

60 YEARS OF NEUROENDOCRINOLOGY

The hypothalamo-GH axis: the past 60 years

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Abstract

At the time of the publication of Geoffrey Harris's monograph on 'Neural control of the pituitary gland' 60 years ago, the pituitary was recognised to produce a growth factor, and extracts administered to children with hypopituitarism could accelerate growth. Since then our understanding of the neuroendocrinology of the GH axis has included identification of the key central components of the GH axis: GH-releasing hormone and somatostatin (SST) in the 1970s and 1980s and ghrelin in the 1990s. Characterisation of the physiological control of the axis was significantly advanced by frequent blood sampling studies in the 1980s and 1990s; the pulsatile pattern of GH secretion and the factors that influenced the frequency and amplitude of the pulses have been defined. Over the same time, spontaneously occurring and targeted mutations in the GH axis in rodents combined with the recognition of genetic causes of familial hypopituitarism demonstrated the key factors controlling pituitary development. As the understanding of the control of GH secretion advanced, developments of treatments for GH axis disorders have evolved. Administration of pituitary-derived human GH was followed by the introduction of recombinant human GH in the 1980s, and, more recently, by long-acting GH preparations. For GH excess disorders, dopamine agonists were used first followed by SST analogues, and in 2005 the GH receptor blocker pegvisomant was introduced. This review will cover the evolution of these discoveries and build a picture of our current understanding of the hypothalamo-GH axis.

Key Words

- ▶ history
- ▶ pituitary
- ▶ growth hormone
- ▶ hypothalamus

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Introduction

The work of Geoffrey Harris in the 1940s and 1950s identified that the function of the anterior pituitary gland was not dependent on a direct nerve supply but was controlled by neurohumoral factors in the portal blood

supply. These 'releasing' factors originated from neurones in the hypothalamus and entered into the portal blood. With regard to the growth hormone (GH) axis, work in the 1960s showed that intra-carotid injection of stalk-median

eminence extracts into rats and guinea pigs led to a reduction of pituitary GH content, implying that the extract had caused release of GH from the pituitary into the blood (Muller & Pecile 1965, Muller *et al.* 1965). The recognition from 60 years ago that there was a hypothalamic–GH connection has led investigators to now establish the major roles of the GH-releasing hormone (GHRH), somatostatin (SST)/somatotrophin release inhibitory factor (SRIF) and ghrelin in the central control of GH secretion from the anterior pituitary somatotrophs. Investigators have also identified the peripheral factors that impact on this central hypothalamic–GH axis and have characterised both murine and human models associated with abnormalities within the central control of GH.

To provide a historical perspective, the discoveries of the key components of the GH axis are shown on a timeline in Fig. 1 and our current understanding of the axis is summarised in Fig 2.

The evolution in understanding the central GH axis

The 'early' years: 1920s–1960s

The lack of a growth-stimulating factor in humans who exhibited severe proportionate short stature has been

recognised throughout history. However, the relationship between the pituitary gland and growth was not apparent until 1921, when Evans & Long (1921) treated rats with extracts from bovine anterior pituitary glands and showed increased growth. In the 1930s pituitary extracts started to be administered to human 'pituitary dwarfs', but it was not until the 1950s that GH extracted from human pituitaries was given to such individuals. In 1958 Raben (1958) reported successful growth promotion in a 'pituitary dwarf' treated with human GH.

The pituitary gland was first linked to metabolism in 1936 when increased sensitivity to insulin was identified in hypophysectomised animals, which reversed when they were injected with pituitary extracts (Houssay 1936). A direct effect of GH was identified in 1965 when it was demonstrated that injection of GH into the brachial artery reduced forearm glucose uptake in both skeletal muscle and adipose tissue and blocked the action of insulin when both hormones were co-administered (Rabinowitz *et al.* 1965).

In this era, the diagnosis of GH deficiency (GHD) was based on clinical knowledge that a state of hypopituitarism existed with a need for the replacement of other pituitary hormones. In 1963 a RIA for GH was developed (Glick *et al.* 1963), and it was then possible to measure

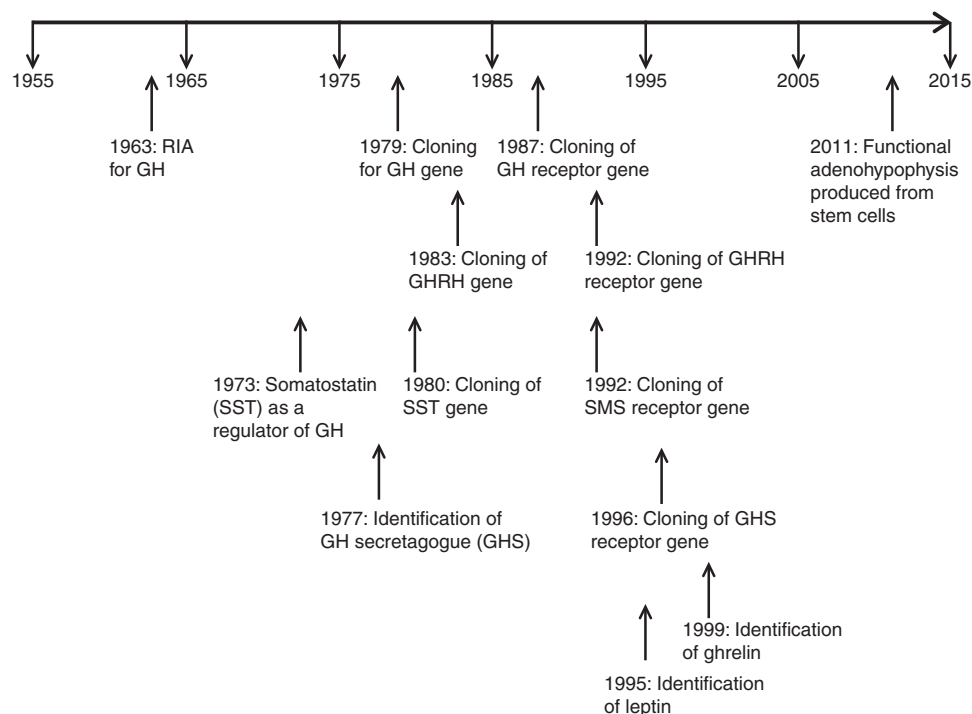
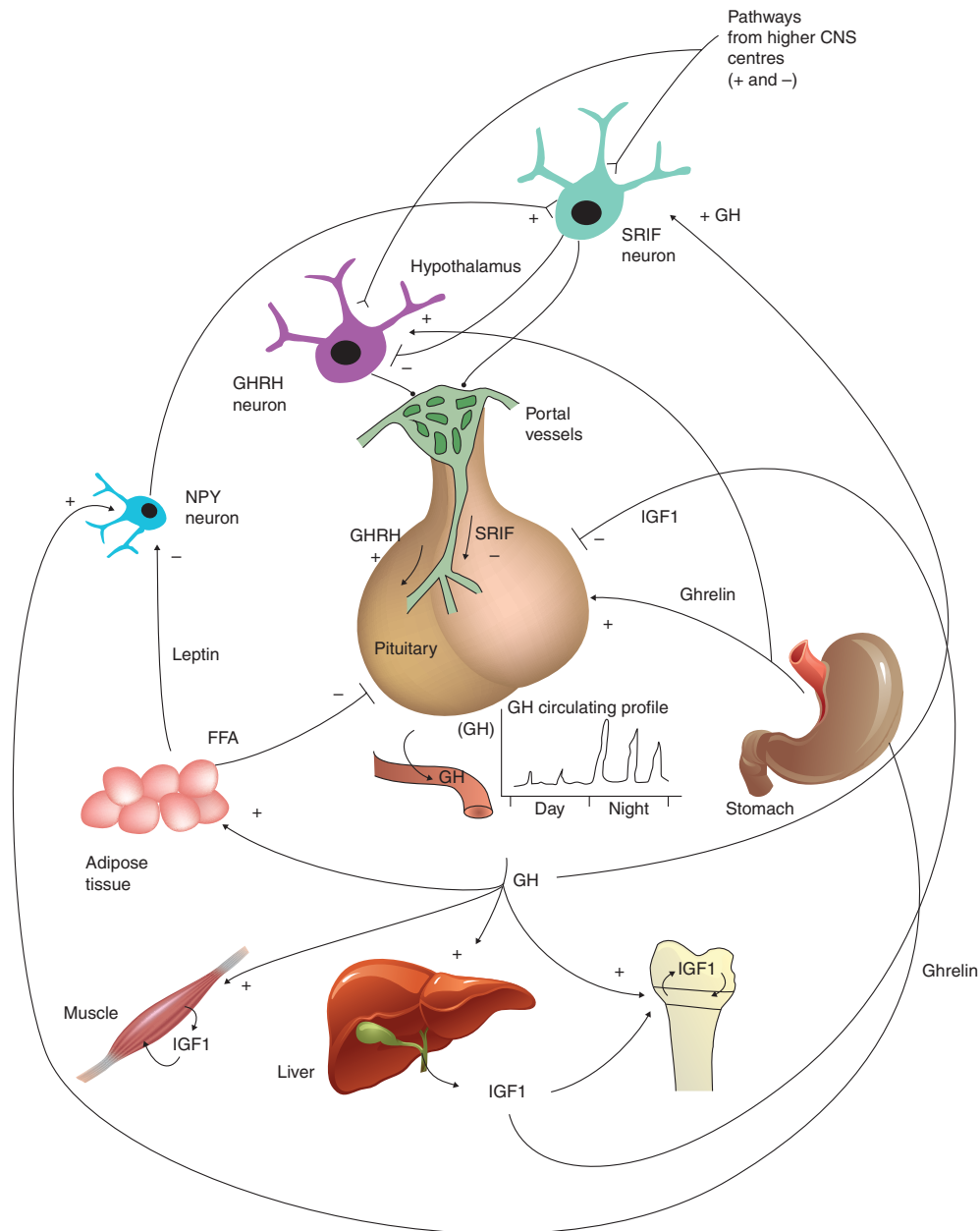


