

# Multiple Effects of Growth Hormone in the Body: Is it Really the Hormone for Growth?

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**ABSTRACT:** In this review, we analyze the effects of growth hormone on a number of tissues and organs and its putative role in the longitudinal growth of an organism. We conclude that the hormone plays a very important role in maintaining the homogeneity of tissues and organs during the normal development of the human body or after an injury. Its effects on growth do not seem to take place during the fetal period or during the early infancy and are mediated by insulin-like growth factor I (IGF-I) during childhood and puberty. In turn, IGF-I transcription is dependent on an adequate GH secretion, and in many tissues, it occurs independent of GH. We propose that GH may be a prohormone, rather than a hormone, since in many tissues and organs, it is proteolytically cleaved in a tissue-specific manner giving origin to shorter GH forms whose activity is still unknown.

**KEYWORDS:** growth hormone, growth, nervous system, gonads, liver, cardiovascular system

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## Introduction

The possible existence of a factor responsible for the longitudinal growth of an organism was first postulated in 1921, when Evans and Long<sup>1</sup> reported that treating rats with extracts obtained from bovine anterior pituitary glands led to an increased growth of these animals. However, the lack of the necessary technology delayed for more than 30 years the identification of the factor putatively responsible of such effect on growth, despite the clinical findings indicating that human dwarfs were able to grow after administering human pituitary extracts. In 1956, a protein present in the anterior pituitary gland could be extracted from human pituitaries and then administered successfully to human dwarfs, a novel treatment first communicated by Raben<sup>2</sup> for a clinical situation that until then had no solution. However, at that time, the existing technical methods were not sufficient for obtaining a pure and safe product for therapeutic purposes; the pituitary extracts had to be obtained from human cadavers, and some of them were contaminated with nervous tissue from the posterior pituitary lobe. This led to the occurrence of a number of cases of iatrogenic Creutzfeldt-Jakob disease in children who received pituitary extracts in which pathogenic prions were present in the nervous tissue of the posterior pituitary lobe.<sup>3,4</sup>

In 1978, Sairam et al<sup>5</sup> reported the isolation of growth hormone (GH) from human pituitary glands in an active form well suited for clinical use. This had been preceded, since 1944, by numerous studies by Li and Dixon<sup>6</sup> in different animal species, including human beings, that culminated

in the identification of the primary sequence of human GH in 1971. After that, the advances in the field of genetical engineering allowed to produce, since 1981, practically unlimited quantities of the hormone, pure and safe, by DNA recombinant technology, first in prokaryotes (*Escherichia coli*) and later in eukaryotes (murine fibroblasts), to be used in GH-deficient (GHD) children. The only difference between the hormone produced in prokaryotes and eukaryotes is the presence of the 20 kDa GH variant in the preparations obtained from eukaryotes, since alternative splicing cannot take place in prokaryotes; however, no differences have been described in terms of growth effectivity or any other effects between the different recombinant GHs in the market. From 1985, the spectrum of therapeutic applications of the recombinant GH was expanded, especially in the pediatric population, such as children born small for gestational age, children with idiopathic short stature, and children with growth delay due to chronic renal insufficiency, Turner syndrome, Prader-Willi syndrome, SHOX gene deficiency, and Noonan syndrome, and also in adults with GH deficiency (in Spain, whenever they have any other pituitary hormone deficiency [excepting prolactin]) and in patients with acquired immunodeficiency syndrome (AIDS) wasting.

While it is clear that the longitudinal growth of a GHD child is defective unless he/she receives the adequate daily dose of the hormone, a number of clinical and experimental findings lead us to ask ourselves if GH is in fact the real GH.

A classic summary of GH effects would describe GH as: “a metabolic hormone that induces hyperglycemia (as a



counterregulatory hormone), lipolysis, and protein anabolism. In addition, GH induces the expression of many other growth factors (mainly insulin-like growth factor I [IGF-I]) and has direct effects on cellular proliferation and differentiation". The GH effects on growth probably occur as a consequence of the metabolic induction of expression of other growth factors and the proliferative actions during a short period of time along the life of an organism.

According to a recent review by Murray and Clayton,<sup>7</sup> human growth is a process that starts after conception and finalizes in adolescence at the time of growth plate fusion. They divide growth into the following four phases: (1) fetal, (2) infancy, (3) childhood, and (4) puberty. These different growth stages have been previously described and widely analyzed by Karlberg et al.<sup>8-11</sup> For instance, they first described that "linear growth during the first three years of life can be represented mathematically using a combination of a quickly decelerating Infancy component with the addition of a slowly decelerating Childhood component that acts from the second half of the first year of life". The appearance of this second component leads to an abrupt increase in growth velocity during the second half of the first year of life. Since a similar increase is observed in GHD children when GH therapy begins, they conclude that the Childhood component defines the age at which GH begins to play a significant physiological influence on linear growth.<sup>8</sup>

Before discussing the involvement of GH in each of these phases, some important concepts need to be analyzed.

**The GH gene family.** Classically, it was thought that proteic hormones exert only their biological effects after interacting with specific membrane receptors. Then, the consequent activation of these receptors would initiate a cascade of intracellular signaling pathways, leading to the appearance of hormone-specific biological effects. That is, the proteic hormones would not enter into cells for inducing, or modifying, any kind of activity, different to what happens with steroids, for instance, which have to enter into cells because their receptors are located in the cytoplasm or in the nucleus of the cell. However, this concept was challenged with the report that insulin and epidermal growth factor could be internalized in living cells,<sup>12</sup> followed by the description of the mechanism by which polypeptide hormones may be internalized and the significance of this finding.<sup>13</sup> Waters et al<sup>14-16</sup> first demonstrated that GH, after interacting with its membrane receptor, is internalized together with its receptor (GHR) via the endosomal pathway and that this mechanism allows the translocation of both GH and GHR to the nucleus where they promote the transcription of a number of genes. On this basis, the detection of GHR in the nucleus of a cell indicates that there has been a previous interaction GH-GHR at the cell membrane level. But apart from this nuclear translocation and its effects on gene transcription, we demonstrated that the internalized GH also suffers a tissue-specific proteolytic processing, influenced by sex and age, which gives origin to a

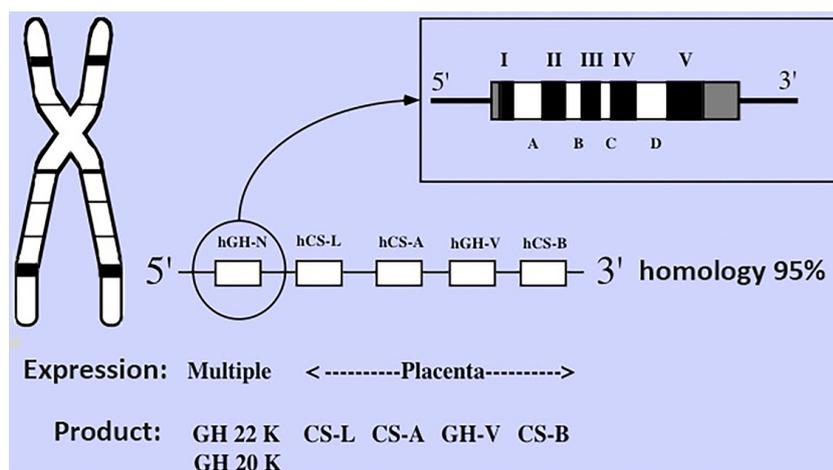
number of different molecular forms, whose actions are largely unknown.<sup>17</sup> The knowledge of these concepts is important for a better understanding of the diverse effects of GH.

Equally important is to know that GH is a pleiotropic hormone, expressed not only at the pituitary level but also in a number of cells and tissues, which plays a number of different roles (endocrine, autocrine/paracrine) far beyond those classically described.<sup>17-20</sup> The hGH/hCS locus contains two GH genes and three CS (chorionic somatomammotropin, now known as placental lactogens) genes spanning 48 kb of DNA in the chromosome 17 in the order: 5'-(hGH-1/hCS-5/hCS-1/hGH-2/hCS-2)-3'.<sup>21</sup> Among them, GH-1 (now known as GH-N) is expressed in the pituitary gland and peripheral tissues, while the other four seem to be expressed only in the placenta, although *N*-glycosylated GH-related peptides have been found in human pituitary extracts, suggesting that the *bGH-2* gene (now known as GH-variant [GH-V]), or other unknown GH-related genes, could be expressed too at the pituitary level, and perhaps in other tissues, since the hGH-N gene lacks the consensus sequence for *N*-glycosylation observed in some nonplacental GH-related products.<sup>22,23</sup>

Figure 1 shows the hGH gene family and the main products of expression of its genes.

However, this is only a schematic description of the hGH gene family, but pituitary and tissue GH heterogeneity is really high.

For instance, in human pituitary, many GH isoforms may be found. Apart from the principal GH products, 22 and the 20 kDa GH form, the later resulting from the alternative mRNA splicing of the GH-N gene, there are a number of posttranslationally modified GH forms (*N*-acylated, deamidated and *O*-glycosylated monomeric GH forms), as well as both noncovalent and disulfide-linked oligomers up to pentameric GH.<sup>24,25</sup> Moreover, in human pituitary extracts, some significant amounts of shorter molecular weight GH variants (17 and 5 kDa) can be found. These are originated by selective cleavage of the bond between amino acids 43 and 44, leading to the production of fragments 1-43 and 44-191 in the own somatotroph cells, and they have also been found in plasma and tissues from human beings and some different species.<sup>17,25-30</sup> Given that these forms can be originated by specific cleavage of the main 22 kDa GH form, it is likely that they appear in tissues as a result of posttranslational modification of the hormone. Interestingly, while human GH 22 kDa has both insulin-like and diabetogenic effects, these fragments proteolytically generated show opposite effects: the short GH 5 kDa potentiates the effects of the insulin, while the 17 kDa form shows diabetogenic activity.<sup>26</sup> Moreover, since these two isoforms can be generated under acidic conditions, it has been postulated that they can play a significant physiological role because the hormone in the cells is exposed to an acidic environment.<sup>26</sup> Similar conclusions regarding the effects of these 17 and 5 kDa GH variants had been reached



**Figure 1.** The products of expression of the hGH gene family share 95% of homology among them. The main GH form corresponds to the 22 kDa GH-N, expressed not only at the pituitary level but also in many different peripheral cells and tissues. About 10% of pituitary GH-N transcripts suffer a physiological posttranscriptional modification, occurred by alternative splicing of exon III, giving origin to a 20 kDa variant. Among placental GH genes, the most important seems to be the GH-V, which differs from 22 kDa GH-N in only eight amino acids.

by Lewis et al at the end of the last century.<sup>31,32</sup> According to their data, these GH fragments, obtained by recombinant DNA technology, have potent *in vivo* effects on glucose homeostasis in rodents, but cannot stimulate body growth.

Once established these important concepts, we will now analyze how growth occurs and what is the role that GH plays in the different steps of this physiological process.

**GH and IGF relationships.** In 1957, Salmon and Daughaday<sup>33</sup> reported the presence of a factor hormonally controlled in serum, which stimulated sulfate incorporation by cartilage *in vitro*. Soon this factor was isolated and was found to be a small peptide very similar to insulin, both in its structure and mechanisms of action. Therefore, the initial name of somatomedin C was substituted by IGF-I. The purpose of this review is not to analyze how IGF-I acts, but some descriptions about it are needed.

