in the AGHD population.

In response to endoplasmic reticulum stress, cells initiate the unfolded protein response (UPR) to balance the endoplasmic reticulum. The UPR has been confirmed to form the basis of chronic metabolic diseases together with inflammation, lipid metabolism and energy control pathways (24). MANF is a class of endoplasmic reticulum stress-activated protective protein factors that stimulate MANF secretion to maintain endoplasmic reticulum homeostasis in response to cellular activation of the UPR (25). According to the literature, AGHD is a special endocrine condition closely related to lipid and glucose metabolism, and its circulating MANF concentration seems to be secreted due to the activation of the UPR to play the role of endoplasmic reticulum balance. However, interestingly, our study found the opposite, a decreasing trend in MANF levels. We speculate that AGHD patients may be in a decompensated state of MANF secretion due to prolonged low levels of growth hormone stimulation, resulting in lower overall circulating levels.

Adipose tissue is an important target for growth hormone action due to the presence of GH receptors, which are extremely sensitive to the stimulatory feedback of growth hormone (26). Obesity and overnutrition induce chronic ER stress in the liver and some other tissues (27). In the present study, significant differences in MANF levels were observed in the nonobese group and in overweight patients with AGHD. Using the WHO recommended BMI=25, serum levels were significantly higher in the obese group of AGHD patients (n=34) than in the nonobese group (n=67). Circulating MANF levels in the obese group were positively correlated with the body weight, TC, Tg, LAP, VAI, and other parameters. The levels of TC and LDL were independently linearly correlated with the level of MANF. This result reflects the close relationship between MANF and lipid metabolism. Several epidemiological trials have validated the LAP and VAI as reliable markers for predicting cardiovascular disease risk in the general population (28–30). Due to the limitation of the sample size, the linear correlation between MANF and LAP, VAI, etc., was not observed in the present study, which needs to be investigated by subsequent case expansion. However, this does not preclude the use of MANF as an independent diagnostic factor in the AGHD population and as an indicator for assessing the risk of cardiovascular morbidity in obese patients with AGHD.

It has previously been shown that MANF triggers insulin resistance by enhancing the activity of phosphatidylinositol 5-phosphate 4-kinase type-2 beta (PIP4k2b, a kinase known to regulate insulin signaling) localized to the endoplasmic reticulum (31). Patients with AGHD usually show varying degrees of insulin resistance. Thus, we divided 101 patients into insulin-resistant and noninsulin-resistant groups according to the HOMA model. Likewise, there was a surprisingly significant difference in MANF levels between the two groups. MANF was significantly higher in the 34 patients with HOMA-IR>2.71 than

in the noninsulin-resistant group, and the TG, waist circumference, hip circumference, waist-to-hip ratio, LAP, and VAI values were also higher in that group than in the noninsulin-resistant group. The most significant positive correlation was between the level of MANF and insulin resistance. Both visceral obesity and insulin resistance increase cardiometabolic disease risk. A 2017 study of a large sample of European populations indicated that the VAI was independently associated with an increased 10-year risk of cardiovascular disease (32). Dysregulation of sympathetic nervous and renin-angiotensin systems resulting in enhanced stimulation of both adrenergic and angiotensin II receptors is a typical feature of heart failure and hypertension and is involved in the pathogenesis of insulin resistance. Angiotensin II acts through the angiotensin receptor to inhibit the actions of insulin in vascular tissue, in part, by interfering with insulin signaling through phosphatidylinositol 3-kinase and downstream protein kinase B signaling pathways *via* generation of reactive oxygen species by nicotinamide adenine dinucleotide phosphate oxidase (33). Evidence suggests that AGHD patients with insulin resistance may have a higher risk of cardiovascular morbidity and that MANF may be a good predictor for cardiovascular risk assessment in AGHD patients.

The main manifestation of AGHD is a decrease in GH secretion, which is accompanied by a decrease in IGF1 and IGFBP3. Regrettably, we have not yet detected the correlation between MANF and IGF1 and IGFBP3. This may be because in the AGHD population, IGF1 levels do not absolutely correspond to the disease condition, and many patients may have a normal level of IGF1. It has also been shown that GH can function independently of IGF1 (34). There are many reasons why there may not be a linear relationship between MANF and IGF1. However, it is still necessary to expand the sample size to confirm this speculation in the future.

MANF has also shown good cardioprotective effects in several studies. In infarction and localized ischemic disease, MANF is secreted in large quantities after sarcoplasmic reticulum/endoplasmic reticulum calcium homeostasis is disrupted to prevent ischemic myocardial injury and has an antihypertrophic effect (35). Other types of secreted proteins do not exhibit such secretory characteristics and efficacy (36). Due to varying degrees of insulin resistance status, abnormal lipid metabolism and visceral adipose deposition lead to vascular endothelial damage, and patients with AGHD have a higher risk of cardiovascular disease (37). MANF exhibits excellent therapeutic potential due to its unique endoplasmic reticulum balancing function.