Figure 1

Timeline of the key events in the understanding of the components that control the hypothalamo-growth hormone axis.

**Figure 2**

Central and peripheral components that regulate the GH axis. NPY, neuropeptide Y; FFA, free fatty acids; GH, growth hormone; IGF1, insulin-like growth factor 1; GHRH, GH-releasing hormone; SRIF, somatotrophin release inhibitory factor.

circulating levels of GH in children and adults with hypopituitarism and acromegaly and in response to physiological changes (e.g., exercise) or metabolic stimuli (e.g., induction of hypoglycaemia or infusion of amino acids, such as arginine). From these observations clinical tests were established in the 1960s, such as the exercise test, the insulin tolerance test and the arginine test, which could help define an individual's ability to acutely release

GH from the pituitary, defining a pharmacological rather than physiological response.

During this time the clinical priority of trying to provide diagnoses and treatments for those with disorders of the GH axis and the lack of tools to dissect the various components of the GH axis dictated that clinical investigations and therapeutics were outstripping physiological studies of how the GH axis functioned and was

controlled. However, as genes were cloned and assays refined, the detailed functional studies of the last 20 years filled in the knowledge gaps, allowing investigations to be refined and new therapies to be developed.

Defining the central components of the GH axis

Somatostatin The first hypothalamic hormone identified to regulate GH secretion was SST in 1973 by Brazeau *et al.* (1973) in the laboratory of Roger Guillemin at La Jolla. After the discovery of the thyrotrophin-releasing hormone and luteinizing hormone (LH), the intended search was for a GH releasing factor. Instead of promoting GH release as expected, hypothalamic extracts (but not other cerebral extracts) were found to inhibit GH release from rat pituitary culture. The substance producing this effect was given the name SRIF. Ion-chromatography using fragments from 500 000 sheep hypothalami led to the identification of a fraction with the capacity to inhibit GH release. Purification and sequencing of this fraction identified a 14-amino acid peptide (SST), which was subsequently confirmed to suppress GH secretion in rats and humans (Siler *et al.* 1973). A broad range of secretagogues including nutrients, neuropeptides, neurotransmitters, hormones, growth factors and cytokines influences the secretion of SST. At the hypothalamus GH, insulin-like growth factor 1 (IGF1) and GHRH stimulate SST secretion while opiates and GABA inhibit SST release. SST acts to reduce GH secretion by inhibiting the response of the pituitary to GHRH (Spoudeas *et al.* 1992, Wagner *et al.* 1998).

After the discovery of SST there were many potential therapeutic indications to be explored. The earliest reports indicated a role for SST to reduce GH secretion in acromegaly (Reichlin *et al.* 1976). Although SST did not affect secretion of prolactin or adrenocorticotrophic hormone (ACTH) in normal individuals, it was noted to reduce prolactin levels in acromegalic patients and ACTH levels in individuals with Nelson syndrome (Reichlin *et al.* 1976). An extrapituitary effect of SST was first noted when it was found to induce hypoglycaemia by suppressing glucagon secretion when administered to fasting baboons (Koerker *et al.* 1974). It was rapidly identified that SST inhibited both insulin and glucagon secretion, and although early reports focused on a potential role in improving control in type 1 diabetes, the effects on insulin secretion ultimately led to a role in the treatment of hyperinsulinism. More recently there has been increasing interest in the use of SST as an antiangiogenic agent in diabetic retinopathy (Boehm 2007). Now SST is known

to be expressed widely and act on multiple targets including the brain, pituitary, gut, pancreas (exocrine and endocrine), adrenals, thyroid and kidney. SST has a biological role as a neurotransmitter in glandular secretion, smooth muscle contraction and cell proliferation. To varying extents SST is capable of inhibiting the secretion of almost every exocrine- and endocrine-secreted hormone or peptide.