Initially it was thought that IGF-I was produced only in the liver and that its hepatic expression was fully dependent on GH. However, we know today, that while liver is the main source of circulating IGF-I, this peptide is produced, as it occurs with GH, practically in all cells and tissues, where it plays an auto/paracrine role. Even IGF-I is expressed within the pituitary gland, where it may act as a paracrine regulator of GH secretion.<sup>34</sup> The original somatomedin hypothesis proposed that GH stimulates hepatic production of IGF-I, which in turn promotes growth directly at the epiphyseal plate.<sup>35</sup> Posteriorly, a dual somatomedin hypothesis was proposed,<sup>36</sup> suggesting that local IGF-I promotes chondrocyte maturation and longitudinal growth in an auto/paracrine manner,<sup>37</sup> while the relationships between GH and IGF-I mainly consist on GH inducing liver IGF-I transcription which, in turns, negatively controls pituitary GH secretion.<sup>38</sup> (For a detailed description about how pituitary secretion of GH is controlled, see Refs. 39 and 40.) However, there are direct actions of GH on bone: GH

induces unilateral epiphyseal growth when injected,<sup>41</sup> or infused locally,<sup>42</sup> and promotes chondrocyte proliferation *in vitro*.<sup>43</sup> Moreover, IGF-I antibodies block the proliferative effects of GH, indicating a local IGF-I action dependent on GH stimulation of local IGF-I production.<sup>44</sup> Taken together, these findings indicate that GH acts directly on chondrocyte stem cells and also promotes chondrocyte proliferation via local IGF-I production. IGF-I then would stimulate clonal expansion of proliferating cells in an auto/paracrine manner.<sup>45</sup>

A direct evidence of the role of IGF-I in the longitudinal growth of the human body proceeds from the first observation by Laron and Kauli, almost 60 years ago, communicating that some short-height children who resembled patients with isolated GHD had high serum levels of GH but a defect in liver GHR that impeded GH signaling for hepatic IGF-I expression.<sup>46,47</sup> Since that first report, a number of publications demonstrated that defective liver GHR led to dwarfism because of the liver's inability to synthesize IGF-I. This situation reverted when IGF-I produced by recombinant DNA technology became available and children with Laron syndrome could be successfully treated. The same occurs in GHR null mice, in which a severe postnatal growth retardation exists, and while plasma IGF-I levels are markedly reduced, plasma GH levels are elevated.<sup>48,49</sup>

According to the concepts described above, IGF-I would really be the GH, while GH would merely be an inducer of its hepatic and chondrocyte expression. However, some clinical evidences indicate that the role of GH as an inducer of hepatic IGF-I expression is dependent on the nutritional status of the organism. In fact, growth velocity in obese children is normal or even higher than that in their age-related normal body weight children, despite that GH secretion is decreased, both physiologically and in response to any provocative stimulation, or even absent in obese children, plasma IGF-I values



are elevated.<sup>50,51</sup> Multiple deconvolution studies have defined deficiencies in both basal and pulsatile GH secretion with increasing body mass index and, consistently, a reduction in both GH pulse amplitude and orderliness of secretion. Reduced frequency of pulses and alterations in the half-life of GH has been variably reported as contributing to the overall reduction in GH secretion.<sup>52</sup>

The opposite situation can be seen in anorexia nervosa patients. In spite of the increased GH secretion, plasma IGF-I values are drastically reduced.<sup>53,54</sup> Looking at the ends of the spectrum of body weight and analyzing how GH and IGF-I behave in these extremes, one might conclude that there is a need for an efficient hepatic metabolism of glucose to GH may induce hepatic expression of IGF-I. Even more, it has been demonstrated, in cultured hepatocytes from perinatal rats, in a serum-free condition, and in the presence of nonstimulatory dose of insulin, that glucose was able to induce a clear increase of IGF-I and IGF-II mRNA. However, administering 2-deoxyglucose, that the liver cannot metabolize, blocks IGF-I gene expression. This agrees with the clinical data described above and suggests that IGFs belong to a family of genes that are positively regulated by glucose.<sup>55</sup> Moreover, we found a high prevalence of low plasma IGF-I values and a clearly delayed growth in children with spastic cerebral palsy.<sup>56</sup> It is feasible to assume that spasticity increases the muscular uptake of glucose, therefore decreasing the liver uptake with the subsequent effects on IGF-I production and longitudinal growth.

Figure 2 shows a schematic overview of how the hepatic expression of IGF-I may be regulated.

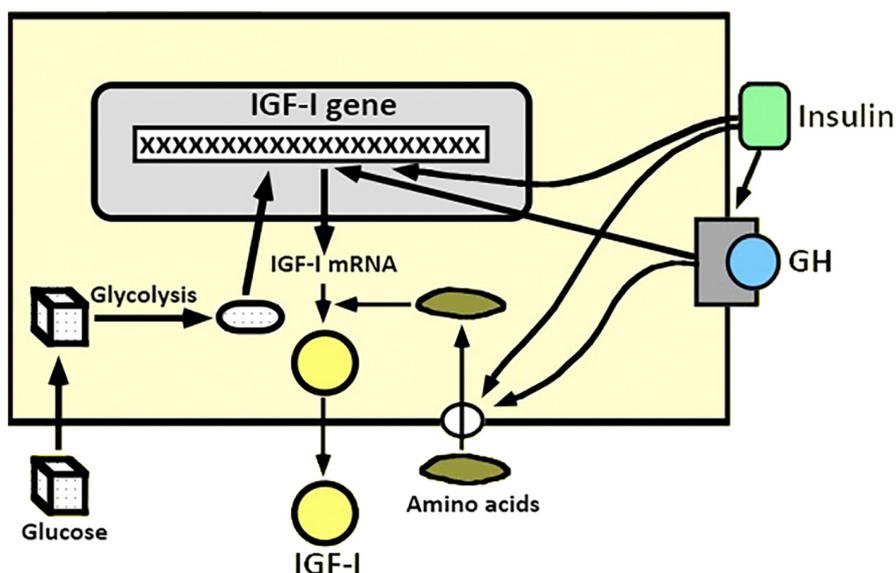
Given that the longitudinal growth of the organism occurs at the epiphyseal growth plate level as a combination of

chondrogenesis and ossification, we will analyze briefly how this process occurs (for a more detailed review, see Ref. 57).

**Growth plate physiology.** In mammals, the growth plate is a cartilaginous tissue with specific functions since growth begins until epiphysis is closed after puberty. It is composed of three different zones: the resting zone, the proliferative zone, and the hypertrophic zone. In the resting zone, there are stem cells that slowly replicate and generate new clones of proliferative chondrocytes.<sup>58</sup> These replicate at a high rate and align themselves in columns oriented parallel to the long axis of the bone.<sup>59</sup> As they are progressively separated from the epiphysis, replication is decreasing until it stops; they then enlarge and alter the extracellular matrix to form the hypertrophic zone. This hypertrophic cartilage attracts the blood vessels, osteoclasts, and differentiating osteoblasts, which remodel the newly formed cartilage into bone tissue.<sup>60</sup>

Progressively, after puberty, the growth plate experiences functional and structural changes, leading to what is known as growth plate senescence. As a consequence, the longitudinal growth of bone slows until ceasing and determining the length or height of the adult body. This process has been postulated to be due to a mechanism intrinsic to the growth plate.<sup>61</sup>

As indicated above, a number of evidences indicate that the GH/IGF-I system regulates longitudinal bone growth at the growth plate, either by GH-induced liver production of IGF-I, which then acts as an endocrine factor to stimulate longitudinal bone growth (this would explain the results obtained when treating with recombinant IGF-I children with Laron syndrome), or by a local action of GH on bone,<sup>41</sup> an effect that may be mediated in part by stimulation of local IGF-I production.<sup>42,43</sup> It has been proposed that GH would act mainly on the resting zone of the growth plate, whereas



**Figure 2.** Possible modulation of the hepatic expression of IGF-I gene. GH and insulin may directly induce the transcription of IGF-I gene, but for that, glucose uptake and metabolism by the liver is needed. Hepatic glucose metabolism may directly induce the transcription of IGF-I gene. In addition, liver uptake of some amino acids, induced by GH or insulin, facilitates IGF-I expression, because these amino acids stabilize the IGF-I mRNA.



IGF-I would act on the proliferative zone.<sup>62</sup> However, this has not been confirmed by other studies.<sup>63,64</sup>

In an elegant study, using manual microdissection and quantitative real-time PCR, Parker et al<sup>57</sup> found that IGF-II, and not IGF-I, is the predominantly expressed IGF in growth plate chondrocytes of young rapidly growing rats. Moreover, they found that in one-week-old rats, GH receptor (GHR) and type 1 IGF receptor (IGFR1) are expressed in all zones of the growth plate, while the expression of IGF-binding proteins (IGFBPs) is very low as compared with that found in other rat tissues. Since growth velocity in rats decreases with age, IGF-II expression drastically decreases while the expression of many of the IGFBPs and the type 2 IGFR increases. Given that the availability of IGFs depends on the amount of IGFBPs, these data indicate that the abundance of free bioactive IGFs in growth plate cartilage decreases with age. Their findings also indicate that IGF-I is expressed at very low levels in growth plates, suggesting that local production of IGF-I by growth plate chondrocytes does not seem to be biologically important. They suggested that IGF-I protein in the growth plate is likely a product that mainly arrives by diffusion from local sources in perichondrium and/or adjacent bone and from liver via the circulation. Contrary to IGF-I, the same study found that IGF-II is highly expressed in the growth plate cartilage,<sup>57</sup> confirming data from previous studies using *in situ* hybridization.<sup>65,66</sup> Moreover, in their study, Parker et al<sup>57</sup> found that expression of IGF-II mRNA is higher in the resting and the proliferative zones than in the hypertrophic zone; this led them to suggest that IGF-II may regulate cell proliferation in the former two zones of the growth plate. With regard to GH, its receptor seems to be expressed at similar levels in all zones of the growth plate, whereas IGFR1 was expressed at threefold higher levels in the proliferative zone than in the hypertrophic zone. These data indicate that GH acts on the three zones of the growth plate, and since the type 1 IGFR is also expressed throughout the growth plate, it can be deduced that IGFs (I and II) regulate all zones of the growth plate. According to the data of the study by Parker et al,<sup>57</sup> the possibility exists that GH may act on growth plate chondrocytes by an IGF-independent mechanism, but the hormone may also exert effects by stimulating IGF-I production in the perichondrium. Interestingly, in their study, they found that mRNA expression of IGFBPs increases with age. IGFBP-1, IGFBP-2, IGFBP-3, and IGFBP-4 increased in perichondrium, and IGFBP-3 and IGFBP-4 increased in metaphyseal bone. This pattern of increased expression of many of the IGFBPs with age in the growth plate, perichondrium, and bone further supports the idea that growth plate senescence may be in part due to decreased availability of IGFs. Interestingly, the most consistent increase in IGFBP expression is seen in the perichondrium where IGF-I mRNA expression is the highest, suggesting an inhibitory effect on IGF-I action while aging.<sup>57</sup>

The study by Parker et al<sup>57</sup> relates GH, IGFs, and IGFBPs to the longitudinal growth of the organism, and their results

in rats partially agree with clinical findings such as those described in children with Laron syndrome. As stated before, this syndrome is a cause of short stature due to an abnormality of GHR that is translated in a complete insensitivity to GH. This leads not only to a very short stature but also to an early hypoglycemia. The lack of GH responsiveness, and also the hypoglycemia, leads to the absence of production of IGFs. Therefore, treatment with recombinant IGF-I is the only therapeutic option that improves the prognosis and the height in these patients. In this case, however, it seems to be impossible that IGF-II could be expressed in the growth plate. The only possible explanation to the improvement in growth velocity and final height in children with Laron syndrome obtained during treatment with IGF-I is that, while its expression in the resting and proliferative zones of the growth plate is physiologically low, the exogenous IGF-I administration may compensate this low expression. Moreover, as described above, type 1 IGFR is expressed throughout the three growth plate zones.

Recently, it has been published that, in mice, GH stimulates growth plate chondrogenesis and longitudinal bone growth directly at the growth plate, even when the local effects of IGF-I and IGF-II are prevented.<sup>67</sup>

In summary, and according to the studies described here, the effects of GH, IGF-I, and IGF-II on growth remain to be fully elucidated; even more, a recent study challenged the importance of IGF-I for endochondral growth, demonstrating, in mice, the critical importance of suppressor of cytokine signaling-2 (SOCS2) in regulating GH ability to promote bone growth via a mechanism that is independent of IGF-I.<sup>68</sup> To better clarify this concept, this study was performed in juvenile mice SOCS2 (-/-). In these animals, but not in wild-type animals, IGF-II is elevated, and the IGF receptor is required to be expressed. Therefore, the possibility exists that in these conditions, GH promotes bone growth via IGF-II acting on type 1 IGFR.

Further studies are needed to clarify the exact role of GH and IGFs on the longitudinal growth of the organism, and also how aging may change the role played by each of these growth factors at bone level.

We will now analyze how plasma GH changes across the life and how growth differentially depends on nutritional and/or growth factors. Before that, we must recapitulate about what are GH effects: the hormone promotes stem cell activation, cell proliferation, differentiation, and survival, either directly or through the induction of IGF-1 and/or many other trophic factors. GH acts via its cell membrane receptor to initiate a cascade of signal transduction pathways downstream of the GHR. The transcription factor STAT5b is regarded as the major signaling pathway by which GH achieves its physiological effects, including the liver expression of IGF-I,<sup>69</sup> and also regulates the expression of a host of other genes, some of which are important growth regulators. In addition to signaling from the cell membrane, the GHR translocates to the



nucleus in a GH-dependent manner, and once in the nucleus it regulates the expression of other cell growth-related genes and sensitizes the cell to the proliferative action of GH.<sup>70</sup> Initially, we will analyze the effects of GH on gonads and gametogenesis (for a more detailed review, see Ref. 71).

