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Horizons in Nutritional Science

The many faces of ghrelin: new perspectives for nutrition research?

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The appetite-modulating peptide ghrelin is predominantly produced and secreted by the stomach and shows a strong growth hormone-releasing activity, which is mediated by the activation of the so-called growth hormone secretagogue type 1a receptor. Ghrelin is involved in the regulation of energy balance by increasing food intake and reducing fat utilization. Additionally, it stimulates lactotroph and corticotroph function, influences the pituitary gonadal axis, inhibits pro-inflammatory cytokine expression, controls gastric motility and acid secretion and influences pancreatic exocrine and endocrine function, as well as impacting on glucose metabolism. This review summarizes the known functions of ghrelin and its role in the regulation of the gut–brain axis.

Ghrelin: Diabetes: Obesity: Anorexia: Energy balance

The discovery of two new hormones has profoundly changed the scientific model of energy balance regulation during the past decade. One of these, the now well-known adipocyte hormone leptin has been studied extensively since its discovery in 1994 (Zhang *et al.* 1994). The other, ghrelin, was discovered in 1999 (Kojima *et al.* 1999) and is also involved in the regulation of food intake and body weight. An impressive number of data have been generated, which consistently suggest a role for ghrelin as a humoral signal from the stomach to inform the brain about acute changes in peripheral energy balance.

However, numerous questions about the function of this hormone remain. The cross-species transferability of ghrelin-related findings, differential roles for the putative brain-derived and peripherally circulating ghrelin, its (in)activation by attachment or cleavage of its octanoyl side chain, and its possibly redundant rather than essential role in the regulation of food intake and body weight regulation all remain unanswered. Non-acylated ghrelin is predominantly produced and, after post-translational octanoylation, secreted by the gastric mucosa. The principal site of ghrelin production is clearly the stomach, which contributes 70 % of the circulating ghrelin concentration (Jeon *et al.* 2004). Additionally, ghrelin has been detected in the small intestine, pancreas, kidneys, lung, placenta, testes, pituitary and hypothalamus (Kojima *et al.* 1999; Date *et al.* 2000; Mori *et al.* 2000; Gualillo *et al.* 2001; Korbonits *et al.* 2001; Volante *et al.* 2002; Cowley *et al.* 2003).

Ghrelin increases food intake, and it may be involved in the regulation of energy balance (Tschöp *et al.* 2000; Horvath *et al.* 2001; Nakazato *et al.* 2001; Wren *et al.* 2001; Zigman & Elmquist, 2003). For example, ghrelin's circadian rhythm indicates that there is a pre-meal increase in ghrelin and postprandial decreases, which may suggest an important role for ghrelin in meal initiation (Cummings *et al.* 2001). Even a more conservative interpretation of the available data suggests that ghrelin may, along with multiple other afferent signals, inform neuroendocrine networks in the central nervous system (CNS) about acute and chronic changes in food intake, metabolism or body fat mass (Tschöp *et al.* 2000; Horvath *et al.* 2001; Cowley *et al.* 2003; Zigman & Elmquist, 2003). These changes may initiate efferent responses that regulate energy homeostasis.

Molecular aspects of ghrelin

Ghrelin is a twenty-eight amino acid peptide cleaved from the precursor preproghrelin and is the first peptide isolated from mammals in which the hydroxyl group (of a specific serine residue) is acylated by *n*-octanoic acid. The octanoylation allows the peptide to bind to its receptor, the growth hormone (GH)

Abbreviations: CNS, central nervous system; GH, growth hormone; GHS-R, growth hormone secretagogue receptor; HPA, hypothalamo-pituitary-adrenal; LH, luteinizing hormone.

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secretagogue receptor (GHS-R) type 1a (Kojima *et al.* 2001). The acylation of the hydroxyl group of serine in position 3 is required for ghrelin's ability to alter energy balance or endocrine function in rodents and man. Intriguingly, it is also required for the hormone to cross the blood–brain barrier (Banks *et al.* 2002; Guallillo *et al.* 2003).

In human serum, non-acylated ghrelin is found in much higher amounts than 'bioactive' (acylated) ghrelin (about 3–4 % of total circulating ghrelin), and this ratio is closely maintained even after meal intake (Lucidi *et al.* 2004). Recent work suggests, however, that the non-acylated form is not completely biologically inactive. For example, it may have cardiovascular and anti-proliferative effects mediated through different GHS-receptor subtypes or completely different, unknown, ghrelin receptors (Date *et al.* 2000; Cassoni *et al.* 2001). Nonetheless, in man, non-acylated ghrelin does not possess the endocrine activities of acylated ghrelin and does not change the secretory patterns at the pituitary or the pancreas (Broglio *et al.* 2003*b*).