The SST gene was first cloned in 1980 (Goodman *et al.* 1980); two genes were identified in fish but in mammals there is only one SST gene with the two distinct SST peptides (SST14 and SST28) derived via alternative splicing. The SST receptor (SSTR) was first cloned in 1992 (Yamada *et al.* 1992) with a total of five different SSTR genes identified and cloned by 1994 (Patel *et al.* 1995). The products (SSTRs 1–5) range in size from 356 to 391 amino acids and are typical G protein coupled receptors (GPCRs) with seven transmembrane domains in addition to extracellular and intracellular domains. Sequence identity ranges from 39 to 57% across the family of receptors. SSTRs 1–4 are partially selective for SST14, binding to it with a two- to threefold higher affinity than SST28, while SSTR5 is SST28 selective, binding to it with a ten- to 30-fold higher affinity than SST14 (Patel *et al.* 1995). The SSTRs are widely expressed with tissue and species specific patterns. In humans SSTR1 is expressed at low levels in jejunum and stomach, while SSTR2 is expressed highly in the brain, gut, liver, colon and jejunum, SSTR3 in the brain, pituitary and pancreatic islets and SSTR4 in the brain, stomach, lungs, kidney, pituitary and adrenals. SSTR5 is, in contrast, expressed exclusively in the pituitary gland in adults. Our understanding of SST has moved from a hypothalamic factor inhibiting GH secretion to a multifunctional peptide widely expressed throughout the body.

GH-releasing hormone The existence of a potential hypothalamic factor regulating GH secretion was first demonstrated in 1961 by Reichlin (1961), who identified that lesions of the ventromedial nucleus in the rat resulted in poor growth and a reduction in GH content of the pituitary. This evidence of a hypothalamic GHRH was strengthened by observations that the addition of rat hypothalamus extract increased GH secretion from cultured rat pituitaries, but rat cerebral cortex extract had no such effect (Deuben & Meites 1964). An association between acromegaly and extrapituitary tumours was recognised as early as the 1960s, and in 1973 extracts from human lung tumours were shown to stimulate GH release from rat pituitaries (Beck *et al.* 1973). It was not until 1982 that three different peptides (GH-releasing

factors, GRF(1–44)NH₂, GRF(1–40)OH and GRF(1–37)), were purified and sequenced from pancreatic tumours removed from patients with acromegaly by two separate groups (Guillemin *et al.* 1982, Rivier *et al.* 1982). Two of these peptides were subsequently identified within the human hypothalamus (Bohlen *et al.* 1983) with the major form being GRF(1–44)NH₂ (Ling *et al.* 1984) and given the name GHRH.

The *GHRH* gene was cloned initially from tumour cDNA in 1983 (Mayo *et al.* 1983), and the human gene mapped in 1985 (Mayo *et al.* 1985). The GHRH receptor (GHRHR) was cloned in 1992 (Mayo 1992); key to this event was the identification that GHRHR was likely to be a GPCR, as GHRH induced pituitary cAMP expression. Mayo (1992) screened rat pituitary cDNA clones for those containing a sequence similar to transmembrane domains 6 and 7 of other known GPCRs identifying rat and human clones encoding GPCRs subsequently shown to bind to GHRH when expressed in HEK293 cells. Shortly after the cloning of *GHRHR*, a point mutation was identified in this gene in the dwarf (*lit/lit*) mouse (Lin *et al.* 1993), which led to an absence of signal transduction via the mutant receptor. The *lit/lit* mouse also helped identify the role of GHRH in the development of the anterior pituitary with GHRH having no impact on initial pituitary ontogeny with a full complement of PIT1, GHRHR and GH cells present, but it had a profound impact on subsequent somatotroph proliferation leading to anterior pituitary hypoplasia.

The first human mutation in *GHRHR* (E72X) was reported in 1996 in two members of a large consanguineous family with severe isolated GHD (IGHD; Wajnrajch *et al.* 1996). Since this report a variety of missense, nonsense, splice site mutations and deletions have been reported (for review, see Alatzoglou & Dattani (2010)), accounting for 10% of the cases of familial IGHD. Affected individuals present with severe short stature and GHD but usually without midface hypoplasia, neonatal hypoglycaemia and microphallus. Magnetic resonance imaging of the pituitary gland invariably identifies anterior pituitary hypoplasia.

The major role of GHRH is to induce the pulses of GH secretion as immunisation against GHRH; the use of GHRH antagonists and destruction of the arcuate nucleus all result in loss of pulsatile GH secretion. Secretion is induced by depolarisation, hypophysectomy, thyroidectomy, hypoglycaemia and α_2 -adrenergic stimulation and is inhibited by SST, IGF1 and the activation of GABAergic function.

GHRH has been used to treat GHD children with the first demonstration of an increase in GH secretion being in a child with a suprasellar germinoma (Sassolas 2000). GHRH may well be a useful therapeutic agent in children

with hypothalamic aetiology for their GHD but clearly will be ineffective when the cause of GHD lies within the pituitary. Overall, there was a great variability in response to GHRH treatment, probably representing variability in the aetiology of the disease, but when patients demonstrate a rise in GH to the first injection, 70% of the patients will display an increase in height velocity of >2 cm/year (Sassolas 2000). The availability and effectiveness of recombinant human GH resulted in GHRH not having a role in the treatment of GHD, but it has found a role in the diagnosis of GHD as with the GHRH test (Takano *et al.* 1984) and, more recently, the combined GHRH–arginine test (Ghigo *et al.* 1996).

Ghrelin There has been a long lead into the identification of ghrelin, beginning with the discovery of synthetic, nonnatural GH-releasing peptides in the 1970s. The enkephalin pentapeptide hormones were isolated from porcine brain (Hughes *et al.* 1975) and identified as having an opioid effect. They were felt to be good candidates for hypophysiotrophic hormones due to their size and distribution within the brain and because the sequence of Met-enkephalin (Tyr-Gly-Gly-Phe-Met) was identical to residues 61–65 of the pituitary hormone β -lipotrophin. Bowers *et al.* (1977) identified an analogue of Met-enkephalin (Tyr-D-Trp-Gly-Phe-Met-NH₂) with GH secretory capacity by incubating the peptides with extracted rat pituitaries. The possibility of a native GH-releasing peptide and its cell surface receptor was hypothesized by Bowers *et al.* (1980). Multiple further GH-releasing peptides were identified, including nonpeptidyl GH secretagogues (GHS; Camanni *et al.* 1998), and demonstrated to have GH secreting capacity *in vivo* (Bowers *et al.* 1990).

The laboratory of Roy Smith identified that GHSs and their mimetics produce intracellular effects similar to hormones acting via a GPCR (increased intracellular calcium, inositol triphosphate and increased protein kinase C activity; Pong *et al.* 1996). *Xenopus* oocytes injected with swine pituitary cRNA intermittently demonstrated an increase in calcium-activated chloride channels when treated with a nonpeptide GHS. Howard *et al.* (1996) used this property in the identification of the GHS receptor (GHS-R). By co-injecting pools of swine pituitary cRNA with cRNA for *GNAS* into *Xenopus* oocytes, they were able to identify a single clone that reliably produced the effect on oocyte chloride currents. This led to the cloning of the GHS-R1a and GHS-R1b receptors, seven transmembrane domain-containing GPCRs. It was not until 3 years after the cloning of the receptors that the endogenous ligand ghrelin was identified (Kojima *et al.* 1999).

By treating Chinese hamster ovary cells stably expressing the rat GHS-R and measuring the changes in intracellular calcium concentration induced by extracts from a variety of rat organs, Kojima *et al.* identified the stomach as the source of ghrelin and then identified and purified the mature peptide and its sequence by purification of the stomach extract by chromatography. A difference in molecular weight between the synthetic and endogenous peptide combined with the inability of the synthetic peptide to induce an increase in intracellular calcium led to the discovery that ghrelin is *O*-*n*-octanoylated at serine 3; this is essential for the activity of the peptide and the first time that octanoylation of a peptide hormone was observed. A prepro-ghrelin of 117 amino acids with an N-terminal 23 residue secretory signal peptide was identified with expression in both the stomach and hypothalamus with readily detectable plasma concentrations in blood. Taken together this suggested a model in which ghrelin is secreted from the stomach and acts on the hypothalamus to regulate GH secretion.