### GH and gonads.

*GH and gonadal function in males.* The testis seems to behave as a small hypophysis, since different to what happens at the nervous system, circulating GH cannot easily access testicular cells within the blood–testis barrier. Therefore, the ligands for the GHRs on these cells are thus likely to be produced within the testis. Supporting this hypothesis, GH gene expression has been detected within the rat, human, and chicken testis.<sup>72</sup> Curiously, the GH-variant (GH-V) gene products, previously thought to be pregnancy specific, are the most abundant GH mRNA isoform in the human testis.<sup>73</sup>

GHR immunoreactivity has been detected in the human testis, and it is concentrated in Leydig cells.<sup>74</sup> Potential GH regulators are similarly expressed in the testis. In rats and human beings, for instance, testicular Growth Hormone Releasing Hormone (GHRH) closely resembles placental GHRH and is capable of stimulating pituitary GH release and Sertoli cell adenylate cyclase activity.<sup>72</sup> GHRH receptors have a wide distribution in human beings, including Leydig cells, Sertoli cells, germ cells, and the prostate gland, suggesting that GHRH may exert testicular actions distinct from GH.<sup>75</sup>

More recently, ghrelin and putative ghrelin receptors have been localized in the testis, and ghrelin alters testosterone synthesis and other testicular parameters.<sup>76,77</sup> In addition, negative regulators of GH secretion and actions, such as somatostatin and its receptors (SSTR 1–5), have been detected in mice Sertoli cells;<sup>78</sup> somatostatin treatment significantly promotes apoptosis of these cells and decreases IGF-I expression together with a dose-dependent suppression in the mRNA level of *kitl* gene, which is important in the regulation of spermatogenesis. These findings suggest that somatostatin and its receptors (mainly SSTR2 and SSTR5) play an important role in the regulation and development of Sertoli cells.<sup>78</sup>

In all, these data indicate that all the components of the hypothalamic–somatotrophic axis exist in the testes, although how it acts and its relationship with the similar endocrine axis are not well known.

However, endocrine GH promotes testicular growth and development and stimulates gametogenesis and steroidogenesis in the adult testes. These actions seem to be mediated by IGF-I, since it can recover testicular differentiation in fetal mice treated with GH antibodies and testicular growth in children with Laron syndrome.<sup>79,80</sup> In addition, GH is a potent steroidogenic factor in vitro that stimulates androgen and estradiol production by Leydig cells in a number of species including human beings. In vivo, GH treatment has been seen to improve the production of testosterone induced by chorionic gonadotropin in fertile GHD subjects.<sup>81,82</sup> However, GH treatment in hypopituitary or moderately obese men decreases

the concentrations of total serum testosterone,<sup>83,84</sup> most likely an effect due to a stimulatory effect on aromatase activity and the resulting conversion of testosterone to estradiol.<sup>85</sup> The effects of GH on testicular steroidogenesis may be due to enhancing testicular LH sensitivity and promoting development of Leydig cells.

The relevance of testicular IGF-I to gonadal function is well established;<sup>86</sup> however, gonadotropins, rather than GH, may be its primary regulator.<sup>87,88</sup> Testicular IGF-I in rats, for instance, responds poorly to changes in the systemic GH concentration.<sup>89</sup> GH does not stimulate IGF-I production in Leydig cells isolated from horse,<sup>90</sup> and in chickens, testicular IGF-I production appears to be entirely GH independent, since it is elevated in GH-resistant dwarf chickens.<sup>91</sup>

In summary, from these data, it is likely that, in a normal man, endocrine GH synergizes with gonadotropins, potentiating the effects of these hormones on testicular cells while the role of the testicular GH axis and its relationships with endocrine GH remain unknown.

*GH and gonadal function in females.* Today it is clear that GH is a necessary hormone for female fertility. Fertility is decreased in GHD women,<sup>92</sup> and GH replacement allows successful pregnancies in previously infertile GHD women.<sup>93</sup>

Normal fertility requires two processes, namely, oogenesis and folliculogenesis, that depend on a complicated system of intracrine, juxtacrine, autocrine, paracrine, and endocrine signals. GH, both of pituitary and ovarian origin, may be a modulatory signal in this complex interplay.

In order to produce a normal viable embryo, steroidogenesis, folliculogenesis, and oocyte maturation have to be significantly linked and optimized.

Pituitary gonadotropins are the primary regulators of ovarian steroidogenesis, but in vitro evidence suggests that GH also modulates progesterone and estradiol release. GH stimulates progesterone and estradiol production from bovine granulosa cells<sup>94,95</sup> and human luteinized granulosa cells.<sup>96,97</sup> GH effects vary throughout the ovarian cycle, since GH stimulates basal progesterone production in porcine corpora lutea, but not follicles,<sup>98</sup> and enhances leptin-induced progesterone production in follicles.<sup>99</sup>

In the presence of FSH, GH promotes early reactions in the steroidogenic pathway via increased local IGF-I, thereby enhancing progesterone synthesis. Conversely, GH inhibits FSH-induced aromatase activity and thus estradiol synthesis, by an IGF-I independent pathway, since IGF-I alone stimulates aromatase activity.

In vivo studies of GH-induced steroidogenesis have produced contradictory results, perhaps because the effects of exogenous GH administration may be modified by other factors, for instance ovarian GH production. However, the fact that GHR-deficient cattle shows a partial progesterone deficiency suggests that GH is physiologically relevant to ovarian steroidogenesis.<sup>100</sup> Many different studies indicate that GH is also important for the development and maintenance of



ovarian follicles. Its administration increases follicular size and number,<sup>101</sup> effects related to the proliferative and antiapoptotic actions of the hormone. In fact, mice with GHR deletion have more primordial follicles and fewer primary, secondary, preantral, and antral follicles, as well as increased follicular atresia.<sup>102,103</sup> These result in lower ovulation and implantation rates, fewer corpora lutea, and smaller litter sizes.<sup>102</sup> The follicular actions of GH may be due to interactions with the bone morphogenetic protein (BMP) system (one of the factors secreted by oocyte), since GH downregulates BMP receptors and upregulates inhibitors of BMP signaling (such as Smad 6/7).<sup>104</sup> In turn, BMP signaling inhibits the formation of GHRs, IGF-I, and IGF-IRs.<sup>104</sup> GH has been shown to promote nuclear maturation of denuded oocytes from mice,<sup>105</sup> and human beings,<sup>106</sup> suggesting that the hormone also acts directly at the oocyte. Moreover, GHR mRNA is readily detectable in oocytes from many species, including human beings,<sup>107</sup> and in mature human ovaries, GHR immunoreactivity has also been detected in the oocyte nucleus.<sup>108</sup> Since, as described before, the translocation of GHR to the nucleus depends on a previous interaction GH–GHR, this finding is consistent with a direct effect of GH on oocytes. Therefore, GH may improve nuclear maturation and thus oocyte quality. This is important for the use of the hormone in *in vitro* fertilization protocols.

At the earlier stages of pregnancy, the maintenance of the corpus luteum, secreting progesterone in the needed amounts for allowing implantation and avoiding abortion, is a key process. During these stages, until the placenta begins to produce progesterone, it seems that GH plays a role in luteal cells, proliferative and antiapoptotic, since GHR mRNA and GHR immunoreactivity have been detected in these cells in a number of species including human beings.<sup>109</sup> While these effects of GH on the ovary are beyond any doubt, the question is to know whether they are produced by endocrine or exogenously administered GH or they depend on the production of GH by their own ovary.

It has been suggested that plasma GH modulates ovarian function;<sup>110</sup> however, granulosa cells and oocytes are avascular, and they are separated from the systemic circulation by the basal lamina.<sup>111</sup> Hence, the ovarian actions of GH are most likely due to the local production of the hormone.

In fact, GH mRNA and immunoreactivity are detectable in ovarian stromal and follicular tissue from numerous species, including human beings.<sup>112</sup> Ovarian GH production is greater in the inner, avascular follicular compartments, since GH mRNA is detectable in granulosa cells and oocytes, but is absent in cumulus cells and is less abundant in or absent in thecal cells, at least in bovines and chicken.<sup>113,114</sup>

GH gene expression is initiated very early in follicular development in human beings, since GH mRNA and immunoreactivity were detected in the oocyte cytoplasm and occasionally the granulosa cells of fetal primordial follicles.<sup>108</sup>

It has been proposed that the GH gene expression increases during follicular development, since GH transcripts have been

detected in mature (but not immature) bovine follicles.<sup>113</sup> The temporal and spatial patterns of follicular GH expression are parallel to those of GHR expression in human follicles,<sup>108</sup> supporting the possibility of auto/paracrine ovarian GH actions.

It is unclear how ovarian GH synthesis is regulated, although GHRH mRNA and GHRH receptor immunoreactivity are present in human beings and rats.<sup>115,116</sup> Most likely, ghrelin may act as the primary ovarian GH secretagogue, since ghrelin increases GH secretion but not synthesis in cultured porcine follicles,<sup>117</sup> and both ghrelin and its receptor have been found in the ovaries of pig<sup>118</sup> and hen.<sup>119</sup> However, the role of ghrelin in human ovaries is not known.

As described before, GH-induced hepatic IGF-I is the mediator of many of the GH effects in different tissues. However, the fact that GHR mRNA has been detected in ovarian follicles in many species suggest that follicular GH actions are independent of hepatic IGF-I,<sup>101</sup> but does not exclude the possibility that a local expression of IGF-I could mediate GH actions on ovaries. In fact, GH stimulates IGF-I expression in porcine<sup>120</sup> and rat granulosa cells.<sup>104</sup> In addition, IGF-I antibodies block GH-induced oocyte maturation in rat follicles,<sup>121</sup> and IGF-I administration improves follicular maturation in GHR knockout mice<sup>102</sup> and the maturation of mouse oocytes nonsynergistically with GH.<sup>105</sup> However, other data suggest that GH and IGF-I act independently, at least in part. Follicular IGF-I levels are normal in GHR knockout mice; despite delayed follicular maturation,<sup>102,103</sup> this may possibly reflect that other hormones (for instance, hCG and estradiol) are responsible for activating IGF-I production.<sup>122</sup> The possibility exists that local IGF-I mediates GH effects on ovaries in some species but not in others, ie, a species-specific manner. Supporting this concept, it has been observed that IGF-I and GH have synergistic effects (hence independent) on the development of ovine preantral follicles.<sup>123</sup> Finally, GH stimulates IGF-II production in cultured human granulosa cells,<sup>124</sup> suggesting that IGF-II, instead of or in addition to IGF-I, may be an important follicular mediator of GH action; the hormone would sensitize the granulosa cells to the IGF-II produced by the cells themselves, which acts through the IGF-I receptor.

In this section, we analyze the multiple effects of GH on gonads. Many aspects of GH effects at this level remain to be well established, and it is likely that some controversial data depend on species specificity. However, it seems to be clear that GH is a key hormone for a normal gonadal function, in both sexes. Whether its effects depend on endocrine GH or on the hormone produced locally is not well known, since in both testes and ovaries there is a GH axis, similar to the hypothalamic–somatotrophic axis. Therefore, it is likely that, at least for some effects, there is an association between systemic GH and the hormone produced in the gonads, as we will analyze later in the central nervous system (CNS).

We will now review how growth occurs along the four stages above described: (1) fetal, (2) infancy, (3) childhood, and (4) puberty.