These results leave much space for investigation. The circulating MANF concentrations in the normal population were significantly higher than those in the AGHD population, which seems to suggest that MANF at physiological concentrations is a protective factor for the body and has a balancing effect on normal lipid and glucose metabolic pathways. However, as a secreted protein, MANF exerts not only an extracellular effect on the circulating paracrine pathway but also intracellular effects of binding to transmembrane receptors to regulate the intracellular signaling cascade (38). Two different modes of action also lead to the possibility that MANF may have different biological efficacies. In the present study, lower tertile MANF concentrations had the highest odds ratio for disease, whereas in the AGHD population, higher MANF was positively associated with insulin resistance and lipid deposition. We speculate that the level of MANF content is a relative concept and that under pathological concentration conditions, relatively high MANF levels instead show negative physiological effects. It has been shown that MANF interacts with PIP4k2b and triggers insulin resistance *via* an unknown pathway other than the inflammatory activation state (39). However, the background of this experiment is that MANF transgenic mice overexpress MANF factor in the hypothalamus and exhibit insulin resistance. However, no further studies have been conducted on a pathological state model with a low dose of MANF. This is the next step in our team's research. We observed that at physiological concentrations, MANF did not differ significantly based on insulin resistance or obesity status.

The different causative factors of AGHD also provided some interesting results. The height, weight, and BMI of the patients in Sheehan's group were significantly lower than those of the AGHD patients with the remaining causative factors. Patients with AGHD due to surgical damage to the pituitary gland had significantly higher levels of TC, LDL, and other parameters than idiopathic patients. However, the exact cause could not be explained yet. This may be due to the limitation of the specimen size in a rare metabolic disease such as AGHD, and we also need to expand the sample size in the future to advance subsequent studies.

Overall, this study leaves us with much uncertainty and many potential research directions. However, it is undeniable that serum circulating MANF levels may be an excellent target for predicting the onset of AGHD and serve as an excellent potential therapeutic factor for cardiovascular disease in AGHD patients. MANF also needs to receive more extensive attention and research.

CONCLUSION

MANF, an endoplasmic reticulum stress-secreting protein, is strongly associated with insulin resistance and abnormal lipid metabolism under AGHD conditions. This factor may be critical in the early diagnosis of AGHD and is involved in the occurrence and development of AGHD. It may have good therapeutic potential for later cardiovascular disease.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the First affiliated hospital of Chongqing medical university. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ZR, DL, and WR jointly conceived and designed this research. ZR and YW performed the data analysis and wrote the manuscript. QC and JL collected the medical records and biochemical data for each subject. RZ, XW, WQ, and YC conducted telephone follow-up interviews of patients and collected relevant physical information and data. DL and WR reviewed the full text and guided revisions. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by the Project of the Science and Technology Committee in Chongqing 2016 (Number: cstc2016jcyjA0025) and the National Natural Science Foundation of China (Grant No. 81370467 to DL).

ACKNOWLEDGMENTS

We thank the staff of the Department of Endocrinology, First Affiliated Hospital of Chongqing Medical University, for the excellent technical service they provided. We are grateful for the support from The Second Affiliated Hospital of Chongqing Medical University, where our work was conducted.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 19 October 2021
doi: 10.3389/fendo.2021.678778

GH Deficiency and Replacement Therapy in Hypopituitarism: Insight Into the Relationships With Other Hypothalamic-Pituitary Axes

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 10 March 2021
Accepted: 27 September 2021
Published: 19 October 2021

Citation:
Profka E, Rodari G, Giacchetti F and Giavoli C (2021) GH Deficiency and Replacement Therapy in Hypopituitarism: Insight Into the Relationships With Other Hypothalamic-Pituitary Axes. Front. Endocrinol. 12:678778. doi: 10.3389/fendo.2021.678778

GH deficiency (GHD) in adult patients is a complex condition, mainly due to organic lesion of hypothalamic-pituitary region and often associated with multiple pituitary hormone deficiencies (MPHD). The relationships between the GH/IGF-I system and other hypothalamic-pituitary axes are complicated and not yet fully clarified. Many reports have shown a bidirectional interplay both at a central and at a peripheral level. Signs and symptoms of other pituitary deficiencies often overlap and confuse with those due to GH deficiency. Furthermore, a condition of untreated GHD may mask concomitant pituitary deficiencies, mainly central hypothyroidism and hypoadrenalism. In this setting, the diagnosis could be delayed and possible only after recombinant human Growth Hormone (rhGH) replacement. Since inappropriate replacement of other pituitary hormones may exacerbate many manifestations of GHD, a correct diagnosis is crucial. This paper will focus on the main studies aimed to clarify the effects of GHD and rhGH replacement on other pituitary axes. Elucidating the possible contexts in which GHD may develop and examining the proposed mechanisms at the basis of interactions between the GH/IGF-I system and other axes, we will focus on the importance of a correct diagnosis to avoid possible pitfalls.

Keywords: growth hormone deficiency, hypopituitarism, central hypoadrenalism, central hypothyroidism, hypogonadotropic hypogonadism

INTRODUCTION

Growth hormone (GH) deficiency in adults (AGHD) is a complex condition characterized by a well-defined clinical phenotype including modified body composition (increased fat mass and loss of lean muscle mass), intermediate metabolism changes, reduced bone mass, compromised aerobic exercise capacity, impaired quality of life and increased cardiovascular risk profile (1–3).

