Published in final edited form as:

Mol Cell Endocrinol. 2011 June 20; 340(1): 10–14. doi:10.1016/j.mce.2011.03.021.

The role of ghrelin in GH secretion and GH disorders

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Abstract

In humans, growth hormone (GH) is secreted from the anterior pituitary in a pulsatile pattern. The traditional view is that this secretory pattern is driven by two counter regulatory neurohormones, GHRH and somatostatin. Ghrelin, the natural ligand for the growth hormone (GH)-secretagogue receptor (GHS-R), is produced in the stomach. Ghrelin is the strongest GH secretagogue known to date, but the role of endogenous ghrelin in the regulation of circulating GH levels remains controversial. The following review examines the evidence suggesting that endogenous ghrelin may be a key regulator of GH peak amplitude and discusses studies of diseases with altered GH levels, where it is found that in these states GH and ghrelin levels change in a similar way.

Keywords

Ghrelin; Growth hormone; Anorexia nervosa; Obesity; Aging

1. Regulation of growth hormone (GH) release

1.1. Physiology

In 1999 ghrelin was discovered as an endogenous ligand of the growth hormone secretagogue receptor (GHSR) (Kojima et al., 1999) and this breakthrough was preceded by a large body of work in the field of growth hormone (GH) secretagogues. Peptidergic and non-peptidergic GH secretagogues had been developed (Bowers, 2001; Thorner et al., 1997), which had then been utilized to characterize and clone their receptor (Howard et al., 1996). This G protein coupled GH secretagogue receptor, now known to be the ghrelin receptor, binds acyl-ghrelin (and secretagogues) and signals via the phospholipase C–inositol triphosphate–PKC signaling pathway (Howard et al., 1996) and also activates MAPK (Nanzer et al., 2004), PKA, Akt and AMPK signaling pathways. The receptor has a strong constitutive activity, with ligand-independent signaling (Holst et al., 2003). Data using ghrelin receptor knockout (KO) animal models confirmed that ghrelin's GH releasing and orexigenic effects are both mediated via this receptor (Sun et al., 2004). Ghrelin is a 28 amino acid peptide which is acylated by the attachment of a medium-chain fatty acid at position 3. This acylation is mandatory for its biologic effects on GH secretion (Smith,

2005) and for binding at the receptor (Kojima et al., 1999). It circulates in two major forms, acylated and des-acylated ghrelin (Kojima et al., 1999). The main site of ghrelin synthesis is the fundus of the stomach, within the oxyntic glands, but ghrelin synthesis has also been documented in the small intestine (van der Lely et al., 2004) and lesser amounts are seen in many tissues including pituitary, hypothalamus, lymphocytes and pancreas.

While earlier studies with GH secretagogues, as well as the studies with ghrelin itself, have shown a significant and reproducible GH releasing effect (van der Lely et al., 2004), the physiological role of circulating ghrelin in the regulation of GH release is still not fully established.

Growth hormone (GH) secretion is pulsatile and it is thought to be controlled by the two neurohormones, growth hormone releasing hormone (GHRH) and somatostatin (Hartman et al., 1991; Thorner et al., 1997). Several lines of evidence suggest that besides GHRH and somatostatin other factors play a role in the regulation of GH release from the pituitary (Wagner et al., 2009). The most reproducible GH pulse is observed shortly after sleep onset (Van Cauter et al., 1998). Several studies observed that the nocturnal rise in GH is not abolished by an infusion of the somato-statin analog octreotide (Dimaraki et al., 2001; Vance et al., 1985; Webb et al., 1984), which indirectly supported the hypothesis that a factor which antagonizes the effects of somatostatin might be involved in the regulation of GH. Patients with inactivating mutations of the GHRH receptor continue to have rhythmic GH secretion, which supports a role for an additional GH releasing factor besides GHRH in the regulation of GH (Maheshwari et al., 2002). Animal studies also support the involvement of an additional factor in the regulation of GH secretion (Muller et al., 1999). Portal blood measurements of GHRH and somatostatin in rodents showed that while the majority of GH pulses were closely related to GHRH pulses, about 30% of GH pulses were completely dissociated (Frohman et al., 1990). Several animal studies support an interaction of the GHRH and ghrelin systems on the hypothalamic level, such as experiments on transgenic rats that have decreased GHS-R expression in the arcuate nucleus of the hypothalamus and decreased GHRH containing neurons (Mano-Otagiri et al., 2006).