Secretion of ghrelin is decreased by food intake and increased by food deprivation, hypoglycaemia and leptin administration (Camina *et al.* 2003). Plasma ghrelin is reduced in obese subjects and increased in malnourished subjects. In addition to stimulating GH secretion, ghrelin also stimulates prolactin and ACTH secretion and exhibits orexigenic and adipogenic effects when injected centrally or peripherally (Bowers *et al.* 1990). Numerous other effects have been documented including those on gut motility, insulin secretion, sleep, response to stress, learning, memory, cardiovascular performance, cell proliferation, cell differentiation, cell survival and immunological response (van der Lely *et al.* 2004). Ghrelin increases GH secretion via a direct effect on pituitary somatotrophs by depolarising cell membrane and increasing GH secretion per cell and by stimulatory actions on GHRH release at the hypothalamus with a weaker element of SST inhibition (Baragli *et al.* 2011).

Given the identified role of ghrelin in GH secretion and appetite stimulation, it was surprising when the ghrelin knockout (KO) mouse was reported to have no change in size, growth rate, food intake, body composition and behaviour compared to WT littermates (Sun *et al.* 2003). More recently there has been a focus on the role of multiple alternative products from the ghrelin gene (e.g., obestatin; Zhang *et al.* 2005), the role of unacetylated ghrelin (comprising 75% of circulating ghrelin) and non-endocrine roles of ghrelin in psychiatric disorders (Wittekind & Kluge 2015), tumour progression (Xu *et al.* 2015), brain injury (Xie *et al.* 2015) and heart failure (Khatib

et al. 2014). Like the multifunctional SST, ghrelin, initially a GHS, is now recognised as a pleiotropic hormone.

Patterns of GH secretion

The stimulatory actions of GHRH and ghrelin on GH secretion and the tonic inhibitory influence of SST were recognised to generate a pulsatile pattern of GH secretion. In the 1980s and 1990s frequent blood sampling techniques with measurements taken every 5–20 min over a 24-h period were used to characterise GH profiles. Original studies demonstrated that approximately eight GH peaks occurred over 24 h, predominantly at night in healthy males and females, defining ultradian rhythms of GH secretion (frequency <24 h). These studies were limited by the use of relatively insensitive GH immunoradiometric assays, in which up to 90% of the daytime GH samples were undetectable (Plotnick *et al.* 1975). The development of increasingly sensitive GH assays and sophisticated deconvolution analyses allowed more detailed interrogation of the patterns of GH secretion. This highlighted the importance of the differential control of baseline GH secretion compared to the frequency and amplitude of the GH peaks over a 24-h period and how these are influenced by endogenous and exogenous stimuli (Veldhuis *et al.* 2008). Trough GH concentrations have been linked to BMI and waist–hip ratio while peak GH concentrations are linked to IGF1 concentration (Hindmarsh *et al.* 1997). In addition to the amplitude and frequency of pulses, it has become apparent that the orderliness (a measure of regularity and uniformity) of the GH pulsatile activity also influences the actions of GH. However, a complete understanding of the significance of orderliness for GH action and factors influencing this orderliness in healthy development remains elusive (Veldhuis *et al.* 2008). There is also evidence for infradian rhythms of GH secretion, i.e., rhythms which occur over a >24-h period. These have been established more recently by measurements of urinary concentrations of GH over time periods of up to 1 week (Clayton *et al.* 2014).

The complexity in patterns of GH secretion necessary for optimal coordination of the growth and metabolic effects of GH has started to be unravelled over the last 60 years but numerous questions remain unanswered.

External regulators of the central GH axis

A multitude of exogenous factors influence the complex regulatory mechanism of the GH axis, for example, age, gender, sleep, nutrition, obesity and exercise (reviewed in

Giustina & Veldhuis (1998)); here we have focused on a few key factors which are crucial to growth and metabolic processes.

Sex steroids Sexual dimorphism of the GH axis exists in humans with postpubertal men having greater diurnal variation (smaller pulses in daylight, more at night) and females having more frequent pulses and higher daily GH production. The significant differences between male and female GH dynamics are driven at least in part by a modulatory effect of gonadal steroids on GH secretion and responsiveness.

Testosterone Men with hypogonadism have reduced overall 24-h GH secretion, and boys who have precocious puberty and an early rise in sex steroids also have an early increase in GH secretion. Administration of exogenous testosterone or pulsed gonadotrophin-releasing hormone in primary or hypogonadotrophic hypogonadism increases spontaneous and stimulated GH secretion and the disorderliness of secretion. However, use of non-aromatisable androgens in hypogonadal boys does not alter GH dynamics, implying that the testosterone effect requires aromatisation to oestrogen (Kerrigan & Rogol 1992, Veldhuis *et al.* 1997). This oestrogen effect is believed to be at a central level as testosterone stimulates increased GH secretion and, in parallel, increased IGF1 production.

Oestrogen The relationship between oestrogen and GH secretion is complex and incompletely understood, particularly in adults. Similar to testosterone, in children there is a correlation between oestrogen status and GH secretion, with reduced GH secretion in hypogonadal girls with Turner's syndrome that is normalised with oestrogen replacement (Veldhuis *et al.* 1997). In contrast, GHRH-stimulated GH secretion was not different between normal adult women and those with premature ovarian failure, replaced or not replaced with oestrogen (Hartmann *et al.* 1997). More recent deconvolution analyses in older women did show a relationship between oestrogen and both basal and pulsatile GH secretion (Hudson *et al.* 2010, Veldhuis *et al.* 2011).

Intriguingly, although the very reproducible effect of testosterone on GH secretion appears to be mediated by the central effects of aromatisation to oestrogen, the evidence for a similar mechanism in women is less clear. IGF1 levels are not consistently elevated as a result of oestrogen replacement.

Oestrogen also directly reduces the sensitivity of the liver to the effects of GH by stimulating *SOCS2* expression and inhibiting GH signal transduction (Leung *et al.* 2003).

Thus premenopausal women have a lower IGF1 concentration for the same level of GH secretion compared to men and postmenopausal women. In keeping with this, GHD women who are oestrogen replete require higher doses of GH to attain the same IGF1 level. At least some of the effects of oestrogen on GH secretion are therefore likely to be related to feedback produced by a reduction in IGF1 levels (Meinhardt & Ho 2006).

In relation to central oestrogen effects, the presence of alpha and beta oestrogen receptors in murine somatotrophs has recently been demonstrated. Despite this, it is unclear whether direct oestrogen effects at the level of the pituitary have a role in mediating the rise in GH with oestrogen as female mice with a KO of somatotroph oestrogen receptors had normal puberty and growth rates (Avtanski *et al.* 2014).

Central sex steroid influences may be more likely mediated at a hypothalamic level; recent elegant studies demonstrate that oestrogen and testosterone supplementation augment ghrelin-stimulated GHRH release and inhibit SST repression of the axis, thus increasing overall GH secretion (Veldhuis *et al.* 2012, Norman *et al.* 2014).