Response to recombinant human growth hormone (rhGH) replacement therapy has a high inter-individual variability and, though several placebo-controlled and observational studies have provided information on its efficacy and safety, the results are still inconclusive, especially regarding quality of life (QoL) improvement and GH specific mortality reduction (2–5).

In adults, growth hormone deficiency (GHD) is often accompanied by other multiple pituitary hormone deficiencies (MPHD), mainly secondary to organic causes (pituitary tumour mass, surgery or radiation, traumatic brain injury, subarachnoid haemorrhage, hypophysitis, Sheehan's syndrome, vascular damage, empty sella, hypothalamic infiltrative/inflammatory diseases or pituitary metastasis). Nonetheless, sometimes adult-onset GHD can be idiopathic, due to an impaired somatotroph function in the absence of an underlying pituitary lesion or defect. In this setting, the diagnosis can be extremely challenging due to its subtle manifestations and only an extended use of dynamic GH test may reveal such condition (6, 7).

Less frequently, adult GHD is of childhood origin, reconfirmed at adult height and after the transitional age. Childhood-onset GHD (CO GHD) is mostly occurring as an idiopathic isolated hormone deficiency, being additional MPHD rarely encountered (8, 9). However, there are cases of CO GHD reconfirmed in adulthood and the association with other MPHD represents an important predictive factor of persistent GHD, especially in the presence of organic lesions (i.e. craniopharyngiomas). Indeed, severe GHD tends to reconfirm in more than 90% of organic CO GHD and around 50% of idiopathic GHD (10). Moreover, among CO GHD associated with MPHD, it is worth mentioning congenital aetiologies due to mutations of the transcription factors involved in the embryologic development of the pituitary, namely PROP1, POU1F1 (PIT-1), HESX1, LHX3, LHX4 or SOX2 (11).

Clinical manifestations of MPHD are insidious and strictly dependent on the degree and severity of hormone deficiencies, the gender, the age of onset and the underlying comorbidities. In case organic cause, signs and symptoms related to mass effect can also be present.

The challenging management of MPHD is due to the complex and multifaceted interplay between the GH-IGF-I and other pituitary hormones axis, in which specific signs and symptoms of GHD often coincide with those of other deficits. Moreover, a condition of untreated GHD may mask other underlying pituitary deficiencies, mainly central hypothyroidism (CHT) and hypoadrenalism (CHA). In this setting, an appropriate diagnosis can be possible only after rhGH replacement. On the other hand, the concomitant reduction of other pituitary hormones can alter GH secretion and response to pharmacological stimuli, thus an appropriate replacement therapy is required in order to avoid GHD diagnosis pitfalls (1).

The impact of these interactions is more than theoretical: for instance, since rhGH start may increase cortisol metabolism in patients with MPHD, it is possible that GH treatment initiation could lead to acute adrenal insufficiency by "unmasking" a condition of unsubstituted CHA or require an adjustment of glucocorticoid replacement dosages.

Moreover, several androgens enhance GH effects in peripheral tissues (12) explaining why men are more responsive than young women to rhGH therapy and supporting a sexual dimorphism of rhGH effects at different end-points of the treatment (13).

As aforementioned, the clinical manifestations of AGHD may also be related to other underlying pituitary deficiencies or suboptimal replacement therapies. Thus, a correct diagnosis of hypopituitarism and the subsequent indication of appropriate replacement therapy can be crucial in the detection of the beneficial effects of GH therapy.

By elucidating the possible context in which GHD may develop and by examining the proposed mechanisms and the basis by which the GH/IGF-I system and other axes interact (**Figure 1**), we will enlighten the importance of reaching a correct diagnosis and establishing a correct management to avoid possible pitfalls.

GH-IGF-I AXIS AND HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

Growth hormone and IGF-I, together with androgens, represent the main anabolic hormones and cortisol the main catabolic one, thus their actions are evidently linked. Several studies have reported a complex relationship between the GH/IGF-I and the hypothalamic-pituitary-adrenal (HPA) axis, both at a central and a peripheral level (14, 15).

At the hypothalamic-pituitary level, altered cortisol and ACTH secretion may affect GH release. Indeed, a substantial body of literature has described that a condition of eucortisolism is required to elicit a GH response to pharmacological testing (16). The clinical importance of this phenomenon is particularly evident in infants with severe ACTH deficiency, when, even in the presence of mutations of transcription factors not involved in GH axis regulation, a severe GHD can resolve with cortisol replacement (17).

At the peripheral level, some studies reported a possible direct effect of GH therapy on cortisol-binding globulin (CBG) levels, but data are contradictory (15, 18–20).

