Ghrelin in Obesity, Physiological and Pharmacological Considerations

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Abstract:

The aim of this review is to summarize the physiological and pharmacological aspects of ghrelin. Obesity can be defined as an excess of body fat and is associated with significant disturbances in metabolic and endocrine function. Obesity has become a worldwide epidemic. In obesity there is a decreased growth hormone (GH) secretion, and the altered somatotroph secretion in obesity is functional. Ghrelin is a peptide that has a unique structure with 28 amino-acids and an n-octanoyl ester at its third serine residue, which is essential for its potent stimulatory activity on somatotroph secretion. The pathophysiological mechanism responsible for GH hyposecretion in obesity is probably multifactorial, and there is probably a defect in ghrelin secretion. Ghrelin is the only known circulating orexigenic factor, and has been found to be reduced in obese humans. Ghrelin levels in blood decrease during periods of feeding. Due to its orexigenic and metabolic effects, ghrelin has a potential benefit in antagonizing protein breakdown and weight loss in catabolic conditions such as cancer cachexia, renal and cardiac disease, and age-related frailty. Theoretically ghrelin receptor antagonists could be employed as anti-obesity drugs, blocking the orexigenic signal. By blocking the constitutive receptor activity, inverse agonists of the ghrelin receptor may lower the set-point for hunger, and could be used for the treatment of obesity. In summary, ghrelin secretion is reduced in obesity, and could be partly responsible for GH hyposecretion in obesity, ghrelin antagonist or partial inverse agonists should be considered for the treatment of obesity.

Keywords: Obesity, ghrelin, appetite regulation, weight homeostasis, gastrointestinal hormones.

1. INTRODUCTION

1.1. Obesity

Obesity can be defined as an excess of body fat. Its clinical management is complex and frequently unsuccessful [1]. A surrogate marker for body fat content is the body mass index (BMI), which is determined by weight (kilograms) divided by height squared (square meters). In clinical terms, a BMI of 25–29.9 kg/m^2 is defined as overweight, while higher BMIs (≥30 kg/m^2) are defined as obesity [2] (Table 1).

Among children and adolescents between the ages of 2 to 19 years, overweight has been defined based on the sex- specific BMI for age growth charts from the Centers for Disease Control and Prevention. Overweight has been defined as a BMI for age at or above the sex-specific 95th percentile [3]. A better way to define obesity should be in terms of the percentage of body fat, defining obesity as 25% or greater in men and 35% or greater in women.

Obesity is a chronic disease like hypertension and diabetes. The aetiology of obesity is an altered balance between the energy ingested and the energy expended. The excess energy is stored in fat cells that enlarge and/or increase in number. This hyperplasia and hypertrophy of fat cells is the pathological lesion of obesity. Enlarged adipose tissue produces the clinical problems associated with obesity, either because of the weight or mass of the extra fat or because of the increased secretion of free fatty acids and peptides from fat cells. The consequences of these two mechanisms are different diseases, such as type 2 diabetes, hypertension, gallbladder disease, osteoarthritis, ischemic heart disease, some types of cancer, social and psychological disabilities [4]. Obesity is associated with an increased risk of diabetes, hypertension, dyslipidemia, cardiovascular disease, osteoarthropathy, non-alcoholic steatohepatitis and cancer [5]. The risk fraction for type 2 diabetes due to obesity is 77% for women and 64% for men [6]. Mortality due to overweight and obesity is a very important public health problem: in Europe 7.7% of all deaths are related to excess weight [7]. The relative risk of death at age 50 with a BMI 2:40 kg/m^2, compared with normal weight, in men and women who never smoke is 3.82 to 3.79 respectively [8]. It has been confirmed that in adults, overweight and obesity are associated with increased mortality, and that all-cause mortality is generally lowest with

a BMI of 20.0 to 24.9 [9]. Direct sanitary costs of patients with a BMI of IMC ≥35 kg/m^2 are three times greater, when compared to patients of normal weight [10].