Ghrelin and endocrine axes

Ghrelin dose-dependently stimulates GH-releasing activity, which may be mediated via the activation of GHS-R type 1a (Kojima *et al.* 2001). We recently reported a potential GH–ghrelin feedback loop between stomach and the pituitary using a hypophysectomized rat model. In that study, we demonstrated that hypophysectomy increased circulating plasma ghrelin levels in rats compared with plasma ghrelin levels from sham-hypophysectomized or normal rats, whereas GH administration decreased circulating ghrelin levels in normal rats (Tschöp *et al.* 2002). In GH-deficient dwarf rats, however, plasma ghrelin concentrations (Kojima *et al.* 1999; Date *et al.* 2000) are not significantly different from those of normal controls (Tschöp *et al.* 2002). Therefore, the lack of pituitary hormones, but not GH deficiency alone, may result in significant increases in circulating ghrelin level in rodents. Furthermore, GH replacement therapy does not seem to significantly modify circulating ghrelin levels; it is, however, effective in changing body fat distribution and body fat mass (Janssen *et al.* 2001). Similar, although slightly controversial, results have recently been reported by Barkan *et al.* (2003), who demonstrated that circulating ghrelin concentrations were not affected by an isolated suppression of GH level in human subjects. Finally, however, Freda *et al.* (2003) found that the surgical removal of a GH-producing tumour in acromegalic patients normalized suppressed serum ghrelin levels.

The GH-releasing effect of ghrelin has been demonstrated numerous times and is independent of gender, although it undergoes an age-related decrease (Broglio *et al.* 2003*a*). Currently, ghrelin is thought to have a modulatory role on GH secretion, rather than a direct effect on the physiological system driving the endogenous production and secretion of GH pulses (Broglio *et al*. 2003*a*; Cummings & Shannon, 2003). Additionally, ghrelin stimulates the lactotroph and corticotroph system, and the systemic administration of synthetic ghrelin increases adrenocorticotrophic hormone and cortisol levels in healthy subjects (Arvat *et al.* 2001; Leal-Cerro *et al.* 2002). Patients with Cushing's syndrome have a hyperresponsiveness of adrenocorticotrophic hormone and cortisol in response to systemic administration of ghrelin (Leal-Cerro *et al.* 2002). Wren *et al.* (2000) suggested that ghrelin stimulates the hypothalamo-pituitary-adrenal (HPA) axis at the hypothalamic level, through the activation of corticotrophin-releasing hormone and vasopressin. We recently demonstrated that endogenously and exogenously induced hypercortisolism led to a significant decrease of plasma ghrelin in human subjects, suggesting a possible feedback mechanism between gastric ghrelin secretion and the HPA axis (Otto *et al.* 2004). In Cushing's syndrome, reduced ghrelin secretion may reflect a compensatory response to the metabolic consequences of chronic hypercortisolism. However, the effect of ghrelin on lactotroph and corticotroph secretion is independent of gender and age (Broglio *et al.* 2003*a*) – factors known to be important in HPA dysregulation.

Sex hormones modulate circulating plasma ghrelin concentration in human subjects and in rats (Pagotto *et al.* 2003; Clegg *et al*. unpublished data) – the data so far available indicate that ghrelin may operate at different levels of the reproductive system (Barreiro & Tena-Sempere, 2004). Testosterone replacement therapy restores normal ghrelin levels in hypogonadal men (Pagotto *et al.* 2003), whereas obese women with polycystic ovary syndrome and hyperandrogenism have lower ghrelin levels than matched obese individuals (Pagotto *et al.* 2002; Schofl *et al.* 2002). Anti-androgen treatment increases circulating ghrelin concentration in obese women with polycystic ovary syndrome (Gambineri *et al.* 2003). Ghrelin was shown to suppress luteinizing hormone (LH) secretion *in vivo*, and to decrease LH-responsiveness to LH-releasing hormone (Barreiro & Tena-Sempere, 2004). Vulliemoz *et al.* (2004) found that a 5 h ghrelin infusion significantly decreased LH pulse frequency in ovariectomized rhesus monkeys and postulated that ghrelin might mediate the suppression of the reproductive system observed in conditions of undernutrition, such as in anorexia nervosa. These findings suggest that there is an interaction between steroid hormones from the HPA as well as the hypothalamo-pituitary-gonadal axis that regulate the orexigenic drive, metabolism and body composition in health and disease.

Ghrelin and energy balance

A solid body of data demonstrates that ghrelin is involved in the regulation of energy balance. Cummings *et al.* (2001) demonstrated that there is a pre-meal increase in plasma ghrelin, suggesting a possible role for ghrelin in meal initiation. Exogenous ghrelin induces weight gain in rodents by increasing food intake and reducing fat utilization (Tschöp *et al.* 2000; Wren *et al.* 2001; Tang-Christensen *et al.* 2004). In cachectic cancer patients, intravenously administered ghrelin had a stimulatory effect on food intake compared with saline infusion (Neary *et al.* 2004).

In rodents, the central administration of ghrelin is relatively more potent in inducing these effects than is peripherally administered ghrelin, suggesting an important central action for these effects (Tschöp *et al.* 2000). Additionally, centrally (interceberebroventricular) administered ghrelin triggered sustained changes in food intake and spontaneous locomotor activity (Tang-Christensen *et al.* 2004) and potently enhanced fat ingestion (Shimbara *et al.* 2004). Finally, centrally administered ghrelin exerts an orexigenic activity through neuropeptide Y and agouti-related protein systems (Chen *et al.* 2004) and also induces immunoreactivity for C-Fos (a marker of neuronal activation) in feeding-related brain areas (Hewson & Dickson, 2000; Olszewski *et al.* 2003), indicating that ghrelin-induced food intake is mediated via the orexin pathway (Toshinai *et al.* 2003).