Moreover, the GH-IGF-I axis interplay can act at a tissue level by modulating the activity of 11beta-hydroxysteroid dehydrogenase (11ßHSD): the well-known cortisol-cortisone shuttle (21). The type 1 isoenzyme (11ßHSD1) can be found in the liver, lung, adipose tissue, gonads, pituitary and central nervous system. It is a low affinity NADP(H)-dependent bi-directional enzyme which interconverts inactive cortisone to active cortisol (22). Conversely, Type 2 isoenzyme (11ßHSD2) is a unidirectional, NAD-dependant dehydrogenase, localized in the kidney, placenta, colon and in the salivary glands and has a dehydrogenase activity which converts active cortisol to cortisone (23). In this context, GH modulates cortisol metabolism mainly by inhibiting 11ßHSD1, thus leading to a reduced cortisone activation into cortisol (18, 24–28). The exact mechanism of modulation is far from being clear: *in vitro* studies indicate a dose-dependent inhibition of 11ßHSD1 activity induced by IGF-I, but not by GH (26). Whatsoever, the result is that in the lack of GH an increased amount of cortisol is locally generated. Indeed, it has been hypothesized that some of the phenotypic features of GHD can be explained by an alteration in 11ßHSD1 activity, especially in the liver and in the adipose tissue. In particular, the increased local 11ßHSD1 activity in adipose tissues (24, 29, 30), resulting in increased local cortisol exposure (29), could promote insulin resistance and visceral adiposity which tend to

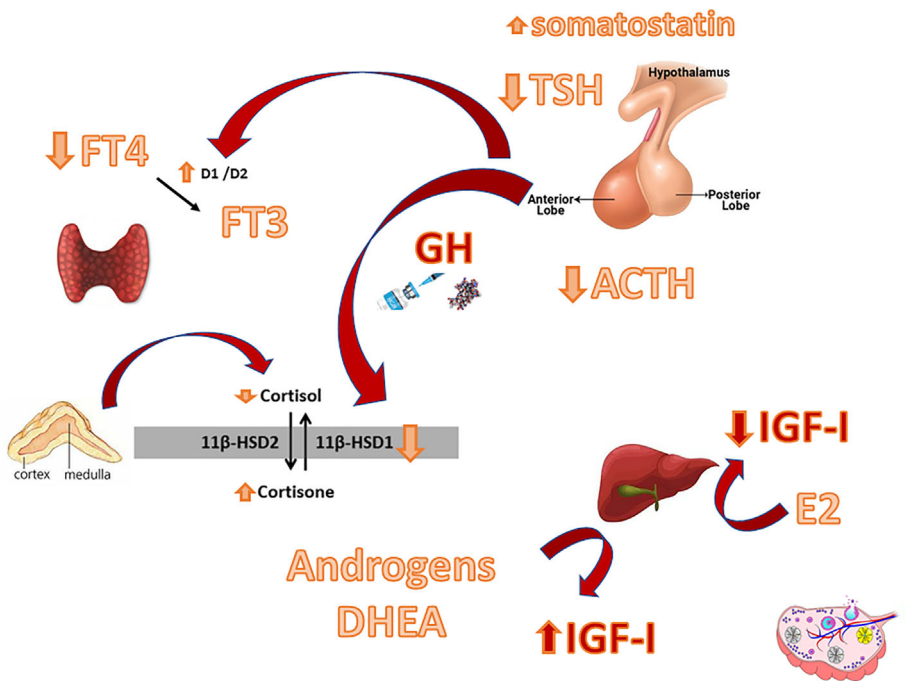

FIGURE 1 | Main interactions between the GH-IGF-I system and other hypothalamic-pituitary axes. D1/D2: Deiodinase type 1/ Deiodinase type 2. 11ßHSD: 11ß-hydroxysteroid dehydrogenase.

reduce after GH replacement (30, 31), possibly explaining its beneficial effects (32). An intriguing observation has been recently made by Agha and colleagues: the authors reported that 11ßHSD1 activity is regulated differently in patients with different aetiologies of hypopituitarism. In particular, they found that patients with a craniopharyngioma had higher 11ßHSD1 activity even during GH therapy compared to a matched group of patients with NFPAs/ prolactinomas, with amplified cortisol production in adipose tissues and liver. The clinical significance of this observation remains unclear but the authors hypothesized that this condition may increase the risk of adverse metabolic outcomes (33). Indeed, regarding metabolic outcomes, patients with craniopharyngiomas seem to have a lower response to GH therapy than those with NFPA (34).

Our group investigated the effect of rhGH on the HPA axis in both adults and children with GHD. The former study was carried-out in 12 patients with adult-onset GHD due to surgically treated pituitary tumours and preserved HPA function, before and during rhGH therapy.

Urinary free cortisol, as well as basal and stimulated serum cortisol levels, were lower on therapy than before and a condition of CHA was unmasked in the majority of subjects. Since no change in CBG was found, the results were mainly ascribed to restoration of 11ßHSD1 activity inhibition induced by GH replacement (27). Nonetheless, in the setting of hypopituitarism, CBG levels need to be considered in women taking oral oestrogen replacement therapy since oestrogens increase CBG and consequently total cortisol levels, but not the unbound active fraction.

The above reported data suggest that in patients with organic hypopituitarism, GH deficiency may mask the presence of CHA. To confirm this observation, the study conducted in 10 children with idiopathic isolated GHD and normal pituitary MRI, showed no changes in the HPA axis during rhGH (35).

