THE ROLE AND INTERACTIONS OF GHRELIN CONCERNING THE NUTRITIONAL AND INFLAMMATORY STATUS

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THE ROLE AND INTERACTIONS OF GHRELIN CONCERNING THE NUTRITIONAL AND INFLAMMATORY STATUS (Abstract): Ghrelin is an important neuroendocrine peptide having as main purpose the stimulation of growth hormone (GH) secretion. It is also an important regulator of the long-term energy balance and short-term nutritional intake. Ghrelin has several other biological actions, among which the capacity to regulate gastrointestinal motility, to modulate the reproductive and stress axes as well as the glucose metabolism, and other well-defined actions within the cardiovascular and renal physiology. Due to its numerous effects, ghrelin is considered on one hand a potential target in the treatment of obesity and on the other, a therapeutic option in other dysfunctions and illnesses. Keywords: GHRELIN, GHRELIN-O-ACYL-TRANSFERASE, INFLAMMATION, OBESITY, CACHEXIA.

The term “ghrelin” originates from the Proto-Indo-European language, where “ghre” means “to grow” and “relin” means “release”. The gene for ghrelin is located on chromosome 3p26-p25, encoding a protein of 117 amino acids named pro-ghrelin (1). This protein is processed post-transcriptionally into a 28 amino acid and 3.3 kDa peptide, synthesized especially in X/A-like cells of the gastric mucosa and to a smaller degree in the entire organism. X/A-like cells are a type of endocrine cells situated in the middle and lower regions of the gastric oxyntic glands that represent about 20-30% of all gastric endocrine cells, therefore occupying the second place within the gastric endocrine cell populations (2).

There are two isoforms of mARN for pro-ghrelin, one for ghrelin and the other one for des-Gln⁰⁴-ghrelin precursors, and they are produced in the rat stomach by an alternative splicing mechanism (3). Des-Gln⁰⁴-ghrelin is found in limited amounts only in the stomach, which proves that the physiologically important peptide is actually ghrelin, being the one released in the general circulation (3).

In the serum, ghrelin is found in 2 forms: non-acylated ghrelin, predominant in the plasma and acylated ghrelin. The latter is produced post-translationally by esterification of the hydroxyl group of the third amino acid residue, serine, by n-octanoic fatty acid, increasing the hydrophobicity of the molecule. This change allows acylated ghrelin to bind to the growth hormone secretagogue receptor 1a...
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(GHS-R1a), manifesting thereby its biological activities including growth hormone secretion, appetite regulation, modulation of insulin secretion, and stimulation of adiposity (4).

The enzyme responsible for ghrelin acylation was found in humans in 2007 as the forth member of the membrane-bound O-acyl-transferase family and was named GOAT (ghrelin-O-acyl-transferase). It is a well-conserved protein across vertebrates with a similar sequence in humans, rat and mouse (74.5% identity and 98.4% similarity). The endocrine cells expressing GOAT are found in the murine stomach, especially in the middle portion of the oxyntic glands, a localization which corresponds to the X/A-like cells that secrete ghrelin (5).

In rats and mice, GOAT has also been found in the plasma, which could suggest an extracellular ghrelin acylation. GOAT concentration after 24 hours of fasting increases significantly and can lead to an increase in acylated ghrelin concentration, enhancing the orexigenic effect of the latter. This represents a supplementary mechanism, apart from the gastric ghrelin mRNA expression increase as a response to fasting, to the decrease in ghrelin concentration in the X/A-like cells and increase of plasma levels of ghrelin in mice, as well as in rats.

Ghrelin has a wide distribution in human tissues and the distribution of GOAT follows the same pattern (6), allowing therefore active ghrelin to manifest its effects. GOAT expression is lower than that of ghrelin, without a statistical correlation between the expression of ghrelin and GOAT mRNA. The mRNA for GOAT is mostly found in the pancreas, stomach and pituitary, where the endocrine, paracrine and autocrine effects of ghrelin have been described.

The importance of GOAT and ghrelin in human physiology was demonstrated by knock-out studies on mice under severe caloric restriction (60%). The absence of ghrelin with the consecutive decrease in growth hormone leads after 7 days to severe hypoglycemia in the hours that precede feeding (7).