Contrary to our prediction, circulating ghrelin levels are decreased in human obesity (Tschöp *et al.* 2001*b*). Therefore, many recent experiments have focused on a possible negative relationship between ghrelin level and BMI, including the fact that weight loss increases the circulating level of ghrelin in healthy individuals (Ravussin *et al.* 2001) and obese subjects (Hansen *et al.* 2002). One interpretation of these findings is that ghrelin secretion is reduced in a state of energy excess (i.e. obesity), possibly to reduce orexigenic stimulation during states of positive energy balance. Further support for this model is derived from studies in patients with anorexia nervosa who have elevated plasma ghrelin concentrations that return to a normal range after partial weight gain (Otto *et al.* 2001). Similarly, in rodents, circulating ghrelin concentrations are decreased in acute states of positive energy balance and are increased in fasting periods or in states of cachexia (Nagaya *et al.* 2001; Tschöp *et al.* 2001*a*; Ariyasu *et al.* 2002).

Controversy remains over to what extent postprandial ghrelin secretion depends on macronutritients, and this field needs further investigations in the future. Gomez *et al.* (2004) found that all nutrient types (i.e. carbohydrates, proteins and fats) inhibited ghrelin secretion equally in the fasted rat, whereas in human subjects ghrelin release was dependent on the ingested macronutrients (less suppression resulting from lipids than carbohydrates), as was described by Erdmann *et al.* (2003) and Overduin *et al.* (2005). Additionally, Callahan *et al.* (2004) concluded that postprandial ghrelin suppression was proportional to ingested energy load but was not a determinant of inter-meal interval. However, Erdmann *et al.* (2004) described a correlation between ghrelin release and the recurrence of hunger.

In summary, it appears that, independently of the (patho)physiological state or species, ghrelin is a signal to the CNS when acute or chronic energy supplies are insufficient (Tschöp *et al.* 2000). Ghrelin-induced positive energy balance is GH-independent and probably involves the modulation of a network of CNS cells, such as leptin-responsive neurones in specific regions of the hypothalamus and the brainstem (Horvath *et al.* 2001; Nakazato *et al.* 2001; Cowley *et al.* 2003; Faulconbridge *et al.* 2003). This is, however, probably not the only site of action of ghrelin. Indeed, there may be direct effects of ghrelin on adipose tissue (Ott *et al.* 2002), on the vagus nerve as a target of peripheral signal regulating energy balance (Asakawa *et al.* 2001), and on the HPA axis (Wren *et al.* 2000), where ghrelin may influence energy balance and adiposity as well.

Ghrelin and glucose homeostasis

Studies in man, as well as in experimental animals, have found a negative association between circulating ghrelin concentrations and insulin secretion (McCowen *et al.* 2002; Möhlig *et al.* 2002; Saad *et al.* 2002; Flanagan *et al.* 2003). Circulating ghrelin concentrations are suppressed independently of glucose during a hyperinsulinaemic clamp (Flanagan *et al.* 2003). These findings support the hypothesis that insulin is an important regulator of plasma ghrelin. One caveat, however, is that most of these studies used a hyperinsulinaemic–euglycaemic clamp, and therefore changes in ghrelin level may be secondary to the duration or concentration of postprandial hyperinsulinaemia. In contrast, Caixas *et al.* (2002) demonstrated that, 30–40 min after an oral glucose load or mixed liquid meal, ghrelin level was decreased by 28 % and 26 %, respectively. The parenteral administration of glucose and insulin (Humalog 0·03 U/kg subcutaneously) did not, however, suppress ghrelin concentrations, suggesting that changes in plasma insulin or glucose are not responsible for changes in ghrelin levels after food intake (Caixas *et al.* 2002, Schaller *et al.* 2003).

Obese patients with type 2 diabetes mellitus have lower fasting plasma ghrelin concentrations than normal-weight patients without diabetes (Shiiya *et al.* 2002). Low plasma ghrelin levels are independently associated with insulin resistance, hypertension and the prevalence of type 2 diabetes (Pöykkö *et al.* 2003). Plasma levels of ghrelin and insulin are similar in acromegalic patients with GH-induced insulin resistance to obese patients (Cappiello *et al.* 2002). Consistent with a ghrelin–insulin interaction, Pagotto *et al.* (2002) described lower ghrelin levels in patients with polycystic ovary syndrome, a syndrome characterized in part by decreased insulin sensitivity. Furthermore, obese human subjects, exhibit insulin resistance in association with elevated ghrelin levels, relative to non-insulin-resistant individuals (English *et al.* 2002). Additionally, Dezaki *et al.* (2004) recently demonstrated that endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca^{2+} signalling β-cells. These findings implicate ghrelin in the integrative regulation of energy homeostasis (Dezaki *et al.* 2004) and suggest that ghrelin might partially underlie some of the mechanisms associated with obesity and type 2 diabetes (Horvath *et al.* 2001).

Two recent reports examined the postprandial regulation of plasma ghrelin in the absence of insulin (patients with type 1 diabetes with and without a basal substitution of insulin analogue). The findings suggest that although ghrelin levels fail to decrease after meals in patients with type 1 diabetes, the ability to suppress postprandial ghrelin can be restored by the replacement of a basal amount of insulin or an insulin analogue (Murdolo *et al.* 2003; Spranger *et al.* 2003). Thus, a postprandial insulin peak does not seem to be required for the reduction in ghrelin secretion normally seen following food consumption, although some insulin is necessary. Collectively, these results suggest that glucose, and possibly NEFA, affect ghrelin secretion by activating the gastric ghrelin-secreting or the nephric ghrelin-clearing cells via an insulin receptor-mediated action.