The major studies so far available on this topic are reported in **Table 1**.

All these observations, taken together, tend to support a strong interplay between the GH/IGF-I and the HPA axis. The clinical impact is particularly evident in patients with MPHD, who may experience a life-threatening adrenal crisis after rhGH initiation in the presence of an untreated CHA.

Thus, in patients with possible MPHD, the integrity of the HPA axis must be evaluated both before GH pharmacological stimulating tests (to avoid diagnostic pitfalls) and after rhGH start. Indeed, an underlying unsubstituted CHA might be unmasked by rhGH therapy, inducing a possible adrenal crisis. Moreover, in patients already under replacement for ACTH deficiency, steroid therapy should be adjusted, especially when using cortisone acetate (1, 36, 37).

GH-IGF-I AXIS AND HYPOTHALAMIC-PITUITARY-THYROID AXIS

It is well ascertained that untreated hypothyroidism is associated with reduced IGF-I and IGF binding protein-3 (IGF-BP-3) and,

TABLE 1 | Modifications of hypothalamic-pituitary- adrenal axis during GH therapy in AGHD.

Study	N	ACTH-def before rhGH therapy	ACTH-def after rhGH therapy	CBG	MCP	UFC	CoM	F/E
Weaver et al. (18)	19	16	16	↓	NA	↓	↓	↓
Rodriguez-Arnao et al. (19)	14	14	14	↓	↓	NA	NA	NA
Gelding et al. (24)	10	7	7	NA	NA	↔	NA	↓
Tschop et al. (20)	22	16	16	↓	↔	NA	NA	NA
Isidori et al. (15)	30	17	17	↔	NA	NA	NA	NA
Giavoli et al. (27)	12	0	9	↔	↓	↓	NA	NA
Toogood et al. (28)	9	9	9	NA	NA	NA	↔	↓

AGHD, adult growth hormone deficiency; ACTH-def, ACTH deficiency; CBG, cortisol binding globulin; MCP, mean cortisol peak; UFC, urinary free cortisol; CoM, urinary cortisol metabolites; F/E, ratio 11-hydroxy/11-oxo cortisol metabolites; ↔, unchanged; ↓, decreased; NA, not available.

indeed, even in subclinical hypothyroidism, these reduced levels of IGF-I increase with Levotiroxine (LT4) replacement therapy (38). This phenomenon is easily noticeable in hypothyroid children whose growth failure is reversible by the introduction of LT4 replacement therapy. Moreover, hypothyroidism induces a decrease in GH pulsatility and blunts GH responses to secretory stimuli, changes that are reversible after LT4 introduction, suggesting a possible underlying driven role of thyroid hormones (39).

Therefore, GH provocative tests as well as rhGH replacement therapy should be performed or administered only after the restoration of a condition of euthyroidism. Indeed, as LT4 accelerates cortisol clearance potentially triggering an Addisonian crisis in the presence of an underlying CHA, glucocorticoid replacement therapy should be started first. Thus, in patients with MPHD, hormone replacement therapies must be introduced following a well-defined order: first hydrocortisone, then LT4 (usually after a week), rhGH and, when indicated, sex hormones (36).

However, we have to consider that rhGH therapy can affect the regulation of the HPT axis and thyroid hormone concentrations by several different mechanisms.

Firstly, at a peripheral level, GH induces the extra-thyroidal conversion of T4 to the active hormone triiodothyronine (T3), reducing the concentrations of the inactive form reverse-T3 (rT3) and increasing the T3/T4 ratio (40–45). The effect of GH on circulating T3 levels has been firstly described in animal models (46–48) where GH stimulates T4 conversion to T3. In untreated GHD patients, there is a decreased conversion of T4 to T3, with increased concentrations of rT3 (49). Even if the exact activation pathway under GH control is still unknown, an upregulation of type 2 iodothyroinine deiodinase by GH has been recently described in humans (50). In MPHD, the activity of type 2 deiodinase is usually increased to counterbalance, with a more efficient T3 production, the initial T4 reduction. This compensatory mechanism would be lacking in hypothyroid patients with GHD (50).

Secondly, an interaction at a central level has also been postulated: increased somatostatinergic tone or T3 negative feedback within the thyrotropes, driven by increased T3 production from T4 deiodination, may inhibit TSH release (40–42, 44, 51, 52). In GH deficient adults on rhGH replacement, a significant blunting of the TSH nocturnal surge has indeed been reported (53, 54). However, other studies failed to find TSH variation during rhGH therapy (49). Moreover, whether the interaction between the GH/IGF-I and the HPT axis is directly mediated by GH or through IGF-I is still to be established. Some studies failed to support the influence of IGF-I administration on serum T3 (55). Furthermore, a much higher T3 increase has been described after GH than after IGF-I therapy in GHD patients suggesting that GH has a more direct potent effect on thyroid hormone metabolism (56).