Clinical studies have come to conflicting results demonstrating: (a) a relationship between GH and circulating ghrelin (Koutkia et al., 2004a), (b) no relationship (Avram et al., 2005), (c) a relationship under certain conditions such as fasting or the time of day (Muller et al., 2002). Most of the available studies measured total ghrelin and did not distinguish between acyl- and desacylghrelin. Koutkia et al. (2004a) studied ghrelin pulsatility with cross-approximate entropy (X-ApEn) analysis and found that there is significant regularity in co-secretion between ghrelin levels and GH in the fasted state during nighttime. Similarly, Misra et al. (2005), using deconvolution analysis for GH and total ghrelin in healthy adolescents and adolescents with anorexia, found that fasting ghrelin is an independent predictor of basal GH secretion and GH secretory burst frequency. Data published by Avram et al. (2005), Espelund et al. (2005), and Norrelund et al. (2002) and did not find a correlation between GH and circulating ghrelin levels. The main criticism of these studies relates to the fact that there were either only few samples collected, the sampling interval was too long when frequent sampling was used or only total ghrelin levels were measured. Studies from our group examining 8 healthy young men (Nass et al., 2008a) showed a

significant correlation between the amplitudes of GH secretory events and the average acyl-ghrelin concentration in the 60 min interval during and before the GH pulse. Based on the similar diurnal rhythm, the results suggest that during fed conditions acyl-ghrelin acts as a positive modifier of GH release (see Fig. 1). MK-677 is an orally active, long acting growth hormone secretagogue which acts as a ghrelin mimetic at the ghrelin receptor. Long-term administration of MK-677 in older adults (Nass et al., 2008b) increased GH pulse amplitude and the pre-existing pulsatile GH profile was preserved. A potential physiological role of ghrelin in the regulation of GH release is supported by the data of Pantel et al. (2006), who could show that, in humans, a mis-sense mutation which impairs the constitutive activity of the GHSR is associated with short stature. Similarly, GHSR-null mice have lower IGF-I levels when compared to wild type animals (Sun et al., 2004) and ghrelin null mice have a tendency to lower IGF-I concentrations when compared to the control animals (Sun et al., 2003). Reasons for the unexpectedly modest changes found in these KO models could be due to the constitutive activity of the receptor which compensates for the lack of ghrelin and also the presence of a complex redundant system. Data by Zizzari et al. (2005) show that application of a GHSR antagonist results in a decrease in GH pulse amplitude in rodents.

2. Potential use of ghrelin or ghrelin antagonists in disorders and conditions of altered GH secretion

There are several disease states where ghrelin and GH levels change in a similar way.

2.1. Obesity

Subcutaneous administration of ghrelin to rodents (Tschoep et al., 2000) has been shown to increase body weight and body fat mass, but in humans, circulating ghrelin levels have been found to be lower in obese adults when compared to lean adults (Tschoep et al., 2001). This decrease may be a physiological adaptation to the positive energy balance associated with obesity. Similarly, circulating GH levels are low and obese subjects have a blunted responsiveness to GH stimuli, which is reversible after the obese subjects lose weight (Williams et al., 1984). GH levels are negatively correlated with body mass index (BMI) and its half-life, amplitude and pulsatility are reduced in obesity (Scacchi et al., 1999). Studies which compare the acute GH stimulatory effects of GHRH and ghrelin in obese and lean adults could show an increased GH response when ghrelin was given alone (24.4 µg/l) or in combination with GHRH (39.9 µg/l), compared to GHRH alone (2.4 µg/l) in the obese subjects. However the response was still decreased when compared to lean subjects (ghrelin: 68.5 µg/l, GHRH and ghrelin: 117.8 µg/l, GHRH: 25 µg/l) (Alvarez-Castro et al., 2004). These data support the speculative conclusion that ghrelin could play a role in the blunted GH secretion in obesity. Other data have suggested that visceral fat mass is a strong predictor of basal ghrelin concentrations (Katsuki et al., 2004; Sondergaard et al., 2009); similar correlations have been found for visceral abdominal fat and 24-h GH release (Clasey et al., 2001). GH has strong lipolytic effects (Nass and Thorner, 2002) and administration of GH for 9 months in middle-aged men with abdominal/visceral obesity has been shown to decrease abdominal visceral fat (Johannsson et al., 1997) and total body fat. Similarly, reduced GH concentrations are observed in men with human immunodeficiency virus (HIV) lipodystrophy, and administration of GHRH for 12 weeks has been shown to result in a

decrease in truncal and visceral fat in these patients (Koutkia et al., 2004b). The concept of increasing GH levels in obese adults via administration of a ghrelin mimetic was tested by Svensson et al. (1998), who administered the oral ghrelin mimetic MK-677 to healthy male obese adults for 2 months. While the ghrelin mimetic was able to activate the GH axis and increase IGF-I levels and increase fat free mass, total and visceral fat mass did not decrease. Results from our own studies (Nass et al., 2008b) in healthy non-obese older adults, show that after 1 year, administration of MK-677, an orally active ghrelin mimetic, results in a sustained activation of the GH-IGF-I axis and an increase in lean body mass, but no change in total fat mass or abdominal visceral fat. Overall these short and long-term results suggest that activation of the GH axis via ghrelin or a ghrelin mimetic in the obese is possible, however, the adipogenic effects of ghrelin make it an unlikely candidate for the treatment of obesity in humans.