Plasma ghrelin levels are decreased following gastric infusions of glucose (Tschöp *et al.* 2000). However, Williams *et al.* (2003) demonstrated that gastric infusions of either glucose or water did not decrease plasma ghrelin level when the pylorus was occluded. One conclusion from these studies is that meal-related plasma ghrelin suppression requires post-gastric absorption, which may be mediated via circulating serum factors rather than by intraluminally acting nutrient components.

Intriguingly, circulating ghrelin levels have been reported to be decreased following gastric bypass surgery (Cummings *et al.* 2002). Although several more recent studies have confirmed these findings, a few others have found no change or even an increase in circulating ghrelin following gastric bypass. The superior efficacy of this bariatric surgery, compared with any other treatment for obesity, has long been speculated to have an underlying endocrine mechanism. In fact, some have suggested that the surgery-induced ebbing of ghrelin from its main source in the stomach may represent such a mechanism.

Broglio *et al.* (2001) found that ghrelin administration induced a significant increase in plasma glucose level followed by a reduction in insulin secretion; the authors suggested that one mechanism of ghrelin action might be a direct stimulatory effect on glycogenolysis and insulin secretion. Additionally, it has been demonstrated that ghrelin blocks the inhibitory effects of insulin on gluconeogenesis (Murata *et al.* 2002). The recent findings of Dezaki *et al.* (2004) implicating the insulinostatic action of pancreatic islet origin, possibly together with that of circulating ghrelin, summarizes the function of ghrelin in regulating glucose metabolism. Through manipulations of the ghrelin–GHS-R system, Dezaki *et al.* (2004) hope to provide new tools to treat patients with hyperinsulinaemia, type 2 diabetes and obesity.

Ghrelin and gastroenteropancreatic function

The gastric hormone ghrelin also acts at the gastroenteropancreatic level, where both GHS-R 1a and 1b are expressed (Date *et al.* 2000). Interestingly, there is a close structural relationship between the precursor peptides of motilin and ghrelin, which share a 36 % sequence homology of the mature peptides (Asakawa *et al.* 2001). Additionally, Feighner *et al.* (1999) first demonstrated a high degree of structural homology between the gastrointestinal motilin receptor 1A and the GHS-R 1a receptor. Collectively, these findings suggest potential similar functions for ghrelin and motilin.

Indeed, ghrelin stimulates gastric acid secretion and gut motility in rats (Date *et al.* 2001; Trudel *et al.* 2002), whereas Masuda *et al.* (2000) demonstrated that these actions are mediated by the cholinergic system and are abolished by pre-treatment with atropine or vagotomy. Date *et al.* (2001) found that even the intracerebroventricular administration of ghrelin increased gastric acid secretion in a dose-dependent manner, suggesting that ghrelin signals from the gut to the brain as well as from the brain to the gut. This finding seems particularly intriguing since there are reports of a well-defined population of bipolar-shaped neurones in the internuclear hypothalamic space around the third ventricle that express small amounts of ghrelin (Cowley *et al.* 2003). Such reports, however, remain controversial as they are based on immunohistochemistry or PCR studies and await confirmation by *in situ* hybridization.

Stomach-derived ghrelin signalling starvation and GH release may be related to the CNS via the afferent vagal nerve (Date *et al.* 2002) as ghrelin given intravenously decreased the gastric vagal afferent (Asakawa *et al.* 2001; Date *et al.* 2002). Importantly, GHS-R 1a may be synthesized in vagal afferent neurones and transported to the afferent terminals (Date *et al.* 2002). Additionally, Sakata *et al.* (2003) described GHS-R-producing cells in the rat nodose ganglion; furthermore, it has been demonstrated that blockade of the gastric vagus abolishes gastric acid secretion (Masuda *et al.* 2000; Date *et al.* 2001) as well as ghrelin-induced food intake and GH secretion in rats (Date *et al.* 2002). In contrast, Sibilia *et al.* (2002) described a centrally mediated inhibitory role of ghrelin and synthetic GH secretagogues on acid secretion in rats. One conclusion from the current data is therefore that ghrelin signalling between the periphery and the brain involves classical endocrine mechanisms (i.e. via circumventicular organs) in hypothalamic and brainstem regions as well as neuronal pathways such as the vagal system. In addition, this communication may be sent from gut to brain as well as brain to gut, as outlined earlier.

Gastrectomy in rats reduces the circulating ghrelin concentration by approximately 80 %, showing that the stomach is the main source of endogenous ghrelin (Date *et al.* 2000). Furthermore, circulating ghrelin levels are correlated with gastric emptying in human subjects (Tschöp *et al.* 2001*a*), and Trudel *et al.* (2002) demonstrated a direct prokinetic effect of ghrelin by administering ghrelin, which reversed a gastric postoperative ileus in rats. Additionally, there was no significant change in ghrelin level after modified sham-feeding or non-nutritive gastric distension (Erdmann *et al.* 2003, Williams *et al.* 2003).