Ghrelin receptor has been known long before the discovery of ghrelin in 1999. The cells of the anterior pituitary present a receptor that, once activated, stimulates growth hormone secretion. This receptor has been named the growth hormone secretagogue receptor (GHS-R). It is encoded by a gene localized on chromosome 3q26.2. There are two types of GHR: GHS-R1a with a more limited distribution and GHS-R1b, with inhibitory effects on the GHS-R1a signaling, much more widely distributed in tissues (8). GHS-R1a is a G-protein coupled receptor that contains 366 amino acids and 7 transmembrane domains.

Ghrelin’s actions on the energy balance are mediated partially through its effects on the centers of appetite, such as the central melanocortin system. Ghrelin activates GHS-R localized in the pituitary and the neurons that contain Growth Hormone Releasing Hormone (GHRH) in the hypothalamic arcuate nucleus, thereby stimulating the secretion of GH. Activating the GHS-R situated on the arcuate and ventromedial neurons leads to an increase in the expression and release of orexigenic peptides neuropeptide Y (NPY) and agouti related-peptide and to the decrease of the expression of pro-opiomedanocortin, with its anorexigenic effects. These effects lead to a decrease in the signaling taking place on the melanocortin-4 receptor and an increase in signaling in the Y1 receptor, re-
sulting in the accentuation of food-seeking behavior and in the decrease of basal metabolic rate (9).

**Ghrelin and other hormones**

The stimulatory effect of ghrelin on GH secretion is specific, potent, dose-dependent and synergistic with that of GHRH. In humans, ghrelin induces a significant and durable increase in the level of circulating GH, an effect more potent than that produced by GHRH itself.

Central regulation of feeding is based on ghrelin and leptin, which signal the nutritional status and the level of energy deposits to the hypothalamic feeding centers. Ghrelin is orexigenic when administered both centrally as well as peripherally and is one of the humoral signals regulating the appetite. Leptin on the other hand is an anorexigenic hormone secreted by the adipose tissue, involved in thermogenesis and the control of several neuroendocrine functions of the hypothalamic-pituitary-adrenal axis.

Knock-out mice for ghrelin as well as knock-out mice for GHS-R are not anorexic dwarfs as expected; their size, growth rate, food intake, body composition, reproduction, behavior and tissue pathology are not different from those of the wild types (10). Fasting leads to an identical decrease in serum leptin and insulinenia in mutant mice as well as in the wild ones, demonstrating that ghrelin does not have a direct regulatory effect on leptin or insulin. Knock-out mice for ghrelin present normal responses to starvation and diet-induced obesity (10). As in wild mice, exogenous ghrelin administration stimulates appetite (10). Insulin-like Growth Factor 1 (IGF1) level and weight of the mature GHS-R null mice are slightly reduced when compared to the wild ones, which demonstrates the effect of ghrelin as an amplifier of GH pulsatility and the supposed role to maintain a certain set-point of IGF1 in the anabolic metabolism. Therefore, ghrelin is not critically necessary for the viability, fertility, growth, appetite, mineral bone density and adipose accumulation and probably is not a direct regulator of leptin and insulin and consequently, ghrelin antagonists are unlikely an anti-obesity solution (10).

The fasting ghrelin level is strongly associated to insulinemia (negative correlation), insulin resistance (negative correlation) and the high density lipoprotein cholesterol levels (positive correlation). Ghrelin suppression induced by food intake is correlated to the postprandial increase in insulin (11).

Ghrelin level is similar in men and women (11) and does not vary with the menopausal status or hormonal substitution therapy, demonstrating that ghrelin level is not regulated by estrogens in women.

**Ghrelin and obesity**

In humans, ghrelin concentrations double preprandially and decrease to basal values one hour postprandially. In negative energy balance situations such as hypocaloric diets, chronic physical exercises, cancer anorexia, anorexia nervosa and Prader-Willi syndrome, ghrelin concentrations are increased (12). In the obese, ghrelin concentrations are reduced, which can be a result of the important caloric intake, while a weight loss in the obese leads to an increase in ghrelin concentrations. Although ghrelin is increased in patients under certain diets, gastric bypass surgery reduces ghrelin concentrations, suggesting that the size of the stomach can be directly related to ghrelin concentra-
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Ghrelin plasma concentrations evolve in a divergent manner in obesity and anorexia nervosa after dietary intervention, suggesting that ghrelin is a good marker of the nutritional status (13).