Consistently, in GHD patients under LT4 replacement therapy, rhGH led to a dose-dependent increase in T4 to T3 conversion and a decrease in immunoreactive TSH levels, probably secondary to the increased free T3 in the thyrotropes or to the increased somatostatinergic tone (56, 57). These findings support the crucial role of GH in the HPT homeostasis. Moreover, in hypothyroid patients under LT4 replacement, another possible underlying mechanism is the GH-driven reduction of T4 half-life and the increase of T3 half-life (58) by affecting thyroxine clearance rate or inhibiting LT4 uptake from the gastrointestinal tract (59–61).

When considering GH deficient adults, the first reported results on the interaction with the HPT were controversial, due to the small sample sizes, different study protocols, biochemical analytic methods and criteria for GHD diagnosis, and the use of pituitary GH occasionally contaminated with TSH (45, 62). Nonetheless, subsequent available studies confirmed that, in GHD adults, as in children, rhGH therapy could unmask an underlying CHT. Indeed, a multicenter study evaluated a quite large cohort of patients with either adult or childhood onset severe GHD (17 euthyroid patients and 49 with central hypothyroidism) treated with different rhGH doses (3-12 mg/kg/day) and observed a significant reduction in FT4 and rT3 levels without changes in TSH, FT3 and TBG levels. Interestingly, the fall of FT4 levels was clinically relevant only in patients with organic hypopituitarism (63). A later study confirmed these assumptions in a group of 243 patients, in which the underlying presence of MPHD was found to be the major predictor for CHT development (40). Similar data have been confirmed in long-term follow-up (5 years of rhGH) (64). All in all, the GH-IGF-I and HPT axes interactions have possible tissue-specific effects: indeed, rhGH efficacy on energy expenditure, substrate use and metabolic plasticity can be attenuated by the fluctuations in thyroid hormone levels (65).

Table 2 summarizes the main studies on this topic.

More uncertainties exist over the effects of GH therapy on thyroid volume (TV) and morphology. Actually, TSH represents the major regulator of both thyroid hormone biosynthesis and

TABLE 2 | Hypothalamic-pituitary-thyroid axis changes during GH replacement therapy in AGHD.

Study	N	CH	TT4/FT4	TSH	TT3/FT3	rT3	% new CH
Jorgensen et al. (45)	21	9	↓/↓	↓NS	↑/↑	↓	0
Amato et al. (62)	9	9	↔/↔	↔	↔/↔	↔	0
Porretti et al. (63)	66	49	NA/↓	↔	NA/↑ transient	↓	47
Agha et al. (39)	243	159	↓NS/↓	↔	↑NS/NA	NA	36
Losa et al. (64)	49	37	NA/↓	↔	NA/↔	NA	17

AGHD, adult growth hormone deficiency; CH, central hypothyroidism, ↔, unchanged; ↓, decreased; ↑increased; NA, not available; NS, not significant.

thyroid growth. However, IGF-I itself has a proliferative role interacting with its own receptors, largely expressed in thyroid cells (66). Indeed, most acromegalic patients have goiter and IGF-I levels are positively correlated with TV, while hypopituitary patients tend to have reduced TV (67–70).

The finding of unchanged TV during rhGH in TSH- and GH-deficient children, adolescents and adults supports the idea that IGF-I has only a permissive role on the mitogenic action of TSH (71, 72). In fact, an increased TV in patients with congenital isolated GHD was found after 6 months of rhGH therapy (73). Finally, Curtò and collaborators, studying patients with childhood and adult onset GHD before and after 5 years of rhGH therapy, found smaller pretreatment TV in GHD patients than in healthy controls, with increased TV only in patients without concomitant CHT (74).

To summarize, organic GHD can frequently mask a state of CHT, thus it is mandatory to assess and carefully monitor thyroid function before and during rhGH administration, in order to start or adjust LT4 replacement when indicated (1, 37, 75). Indeed, while it is recommended to maintain FT4 in the mid-normal range in patients with CHT, in the presence of a concomitant untreated GH deficiency it would be sensible to aim for higher FT4 levels, given the underlying impairment of T4 to T3 conversion (76, 77). Moreover, most of FT4 variations occur within the first 6 months of therapy, thus the importance of an early revaluation of thyroid function after rhGH initiation (64, 78).

GH-IGF-I AXIS AND HYPOTHALAMIC-PITUITARY-GONADAL AXIS

In order to understand the complex interaction between the GH/IGF-I and the hypothalamic-pituitary-gonadal (HPG) axis it is crucial to take into consideration the sexual dimorphism of endogenous GH secretion. Indeed, during the pre-pubertal period, GH and IGF-I levels are similar between boys and girls (79) but in adults spontaneous 24-h GH secretion is approximately two-fold higher in women than in men, mostly due to increased pulse amplitude without a difference in pulse frequency (80). The first gender divergences, indeed, occur during puberty, when pulse GH amplitude in girls tend to precede the one in boys, according to the different timing of the pubertal growth spurt in the two sexes (81). Moreover, GH production declines more quickly with age in women than in men and during menopause this is usually associated with a significant gain in visceral fat mass (82).

Despite this important sexual dimorphism of GH levels, cross-sectional studies have found no difference in serum IGF-I concentrations between women and men (83), though in women a moderate raise of IGF-I levels related to increased GH secretion has been reported in the early follicular and periovulatory phase (82, 84).