In human subjects, ghrelin (bolus injection of 3·3 μg/kg) stimulates circulating somatostatin and pancreatic polypeptide release by increasing glucose and decreasing insulin levels (Arosio *et al.* 2003). Additionally, ghrelin is a potent inhibitor of cholecystokinin-induced pancreatic exocrine secretion *in vivo* (rats) and *in vitro* (pancreatic lobules) (Zhang *et al.* 2001). This inhibitory action of ghrelin is indirect and exerted at the level of the intrapancreatic neurones (Zhang *et al.* 2001). Egido *et al.* (2002) stimulated insulin secretion from isolated rat pancreas with glucose, arginine and carbachol (acting via different mechanisms on the pancreatic β-cell) and found a blunted insulin response by exposure to ghrelin, as well as a reduced somatostatin response to arginine. These results suggest that endogenous ghrelin in islets acts on pancreatic β-cells to restrict glucose-induced insulin release, which was recently confirmed by Dezaki *et al.* (2004). These findings are consistent with the negative correlation between insulin and ghrelin plasma levels in human subjects (Cummings *et al.* 2001; Tschöp *et al.* 2001*a*; Möhlig *et al.* 2002). In summary, the involvement of ghrelin in multiple gastroenteropancreatic processes further supports the notion that this unusual hormone plays a physiologically relevant role in the endocrine control of energy balance.

Ghrelin – an important endogenous regulator of energy balance?

It remains to be shown whether the orexigenic and anabolic effects of ghrelin and the changes in its secretion are physiologically relevant to the control of body weight. Similarly, it is unclear whether the findings described here are simply artefacts arising from artificial administration routes, supraphysiological doses, non-human experimental models or non-specific detection methods. What is clear, however, is that very substantial experimental evidence suggests that ghrelin has a significant role in the regulation of metabolic processes. This is further supported by the fact that the molecular structure of ghrelin is well conserved throughout numerous species, indicating that the peptide might be essential for at least one of the biological functions on which survival is dependent. Once potent and specific pharmacological antagonists become available, significant progress in answering these open questions can be made. Apart from determining the main physiological role of ghrelin, modification of the ghrelin pathway with receptor antagonists and agonists might pave the way for new drug treatments for diseases such as obesity, diabetes, cachexia, chronic inflammation, heart failure and cancer.

References

Ariyasu H, Takaya K, Hosoda H, *et al.* (2002) Delayed short-term secretory regulation of ghrelin in obese animals: evidenced by a specific RIA for the active form of ghrelin. *Endocrinology* **143**, 3341–3350.

Arosio M, Ronchi CL, Gebbia C, Cappiello V, Beck-Peccoz P & Peracchi M (2003) Stimulatory effects of ghrelin on circulating somatostatin and pancreatic polypeptide levels. *J Clin Endocrinol* **88**, 701–704.

Arvat E, Maccario M, Di Vito L, *et al.* (2001) Endocrine activities of ghrelin, a natural growth hormone secretagogue (GHS), in humans: comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. *J Clin Endocrinol Metab* **86**, 1169–1174.

Asakawa A, Inui A, Kaga T, *et al.* (2001) Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* **120**, 337–345.

Banks WA, Tschöp M, Robinson SM, & Heiman ML (2002) Extent and direction of ghrelin transport across the blood-barrier is determined by its unique primary structure. *J Pharmacol Exp Ther* **302**, 822–827.

Barkan AL, Dimaraki EV, Jessup SK, Symons KV, Ermolenk M & Jaffe CA (2003) Ghrelin secretion in humans is sexually dimorphic, suppressed by somatostatin, and not affected by the ambient growth hormone levels. *J Clin Endocrinol Metab* **88**, 2180–2184.

Barreiro ML & Tena-Sempere M (2004) Ghrelin and reproduction: a novel signal linking energy status and fertility? *Mol Cell Endocrinol* **226**, 1–9.

Broglio F, Avat E, Benso A, Gottero C, Muccioli G, Papotti M, van der Lely AJ, Deghenghi R & Ghigo E (2001) Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *J Clin Endocrinol Metab* **86**, 5083–5086.

Broglio F, Arvat E, Benso A, Papotti M, Mucciolo G, Deghenghi R & Ghigo E (2002) Ghrelin: endocrine and non-endocrine actions. *J Pediatr Endocrinol Metab* **15**, 1219–1227.

Broglio F, Benso A, Castiglioni C, *et al.* (2003*a*) The endocrine response to ghrelin as a function of gender in humans in young and elderly subjects. *J Clin Endocrinol Metab* **88**, 1537–1542.

Broglio F, Benso A, Gottero C, Prodam F, Gauna C, Filtri L, Arvat E, van der Lely AJ, Deghenghi R & Ghigo E (2003*b*) Non-acylated ghrelin does not possess the pituitaric and pancreatic endocrine activity of acylated ghrelin in humans. *J Endocrinol Invest* **26**, 192–196.

Caixas A, Bashore C, Nash W, Pi-Sunyer F & Laferrere B (2002) Insulin, unlike food intake, does not suppress ghrelin in human subjects. *J Clin Endocrinol Metab* **87**, 1902–1906.