Resistance to insulin and hyperinsulinemia are inversely correlated to the ghrelin concentration, which can represent a part of the feedback mechanism that regulates body weight in humans.

A study that has followed the dynamics of ghrelin in obese patients that were subject to a diet for 6 months and in obese patients operated through gastric bypass has shown that the weight loss induced by diet of 17% of the initial weight was associated to a significant increase in ghrelin, demonstrating its role in long-term regulation of body weight. This role is supported by studies on murine models that prove that continuous ghrelin administration increases in a durable manner the body weight (14). Not only does the caloric intake increase, but with the administration of exogenous ghrelin there is also a decrease in the metabolic rate and of the catabolism of fat (14), leading to modifications of all the energy regulation systems that may eventually lead to weight gain. Blocking the cerebral action of ghrelin leads to a reduction in food intake, which suggests that endogenous ghrelin is necessary to maintain a normal appetite. It appears that the increase in ghrelin levels contributes to the adaptive responses that limit weight gain.

In operated patients with a weight loss of 36% of the initial weight, ghrelin level is significantly lower when compared to the obese subjects that represented the controls, as well as when compared to normal-weight subjects. Normal ghrelin fluctuations related to meals and the diurnal secretion rhythm are suppressed after gastric bypass (15). This suggests the role of ghrelin decrease in the weight loss and appetite reduction in subjects operated by gastric bypass, effects that cannot only be explained by the gastric restriction created through the surgical intervention. These patients have a diminished sense of hunger and they spontaneously reduce their intake of high-caloric foods, although they are still capable to appreciate the taste of food. The explication that the authors propose (15) for the decrease in ghrelin levels could be an override inhibition: normally, the absence of nutrients in contact with the gastric mucosa stimulates ghrelin secretion. In the case of gastric bypass, the permanent absence of food in the stomach and duodenum leads to a continuous stimulatory signal that eventually suppresses the production of ghrelin by a process named override inhibition.

Another study (16) has investigated the evolution of ghrelin in obese and anorexia nervosa patients, in obese and normal-weight type 2 diabetics, as well as ghrelin evolution in response to the oral glucose uptake test in normal subjects and to a test meal in diabetics. Ghrelin concentrations were higher in patients with anorexia nervosa and lower in the obese when compared to normal-weight control subjects. Tschop et al. (17) have reported that fasting ghrelin is negatively correlated to the percentage of body adiposity, fasting insulin and leptin concentrations. The study conducted by Shiiya et al. (16) has proven that fasting ghrelin in normal subjects, in those with anorexia nervosa, obesity or type 2 diabetes is negatively correlated to the body mass index of each group. Oral or intravenous glucose administration in normal subjects leads to a decrease in serum
levels of ghrelin, while the administration of a similar volume of water does not, proving that the effect on ghrelin concentration is not related to gastric expansion.

**Ghrelin and cachexia**

Cachexia is a constellation of symptoms that lead to the wasting syndrome, an increase in the metabolic rate that favors the loss of lean and fat mass and a paradoxical decrease in appetite, persistent in spite of the depleted energy stores. Cachexia appears in the pathophysiological context of various chronic illnesses, such as cancer, cardiac failure, chronic kidney disease and acquired immunodeficiency syndrome. Cachexia is a negative prognostic factor for the underlying illness. In this context, a promising pharmacologic agent is represented by the ghrelin receptor analogue. Ghrelin binds to the GHS-R1a in the cerebral appetite regulatory centers, leading to the increase in expression of Y neuropeptide and agouti-related peptide during short-term treatment. It also presents anti-inflammatory properties, which is even more important when taking into consideration the fact that another factor producing cachexia is the inflammation induced by the initial pathology. In animal studies, the efficacy of GHS-R1a agonists was demonstrated in the treatment of cancer-induced cachexia, chemotherapy and chronic renal disease. Human studies using ghrelin or ghrelin receptor analogues in cancer or cardiac diseases have proven an improvement in appetite and body weight during short-term treatment.