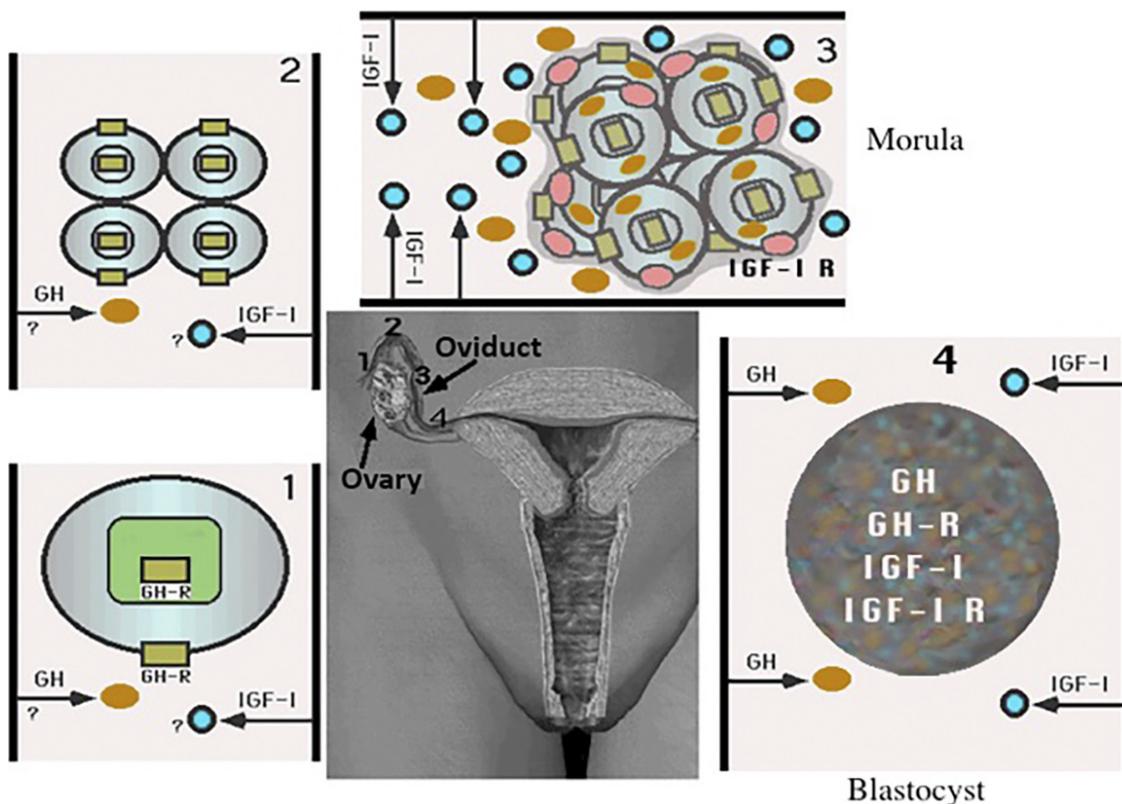
### GH and growth.

*Growth during the fetal period.* The relationship between GH and gonads has been widely analyzed, and it is clear that GH/IGFs play regulatory roles in reproduction. Supporting these data, many women with GH deficiency suffer gonadal deficiencies or insufficiencies.<sup>92,125</sup> Clinically, hypogonadotropic hypogonadism women, who responded poorly to high-dose gonadotropin treatment, achieved more oocytes, higher fertilization, and pregnancy rates after being co-stimulated with GH.<sup>126</sup> It was reported that the use of GH reduced the human menopausal gonadotropin dose and duration required for ovulation induction and improved success rates.<sup>127,128</sup>

Interestingly, coinciding with the progressive increase of ovarian production of estradiol during the late follicular phase of a menstrual cycle, usually ending with ovulation, both the amplitude and frequency of plasma GH peaks reach very high values.<sup>40</sup> While their origin is related to the effect of estradiol on pituitary GH secretion, it is likely that the objective of this increased GH release is related to the upregulation of the GHR in the oocyte. The GHR has been found in the nucleus of the zygote, therefore indicating that a GH–GHR membrane interaction had to occur after fertilization, as shown in Figure 3.

GHRs are expressed in the two-cell embryo, and GH in the blastocyst.<sup>129</sup> GH-cultured blastocysts contain more blastomeres.<sup>130</sup> GH mRNA is expressed consistently between the two-cell and blastocyst stages;<sup>131</sup> hence, in these stages, local GH may be acting in an autocrine or a paracrine manner. Paracrine GH has been implicated in the enhanced growth of two-day embryos, since GH antibodies retard embryonic growth.<sup>130</sup> Of the two major cell populations in the blastocyst, GH appears to directly target the trophoblasts rather than the inner cell mass,<sup>132</sup> despite that GHRs are present in both cell types.<sup>133</sup> In turn, IGF-I most likely acts on the inner cell mass, independent of GH.<sup>132</sup>

For a successful embryonic implantation and placental formation, there is the need of maternal modifications, as it occurs in the embryo. In this regard, it has been shown that two-cell embryos incubated to the blastocyst stage in the presence of GH show higher levels of *in vivo* implantation.<sup>130</sup> This is because the early blastocyst needs to escape from the zona pellucida that surrounds it, a protective matrix composed of glycoproteins and carbohydrates, before it can implant into the endometrium. GH improves the rate of this escaping in bovine,<sup>134</sup> and murine,<sup>135</sup> blastocyst populations. Therefore,



**Figure 3.** Preimplantation period. (1) After interacting with GH, most likely secreted from the oviduct, the GHR is translocated to the nucleus of the zygote, promoting the transcription of genes involved in cells proliferation. GH induces the expression of the glucose transporter Glut 1, which leads to glucose uptake by the zygote therefore providing the substrate needed for the production of the energy necessary for beginning proliferation processes shown in (2) It is unclear if IGF-I is secreted from the oviduct in 1 or 2. (3) While the newly formed embryo is progressing along the oviduct to the uterus, there is a clear secretion of GH and IGF-I from the oviduct walls; IGF-I receptor appears now together with GHR in the membrane of cells constituting the morula, facilitating cell proliferation. (4) In a further step, the blastocyst is formed and their cells begin to express GH, GHR, IGF-I, and IGF-I R. This precedes the implantation in the uterus.

GH facilitates implantation, perhaps by increasing the production of matrix metalloproteinases and also by selectively stimulating trophoblast cell proliferation, since these cells participate in blastocyst cavity formation and invasion of the maternal endometrium.<sup>132</sup> Moreover, placental GH stimulates the invasive activity of these trophoblasts, and GHR expression is correlated with the degree of invasiveness. An autocrine/paracrine interaction is possible, since invasive extravillous cytotrophoblast cells express GH-V mRNA and secrete placental GH.<sup>136</sup>

Together with its effects on the zygote and blastocyst, GH also facilitates the development of the most appropriate maternal environment. This has to be initiated very early in gestation.

GH and GHR are expressed in pregnant and nonpregnant uteri, and GHR is differentially regulated during pregnancy and the menstrual cycle. GHR is expressed in glandular cells of the human endometrium and decidua (but not stromal cells) during the mid and late luteal phases but not during the proliferative or early luteal phases.<sup>137</sup>

Both endometrial gland development and secretory capacity are stimulated by the hormone which, furthermore, increases the amniotic glucose concentration and endometrial protein synthesis. This suggests a positive effect on nutrient transfer to the embryo. In addition, GH promotes the growth of the uterus, according to the fact that pregnancy maintenance requires significant uterine hypertrophy. In fact, GHD women have smaller uteruses than non-GHD women, most likely reflecting that a normal GH axis may be necessary for estrogen-induced uterine hypertrophy, since uterine GHR mRNA abundance is strongly correlated with estrogen-induced uterine growth.<sup>138</sup> In summary, although most of the GH effects described in this section proceed from studies carried out in bovine and murine species, it is likely that similar effects occur in human beings and GH actions already begin in the zygote and continue in preimplantation embryos by stimulating glucose uptake and glycogen utilization. This would provide the needed production of energy for the high rate of cellular proliferation in this stage.<sup>129,139</sup>

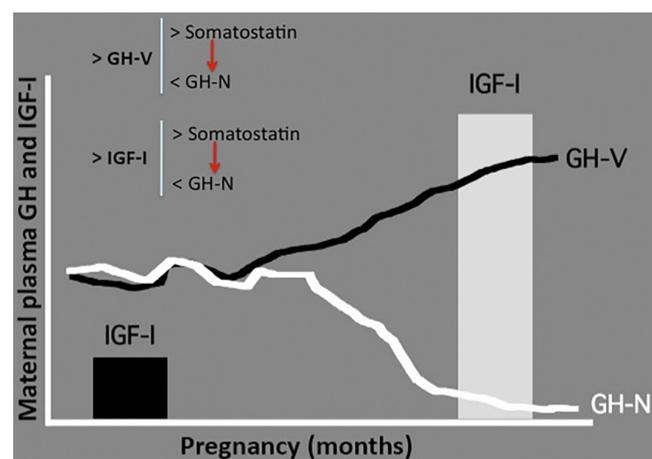
On this basis, it would be feasible to affirm that during the preimplantation period, growth is controlled, directly or indirectly, by GH and IGF-I. It is not known whether IGF-I expression during this period is dependent on GH, but in uterus it is not.

Once implantation occurred and the placenta commences to develop, placental GH genes begin to be expressed. Among them, giving its similitude with the pituitary GH-N, GH-V protein seems to play the most important role. Placental GH appears to be secreted selectively into the maternal compartment.<sup>140</sup> Therefore, a progressive increase in maternal plasma levels of placental GH starts from 8 to 10 weeks of pregnancy,<sup>141</sup> leading to a clear increase in maternal plasma levels of IGFs. The negative feedback that GH exerts on its own pituitary secretion (via stimulating hypothalamic

somatostatin release),<sup>39</sup> added to the inhibitory effect that IGF-I also exerts on pituitary GH release, both directly at the pituitary level and by stimulating somatostatin,<sup>39</sup> leads to a progressive decrease in maternal plasma levels of GH-N, parallel to the increase in GH-V concentrations. Moreover, placental GH-V, different to GH-N secretion, is not pulsatile;<sup>142</sup> thus, the continuous negative feedback exerted by GH-V will completely block maternal GH-N release, but this does not seem to affect fetal growth. For a better understanding of these concepts, see Figure 4.

Fetal growth is not affected if pituitary maternal GHD exists;<sup>143</sup> however, intrauterine growth retardation appears when maternal plasma levels of IGF-I are low or placental GHs production is deficient.<sup>144</sup> In the fetal pituitary, GH is detected at week 10 of gestation, and the hormone begins to be released to fetal plasma two weeks later,<sup>145</sup> peaking during weeks 20–24 and then declining. GHR expression in fetal liver and other tissues parallels the pattern of GH secretion. However, fetal GH production does not seem to have a significant impact on fetal growth, since, for instance, the uterine growth of anencephalic fetuses is not different from that in normal fetuses, despite the absence of the hypothalamo–hypophyseal axis.<sup>146</sup> Currently, it is thought that the fetal GH accomplishes an important developmental programming role in virtually all tissues and organs,<sup>147</sup> but does not act as a fetal “growth hormone”.

In fact, three mouse strains (Ames, Jackson, and Snell dwarf mice), all with spontaneously occurring autosomal recessive mutations, have a normal size at birth, despite all having pituitary hypoplasia, and GH synthesis cannot be detected in their pituitary glands.<sup>148</sup>



**Figure 4.** Evolution of maternal plasma levels of GH (N and V) and IGF-I during pregnancy. As pregnancy progresses, placental GH-V increases in the maternal blood. This leads to increased IGF-I levels and decreased GH-N levels. The decrease of plasma GH-N is due to the negative feedback that GH-V and IGF-I directly exert on pituitary GH-N secretion and also due to the fact that both GH-V and IGF-I stimulate hypothalamic somatostatin secretion that, in turn, negatively controls pituitary GH-N release. The maternal increase in plasma levels of IGF-II is not shown in the figure. Red arrows indicate negative control.

Going back to the role that GH-V may play in the maternal blood, it is important to know that its somatogenic properties are stronger than that of GH-N. It would explain its effects on maternal IGFs which, in turn, would play a critical role for the supply of nutrients to the fetus, particularly glucose and amino acids. Increased fetal glycemia would lead to increased release of insulin from the fetal pancreas. Then, insulin, directly or through an IGFs-mediated action would be the hormone responsible for the fetal growth, that is, insulin would be the real “growth hormone”. In support to this concept gestational diabetes usually leads to the birth of macrosomic children, as it occurs when nesidioblastosis (fetal hyperinsulinism due to hyperplasia in pancreatic beta cells) exists.