The gender-independence of IGF-I levels in healthy adults, despite significantly higher GH concentrations in females, supports the presence of compensated GH resistance in women. This phenomenon is due to a direct inhibitory effect of oestrogen on hepatic but not peripheral IGF-I production. Underlying mechanisms that contribute to this liver sexual dimorphism are pituitary-independent and related to the interaction of oestrogens with their receptors. Namely, the induction of suppressor of cytokine signalling (SOCS)-2 and the inhibition of GHR-Janus kinase (JAK)-2-signal transducer and activator of transcription (STAT)-5 signalling pathway in the liver (85, 86) reduce IGF-I secretion from hepatocytes (87).

Moreover, oral administration of oestrogens introduces an open-loop feedback system during which the continuous and un-physiological suppression of hepatic IGF-I production and release, due to a first pass hepatic effect of oral oestrogen (82), is only partially compensated by increased pituitary GH secretion. In this context, oestrogen replacement discontinuation or omission tends to solve the resistance to GH administration. Serum IGF-I in the GH-deficient state is further lowered by oral oestrogen, but results unaffected by transdermal therapy (88, 89). This phenomenon can explain why IGF-I levels are lower in hypopituitary women than men, despite a similar degree of impaired GH secretion (90). Moreover, women with hypopituitarism tend to be more susceptible to the hepatic effects of oral oestrogens due to the lack of feedback in GH response. Cook et al., indeed, observed that GH requirements in men were not different from those in women not taking oestrogens, but that women taking oral oestrogens required at least a two-fold greater dosage of GH (91).

Interestingly, even in males, many reports have provided robust evidence that oestradiol, rather than testosterone itself, increases GH secretion *via* oestrogen receptor (92, 93) after aromatization from testosterone. In fact, recently, Birzniece and colleagues have shown that the stimulatory effect of testosterone on GH is completely hampered by oestrogen receptor antagonists and by aromatase inhibitors (94).

On the other hand, a study in males revealed that the association of hypogonadotropic hypogonadism (HH) and GHD has an additional lowering effect on testosterone, DHT and oestradiol levels *versus* that seen in isolated HH. This

phenomenon supports a synergistic effect of GH/IGF1 on Leydig cell (LC) function (95). In this context, one would have expected an increase in testosterone levels with rhGH therapy. However, the literature available on this topic reported contrasting data. The only studies that showed an increase in testosterone levels included azoospermic (96) or hypogonadal patients (97) with GH and gonadotropin co-treatment. In contrast, in a double-blind placebo controlled trial performed in young males with childhood-onset GHD, Juul et al. (98) concluded that rhGH administration does not influence the HPG axis. Conversely, another study (99) carried out in males with idiopathic isolated GHD, showed that rhGH treatment displays an effect on LC function, increasing testosterone response to chorionic gonadotropin (CG). However, these studies included patients with either idiopathic or organic GHD or varied HPG axis status, being either normogonadic or hypogonadic under treatment with testosterone. Moreover, the high rhGH doses employed in these studies make it difficult to distinguish physiological and pharmacological rhGH effects. In another study on adult males with organic GHD and normal HPG axis we reported a significant decrease in serum testosterone levels strictly related to Sex Hormone Binding Globulin (SHBG) reduction. This suggests the importance of the evaluation of the HPG axis during rhGH treatment, utilizing free calculated testosterone, rather than total testosterone, in order to avoid unnecessary replacement therapy (100).

Moreover, some literature is available on the impact of rhGH treatment on infertility. Males with HH who failed to respond adequately to conventional infertility treatment showed increased testosterone secretion and improved fertility outcomes and sperm production after rhGH adjuvant therapy with gonadotropins (101). In addition, a prospective, open-label, non-randomized observational study of 14 men (26 to 35 years) with normogonadotropic idiopathic oligoasthenospermia found beneficial effects of six months of rhGH treatment on semen volume, count, and motility (102). On the contrary, in a small group of hypogonadotropic hypogonadal azoospermic patients, rhGH replacement therapy for six months, following a previous period of six months of gonadotropin treatment, while increasing testicular volume and testosterone levels, failed to induce the appearance of spermatozoa in the sperm (97). Undoubtedly, the interaction between the GH-IGF-I and the HPG axis plays a role in reproduction and fertility. However, data on the impact of rhGH therapy in non-GHD males are scanty and data on spermatogenesis and fertility in GHD adults, either treated or untreated, are missing.

Similarly, in females, the presence of GH receptors on oocytes suggests a direct action of GH at this level (103). Yet, IGF-I could mediate the reproductive effects of GH, being present in follicular fluid and involved in the cytoplasmic maturation, oocyte capability and granulosa cell function (104). Clinical studies evaluating female patients with suboptimal response to *in vitro* fertilization (IVF), have shown that the co-administration of GH with gonadotropin for controlled ovarian stimulation was associated with a reduction in the gonadotropin requirement, with a higher proportion of successful embryo transfer stage, higher pregnancy and live births rate (105, 106). These outcomes bring to light a possible role for rhGH treatment in oocyte and embryo quality improvement. However, in these patients, endogenous GH secretion was not investigated. When considering GHD, a study by De Boer and Coll reported decreased fertility even in patients without associated hypogonadism (107), suggesting the contribution of GHD to infertility. Giampietro et al. (108) presented four cases of infertility in women with isolated GHD and normal HPG function, in which initiation of rhGH led to efficacious conception and pregnancies. Similarly, in a recent case report by Albu et al, GH therapy contributed to IVF success by improving oocyte competence in a GHD patient. The author concluded that the influence of GH in enhancing oocyte quality should be taken into account in all infertile females with GHD, in order to improve treatment outcome especially when facing previous treatment failure (109). Nevertheless, the responsible mechanisms of GH action on fertility are not fully understood.