Body composition The close relationship between body composition and GH secretory dynamics is well documented but complex. There is an established association between a higher BMI and a reduced overall GH 24-h production rate in healthy men and women. For example, in healthy men, a 50% drop in GH production for a male with a BMI of 28 kg/m² compared to a male with a BMI of 21 kg/m² has been described (Iranmanesh *et al.* 1991). Transgenic mouse models support a direct role for GH in regulating adiposity with GHR^{-/-} mice and mice expressing a GH antagonist displaying substantial increases in subcutaneous white adipose tissue, whereas mice over-expressing bovine GH have a reduced percentage of body fat (Berryman *et al.* 2004). Changes in fat mass relate to tissue specific roles of GH with liver specific deletion of GHR resulting in no change in fat mass, whereas an increase is seen in mice with a skeletal muscle GHR deletion (Berryman *et al.* 2011).

Multiple deconvolution studies over the last 30 years have defined deficiencies in both basal and pulsatile GH secretion with increasing BMI 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 (Pijl *et al.* 2001).

Adiposity is related to not only spontaneous GH secretion but also the response of GH secretion to GHRH, arginine and glucagon; these are all reduced with increasing BMI, independent of sex and age (Makimura *et al.* 2008). The mechanisms underlying this relationship to adiposity are unclear and whether reduced pituitary GH secretion drives adiposity, in particular visceral fat, or whether the low GH secretion is a result of adiposity remains to be established. Initial data demonstrating massive weight loss leading to normalisation of GH secretory dynamics led to the belief that the altered GH dynamics were a result of the changes in adiposity (Rasmussen *et al.* 1995). However, these results were not confirmed in a study of weight loss in viscerally obese women (Pijl *et al.* 2001), and interestingly, recent studies of overeating show an acute reduction in pituitary GH secretion as a result of reduced pulse amplitude before any change in body mass occurs (Cornford *et al.* 2011).

A number of factors have been postulated as being responsible for mediating this relationship between adipose depots and the GH axis. Free fatty acids (FFA) have long been shown to directly suppress GH secretion, and this relates to changes in mRNA levels of both pituitary GH and the GHRHR, at least in primates (Luque *et al.* 2006). Inhibition of lipolysis and FFA production using acipimox in obese patients restored GH response to GHRH (Cordido *et al.* 1996). These experimental results have to be measured against the fact that plasma FFA levels are not consistently elevated in obesity, and a simple relationship between FFA and obesity has been called into question (Karpe *et al.* 2011).

The acute suppression of GH secretion in overeating is likely related to hyperinsulinemia, and insulin has been shown *in vitro* to directly reduce mRNA for GH and the GHRHR. High levels of insulin have also been demonstrated to reduce GH response to GHRH *in vivo*. How these acutely elevated insulin levels as a result of overeating relate to the longer term changes in GH secretion in obesity have not been determined (Cornford *et al.* 2011, 2012).

The identification and cloning of leptin in rodent adipose tissue in 1994 and the discovery of leptin receptors in the arcuate and periventricular hypothalamic nuclei shortly afterwards stimulated much interest in leptin being the potential link between adiposity, nutrition and GH secretion. In experimental animals leptin has been shown to influence GH secretion, with leptin antiserum leading to a significant decrease in spontaneous GH secretion (Carro *et al.* 1997) and leptin infusions into rat brains generating an increase in spontaneous GH secretion

and the response to GHRP6, with this being mediated by effects on SST (Tannenbaum *et al.* 1998).

These data led to the speculation that leptin may be the signal for the reduced GH secretion seen in obesity; however, the relationship between obesity and leptin levels in humans is rather more complex, with simple forms of obesity leading to elevated leptin levels and a presumed central resistance to its effects. Furthermore, an elegant study in leptin deficient patients demonstrated that the response to GHRH stimulation of these patients, patients with high leptin obesity and healthy controls was related directly to the degree of adiposity and not the leptin levels themselves (Ozata *et al.* 2003).

Adiponectin was first identified in 1995 and acts to increase muscle fatty acid oxidation, suppress hepatic glucose production and decrease serum glucose levels (Scherer *et al.* 1995). Obesity and insulin resistance are associated with decreased adiponectin levels (Weyer *et al.* 2001). In acromegaly, with raised GH levels and insulin resistance, adiponectin levels have been reported to be decreased (Lam *et al.* 2004). GH suppresses adiponectin secretion in cultured human adipose tissue, and in transgenic mice overexpressing GH adiponectin is decreased, while in GHR^{-/-} mice adiponectin is increased (Nilsson *et al.* 2005). This suggests therefore that, in part, the metabolic effects of GH on insulin resistance are mediated via adiponectin.

There is still much work to be done to understand this complex interplay between adiposity, nutrition and GH secretion; intriguing questions remain unanswered, such as why obese children with low GH levels equivalent to those of GHD patients do not have short stature. There have been over 20 clinical trials with GH or GHSs in obesity but its efficacy remains to be established, and there are concerns around hyperglycaemic side effects (Berryman *et al.* 2013).

The impact of cranial irradiation on the GH axis

The impact of cranial irradiation on the GH axis has provided an interesting model to dissect the relationship between the hypothalamus and pituitary. Cranial irradiation at doses as low as 18 Gy has been associated with the induction of a GHD state and subsequent poor growth if delivered in childhood. Doses of cranial radiotherapy <40 Gy often lead to IGHD, with larger doses increasing the likelihood of multiple pituitary hormonal defects.

A subgroup of children cranially irradiated as part of treatment for acute lymphoblastic leukaemia in the 1980s

were noted to have poor growth despite normal GH responses to the provocative testing of the GH axis with arginine or GHRH. Quantification of 24-h GH secretion, however, did demonstrate reduced spontaneous GH secretion and a reduction in GH pulse amplitude and frequency (Blatt *et al.* 1984). GH response to insulin-induced hypoglycaemia was, however, subnormal (Blatt *et al.* 1984, Romshe *et al.* 1984, Ahmed *et al.* 1986). This combination of GH axis responses was also demonstrated in two monkeys cranially irradiated with 40 Gy that showed impaired spontaneous GH secretion and a reduced GH peak to insulin-induced hypoglycaemia but an adequate GH peak response with arginine (Chrousos *et al.* 1982).

Thus, cranial radiation was apparently associated with a phenomenon known as neurosecretory dysfunction, a condition in this case thought to be caused by radiation-induced hypothalamic damage leading to reduced spontaneous GHRH and GH production but a normal somatotroph response to direct stimulation. Neurosecretory dysfunction had first been described in a subset of short, slowly growing children with normal GH responses in stimulation tests (Spiliotis *et al.* 1984).

More recent studies in adult survivors of childhood brain tumours suggest the picture is not so straightforward, with patients having not only reduced spontaneous GH secretion as previously described but also a more subtle reduced overall response to provocative testing. It has been proposed that cranial irradiation causes damage to both the hypothalamus and pituitary, a combination that leads to the overall GHD and poor growth (Darzy *et al.* 2007).

The GH axis through life

In infancy, growth is predominantly nutrition driven. The impact of GH on growth increases over the first years, and from approximately 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. At this stage around 200–600 µg of GH are produced per day, and there is a relationship between the absolute amount of GH release over 1 day and height accrual. Although low GH secretion rates are therefore unsurprisingly associated with growth failure, as GH levels increase, the effect of total GH secreted on overall growth parameters reduces (Clayton *et al.* 2014).