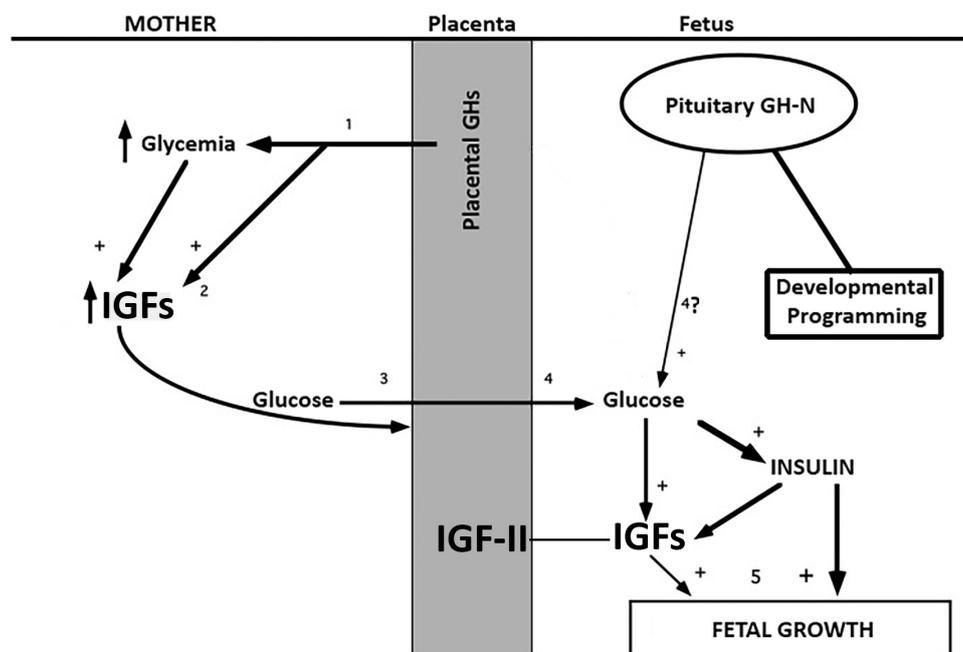
These concepts are schematically depicted in Figure 5.

Despite the known role of IGF-I during this stage of development, IGF-II is the most abundant fetal IGF. It acts as a paracrine growth regulator synthesized by multiple fetal tissues, independent of GH-N.<sup>149</sup> Effects of IGFs on fetal growth are additive, as deletion of the receptor for IGF-I, through which both IGFs act, reduces birth weight to a greater extent than deletion of either IGF-I or IGF-II genes alone.<sup>150</sup> IGF-II is thought to provide the stimulus for fetoplacental growth, while, as described before, IGF-I appears to regulate fetal growth in response to nutrient availability. IGF-II increases in maternal plasma during pregnancy and declines toward term. In all species studied, the placenta synthesizes IGF-II, at least

in early pregnancy, being produced by the syncytiotrophoblast layer in direct contact with the maternal blood.<sup>151,152</sup> Curiously, in pregnant women, IGF-II is secreted as a biologically inactive pro-peptide, which undergoes proteolytic cleavage to become active; this proteolytic activation increases during pregnancy and correlates with maternal IGF-II plasma concentrations.<sup>153,154</sup> In addition to IGF-I and insulin receptors (InsR), the placenta expresses a hybrid receptor IGF1R-InsR; in women, these receptors are localized to the syncytiotrophoblast layer bathed in maternal blood;<sup>152</sup> this permits endocrine regulation of placental growth and function by maternal IGFs.

In summary, IGFs in the maternal circulation are important regulators of fetal growth via their actions on both the mother and the placenta. They influence maternal tissue growth and metabolism and, thereby, modulate nutrient availability for conceptus growth. They also regulate placental morphogenesis, substrate transport, and hormone secretion, which influence fetal growth either via indirect effects on maternal substrate availability, or through direct impacts on fetal nutrient supply and its endocrine environment.

Maternal IGF-I and IGF-II may have complementary, but overlapping, roles in optimizing fetal nutrient acquisition for growth and survival. Maternal IGF-I appears to act predominantly on maternal tissues to influence substrate availability, whereas maternal IGF-II acts on the placenta to influence substrate delivery to the fetus. In turn, this increased



**Figure 5.** Fetal growth. (1) Placental GHs, particularly GH-V, are progressively increasing in maternal blood inducing an increase in plasma levels of glucose. This, and presumably (2) the effect of GH-V on liver, increases the hepatic production of IGFs, which optimizes the supply of nutrients to the fetus (3), particularly glucose. The resulting increase in fetal glycemia (4) induces increased release of fetal pancreatic insulin, and the hepatic production of IGFs, which also can be induced by insulin. Despite pituitary fetal GH-N secretion, it is unlikely (4?) that it contributes to fetal hyperglycemia. For its part, the placenta significantly contributes to the increase in fetal plasma concentrations of IGFs by producing IGF-II. Fetal GH most likely plays a role on the developmental programming of virtually all tissues and organs. (5) Insulin, mainly, and IGFs are the hormones that the fetus needs for growing.

**Abbreviation:** +, stimulates.

supply of nutrients, particularly glucose, positively regulates expression of IGFs in fetal liver.<sup>55</sup>

According to these extensive data, growth before birth depends on a number of hormonal and nutritional factors. Among them, GH seems to play a key role in the early stages of development, since the zygote is formed until the blastocyst is implanted in the uterus. Then, when the placenta begins to develop and placental GHs are secreted, they act mainly on maternal increase of IGFs; these contribute to the further development of the placenta and also act on maternal tissues for improving the supply of nutrients to the fetus. Therefore, plasma glucose increases in the fetus leading to increased insulin release that, in addition to glucose, stimulates the liver production of IGFs. Insulin and IGFs would then be the main regulators of fetal growth as pregnancy progresses.

*Growth during the early infancy.* Nutrition plays a key role for growing during the first months of postnatal growth, even when pituitary GH secretion is normal. After birth, growth continues at the same rate as is seen in utero, despite the onset of pulsatile release of GH occurs soon after birth. This high rate of growth occurs until 6–10 months of age, after which growth slows. Therefore, while growth velocity in utero follows an exponential curve, it changes to a double exponential curve during the first year of life (Fig. 6). This is indicative that two kinds of different factors influence growth velocity during this first year of life: nutritional factors (again, the glucose–IGF–I–insulin axis seem to play the role of a “growth hormone”) and GH–IGF-I. Therefore, during this first period of life, GH does not seem to play a significant role on the longitudinal growth of the organism. Thus, when a GHD exists in neonates, growth failure is usually detected by 6–10 months of age. However, there are also data indicating that congenital GHD may present

with typical clinical signs and symptoms (for instance, hypoglycemia) very early in postnatal life, among them is decreased growth velocity.<sup>155,156</sup> It would be interesting to know if the decreased growth velocity observed in these congenital GHD children depends on the absence of GH secretion, or on the lack of induction by glucose of the liver production of IGF-I.

*Growth during childhood.* The impact of GH on growth increases over the first years, and approximately from the age of 3 years until puberty, GH and thyroxine predominate as the major influences on growth, leading to normal growth velocities of 5 cm/year (Fig. 6). Thus, GH is the growth hormone during this stage of life, but as described before, its effects on the longitudinal growth are dependent on hepatic or local (chondrocytes) IGF-I production, although the possibility exists that GH may act on growth plate chondrocytes by an IGF-independent mechanism.<sup>41,57</sup>

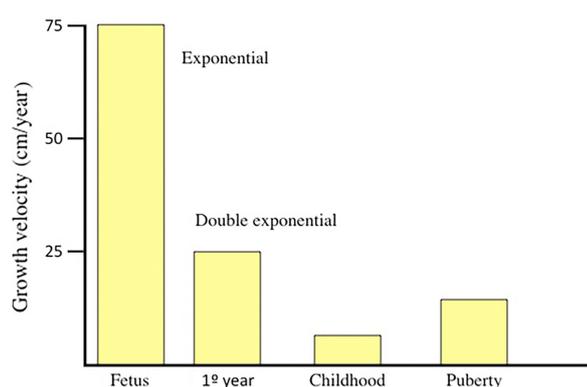
Vertebral growth mainly depends on sex steroids.

*Growth at puberty.* As puberty approaches, growth velocity increases, until reaching values of 10–12 cm/year (Fig. 6). This is a consequence of increased GH secretion and the associated IGF-I increase. The reasons for these changes are due to the effects of sex steroids on the hypothalamic–pituitary axis; sex steroids act on catecholamine pathways that negatively act on somatostatin secretion and facilitate hypothalamic GHRH release.<sup>39</sup> However, this increased growth velocity lasts for a short time, because the own sex steroids (estradiol acting directly on growth plates and producing its ossification, while testosterone is locally transformed in estradiol by the action of an aromatase) lead to the epiphyseal fusion once puberty concludes. Longitudinal growth then ends.

However, and despite that no longitudinal growth already exists, GH secretion continues, although an exponential decline in 24-hour plasma GH concentrations can be seen in both sexes, starting from 18 to 30 years of age. It is a curious event, since sex steroids secretion continues, but GH release progressively declines until almost undetectable values while the subject is aging (from 60 years old, more or less). The fact that GH secretion is not brusquely interrupted, once puberty ends, indicates that the hormone plays other important roles in the organism, basically related, but not only, to its metabolic actions, and suggesting that GH is not “the growth hormone”, although in some periods of the life, as described, its role on growth is important.

**Other effects of GH.** Besides its actions on metabolism, GH as a pleiotropic hormone plays a number of endo-, auto-, and paracrine actions on practically any tissue or organ in the organism. We will try to summarize most of these actions with a particular emphasis on its actions to nervous level (central and peripheral).

*GH and liver.* The liver is an important target of the actions of GH. Mice with liver-specific knockouts for critical GH signaling proteins, GH receptor (GHR), Janus kinase (JAK) 2, and Stat5 share a common phenotype of hepatic steatosis,<sup>157–159</sup> demonstrating that GH plays an important



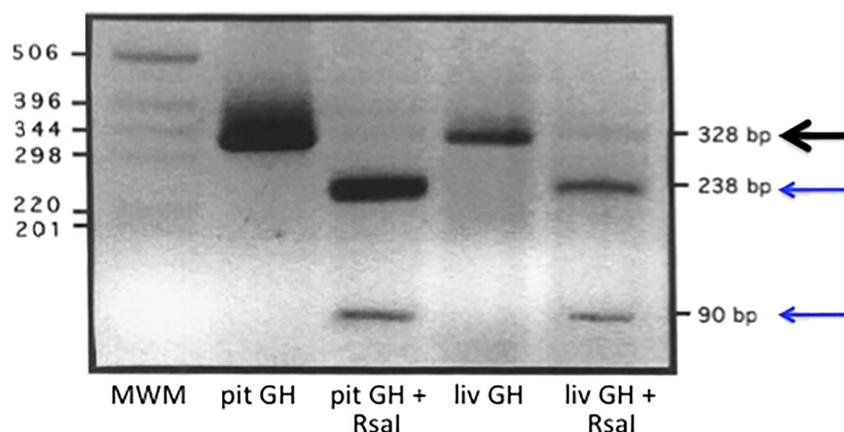
**Figure 6.** Growth velocity along life. The maximum growth velocity is observed during the fetal life, reaching 75 cm/year and following an exponential curve. After birth, growth velocity follows a double exponential curve; the consequence is that the high growth velocity observed after birth progressively slows since 6–10 months of age, indicating that two different kinds of factors are acting in this period. During childhood, growth velocity (lineal now) decreases to 5 cm/year, and when puberty begins, a number of factors (mainly sex steroids) acting on pituitary GH synthesis and release again increase growth velocity until 10–12 cm/year.

physiological role in hepatic triglyceride secretion. Steatosis leads to hepatic degeneration, which may be corrected by GH administration. Even more, a high prevalence of liver dysfunction has been reported recently in adult GHD patients.<sup>160</sup> GH replacement therapy significantly reduced serum liver enzyme concentrations in these patients and improved the histological changes in the liver.<sup>160</sup> However, the liver produces its own factor of regeneration: hepatocyte growth factor (HGF) was first identified in the sera of 70% hepatectomized rats, as a mitogen of adult rat hepatocytes.<sup>161,162</sup> Indeed, during hepatic injuries, blood and liver HGF levels markedly increase, via both extrahepatic and intrahepatic pathways.<sup>163–165</sup> Animal studies using either anti-HGF antibody or *c-Met* gene destruction techniques revealed that both the endocrine and paracrine effects of HGF are involved in liver growth after 70% hepatectomy, and for recovery from hepatitis, respectively.<sup>163–166</sup> HGF has a positive regenerative and protective effect in numerous organs and diseases.<sup>167,168</sup> In spite of its liver production and its strong liver regenerative properties, it was found that in hypophysectomized rats treated with GH, HGF mRNA levels were increased three hours after partial hepatectomy and reached peak levels after five hours. In rats with intact pituitaries and in hypophysectomized rats not given GH treatment, HGF mRNA levels in liver were unchanged during the first 5 hours following hepatectomy and reached peak levels after 10–18 hours. DNA synthesis in the liver of GH-treated rats increased from low levels, 10 hours after hepatectomy, to peak levels, after 18 hours. In rats without GH treatment, the synthesis of DNA was still low, 18 hours after hepatectomy, and was increased, after 26 hours. HGF mRNA levels were constantly lower after sham hepatectomy than after partial hepatectomy. In summary, in hypophysectomized

rats, the responses of hepatic HGF gene expression and DNA synthesis to partial hepatectomy were accelerated by treatment with GH.<sup>169</sup> Whether GH stimulates the transcription of HGF or facilitates it is not known, but we found that GH is expressed in the liver of hypophysectomized rats subjected to partial hepatectomy (Fig. 7) and that this GH promotes the hepatic regeneration, directly or via HGF induction. Moreover, the analysis of the products obtained with the enzyme of restriction *RsaI* demonstrated that the hepatic GH gives origin to two bands in the expected molecular weight position (238 and 90 bp), identical to the bands obtained from pituitary rat GH (Fig. 7).<sup>19</sup> From these data, it is clear that there is a hepatic expression of GH that contributes to, or determines, the high degree of regenerating ability of the liver, apart from playing important metabolic functions in this organ.