2.2. Conditions of severe caloric restriction

While the pharmacologic effects of ghrelin administration have been characterized, studies to define the effects of ghrelin deficiency have been less clear. A remarkable study by Zhao et al. (2010) tested the metabolic consequences in mice that lack the ghrelin O-acyltransferase (GOAT) gene. The authors found that under conditions of 60% caloric restriction the mice failed to maintain their blood sugar levels and became moribund after 7 days, while the wild-type (WT) mice sustained their blood glucose levels. The authors showed that the main difference between these animal groups was the increase in GH levels at day 5 in the WT mice while the caloric restricted GOAT KO mice did not show an increase in GH levels at day 5. At day 8 the GH levels in the GOAT KO were still only one-third of the levels seen in the WT mice. Importantly, restoration of GH or ghrelin levels via infusion to the caloric restricted GOAT KO mice resulted in the restoration of blood glucose levels similar to the WT mice and the prevention of death. While these data seem to contradict clinical studies in humans (Liu et al., 2008), which show that long-term fasting results in low acyl-ghrelin levels, it is important to note that the GOAT KO mice were on caloric restriction (40% of normal levels), but were not completely food deprived. These data suggest a potential physiological role of the ghrelin–GH axis under conditions of severe caloric restriction and the maintenance of blood sugar levels in the undernourished state. While one has to be cautious in extrapolating from animal studies to humans, these findings are similar to humans with caloric restriction due to anorexia nervosa, who also have elevated ghrelin and GH levels, but reduced IGF-I levels (Misra et al., 2005).

2.3. Anorexia

Anorexia nervosa (AN) is associated with increased circulating total ghrelin levels. The mechanisms leading to this increase are thought to be related to a decreased post-prandial decline in circulating ghrelin, or the consequence of ghrelin resistance in these patients (Otto et al., 2001). AN patients also have increased GH levels, but low IGF-I levels, due to GH resistance possibly mediated via fibroblast growth factor-21 (Fazeli et al., 2009). As mentioned before, recent animal data by Zhao et al. (2010) suggest that circulating ghrelin might be essential in maintaining GH levels and thus maintaining blood glucose levels under conditions of chronic severe caloric restriction. A similar clinical situation can be found in patients with AN, a patient model with caloric restriction, and high GH levels. However, the

results of Fazeli et al. (2010) did not show a significant increase in glucose or IGF-I levels in patients with AN when given GH in supraphysiological doses (5 times higher than doses used to overcome GH resistant states).

Published data on the potential role for ghrelin in increasing circulating GH levels in patients with AN are conflicting. Misra et al. (2004) could not find a correlation between GH and ghrelin in the post oral glucose tolerance test (OGTT) phase in adolescent girls. In another study, Tolle et al. (2002) did not find a correlation between GH and circulating ghrelin levels, albeit the interval between sampling was only 4 h. Interestingly, the GH response to GHRH was increased in the patients with AN, which could suggest a role of the increased ghrelin levels in the GHRH-induced GH release in these patients. Ghrelin infusion studies by Miljic et al. (2006) support the existence of ghrelin resistance in AN patients. In this study, IV administration of ghrelin to AN patients with low BMI did not result in an increase in appetite when compared to controls. Total ghrelin levels were increased, but the GH response to the ghrelin bolus was blunted (Miljic et al., 2006); however, the study was not placebo controlled and the number of participants was small.

Supporting a relationship between ghrelin and GH in AN patients, Misra et al. (2005) analyzed the secretory dynamics between ghrelin and GH using a shorter sampling interval. These data showed that circulating total ghrelin levels predict GH burst frequency during a 12-h nocturnal blood sampling (sampled every 30 min) in adolescent girls with AN.