Cachexia is a common characteristic in malignancies, found in up to 85% of the patients with several types of cancer and contributing to over 20% of deaths by cancer, underlining thereby the necessity of an efficient treatment. Weight loss presented by these patients can be severe, with the loss of up to 75% of the lean mass, although even the smallest degrees of weight loss and anorexia are associated to the worsening of prognosis, of the response to chemotherapy and an increased morbidity. Loss of appetite can be a sign of particular importance seeing as in a study on patients with cancer in terminal phases it was proven that the presence of nausea and vomiting is associated to a 68% decrease in survival and contributes to a substantial decrease in the quality of life (18). As in most illnesses associated with cachexia, the presence of underlying inflammation plays an important role in producing cancer cachexia and can represent a target of ghrelin action. Proinflammatory cytokines including IL-1β, IL-6 and TNF-α can be produced by the tumor cells and as well by the host as a response to the tumor. Up to 50% of cancer patients present inflammatory signs at diagnosis and the associated increase in cytokines is involved in the appearance of anorexia, at least partly through its action on the melanocortin central system.

The level of ghrelin was already demonstrated to be increased with approximately 25% compared to normal values in a variety of cancers associated with cachexia, including pulmonary, breast, colon and prostate neoplasia. Although there may be ghrelin secreting neoplasias, the levels of acyl-ghrelin are 50% higher in cancer patients with cachexia than those without cachexia and 80% higher than in non-cancer patients (19). The presence of anorexia despite higher concentrations of acyl-ghrelin suggests either the presence of a certain resistance to the orexigenic properties of ghrelin in the context of cancer cachexia, or due to the exaggerated anorexia-
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genic effects of other processes. The increase of these values can be associated with a saturation of ghrelin receptors, making the pharmacological doses inefficient. Moreover, there is the possibility that these high levels of ghrelin produce unwanted effects such as tumor growth.

The majority of the interventions that administered ghrelin in models of cancer-induced cachexia have shown the improvement of food intake and weight gain, especially due to increase in lean mass. The attempts to treat cancer cachexia with ghrelin have required supraphysiological doses, which have had positive effects on appetite, suggesting that the effect of ghrelin on the appetite regulation centers is not saturated in cancer cachexia. The use of ghrelin and other GHS-R1a agonists on animal models of cancer cachexia has shown a 20-37% increase in food intake, a 10% increase in body weight and a reversal of the losses of lean and fat masses. In humans, the long-term treatment using a GHS-R1a agonist called RC-1291 for a 12-week period has lead to weight gain and increase of the lean mass when compared to placebo (20). However, when measuring the quality of life – important criteria in the treatment of any advanced cancer – there have been no differences between the group treated with the agonist and the placebo group. The problem that is raised is that of the risk of tumor growth that could be stimulated by the increase in GH secretion as a response to GHS-R1a agonists of ghrelin. The study conducted by Garcia (20) has reported a significant growth in the level of IGF1 and IGF1-binding protein 3 in subjects that received the ghrelin receptor analogue compared to the placebo group, without it being clear which type of cancer is more at risk of being developed due to high levels of IGF1. In acromegalic patients with chronic high levels of IGF1, the only more frequently encountered neoplasia is the colonic one.

Another study conducted on 31 patients with progressive, non-responsive gastrointestinal cancers (21) that received low or high doses of ghrelin during an 8-week period has shown that subjects that received high doses reported an increase in appetite, although food intake remained the same. The group receiving high doses of ghrelin presented a decrease in fat mass and a tendency to increase the lean mass, as well as a positive energy balance. Subjects in both groups had a decrease between 9-28% of IGF1, proving that the effect of ghrelin on the body weight can be achieved without an increase in food intake or IGF1 levels.

Ghrelin treatment after the gastric tumor resection has determined a decreased weight loss, although this can be partially due to the replacement of the normal endogenic secretion of ghrelin in the stomach. Another problem is the possibility that a certain neoplasia might express GHS-R that could respond to the treatment with ghrelin. In a study lead by Wang et al. (22), 51-80% of various types of gastrointestinal cancers expressed GHS-R, but it is unclear whether ghrelin can actually induce tumor growth by stimulating these receptors.

CONCLUSIONS

Ghrelin is a recently-discovered hormone with essential effects on the nutritional status. The understanding of its physiology can help explain the disturbances found in several nutrition-related pathologies and can support the development of therapeutic possibilities using ghrelin or its analogues.
REFERENCES