**GH and adrenal glands.** It has been shown that GH and IGF-I enhance steroidogenesis responsiveness to ACTH in cultured adrenal cells and that adrenal steroid responsiveness to ACTH increases in Turner syndrome after long-term treatment with high GH doses.<sup>170</sup> GH is an important modulator of the activity of 11 $\beta$ -hydroxysteroid dehydrogenase type 1 enzyme in the adrenal gland,<sup>171</sup> as indicated by the fact that plasma DHEAS levels are significantly lower in GHD patients (even in the patients with normal ACTH secretion) than in age-matched controls. GH replacement therapy in these GHD patients significantly increases DHEAS plasma levels. This suggests that, in the permissive presence of ACTH, GH stimulates adrenal androgen secretion.

GHR is strongly expressed in the ovine fetal adrenal gland.<sup>172</sup> However, in normal subjects or laboratory animals, the stimulation of adrenal steroidogenesis by GH seems to be restricted to the fetal period.<sup>173</sup>



**Figure 7.** Hepatic expression of GH. GH mRNA from the pituitary and liver (partially hepatectomized rats) was retrotranscribed with specific primers and the resultant cDNAs were resolved in 2% agarose and stained with ethidium bromide, before and after using the enzyme of restriction *RsaI*. As the figure shows pituitary (pit) and liver (liv), GH was detected with the expected molecular weight: 328 bp, because the primers used flanked a region situated between exons 4 and 5 of rat GH gene. The bands obtained after cutting the main GH amplified with *RsaI* led to the appearance of two bands in the molecular weight expected (238 and 90 bp), both in pituitary and liver GH. Black arrows indicate the main GH product. Blue arrows indicate the products obtained after cutting with *RsaI*. Adapted from the work carried out by Dr. Ramón Ríos, for obtaining his Bachelor degree in Pharmacy, under the direction of Prof. J. Devesa.

**Abbreviations:** MWM, molecular weight markers; bp, basepairs.

In a previous study, in rats, we demonstrated that the compensatory adrenal hypertrophy that follows a unilateral adrenalectomy seems to be mediated by adrenal GH expression. In that study, one adrenal gland was surgically removed and weighed, and 24 hours later, the contralateral gland was removed and weighed, and mRNA GH was extracted from both glands for cDNA RTPCR. Our results show that the contralateral gland had increased its weight and GH expression was detected in 2% agarose gel stained with ethidium bromide, while basal GH expression in the control gland was very low, as shown in Figure 8.<sup>19</sup>

These results indicate that apart from the putative effects of GH on adrenal steroidogenesis, the hormone may also play a trophic regenerative role (as in liver) on the adrenal glands.

**GH and the cardiovascular system.** GH acts directly on myocardial growth and heart function during the fetal development.<sup>174</sup> GH (or myocardial GH-induced IGF-I) induces mRNA expression for specific contractile proteins and cardiomyocyte hypertrophy. In addition, and perhaps even most important, GH increases the force of contraction and leads to myosin phenocopy toward the low ATPase activity V3 isoform.<sup>174</sup> The prevalence of V3 isoform increases the number of actin–myosin cross-bridges and their attachment time, enhances protein calcium sensitivity and calcium availability, and allows the myocardium to function at lower energy cost. In these conditions, the fetal heart is able to beat at high frequency without spending too much energy. After birth, this changes, and a hypertrophy and myocardial remodeling occurs; the V1 myosin is then expressed, which implies a higher ATPase activity. However, if an excess of GH exists, such as that which occurs in acromegaly, the fetal genetic pattern reappears because the V3 isoform is then mostly expressed again; this could lead to a myocardial insufficiency.

The GH–IGF-1 axis may also regulate cardiac metabolism, by increasing amino acid uptake, protein synthesis, cardiomyocyte size, and muscle-specific gene expression. GH increases the collagen deposition rate in the heart,<sup>175–179</sup>

while GH-induced IGF-1 influences the trophic status of myocardium by reducing apoptosis of cardiomyocytes, thus preventing myocyte loss.<sup>175–181</sup>

The important role that GH plays at the myocardial level can easily be deduced by analyzing what happens in untreated GHD children. They show cardiac atrophy with a significant reduction in the left ventricle mass, relative wall thickness, and cavity dimensions, compared with age-, sex-, and height-matched controls.<sup>182–186</sup> Moreover, patients have a low ejection fraction, low cardiac output, and high peripheral vascular resistance.<sup>182,184–187</sup> These alterations are more pronounced during physical exercise; they reduce exercise intensity, and duration is reduced in these children. In turn, adult-onset GHD does not produce a reduction in cardiac mass, but cardiac performance and exercise capacity are impaired.<sup>188–190</sup>

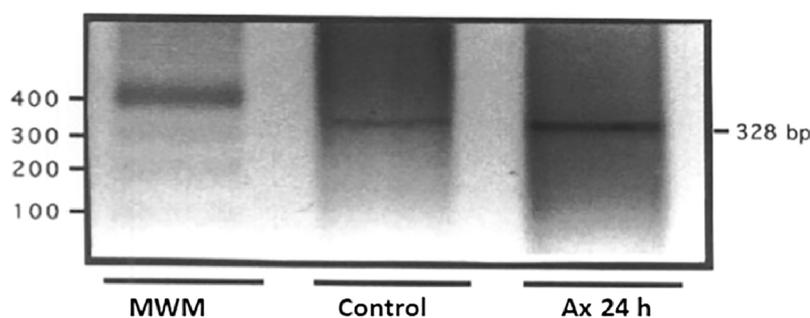
GH replacement trials show an increase in left ventricular mass and improvement in cardiac performance, diastolic filling, and systolic function after GH treatment is given to these children or adults.<sup>182–184,187,190</sup> As a consequence of its positive effects on heart, it may be speculated that GH treatment might be useful in patients with heart failure, mainly if they are GHDs or GH secretion is insufficient.

Apart from its direct or IGF-I-mediated GH effects on heart, the hormone also acts at the vascular level.

In vascular system, the GH–IGF-1 axis activates the nitric oxide (NO) system and regulates nonendothelial-dependent actions.<sup>191–196</sup> NO production relaxes arterial smooth muscle cells, thereby reducing vascular tone. Furthermore, NO inhibits proliferation and migration of smooth muscle cells, reduces platelet adhesion, and decreases lipoxygenase activity and oxidized LDL-cholesterol.<sup>191–196</sup>

Another possible mechanism for the GH/IGF-I influence on vascular tone involves the regulation of gene expression of the vascular smooth muscle KATP channel.<sup>197</sup>

Some vasoactive effects of GH may have central origin. In fact, GHD patients have markedly increased muscle sympathetic nerve activity.<sup>198</sup> Instead, one year of substitution



**Figure 8.** Adrenal expression of GH in rats. GH mRNA from an adrenal removed (control) and from the contralateral adrenal gland (24 hours after unilateral adrenalectomy) was retrotranscribed with specific primers, and the resultant cDNAs were resolved in 2% agarose and stained with ethidium bromide. GH products were detected in the expected molecular weight: 328 bp. Adrenalectomy markedly increased the expression of GH in the contralateral gland 24 hours after the adrenalectomy (Ax 24 h). This may indicate that, apart from ACTH action, GH is overexpressed in the adrenal gland for mediating the compensatory hypertrophy. Adapted from the work carried out by Dr. Ramón Ríos, for obtaining his Bachelor degree in Pharmacy, under the direction of Prof. J. Devesa.

**Abbreviation:** MWM, molecular weight markers.



therapy with GH had a significant effect on decreasing sympathetic nerve activity to the muscle vascular bed.<sup>199</sup> This suggests that GH may regulate the central sympathetic outflow, affecting peripheral resistance.

Many evidences indicate that GH plays a significant role on angiogenesis, contributing to regulate vascular growth and function. GHR has been found in many different blood vessels,<sup>200–203</sup> and in cultured endothelial cells,<sup>204,205</sup> where GH stimulates endothelial cell proliferation<sup>206,207</sup> and tube formation.<sup>208,209</sup> Treatment with GH increases the number of cerebral cortical arterioles in aging rats,<sup>210</sup> augments VEGF expression and angiogenesis in the rat myocardium after infarction,<sup>211,212</sup> stimulates wound angiogenesis in diabetic rats<sup>213</sup> and mice,<sup>214</sup> and enhances vascularization by mobilizing bone marrow-derived endothelial progenitor cells into the blood.<sup>215,216</sup> In agreement with these findings, the skin of adult GHD patients shows reduced capillary density and permeability that improves after treatment with GH,<sup>217</sup> and GHD adults and children have reduced retinal vascularization.<sup>218,219</sup> As in other territories, IGF-I may mediate the proangiogenic actions of GH, as IGF-I receptors are widely expressed in endothelial cells, and IGF-I has been shown to stimulate angiogenesis *in vivo* and *in vitro* (for review, see Ref. 220). However, some actions of GH on endothelial cells may be independent of IGF-I, since GH is unable to increase the transcription of IGF-I in endothelial cells. In addition, systemic<sup>221</sup> or local infusions of GH<sup>222</sup> acutely increase forearm blood flow and NO release in healthy human beings without significantly raising plasma IGF-I levels or muscle IGF-I mRNA expression. While these observations argue in favor of an autonomous GH action mediated by NO, IGF-I is also vasoactive due to its activation of eNOS.

In addition, GH may have context-dependent vascular actions influenced by other angiogenic agents, including IGF-I, NO, and VEGF, and GH itself. GH is produced by endothelial cells, and endothelium-derived GH stimulates the proliferation, migration, survival, and capillary formation of endothelial cells in an autocrine manner.<sup>208</sup> Therefore, the lack of action on angiogenesis, sometimes observed, of exogenous GH may relate to endothelial cell GHRs being already occupied by the endogenous hormone. Other important factors affecting GH actions on angiogenesis include its local proteolysis to vasoinhibins, which inhibit blood vessel dilation, permeability, growth of new vessels, and survival of newly formed. Finally, human GH has the ability to activate PRL receptors, which can also mediate proangiogenic signals.

Despite these proangiogenic effects, increased GH levels are not always related to angiogenesis. The best example are GHD patients (children or adults) receiving GH treatment for long term: the risk of retinopathy is not increased in them.<sup>219</sup>

For a more detailed comprehension of GH effects on the cardiovascular system, see Refs. 223–225.

*GH and the adipose tissue.* A typical finding in untreated GHD children and adults is the increase in fat mass,<sup>226–227</sup>

preferentially visceral fat, which is mainly due to the fact that GH stimulates lipolysis by increasing lipase activity,<sup>228</sup> although changes in the secretion of certain adipokines, such as adiponectin, have also been suggested as mediators of the increased adiposity in GHD states.<sup>229</sup> The administration of GH replacement therapy reverts this increased adiposity,<sup>226,227</sup> therefore confirming the relationship between GHD and increased fat mass. Among other factors, since GH secretion is decreasing from puberty to elderly, it is likely to speculate that the progressive increase in fat stores usually seen along aging might be due to the deficient or insufficient GH secretion.