Based on the above-mentioned interventional study it seems that AN patients are not only GH resistant, but also ghrelin resistant. On the other hand, data from indirect studies analyzing profiles of ghrelin and GH in AN patients suggest a possible relationship between ghrelin and GH (Misra et al., 2005). Most of these AN studies measured only total ghrelin and have not distinguished between acyl- and desacyl-ghrelin. In studies by Hotta et al. (2004) both total and acyl-ghrelin levels were measured in AN patients. They found that while total ghrelin levels were elevated, acyl-ghrelin levels were not elevated relative to controls. More studies with validated assays distinguishing between the acyl- and desacyl-ghrelin are necessary in this patient population to come to a definitive conclusion regarding the levels of acyl and desacyl ghrelin and their relationship to GH secretion in AN.

3. GH and ghrelin levels in the elderly

Data from our group show that mean 24-h acyl-ghrelin levels are lower in a group of adults age 60 years and older when compared to a group of young adults age 30 years and younger where both groups had a similar BMI (Nass et al., 2008c). The results from several other groups support an age-dependent decline of ghrelin (Rigamonti et al., 2002). Aging is also associated with a decrease in circulating GH levels with the decline starting in the second decade and with a half life of 7 years (Nass et al., 2009). The pituitary ghrelin receptor content does not decline with aging (Sun et al., 2007) and the secretory response of the pituitary to ghrelin and GH secretagogues is sustained in the elderly (Broglio et al., 2003).

Ghrelin or ghrelin mimetics have been shown to enhance the pre-existing pulsatile GH release (Smith, 2005) and several short-term studies demonstrate a GH increasing effect in the elderly. Chapman et al. (1997) showed in a 4-week double blind placebo controlled

study, that once daily oral MK-677, given to healthy older adults, enhances pulsatile GH release and IGF-I concentrations reaching levels seen in young adults.

In a multicenter, randomized, double blind placebo-controlled 18-month study in 292 postmenopausal women, MK-677 given in combination with alendronate (a bisphosphonate used for treatment of osteoporosis), increased bone mineral density (BMD) at the femoral neck vs. alendronate alone, however at the lumbar spine, total hip or total body MK-677 did not improve BMD more than alendronate alone (Murphy et al., 2001). The authors concluded that while MK-677 in combination with alendronate has significant positive effects on the bone formation at the femoral neck, the lack of enhancement at other sites made it a less attractive choice when compared to alendronate alone (Murphy et al., 2001). Bach et al. (2004) showed that MK-677, when given to hip fracture patients after hip surgery in a group of patients age 65 years and older, resulted in a greater improvement relative to placebo in 3 of 4 lower extremity functional performance measures. A 2-year study using an orally active ghrelin mimetic in healthy older adults was published in 2008 (Nass et al., 2008b). This study found that 1 year treatment with MK-677 resulted in an increase in fat-free mass by 1.1 kg and that the effect was maintained through the second year. No significant increase in total body fat was found, however, there was a significant increase in subcutaneous limb fat. The changes in muscle mass did not result in measurable changes in muscle strength or function. This study included mainly physically fit, active, healthy older adults. This may explain the apparent discrepancy between the increase in muscle mass and lack of detectable change in function and strength, while in a similar study examining frail subjects, functional improvements were detectable (Bach et al., 2004). Similar changes in body composition were found with the pyrazoline-piperidine ghrelin mimetic capromorelin. Studies showed that this ghrelin mimetic was able to increase lean body mass by 1.4 kg in a group of older adults with mild functional limitation. They also found an improvement in tandem walk and stair climb. However, the study utilized different dosing groups which had to be pooled to demonstrate significance (White et al., 2009). Overall these data suggest a potential role for ghrelin or ghrelin mimetics in the treatment of the anorexia/sarcopenia of aging.

4. Conclusion

The overall role of circulating ghrelin levels in the regulation of GH release is still controversial. Data from healthy adults suggest that in addition to somatostatin and GHRH, acyl-ghrelin plays a role in the regulation of GH secretion in the fed state and modulates the pre-existing pulsatile GH secretion pattern. Both in anorexia and obesity the altered ghrelin levels are most likely to be an adaptation to the change in energy balance and/or ghrelin resistance. Currently published data are controversial and have not excluded or proven a correlation between circulating GH and ghrelin levels under these conditions. Whether the ghrelin-GH axis plays a role in specific diseases which are associated with altered GH levels is unclear to date and requires further investigation.

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Fig. 1. Mean ($\pm$ S.E.M.) plasma acyl-ghrelin (pg/mL) and serum GH levels (μ g/L, shown on log scale); $n = 8$ young men: (A) fed admission; (B) fasted admission. Fig. 2 from Nass et al. (2008a), reproduced with permission; Copyright 2008, The Endocrine Society.