Callahan HS, Cummings DE, Pepe MS, Breen PA, Matthys CC & Weigle DS (2004) Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. *J Clin Endocrinol* **89**, 1319–1324.

Cassoni P, Papotti M, Gehe C, Catapano F, Sapino A, Graziani A, Deghenghi R, Reissmann T, Ghigo E & Muccioli G (2001) Identification, characterisation, and biological activity of specific receptors for natural (ghrelin) and synthetic growth hormone secretagogues and analogs in human breast carcinomas and cell lines. *J Clin Endocrinol Metab* **86**, 1738–1745.

Cappiello V, Ronchi C, Morpurgo PS, Epaminonda P, Arosio M, Beck-Peccoz P & Spada A (2002) Circulating ghrelin levels in basal conditions and during glucose tolerance test in acromegalic patients. *Eur J Endocrinol* **147**, 189–194.

Chen HY, Trumbauer ME, Chen AS, *et al.* (2004) Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. *Endocrinology* **145**, 2607–2612.

Cowley MA, Smith RG, Diano S, *et al.* (2003) The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* **37**, 649–661.

Cummings DE & Shannon MH (2003) Roles for ghrelin in the regulation of appetite and body weight. *Arch Surg* **138**, 389–396.

Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE & Weigle DS (2001) A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* **50**, 1714–1719.

Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP& Purnell JQ (2002) Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med* **346**, 1623–1630.

Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K & Nakazato M (2000) Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* **141**, 4255–4261.

Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, Kangawa K & Nakazato M (2002) The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* **123**, 1120–1128.

Date Y, Nakazato M, Murakami N, Kojima M, Kangawa K & Matsukura S (2001) Ghrelin acts in the central nervous system to stimulate gastric acid secretion. *Biochem Biophys Res Commun* **280**, 904–907.

Dezaki K, Hosoda H, Kakei M, Hashiguchi S, Watanabe M, Kangawa KJ & Yada T (2004) Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca^{2+} signaling in β-cells. *Diabetes* **53**, 3142–3151.

Egido EM, Rodriguez-Gallardo J, Silvestre RA & Marco J (2002) Inhibitory effect of ghrelin on insulin and pancreatic somatostatin secretion. *Eur J Endocrinol* **146**, 241–244.

English PJ, Ghatei MA, Malik IA, Bloom SR & Wilding JP (2002) Food fails to suppress ghrelin levels in obese humans. *J Clin Endocrinol Metab* **87**, 2984–2987.

Erdmann J, Lippl F & Schusdziarra V (2003) Differential effect of protein and fat on plasma ghrelin levels in man. *Regul Pept* **116**, 101–107.

Erdmann J, Töpsch R, Lippl F, Gussmann P & Schusdziarra V (2004) Postprandial response of plasma ghrelin levels to various test meals in relation to food intake, plasma insulin, and glucose. *J Clin Endocrinol Metab* **89**, 3048–3054.

Faulconbridge LF, Cummings DE, Kaplan JM & Grill HJ (2003) Hyperphagic effects of brainstem ghrelin administration. *Diabetes* **52**, 2260–2265.

Feighner SD, Tan CP, McKee KK, *et al.* (1999) Receptor for motilin identified in the human gastrointestinal system. *Science* **284**, 2184–2188.

Flanagan DE, Evans ML, Monsod TP, Rife F, Heptulla RA, Tamborlane WV & Sherwin RS (2003) The influence of insulin on circulating ghrelin. *Am J Physiol Endocrinol Metab* **284**, E313–E316.

Freda PU, Reyes CM, Conwell IM, Sundeen RE & Wardlaw SL (2003) Serum ghrelin levels in acromegaly: effects of surgical and long-acting octreotide therapy. *J Clin Endocrinol Metab* **88**, 2037–2044.

Gambineri A, Pagotto D, Tschöp M, Vicennati V, Manicardi E, Carcello A, Cacciari M, De Iasio R & Pasquali R (2003) Anti-androgen treatment increases circulating ghrelin levels in obese women with polycystic ovary syndrome. *J Endocrinol Invest* **26**, 629–634.

Gomez G, Englander EW & Greeley GH Jr (2004) Nutrient inhibition of ghrelin secretion in the fasted rat. *Regul Pept* **117**, 33–36.

Gualillo O, Caminos J, Blanco M, Garcia-Caballero T, Kojima M, Kangawa K, Dieguez C & Casanueva F (2001) Ghrelin, a novel placenta-derived hormone. *Endocrinology* **142**, 788–794.

Gualillo O, Lago F, Gomez-Reino J, Casanueva FF & Dieguez C (2003) Ghrelin, a widespread hormone: insights into molecular and cellular regulation of its expression and mechanism of action. *FEBS Letters* **552**, 105–109.

Hansen TK, Dall R, Hosoda H, Kojima M, Kangawa K, Christiansen JS & Jorgensen JO (2002) Weight loss increases circulating levels of ghrelin in human obesity. *Clin Endocrinol* **56**, 203–206.

Hewson AK & Dickson SL (2000) Systemic administration of ghrelin induces Fos and Egr-1 proteins in the hypothalamic arcuate nucleus of fasted and fed rats. *J Neuroendocrinol* **12**, 1047–1049.

Horvath TL, Diano S, Sotonyi P, Heiman M & Tschöp M (2001) Minireview: ghrelin and the regulation of energy balance – a hypothalamic perspective. *Endocrinology* **142**, 4163–4169.