There are significant differences in growth patterns between children with similar overall normal daily GH secretion, and it is likely that subtle differences in the GH

infradian rhythms and degree of disorder of GH secretion (measured by approximate entropy) relate to height velocity and stature (Gill *et al.* 2001). This has implications for GH replacement regimens, particularly in light of the weekly GH preparations currently in clinical trials (Gill *et al.* 2001, Clayton *et al.* 2014).

At puberty, growth velocity peaks with a potential for growth >8 cm/year; this reflects increased GH secretion, which is 1.5- to threefold greater at this time than in the prepubertal years and is associated with a commensurate increase in serum IGF1 levels. The significant increase in 24-h GH arises as a result of greater pulse amplitude and GH mass secreted in both boys and girls. This is likely related to the activation of the hypothalamic–pituitary–gonadal axis at this time with oestrogen levels in girls and testosterone in boys correlating with total GH secretion. Although the subtleties of this interaction are still not fully elucidated, increased GHRH, reduced SST tone and reduced sensitivity at a central level to the rising IGF1 are all likely to play a role. Alongside increases in GH secretion during puberty, sensitivity to the action of GH also increases (Giustina & Veldhuis 1998).

The mechanism of activation of the hypothalamic–pituitary–gonadal axis at puberty has long been investigated with recent work establishing the role of kisspeptin, and therefore, the question of whether kisspeptin itself may influence GH dynamics has naturally arisen. Animal studies in calves and female baboons have demonstrated the ability of kisspeptin to stimulate GH secretion, but recent data from adult human females failed to demonstrate any effect of s.c. bolus injections of kisspeptin at doses known to potently stimulate gonadotrophins. Its role at the time of puberty has not been studied (Jayasena *et al.* 2014).

Following attainment of adult height, there is a continuous decrease in overall GH secretion with age, with an initial exponential decline to around a quarter of the pubertal levels in adults over 20 years of age. In boys, cross-sectional studies demonstrated that increased disorderliness in GH secretion was maximal during mid- to late puberty and potentially explains the maximum growth rate seen at this time. There is then a rapid and steep decline towards orderly secretion at the point of reproductive maturity (Pincus *et al.* 2000). There is no current mechanistic explanation for these phenomena.

After this rapid decline at the end of puberty, GH secretion slowly declines throughout life. Interestingly, this is associated with an increase in approximate entropy and disorderliness. Although the most likely explanations relate to declining sex steroid levels and changes in body composition, interventional studies are few, with our

understanding relying on cross-sectional data that makes causation difficult to determine. Men have a GH secretion pattern that reflects testosterone status and therefore a gradual reduction throughout life. Premenopausal women, in contrast, have higher 24-h GH levels than post-menopausal women, and it has been demonstrated since the 1960s that this varies with stages of the menstrual cycle; in the late follicular phase increased oestrogen levels correlate with a doubling in plasma GH secretion (Frantz & Rabkin 1965).

GH secretion over 24 h in adults does show good correlation to the measures of total body fat, visceral fat and physical fitness, with total and visceral fat increasing with age and physical fitness declining. These data and the observation that GH levels can drop to a level consistent with the diagnosis of GHD after the age of 60 have led to the popular concept that GH treatment could be used as an anti-aging agent. A recent meta-analysis of trials of GH therapy in adults over 60 shows significant reductions in fat mass and increases in lean mass with treatment; however, no beneficial effects on function or strength and a significant risk of adverse effects in terms of oedema, arthralgia and impaired glucose tolerance have been reported (Liu *et al.* 2007).

The role of ghrelin in modulating the GH axis in humans remains somewhat controversial. A number of studies demonstrate no link between ghrelin levels and GH secretion although these studies have mainly measured acyl-ghrelin rather than the more sensitive desacyl-ghrelin. With regards to aging, levels of desacyl-ghrelin are reduced in elderly men and the use of MK-677, a ghrelin mimetic, in this age group increases GH pulse amplitude of GH (Nass 2013).

Human and murine mutations affecting the central GH axis

In parallel with the many physiological and pathophysiological studies outlined above that have defined the status of the GH axis in health and disease, our understanding of the significance of key factors within the hypothalamo-pituitary axis (HPA) has been aided greatly by the study of naturally occurring and transgenic murine models as well as the study of patients from families affected by developmental disorders of the HPA.

Three mouse strains (Ames, Jackson and Snell dwarf mice), all with spontaneously occurring autosomal recessive mutations leading to normal size at birth but subsequent growth failure and adult size one-third of wild type, were among the first murine models of genetic

pituitary disorders. All three mouse strains had pituitary hypoplasia with failure to detect synthesis of GH, thyroid-stimulating hormone (TSH) or prolactin and the absence or markedly reduced numbers (in the case of Ames mouse) of mature somatotroph, thyrotroph or lactotroph cells. The pituitary transcription activator POU1F1 (initially referred to as PIT1) was identified to activate both GH and prolactin promoters in 1988 (Ingraham *et al.* 1988). In 1990 the Jackson and Snell mice were identified to have mutations affecting *POU1F1/PIT1*. Li *et al.* (1990) identified the mouse chromosomal location of *PIT1* and then identified an inversion or insertion of a 4 kb fragment in *PIT1* via an alteration in a restriction fragment length polymorphism (RFLP) pattern in the Jackson mouse. The RFLP pattern for the Snell mouse was normal and Li *et al.* (1990) proceeded to clone and then sequence *PIT1* cDNA from the Snell mouse pituitary glands, identifying a homozygous missense mutation p.W261C, which was subsequently identified to affect DNA binding.

The mutation in the Ames mouse was thought to be epistatic to *POU1F1/PIT1* and although *PIT1* expression was absent in the pituitary and the phenotype of the mouse was nearly identical to that of the Snell/Jackson mice, the mutation mapped to chromosome 11. The Prophet of *PIT1* (*PRO1*) gene containing the mutation responsible for the Ames dwarf phenotype was cloned in 1996 using genetically directed representational difference analysis (Sornson *et al.* 1996). A single point mutation was identified in the first α -helix of the homeodomain of *PRO1*, p.S82P, leading to decreased transcriptional activity (Sornson *et al.* 1996). The expression of *PRO1* within the anterior pituitary is first detected at e10–e10.5 with maximal expression at e12.0, prior to the expression of *POU1F1* at e13.5. Thus, *PRO1* was identified as a key regulator of *POU1F1* and essential for the ultimate proliferation of somatotrophs, lactotrophs and thyrotrophs.

Life expectancy was noted to be increased for Ames and Snell mice by ~40–60% (Brown-Borg *et al.* 1996) and for *GHR^{-/-}* mice by 40–55% (Coschigano *et al.* 2000). When fed a normal diet *lit/lit* mice become obese and display no increase in life expectancy, but when fed a low-calorie diet, they live 25% longer than sibling controls fed the same diet (Flurkey *et al.* 2001). Proposed mechanisms through which reduced GH leads to increased longevity include reduced secretion of insulin, increased hepatic sensitivity to insulin action, reduced plasma glucose, reduced generation of reactive oxygen species, improved antioxidant defences, increased resistance to oxidative stress and reduced oxidative damage (Bartke & Brown-Borg 2004). Laron syndrome (due to the loss of function

mutations in the GHR) is associated with a decreased risk of diabetes and cancer (Guevara-Aguirre *et al.* 2011), and untreated GHD has been linked to a reduced life expectancy (Besson *et al.* 2003).