Apart from this metabolic effect, GH plays a role in pre-adipocyte proliferation, differentiation, and senescence.<sup>230</sup>

*GH and skeletal muscle.* The GH-IGF-1 axis constitutes an important physiological regulatory mechanism for coordinating postnatal skeletal muscle expansion and hypertrophy. Administration of GH to both animals and GHD human beings improves muscle strength and reduces body fat.<sup>231–233</sup> A number of studies have shown that mice globally deficient in GHR have reduced muscle mass with defective myofiber specification and growth.<sup>234</sup> This seems to be logic, given the anabolic effects of GH; however, during a time it has been discussed if this GH effect would be restricted to inducing enhanced uptake of amino acids by muscle, while the effects on muscle protein synthesis would be dependent on GH-induced IGF-I expression, mediated by STAT5b. In fact, recent *in vitro* studies show that treatment of primary myoblasts with GH quickly increases IGF-I mRNA, while administration of IGF-I leads to a significant increase in primary myoblasts proliferation.<sup>235</sup> Therefore, the role of GH on muscle would be dependent on its induction of production of IGF-I by myoblasts, and IGF-I would then be responsible for stimulating myoblasts proliferation in an autocrine manner. However, GH affects myofiber development, in a mechanism independent of IGF-I, by stimulating accumulation of additional myonuclei into nascent myotubes; however, the increased myoblast fusion is an IGF-I effect, although mediated by IGF-I expression induced by GH.<sup>235</sup> The disruption of GHR in skeletal muscle and the consequent histomorphometric changes in myofiber type and size and myonuclei number result in functionally impaired skeletal muscle. In agreement with these effects, the histology of muscles of untreated GHD patients is strongly altered, and glucose and triglyceride uptake and metabolism in skeletal muscle of GHR mutant mice are affected.

A recent study demonstrates that a single bolus of GH induces gene expression of regulators of substrate metabolism and cellular growth in human skeletal muscle *in vivo*. While some of these genes, for instance GISH gene, seem to be directly induced by GH, others, like ANGPTL4 gene,<sup>236</sup> seem to be expressed in relation to the subsequent increase in free fatty acid levels induced by GH-dependent lipolysis. These results agree with the role that GH plays on lipid metabolism.

With regard to the putative effects of GH on muscle strength, GH use has been speculated to improve physical



capacity in subjects without GHD through stimulation of collagen synthesis in the tendon and skeletal muscle, which leads to better exercise training and increased muscle strength. In this context, the use of GH in healthy elderly should be an option for increasing muscle strength. However, a clinical trial showed that after six months of therapy, muscle strength in the bench press responsive muscles did not increase in groups treated with GH (no GHD) or placebo and showed a statistically significant increase in the leg press responsive muscles in the GH group. The study demonstrated an increase in muscle strength only in the lower body part (quadriceps, for instance) after GH therapy in healthy men.<sup>237</sup> Therefore, GH administration does not provide significant improvements in increasing muscle power, except when GHD exists.

Of interest, sarcopenia appears while aging or after a prolonged immobilization. Although most likely this is a multifactorial process, a predominant role is played by myostatin, a muscular hormone that inhibits cell cycle progression and reduces levels of myogenic regulatory factors, thereby controlling myoblastic proliferation and differentiation during developmental myogenesis, as we and others demonstrated.<sup>238–240</sup> GH-induced muscular expression of the IGF-I-Akt-mTOR (mammalian target of rapamycin) pathway, which mediates both differentiation in myoblasts and hypertrophy in myotubes, has been shown to inhibit myostatin-dependent signaling. Blockade of the Akt-mTOR pathway, using siRNA to RAPTOR, a component of TORC1 (TOR signaling complex 1), facilitates myostatin's inhibition of muscle differentiation because of an increase in Smad2 phosphorylation.<sup>241</sup> Therefore, GH administration in these conditions of muscle wasting may be useful for recovering muscle mass at expenses of inhibiting myostatin signaling.

*GH and bone.* The actions of the GH-IGF-I axis at the growth plate to promote longitudinal growth have already been described. However, this axis also regulates skeletal development and mineral acquisition.<sup>242</sup> Mouse models with disruptions of GH-IGF-I axis show a clear deterioration in parameters of bone health, dependent on GH-induced IGF-I expression, which increases bone mineral density.<sup>243–245</sup> Apart from GH, other GH-independent mechanisms regulate bone IGF-I expression, for instance parathormone (PTH).<sup>246</sup> Experimental mouse models reveal that osteoblast-derived IGF-I is a key determinant of bone mineralization. Targeted osteoblast-specific overexpression of *Igf1* via the osteocalcin promoter produced a phenotype of increased bone mineral density and trabecular bone volume,<sup>247</sup> whereas knockout of the gene in bone (and muscle) but not liver via Cre recombinase expressed by the collagen type 1 $\alpha$ 2 promoter included a phenotype of reduced bone accretion.<sup>248</sup>

*GH and kidney.* GH affects renal function and kidney growth. GH, acting via IGF-I, increases glomerular filtration rate (GFR) and renal plasma flow (RPF) in GHD patients as well as in normal adults. Furthermore, GFR and RPF are low in hypopituitarism and elevated in acromegaly.

IGF-I is implicated in compensatory renal hypertrophy after unilateral nephrectomy or ischemic renal degeneration.<sup>249</sup> Disordered regulation of the IGF system has been implicated in a number of kidney diseases. IGF activity is enhanced in early diabetic nephropathy and polycystic kidneys, whereas IGF resistance is found in chronic kidney failure. IGFs have a potential role in enhancing stem cell repair after a kidney injury.<sup>250</sup> Importantly, children with chronic kidney disease show growth failure that can be safely treated with GH, as a recent clinical trial demonstrated.<sup>251</sup> Regarding other renal effects, GH increases sodium retention and increases plasma bicarbonate concentration; therefore, it seems to be useful for the treatment of metabolic acidosis.<sup>252</sup>

*GH and the hematopoietic system.* Several reports suggest a role of GH in the regulation of the hematopoietic system: normal differentiation and function of blood cells. An intriguing study shows that after one year of GH treatment in non-GHD children, erythropoietin (Epo) plasma levels significantly decreased and granulocyte-colony stimulating factor (G-CSF) levels increased from basal to 12 months of therapy, whereas in GHD children, they did not change significantly. Circulating levels of G-CSF are significantly lower in GHD than in non-GHD children. In non-GHD children, the number of red blood cells, hemoglobin (Hb), and hematocrit values significantly increased after one year of GH treatment. GH therapy influences Epo and G-CSF levels in short non-GHD children, while it shows no effects in GHD children.<sup>253</sup> These findings need further clarification, since GH increases plasma Epo levels and Hb in adult GHD patients,<sup>254</sup> and increases plasma G-CSF levels and neutrophil counts in adult GHD patients.<sup>255</sup> Another study carried out in GHD patients treated with GH showed that the replacement therapy exerted a beneficial effect leading to a significant increase of erythrocyte parameters and recovery from anemia (typical of GHD patients during childhood), without affecting the number of leukocytes or platelets.<sup>256</sup> In all, these data indicate that GH exerts a positive role on the hematopoietic system, similar to that played by G-CSF.<sup>257</sup>

In recent years, it has been proposed that GH has a strong influence on the immune system. The production and action of immune cell-derived GH is now well known, although its important role in immunity is still emerging. A number of data reveal the production of GH, GHRH, and IGF-I and its receptor on cells of the immune system and their influence via endocrine/autocrine/paracrine and intracrine pathways on immune function.<sup>258</sup> The intracellular mechanisms of action of immune cell-derived GH are not well known, but, for instance, GH promotes the maturation and activation of dendritic cells that, as antigen-presenting cells, participate in the immune response of the organism.<sup>259</sup>

Throughout many years, Weigent et al published data indicating that GH produced in lymphocytes plays a role for lymphocyte growth, survival, and production of cytokines.<sup>260–269</sup> Lymphocyte GH appears to stimulate IFN $\gamma$  production with



a small positive effect on IL-10 production.<sup>269</sup> Both norepinephrine and cortisol inhibit lymphocyte GH production, and the studies suggest that lymphocyte GH may be an important mediator of cellular immune function mediated by the TH-1 pathway.<sup>269</sup> Treatment of rat lymphocytes with a specific GH antisense oligodeoxynucleotide decreased the amount of lymphocyte GH synthesized and, at the same time, reduced lymphocyte proliferation.<sup>260</sup> By using neutralizing antibodies to GH, the number of cells positive for IGF-I decreased two-fold, supporting an important role for endogenously produced GH in the induction of lymphocyte-derived IGF-I.<sup>261</sup> The small amounts of GH synthesized and secreted by cells of the immune system,<sup>270</sup> together with data showing that the same cells that synthesize GH also produce IGF-I,<sup>271</sup> suggest that GH may be classified as an intracrine hormone, acting primarily intracellularly.<sup>272</sup> More recently, it has been shown that overexpression of GH in a lymphoid cell line, devoid of the GHR, decreases the production of superoxide and increases the production of nitric oxide and the expression of IGF-I and IGF-IR, resulting in protection from apoptosis by a mechanism most likely involving an increase in the production of Bcl-2.<sup>262–265</sup>

These results, together with other data, suggest that a complex intracrine/autocrine regulatory circuit may be important for the production and function of leukocyte-derived GH and IGF-I within the immune system. Therefore, this circuit could fulfill local tissue needs for these hormones independent of the pituitary or liver without disrupting homeostasis of other organ systems. For example, cells of the immune system would recognize the association of bacteria, virus, and tumors as an oxidative stress event and signal the release and transport of a lower molecular weight isoform of GH into the nucleus. Once in the nucleus, GH would be free to influence transcriptional responses to the stress event and to defend the cell against oxidative damage. The results provided by Weigent<sup>273</sup> support the concept that changes in the cellular redox status influence the intracellular levels of lymphocyte GH, which may exert effects on elements mediating the oxidative stress response.

Interestingly, in his study,<sup>273</sup> Weigent provided evidence that high molecular weight forms of GH (100 K, 65 K, and 48 kDa isoforms) are present in cells of the immune system. However, the main 22 kDa GH was not detected. Under normal culture conditions, lymphocyte GH exists in the cytoplasm primarily as an isoform of approximately 100 kDa and in the nucleus as an isoform of approximately 48 kDa (EL4 cells) or 65 kDa (primary spleen cells). That is, GH protein isoforms appear to reside in different compartments; however, the actual site(s) at which it exerts its function and the physiological significance of these isoforms remain unknown. However, it is likely that they represent an important regulatory function in mediating intracellular GH activity. This may be supported by the fact that higher levels of the cytoplasmic 100 kDa isoform and particularly the nuclear 65 kDa isoforms of GH were found in B-cells compared to T-cells. Moreover,

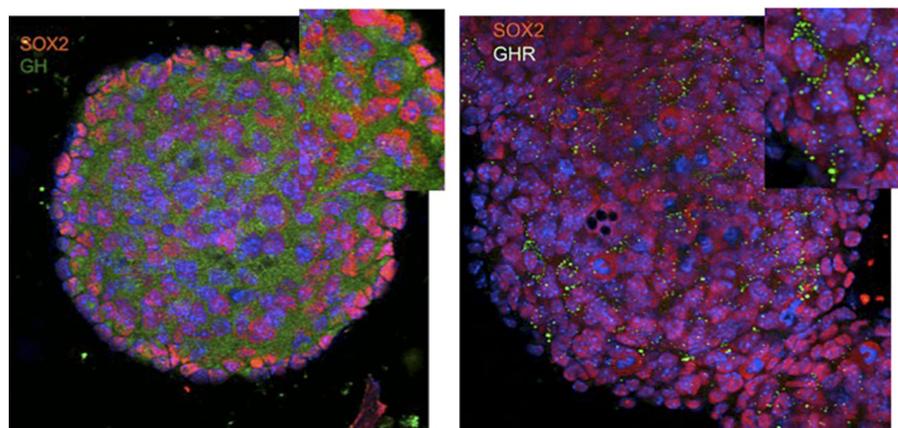
data from this and other studies suggest the possibility that the GHR and/or the GH-binding protein (GHBp) may serve particular roles in the intracrine actions of lymphocyte-derived GH in cells of the immune system.