Janssen JA, van der Toorn FM, Hofland LJ, van Koetsveld P, Broglio F, Ghigo E, Lamberts SW & van der Lely AJ (2001) Systemic ghrelin levels in subjects with growth hormone deficiency are not modified by one year of growth hormone replacement therapy. *Eur J Endocrinol* **145**, 711–716.

Jeon TY, Lee S, Kim HH, Kim YJ, Son HC, Kim DH, Sim MS (2004) Changes in plasma ghrelin concentration immediately after gastrectomy in patients with early gastric cancer. *J Clin Endocrinol Metab* **89**, 5392–5396.

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H & Kangawa K (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402**, 656–660.

Kojima M, Hosoda H & Kangawa K (2001) Purification and distribution of ghrelin: the natural endogenous ligand for the growth hormone secretagogue receptor. *Horm Res* **56**, 93–97.

Korbonits M, Kojima M, Kangawa K & Grossman AB (2001) Presence of ghrelin in normal and adenomatous human pituitary. *Endocrine* **14**, 101–104.

Leal-Cerro A, Torres E, Soto A, Dios E, Deghenghi R, Arvat E, Ghigo E, Dieguez C & Casanueva FF (2002) Ghrelin is no longer able to stimulate growth hormone secretion in patients with cushing's syndrome but instead induces exaggerated corticotropin and cortisol responses. *Neuroendocrinology* **76**, 390–396.

Lucidi P, Murdolo G, Di Loreto C, *et al.* (2004) Meal intake similarly reduces circulating concentrations of octanoyl and total ghrelin in humans. *J Endocrinol Invest* **27**, RC12–RC15.

McCowen KC, Maykel JA, Bistrian BR & Ling PR (2002) Circulating ghrelin concentrations are lowered by intravenous glucose or hyperinsulinemic euglycemic conditions in rodents. *J Endocrinol* **175**, R7–R11.

Masuda Y, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, Hosoda H, Kojima M, Kangawa K (2000) Ghrelin stimulates gastric acid secretion and motility in rats. *Biochem Biophys Res Commun* **276**, 905–908.

Möhlig M, Spranger J, Otto B, Ristow M, Tschöp M & Pfeiffer AF (2002) Euglycemic hyperinsulinemia, but not lipid infusion, decreases circulating ghrelin levels in humans. *J Endocrinol Invest* **25**, RC36–RC38.

Mori K, Yoshimoto A, Takaya K, *et al.* (2000) Kidney produces a novel acylated peptide, ghrelin. *FEBS Lett* **486**, 213–216.

Murata M, Okimura Y, Iida K, Matsumoto M, Sowa H, Kaji H, Kojima M, Kangawa K & Chihara K (2002) Ghrelin modulates the downstream molecules of insulin signaling in hepatoma cells. *J Biol Chem* **277**, 5667–5674.

Murdolo G, Lucidi P, Di Loreto C, Parlanti N, De Cicco A, Fatone C, Fanelli CG, Bolli GB, Santeusanio F & De Feo P (2003) Insulin is required for prandial ghrelin suppression in humans. *Diabetes* **52**, 2923–2927.

Nagaya N, Uematsu M, Kojima M, *et al.* (2001) Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. *Circulation* **104**, 1430–1435.

Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K & Matsukura S (2001) A role for ghrelin in the central regulation of feeding. *Nature* **409**, 194–198.

Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, Frost GS, Ghatei MA, Coombes RC & Bloom SR (2004) Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. *J Clin Endocrinol Metab* **89**, 2832–2836.

Olszewski PK, Li D, Grace MK, Billington CJ, Kotz CM & Levine AS (2003) Neural basis of orexigenic effects of ghrelin acting within lateral hypothalamus. *Peptides* **24**, 597–602.

Ott V, Fasshauer M, Dalski A, Meier B, Perwitz N, Klein HH, Tschop M & Klein J (2002) Direct peripheral effects of ghrelin include suppression of adiponectin expression. *Horm Metab Res* **34**, 640–645.

Otto B, Cuntz U, Fruehauf E, Wawarta R, Folwaczny C, Riepl RL, Heiman ML, Lehnert P, Fichter M & Tschop M (2001) Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. *Eur J Endocrinol* **145**, 669–673.

Otto B, Tschöp M, Heldwein W, Pfeiffer AFH & Diederich S (2004) Endogenous and exogenous glucocorticoids decrease plasma ghrelin levels in humans. *Eur J Endocrinol* **151**, 113–117.

Overduin J, Frayo RS, Grill HJ, Kaplan JM & Cummings DE (2005) Role of the duodenum and macronutrient type in ghrelin regulation. *Endocrinology* **146**, 845–850.

Pagotto U, Gambineri A, Pelusi C, Genghini S, Cacciari M, Otto B, Castaneda T, Tschop M & Pasquali R (2003) Testosterone replacement therapy restores normal ghrelin in hypogonadal men. *J Clin Endocrinol Metab* **88**, 4139–4143.

Pagotto U, Gambineri A, Vicennati V, Heiman ML, Tschöp M & Pasquali R (2002) Plasma ghrelin, obesity, and the polycystic ovary syndrome: correlation with insulin resistance and androgen levels. *J Clin Endocrinol Metab* **87**, 5625–5629.