Four separate groups (Ohta *et al.* 1992, Pfaffle *et al.* 1992, Radovick *et al.* 1992, Tatsumi *et al.* 1992) identified human mutations in *POU1F1* in 1992 associated with anterior pituitary hypoplasia and deficiency of GH, TSH and prolactin. Deficiency in GH and prolactin are complete and present early in life but deficiency of TSH is highly variable. The majority of identified mutations are autosomal recessive but dominant mutations have also been identified. The first human *PRO1* mutation was identified in 1998 (Wu *et al.* 1998) and mutations in this gene are now thought to be the most common genetic cause of familial-combined pituitary hormone deficiency accounting for up to 50% of the cases. Inheritance in all of the cases to date has been autosomal recessive, and the condition is characterised by deficiency of GH, TSH, prolactin and gonadotrophins. GHD is typically present from early life but the other deficiencies evolve with age, including ACTH deficiency in some cases in later life. Anterior pituitary size is typically hypoplastic or normal but there can be a waxing and waning enlargement of the gland that later evolves into hypoplasia. In murine models, *PRO1* is responsible for the migration of progenitor cells from Rathke's pouch into the developing anterior pituitary. One suggestion for the enlarged gland is that it is due to undifferentiated cells collecting in the periluminal area.

After this era of studying spontaneous mutants, targeted gene disruption in mice was used to describe the phenotypic features of a larger number of gene disruption models. The identification of the early role of *LHX3* in pituitary development is an example of this technology. *LHX3* is expressed from e8.5 within the developing Rathke's pouch, and in 1996, targeted disruption in mice (Sheng *et al.* 1996) demonstrated the development of pituitary aplasia with the subsequent identification of human mutations in *LHX3* associated with hypopituitarism and a stiff neck in 2000 (Netchine *et al.* 2000). There are now many genetic defects of early pituitary development resulting in impaired GH secretion that have been described in mice by targeted gene disruption and in humans by candidate gene sequencing including *HESX1*, *SOX2*, *SOX3*, *LHX4*, *PITX2* and *OTX2* (for review, see Kelberman *et al.* (2009)).

Increasingly, there has been recognition that genes involved in other disorders of forebrain development may also be responsible for pituitary phenotypes including GHD. One example of this is where the screening of

hypopituitary patients for mutations in genes known to be associated with Kallman syndrome identified mutations in *FGFR1*, *FGF8* and *PROKR2* (Raivio *et al.* 2012). Whole exome sequencing is replacing candidate gene sequencing in the identification of novel causes of genetic disease and identified loss of function mutations in *ARNT2* as a cause of hypopituitarism in one family (Webb *et al.* 2013).

The key information gained on the genetic control of pituitary development was used in 2011 to develop a technique whereby cultures of mouse embryonic stem cells could be stimulated to develop into a functioning adenohypophysis (Suga *et al.* 2011). When grafted *in vivo*, these adenohypophyses were able to secrete ACTH in sufficient amounts to recover glucocorticoid secretion in hypopituitary mice. Such stem cell-based therapies may allow reconstitution of pituitary function in patients in the future.

In addition to defects affecting transcription factors involved in pituitary development, pathological genetic variants causing GHD have also been identified. The molecular basis of IGHD type 1A (an autosomal recessive disorder characterised by severe GHD with undetectable serum GH levels and anti-GH antibodies when treated with GH therapy) was described in 1981. A 7.5 kb deletion in *GHI* was identified using restriction nuclease digestion of genomic DNA and hybridisation to a ³²P-labelled probe derived from a *GHI* cDNA clone. Subsequent to this, multiple additional deletions as well as nonsense mutations have been identified in families with IGHD type 1A (for review, see Alatzoglou *et al.* (2014)). In contrast to children with IGHD type 1A, those with type 1B deficiency have low but detectable levels of GH in serum, do not develop antibodies to GH therapy and respond well to treatment. Subsequent to the identification of deletions in *GHI*, splice site, nonsense and frameshift mutations were identified in children with IGHD type 1B (Alatzoglou & Dattani 2010). Mutations in *GHRHR* (discussed above) are another cause of IGHD type 1B. Autosomal dominant type 2 IGHD was identified to be caused by a single base pair mutation in the first 6 bp of intron 3 resulting in the skipping of exon 3 (Cogan *et al.* 1994) and the production of a 17.5 kDa GH variant that exerts a dominant negative effect on GH secretion (Lee *et al.* 2000). In 2003 the mechanism through which the splice variant affected GH secretion was described (McGuinness *et al.* 2003) by identifying that the 17.5 kDa isoform destabilised the production of secretory vesicles, and endogenous GH accumulated in toxic aggregates in the cytosol resulting in pituitary hypoplasia and multiple pituitary hormone deficiency. A number of additional mutations affecting

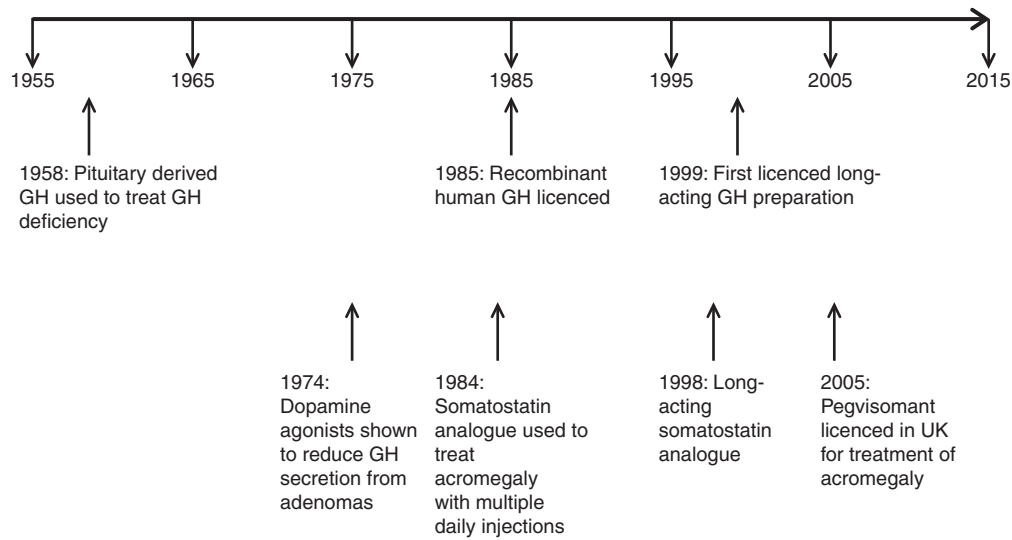


Figure 3
Timeline of key events in the evolution of therapies for GH deficiency and GH excess.

splicing or GH secretion have subsequently been identified (Alatzoglou & Dattani 2010).