*GH and the nervous system.* Since the pioneer discovery of the presence of the GHR in cerebral areas known to be actively involved in neurogenesis within the juvenile brain, including the subgranular zone (SGZ) of the dentate gyrus and subventricular zone (SVZ), by Lobie et al,<sup>274</sup> a number of studies during the last years revealed that GH exerts many different and positive effects on the central and peripheral nervous system. GH immunoreactivity has been detected in the first stages of neural development,<sup>275</sup> in accordance with the key roles that both, GH and IGF-I, play in the early brain development, maturation, and function.<sup>276</sup> Despite GH-binding sites have been found in the choroid plexus, indicating that they may act as carriers for introducing plasma GH into the CNS,<sup>277</sup> they have also been found in a number of brain structures, such as hippocampus, putamen, and thalamus.<sup>277</sup> This is consistent with the finding that GH is also synthesized in the CNS and is regulated differentially to its hypophyseal counterpart. IGF-I is synthesized in the CNS, and in the early postnatal period it is regulated by peripherally secreted GH.<sup>278</sup> However, IGF-I expression has also been detected in neural stem cells (NSCs) obtained from fetal human forebrains induced by exogenously administered GH.<sup>279</sup> Both GH and IGF-I alter the size and morphology of the CNS during development and affect differentiated cell function in the CNS, with consequent modulation of cognitive function. Differential utilization of the same signal transduction molecules indicates that GH and IGF-I possess distinct overlapping roles in CNS function.<sup>278</sup> Apart from its role during fetal development, GH plays a significant modulatory function on brain and seems to play a key role for brain repair after an injury.

The hypothesis that GH and IGF-I play a role on brain repair after an injury has been postulated years ago.<sup>280</sup> This role may be played by inducing neurogenesis in more brain areas than previously established (SGZ and SVZ). As described, GHR is expressed in regions of the brain in which neurogenesis occurs during embryonic brain development<sup>281,282</sup> and in neurogenic regions of the postnatal rat brain.<sup>274</sup> GH itself is also found in cells of the ventricular zone during embryonic neurogenesis<sup>282</sup> and is produced endogenously within the postnatal hippocampus (Fig. 9).<sup>283–286</sup>

GH gene expression within the hippocampus is increased by some factors known to increase neurogenesis,<sup>287</sup> including learning.<sup>283</sup> Studies of the effects of GH on embryonic rat cerebral cortical,<sup>288</sup> and hippocampal neuronal cultures of aged mice,<sup>289–290</sup> found that it induces the proliferation and differentiation of these cells.<sup>289–291</sup> The fact that the blockade of GHR impedes the proliferation and survival of these NSCs indicates that the effect of GH is highly specific.<sup>292</sup>

It has been demonstrated that exogenously applied GH and PRL promote the proliferation and migration of NSCs



**Figure 9.** GH and GHR are expressed in NSCs from mice. NSCs obtained from nine-day-old mice cultured in proliferation media, free of GH, form neurospheres in which confocal microscopy immunoreactivity for SOX2 in the nuclei indicate that these cells are in an early phase of self-renewal. These cells show high immunoreactivity (green labeling) for GH (left) and its receptor (right), indicating that both GH and GHR are expressed in NSCs. Magnification: 40 $\times$ . Adapted from the Doctoral Thesis of Dr. Pablo Devesa.

**Abbreviation:** NSCs, neural stem cells.

derived from fetal human forebrains in the absence of epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF).<sup>279</sup> This agrees with previous preclinical data derived by us and others, demonstrating that exogenous GH administration promotes the proliferation of hippocampal neural precursors after brain injury induced by kainate administration,<sup>291</sup> and in a number of zones in the intact adult rat brain.<sup>293</sup>

According to these evidences, it is likely that GH may facilitate the proliferation, differentiation, survival, and migration of new neurons in response to brain injury. However, to date, only few studies in human beings explore such a possibility. While these studies indicate a positive effect for GH treatment together with specific neurorehabilitation, both in children with cerebral palsy<sup>294,295</sup> and in TBI patients,<sup>296,297</sup> or in a patient suffering from a neurogenic dysphagia after oncological brain surgery,<sup>298</sup> all patients in these studies had GHD most likely occurring as a consequence of their brain damage. However, we recently demonstrated that GH administration, together with specific rehabilitation, after an important brain injury, is able to recover laboratory animals<sup>299</sup> and patients without GHD.<sup>300,301</sup> Similar results have been obtained after a stroke. In rats, delayed and chronic treatment of stroke with central GH may accelerate some aspects of functional recovery and improve spatial memory in the long term,<sup>302</sup> and a pilot study in human patients who suffered a stroke showed improvements after administering GH.<sup>303</sup> In line with these, the expression of both GH and GHR is strongly upregulated after brain injury and specifically associated with stressed neurons and glia.<sup>280,304,305</sup> From these and other studies, it is now clear that GH plays a key role in both physiologic and reparative neurogenesis,<sup>306</sup> being its effect specially marked on cognitive functions,<sup>307</sup> most likely throughout the interaction of the hormone with GHRs expressed in the hippocampus and frontal cortex,<sup>308,309</sup> brain areas especially related to memory and cognition, respectively.

It is already well known that GHD adults have impaired psychological well-being, including energy, motivation, emotion, memory, and cognition.<sup>310–312</sup> However, all these abnormalities improved during GH replacement therapy,<sup>310,312–314</sup> which leads to marked improvements in the quality of life.<sup>315</sup> The same occurs in GHD after being treated with GH.<sup>316</sup> Attention, perception, and cognitive capacity improve in them.<sup>317</sup> Cognitive impairments and mood disturbances are common findings in patients with GH deficiency.<sup>318,319</sup> GH treatment significantly improves memory and cognitive functions in these patients, as shown by functional MRI studies.<sup>319,320</sup> As described before, similar results have been obtained recently by us and others, both in adult GHD patients<sup>296,297</sup> and in patients with normal GH secretion.<sup>300,301</sup>

These effects may be due to the fact that GH may be an important regulator of hippocampus-dependent spatial learning and memory, as it is able to counteract memory deficits related to alterations in cholinergic neurons and an imbalance in hippocampal glutamatergic and GABAergic synapses.<sup>321</sup>

Several studies have indicated that GH affects cognitive functioning by enhancing excitatory synaptic transmission through NMDA receptors.<sup>308,322–324</sup> Furthermore, the hormone might potentially affect the levels of the various receptor subunits in the NMDA receptor complex.

Several studies indicate that the neurobiological consequences of the decline in GH/IGF-I, that occurs physiologically during aging, include decreased neurogenesis in the dentate gyrus of the hippocampus,<sup>325,326</sup> where IGF-I appears to affect primarily the survival of newborn neurons and may also influence the maturation and differentiation of newborn cells.<sup>327,328</sup>

Despite that neurogenesis is dramatically reduced during senescence, newborn granule cells in the aged dentate gyrus retain the capacity for participation in functional hippocampal



networks;<sup>329</sup> this is in agreement with the aforementioned hypothesis, suggesting that it is the age-associated lack of neurotrophic factors (GH/IGF-I?), the cause of decreased neurogenesis. In fact, GH prevents neuronal loss in the aged rat hippocampus.<sup>330</sup>

The effects of the GH-IGF-I axis on cell turnover in the adult brain probably are not limited to neuronal progenitors, since IGF-I can promote proliferation of oligodendrocyte progenitor cells and differentiation and survival of oligodendrocytes,<sup>331,332</sup> an effect also induced by EGF,<sup>333</sup> whose expression and that of its receptor may also be induced by GH.<sup>334</sup> Thus, it is likely that the aging-related decline in GH/IGF-I and dependent changes in oligodendrocyte genesis and/or maturation may contribute to impaired remyelination in the CNS of aged individuals<sup>335</sup> and to a decline in normal cognitive function.

Moreover, apart from IGF-I and EGF and its receptor, GH induces a number of neurotrophic factors that may act as neuroprotective and neuroregenerative. Among them, EPO, VEGF, FGF, BDNF, and several cytokines, as described before, facilitate the gonadal production of testosterone and estradiol.

In addition, GH affects most of the major neurotransmitters differently in several brain regions, including monoamines (serotonin, noradrenaline), the dopaminergic system, the glutamatergic system (at the receptor level), the opioid system, and the cholinergic system.

For instance, it has been shown that GH affects the monoamine levels within 15 minutes after intraperitoneal administration in rats, and its effects are different in normal and hypophysectomized rats.<sup>336</sup> In normal rats, GH decreases the levels of both 5-hydroxyindoleacetic acid (5-HIAA) and norepinephrine in the diencephalon and brainstem, without affecting telencephalic concentrations. In hypophysectomized rats, however, GH produces significant elevations of norepinephrine and 5-HIAA levels in all brain regions.<sup>336</sup> Similarly, bGH transgenic mice evidence increased tissue levels of serotonin and 5-HIAA acid in several brain regions.<sup>337</sup> These two different experimental conditions show similarities with respect to the serotonin system.

GH also has been shown to affect the dopaminergic system. GH reduces dopamine (DA) release in the rat subependymal layer and median eminence after intravenous administration.<sup>338</sup> Perhaps this is a mechanism for increasing somatostatin release and therefore for reducing pituitary GH secretion.<sup>39</sup> In line with this, bGH transgenic mice show decreased DA levels in the brain stem and decreased levels of the DA metabolite 3,4-dihydroxyphenylacetic acid in the mesencephalon and diencephalon.<sup>336</sup> With regard to the glutamatergic receptor system, it is present throughout the brain in both neurons and astrocytes. Three major types of receptors are known, one of these being the NMDA receptor. The NMDA receptor subunits NR1 and NR2B are thought to be involved in memory formation in the hippocampus, and interestingly, GH appears to affect these transcripts. GH treatment in young adult hypophysectomized rats decreases

the hippocampal mRNA expression of NR1, but increases the NR2B subunit,<sup>323</sup> whereas in elderly rats, GH increases both the NR1 and NR2A transcripts. Similar results have been obtained with IGF-I therapy.<sup>339</sup> The results are particularly interesting as it appears that the ratio of NR2B to NR2A mirrors the potential for synaptic plasticity.

Finally, it appears that the cholinergic system has functional links with GH and IGF-I. Functional stimulation of muscarinic transmission by the cholinesterase inhibitor pyridostigmine may enhance GH release from the pituitary under certain conditions.<sup>340,341</sup> Moreover, IGF-I affects potassium-evoked acetylcholine (ACh) release in tissue slices of the adult hippocampus and cerebral cortex. In slices from the adult hippocampus, IGF-I decreases the release of ACh.<sup>342</sup>

In all, there are many evidences that GH and IGF-I affect several of the major neurotransmitter systems. Many of these effects act directly via neurons and can be observed after only a short exposure. However, some of these effects also interact with the other main cell types of the brain, the glial cells.

The effects of GH also take place on central and peripheral nerves. Delayed conduction from the retina to the occipital cortex is a common finding in children with cerebral palsy, but it can be corrected with GH treatment and specific visual stimulation.<sup>343</sup> This indicates that the hormone increases the number of fibers in the optical nerve and also promotes myelination. The same happens in peripheral nerves. We and others have been able to regenerate the sciatic nerve, after its section in rats, by using GH and rehabilitation, promoting muscle reinnervation and inducing a highly proliferative state of Schwann cells responsible for remyelination.<sup>344,345</sup> Therefore, GH is also a promising therapy for peripheral nerve injuries.

## Conclusions

We have shown a number of actions of GH exerted on multiple tissues and organs far beyond the classic effects of the hormone on the intermediate metabolism and growth. The high diversity of actions of GH can be explained only by the fact that the hormone plays many different roles by activating a high number of proteins involved in cell signaling and displaying different mechanisms of action, as recently demonstrated by Ray et al<sup>346</sup> and Carter-Su et al.<sup>347</sup> The possibility exists that, rather than a hormone, GH is a prohormone that depending on the tissue may be proteolytically cleaved giving origin to different and shorter GH derivatives with tissue-specific properties. In addition, GH may activate the proliferation of tissue-specific stem cells that then would act in tissue repair after an injury. In this sense, GH is safe if administered in the appropriate doses and frequency. A classical GH-dependent adverse effect such as hyperglycemia is not important if the hormone is administered before physical exercise. Moreover, recently, it has been reported that GH is able to induce the rescue of pancreatic  $\beta$ -cell and function in streptozotocin-treated mice and, therefore, may be of interest in the treatment of type 1 diabetes.<sup>348</sup>



## Author Contributions

Conceived and designed the experiments: JD. Analyzed the data: PD, CA and JD. Wrote the first draft: JD. Contributed to the writing: PD and CA. Agree with results and conclusions: JD, PD, CA. Jointly developed the structure and arguments: JD, PD, CA. Made critical revisions and approved final version: JD, CA and PD. All authors reviewed and approved of the final manuscript.

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