Pöykkö S, Kellokoski E, Hörkkö S, Kauma H, Kesäniemi YA & Ukkola O (2003) Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. *Diabetes* **52**, 2546–2553.

Ravussin E, Tschöp M, Morales S, Bouchard C & Heiman ML (2001) Plasma ghrelin concentration and energy balance: overfeeding and negative energy balance studies in twins. *J Clin Endocrinol Metab* **86**, 4547–4551.

Saad MF, Bernaba B, Hwu C, Jinagouda S, Fahmi S, Kogosov E & Boyadjian R (2002) Insulin regulates plasma ghrelin concentration. *J Clin Endocrinol Metab* **87**, 3997–4000.

Sakata I, Yamazaki M, Inoue K, Hayashi Y, Kangawa K & Sakai T (2003) Growth hormone secretagogue receptor expression in the cells of the stomach-projected afferent nerve in the rat nodose ganglion. *Neurosci Lett* **342**, 183–186.

Schaller G, Schmidt A, Pleiner J, Woloszczuk W, Wolzt M & Luger A (2003) Plasma ghrelin concentrations are not regulated by glucose or insulin: a double-blind, placebo-controlled crossover clamp study. *Diabetes* **52**, 16–20.

Schofl C, Horn R, Schill T, Schlosser HW, Muller MJ, Brabant G (2002) Circulating ghrelin levels in patients with polycystic ovary syndrome. *J Clin Endocrinol Metab* **87**, 4607–4610.

Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe S, Hosoda H, Kangawa K & Matsukura S (2002) Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab* **87**, 240–244.

Shimbara T, Mondal MS, Kawagoe T, Toshinai K, Koda S, Yamaguchi H, Date Y & Nakazato M (2004) Centrally administration of ghrelin preferentially enhanced fat ingestion. *Neurosci Lett* **369**, 75–79.

Sibilia V, Pagani F, Guidobono F, Locatelli V, Torsello A, Deghenghi R & Netti C (2002) Evidence for a central inhibitory role of growth hormone secretagogues and ghrelin on gastric acid secretion in conscious rats. *Neuroendocrinology* **75**, 92–97.

Spranger J, Ristow M, Otto B, Heldwein W, Tschop M, Pfeiffer AF & Mohlig M (2003) Postprandial decrease of human plasma ghrelin in the absence of insulin. *J Endocrinol Invest* **26**, RC19–RC22.

Tang-Christensen M, Vrang N, Ortmann S, Bidlingmaier M, Horvath TL & Tschop M (2004) Central administration of ghrelin and agouti-related protein (83–132) increases food intake and decreases spontaneous locomotor activity in rats. *Endocrinology* **145**, 4645–4652.

Toshinai K, Date Y, Murakami N, *et al.* (2003) Ghrelin-induced food intake is mediated via the orexin pathway. *Endocrinology* **144**, 1506–1512.

Trudel L, Tomasetto C, Rio MC, Bouin M, Plourde V, Eberling P & Poitras P (2002) Ghrelin/motilin-related peptide is a potent prokinetic to reverse gastric postoperative ileus in rat. *Am J Physiol* **282**, G948–G952.

Tschöp M, Flora DB, Mayer JP & Heiman ML (2002) Hypophysectomy presents ghrelin-induced adiposity and increases gastric ghrelin secretion in rats. *Obes Res* **10**, 991–999.

Tschöp M, Smiley DL & Heiman ML (2000) Ghrelin induces adiposity in rodents. *Nature* **407**, 908–913.

Tschöp M, Wawarta R, Riepl RL, Friedrich S, Bidlingmaier M, Landgraf R & Folwaczny C (2001a) Post-prandial decrease of circulating human ghrelin levels. *J Endocrinol Invest* **24**, RC19–RC21.

Tschöp M, Weyer C, Tataranni AP, Denarayan V, Ravussin E & Heiman ML (2001b) Circulating ghrelin levels are decreased in human obesity. *Diabetes* **50**, 707–709.

Volante M, Fulcheri E, Alla E, Cerrato M, Pucci A & Papotti M (2002) Ghrelin expression in fetal, infant, and adult human lung. *J Histochem Cytochem* **50**, 1013–1021.

Vulliemoz NR, Xiao E, Xia-Zhang L, Germond M, Rivier J & Ferin M (2004) Decrease in luteinizing hormone pulse frequency during a five-hour ghrelin infusion in the ovariectomized rhesus monkey. *J Clin Endocrinol Metab* **89**, 5718–5723.

Williams DL, Cummings DE, Grill HJ & Kaplan JM (2003) Meal-related ghrelin suppression requires postgastric feedback. *Endocrinology* **144**, 2765–2767.

Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA & Bloom SR (2001) Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* **86**, 5992–5995.

Wren AM, Small CJ, Ward HL, *et al.* (2000) The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* **141**, 4325–4328.

Zhang W, Chen M, Chen X, Segura BJ & Mulholland MW (2001) Inhibition of pancreatic protein secretion by ghrelin in the rat. *J Physiol* **537**, 231–236.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L & Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**, 425–432.

Zigman JM & Elmquist JK (2003) Minireview: from anorexia to obesity - the yin and yang of body weight control. *Endocrinology* **144**, 3749–3756.