Therapies for abnormalities in the central GH axis

Treatments for GH axis disorders have not changed greatly over the last 60 years. GH has been used as a replacement for deficient states throughout this time, and it is only over the last 10–15 years that modifications to the GH molecule have been considered to make therapeutic advances. Given the physiology, it is surprising that GHSs have not been successful in, for example, growth promotion. Similarly, medical treatments for GH excess with SST analogues have been used over many years, and it is only over the last decade that the development of a GH receptor antagonist has occurred. It is interesting to speculate whether further therapeutic developments focussing on molecules that will influence the function of GH axis receptors or post-receptor actions may be explored. The timeline of the development of the therapies for GH deficiency and GH excess is seen in Fig 3.

GH deficiency

Human GH was first used to treat a patient with hypopituitarism in 1958 when Raben (1958) treated a 17-year-old, short, prepubertal male with 2 mg of i.m. pituitary-derived GH three times per week for 10 months and observed a sustained increase in height. The supply of

pituitary-derived GH was limited and initially one pituitary supplied only ~1 mg of GH, sufficient to treat one patient for 1 day. Because of the limited supply, there was interest in producing GH in bacteria via recombinant DNA technology, and this was first achieved by Genentech in 1978 with a subsequent series of clinical trials resulting in FDA approval for recombinant human GH to treat GHD in 1985 (Cronin 1997). The timing of the availability of recombinant human GH coincided with the first reports of Creutzfeldt–Jakob disease (CJD) in recipients of pituitary-derived GH (Koch *et al.* 1985). Shortly after the first reports of CJD, distribution of pituitary-derived GH was stopped. Of the 7700 patients treated with pituitary-derived GH between 1958 and 1985 in the USA, 26 (0.34%) are known to have developed CJD (Blizzard 2012). Recombinant human GH replaced pituitary-derived GH in 1985, and by the 1990s the indications for GH treatment began to expand beyond GHD with approval for the following conditions (with variation in licences between countries): chronic renal insufficiency (FDA approved in 1993), adult GHD (FDA approved in 1996), Turner syndrome (FDA approved in 1996), Prader–Willi syndrome (FDA approved in 2000), children born small for gestational age (FDA approved in 2001), children with idiopathic short stature (FDA approved in 2003), AIDS wasting (FDA approved 2003), *SHOX* deficiency (FDA approved in 2006) and Noonan syndrome (FDA approved in 2007).

GH therapy requires daily s.c. injections, and adherence rates are highly variable. Attempts have therefore been made to develop long-acting preparations of GH and

to develop alternative methods of delivery. A biodegradable microsphere preparation of GH was commercially available as Nutropin Depot until 2004 and required twice monthly injections in childhood (once monthly for adults; Kemp *et al.* 2004). It was withdrawn in 2004 due to the significant resources required by the companies to continue the manufacturing and commercialising of the product. LB03002 is a sustained release preparation of GH incorporated into sodium hyaluronate microparticles that has demonstrated similar efficacy to daily GH in pre-pubertal GH naïve children with GHD (Khadilkar *et al.* 2014). There are many other long-acting GH preparations under development utilising hydrogels, implants and modifications of GH by pegylation, conjugation with albumin, conjugation with specific amino acid sequences (XTEN) and fusion to Fc domains of human immunoglobulin (Cai *et al.* 2014). In view of the discussions above regarding the significance of pulsatility of GH secretion associated with both ultradian and infradian rhythms, it will be interesting to understand how the efficacy of these long-acting GH preparations relates to normal physiology.

Alternative routes of administration that have been studied include intranasal, transdermal and pulmonary routes. Intranasal administration has been limited by poor bioavailability, whereas transdermal administration with microneedle patches and self-dissolving micropiles has demonstrated high bioavailability but at the expense of localised skin irritation (Cai *et al.* 2014). Pulmonary delivery requires the development of uniform low density powders to reach the lower lung; initial reports suggested low bioavailability but addition of dimethyl- β -cyclodextrin substantially increased bioavailability in rats (Jalalipour *et al.* 2008). In summary, long-acting preparations of GH are likely to become available for clinical use in the near future, and transdermal delivery appears to be the most promising route of administration.

GH excess

Excess GH production, which is predominantly caused by GH-secreting pituitary adenomas, results in gigantism if it occurs prior to epiphyseal fusion and acromegaly after this time. It is associated with significant morbidity and excess mortality (Orme *et al.* 1998). The gold standard of treatment for this condition is transsphenoidal hypophysectomy of the adenoma, which can normalise GH dynamics if successful. Surgical cure rates vary; using the most up-to-date endoscopic techniques and an expert surgeon generates remission rates of up to 70% overall (Abu Dabrh *et al.* 2014). Traditionally, surgical cure rates

have been much less impressive, and commonly, pituitary radiotherapy and medical therapy were required to control the GH excess.

The first medical therapy available for acromegaly was a dopamine agonist. In the 1970s it was discovered that the administration of L-DOPA to patients with acromegaly paradoxically reduced GH levels, as it caused an increase under normal physiological conditions. Subsequently, D2 receptors were discovered on somatotroph adenomas. Although an effective treatment, IGF1 levels decrease by a mean of 30%, with only one-third of the patients treated with normalising IGF1 (Sandret *et al.* 2011). The discovery that SST was the predominant negative regulator of GH secretion naturally led to the investigation of its use as an effective treatment for acromegaly. Initial use was hindered by the naturally short half-life of native SST, around 3 min, necessitating constant i.v. infusion for it to be an effective medication. Modified forms of SST, including octreotide, a synthetic cyclical octapeptide, and lanreotide, a six amino acid analogue, increased the half-life up to 100 min. These two forms, both of which have been licenced and are available as monthly depot preparations since the 1990s, control GH secretion in ~60% of the patients with active acromegaly (reviewed in Murray & Melmed (2008)). More recently, SOM-230 (parsireotide), which has an increased affinity at SSTRs 1, 2, 3 and 5, potentially has some benefit over octreotide and lanreotide in terms of reduced GH secretion and reduction in pituitary adenoma growth, but it is limited by expense and incidence of hyperglycemia (Gadelha *et al.* 2014).

A novel approach to treating acromegaly was developed in the 1990s when a transgenic mouse designed to produce a modified GH intended to have greater activity at the GHR actually produced a dwarf phenotype (Chen *et al.* 1991). Over the following 10 years, modification and pegylation of this protein led to the development of pegvisomant, a molecule which is a competitive antagonist with GH at the GHR and was licenced in the USA in 2003 and in the UK in 2005 for the treatment of acromegaly (Kopchick *et al.* 2002, Higham & Trainer 2008). It effectively reduces IGF1 in up to 97% of the patients treated and is now used both as a single agent and in combination with SST analogues (Madsen *et al.* 2011).

Conclusions

The last 60 years has seen our understanding of the GH axis move from the recognition that the hypothalamus communicated with the pituitary to generate a growth factor to our

present knowledge of the multiple central and peripheral factors that lead to the generation of a pulse of GH. The main components of the axis have been isolated, their genes cloned and their functions elucidated. However, new hormones and certainly new mechanisms that will influence the GH axis may still be discovered. It is notable that, although postulated for many years, ghrelin was only characterised in 1999. We continue to increase our knowledge about genes that influence pituitary function and GH secretion by finding humans with mutations, and in the era of exome and whole genome sequencing, this is the field that is most likely to extend our physiological knowledge and point to new therapeutic possibilities.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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