Differently from gonadal steroids, in females, DHEA influences the GH/IGF-I axes by increasing IGF-I response thus reducing GH requirement. On the other hand, no rhGH dose adjustment has been necessary in the male group taking testosterone replacement therapy (108, 109). The exact mechanism by which DHEA causes an increase in serum IGF-I levels is still unclear. Some authors have suggested a possible direct stimulatory effect of DHEA on IGF-I hepatic production or an inhibition of IGF-I clearance. On the other hand, DHEA could also enhance GH efficacy acting directly on GH receptors or through testosterone metabolism (110, 111).

To conclude, when treating hypopituitary patients, the gender differences in GH sensitivity and responsiveness are important aspects to take into consideration in clinical practice. In fact, GHD men are more responsive than young women to rhGH therapy, supporting a sexual dimorphism of rhGH effects in different end-points of the treatment. Female patients, indeed, usually require higher rhGH doses to normalize IGF-I levels, especially when receiving oral oestrogen. For this reason, in women with GHD and hypogonadotropic hypogonadism, a transdermal route of oestrogen replacement should be preferred for a cost-effective rhGH treatment.

On the contrary, in males, despite the GH-induced increase in circulating IGF-I by testosterone therapy may suggest the need of lower doses of rhGH, no clinical data have supported a dose reduction during testosterone treatment (112). Moreover, given the above mentioned studies, it is possible to conclude that rhGH treatment does not significantly change the hypothalamic-pituitary-testicular axis metabolism. In this contest, no adjustment of rhGH or testosterone therapy is needed.

Likewise, the reported preliminary data on the influence of the GH/IGF-I axis on fertility does not achieve at present sufficient consensus to be considered in clinical practice.

CONCLUSIONS

In conclusion, the experience developed during the last decades strengthens the view that rhGH replacement therapy is effective

TABLE 3 | GH deficiency and therapy in multiple pituitary hormone deficiency: interactions and dose adjustments.

GH and:	INTERACTION	DOSE ADJUSTEMENT	PITFALL IN DIAGNOSIS
HPA	GH suppresses cortisone to cortisol conversion, GH deficiency may result in higher cortisol levels	Re-assess adrenal function through proper dynamic testing after rhGH start	In the presence of ACTH deficiency GH secretion and response to provocative stimuli may be blunted
HPT	GH influence T4 to T3 conversion A wide proportion of both euthyroid and hypothyroid patients developed low fT4 levels after rhGH initiation	Increase or start L-T4 after treatment initiation of GH replacement therapy	IGF-I levels are reduced in hypothyroid patients and GH stimulation tests may be blunted
HPG **-oestrogens** **-testosterone** **-DHEA**	GH has no clinical influence on gonadal hormone production. -Oestrogens decrease IGF-I hepatic synthesis -Androgens enhance GH effects in peripheral tissues. -DHEA increases the IGF-I levels only in females.	Increase GH dose in women after initiation of oral oestrogen therapy. Decrease GH doses in females after initiation of DHEA therapy. In women with GHD and hypogonadotropic hypogonadism, a transdermal route of oestrogen replacement should be preferred for a cost-effective rhGH treatment.	IGF-I levels are reduced in female patients taking oral oestrogen therapy.

HPA, hypothalamic-pituitary- adrenal axis; HPT, hypothalamic-pituitary-thyroid axis; HPG, hypothalamic-pituitary-gonadal axis.

and safe in treating GHD in adulthood. Nonetheless, the adult with GHD is a complex patient, in whom the deficit is almost always part of a picture of MPHD. In this context, interactions between replacement therapies have to be taken into account, not only to tailor the best hormonal substitutions, but also to achieve a prompt and accurate diagnosis of hypopituitarism, that is of paramount importance in the management of these patients (**Table 3**). In particular, the state of untreated GHD may mask in a consistent manner a number of cases of central hypoadrenalism and/or hypothyroidism, whose diagnosis becomes possible only after rhGH replacement. Hence, the most recent Guidelines suggest the re-assessment of thyroid and adrenal function during rhGH therapy in patients with organic GHD. Similarly, in patients already under glucocorticoid and LT4 replacement, dosages should be adjusted and usually appropriately increased after rhGH start. In the same context, it is recommended using higher rhGH doses to normalize IGF-I levels in women receiving oral oestrogen and lower doses in women taking DHEA supplement. When possible, a transdermal route of oestrogen replacement should be preferred for a cost-effective rhGH treatment (1, 37).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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