Obesity and overweight are worldwide epidemic. The prevalence of excess weight is increasing rapidly across United States (USA), and close to 65% of the adult population is overweight or obese [11]. If we compare the period 1976-1980 with 1999-2000 [11], the prevalence of overweight has increased by 40% (from 46 to 64.5%) and the prevalence of obesity has risen by 110% (from 14.5 to 30.5%). We are also witnessing an alarming increase in weight among young people. More than 10% of 2-5 year olds and 15% of 6-19 year olds are overweight (BMI> 95th percentile for age and gender) [3]. This represents nearly twice the number of overweight children and a nearly three times the number of overweight adolescents over the last two decades.

International data indicate that the epidemic is a global health problem. The prevalence of obesity in Europe is 15-25% for women and 10-20% for men, and nearly half of all Europeans are overweight or obese (BMI ≥25 kg/m^2) [12]. The prevalence of extreme or morbid obesity (BMI ≥40 kg/m^2) in the USA in 2002 was 1.8%, 60% of whom were women and 63% of whom were between the ages 18 and 49 years [13]. The prevalence of extreme obesity in the USA continues to rise, with more recent data indicating a figure of 4.8% [3]. The prevention of obesity must be a public health priority; it has been found that specific lifestyle and dietary factors are associated with marked weight gain, with a substantial aggregate effect and implications for strategies to prevent weight gain [14]. In Spain recent studies have found that the prevalence of obesity is 22.9% (24.4% in men and 21.4% in women). About 36% of adults (32% of men and 39% of women) have abdominal obesity (defined as a waist circumference >102 cm in men and > 88 in women). The frequency of obesity and of abdominal obesity increases with age, respectively affecting 35% and 62% of persons aged 65 and over. The frequency of obesity and abdominal obesity decreases with increasing educational level. For example, 29% of women with primary education or less are obese compared to only 11% of those with university studies [15].

A variety of endocrine and metabolic changes are associated with overweight and obesity [16,17] (Table 2). Most of these changes are secondary because they can be induced by overfeeding and reversed by weight loss. It is not completely clear if some of the hormonal changes may contribute to the pathophysiology or to perpetuate the obese state.

1.2. Ghrelin

In 1976, it was found that modified opioid peptides had low GH secretory activity [18]. Since then, many efforts have been made to develop and improve potential applications of these GH secretagogues (GHSs) [19-23]. GHSs act on the pituitary and hypothalamus to release GH through the GH secretagogue receptor (GHS-R) [24,25]. These facts indicated that an unknown endogenous ligand for GHS-R should exist. In 1999, ghrelin was identified as the endogenous ligand for the GHS-R [26].

Ghrelin is a peptide that is predominantly produced by the stomach that has a unique structure with 28 amino-acids and an n-octanoyl ester at its third serine residue, which is essential for its potent stimulatory activity on somatotroph secretion. It displays strong growth hormone-releasing activity [26], mediated by GHS-R. The unacylated form of ghrelin does not have any direct endocrine activity and does not show any GH-releasing activity [26-28]. Apart from stimulating GH secretion, ghrelin has other endocrine and nonendocrine actions including the stimulation of corticotroph and lactotroph secretion, inhibition of the gonadal axis, stimulation of appetite, control of gastric motility, influencing both endocrine and exocrine pancreatic function, cardiovascular actions, influencing on sleep and behavior, and modulation of the immune system and cell proliferation [29].

The X/A-like cells of the gastric oxyntic glands of the stomach are the most abundant source of circulating ghrelin. The small intestine also synthesizes ghrelin to a lesser extent, with the amount of ghrelin produced diminishing with increasing distance from the pylorus [30]. Ghrelin is acylated with an eight-carbon fatty acid. This post-translational modification is mediated by ghrelin O-acyltransferase (GOAT) [31] and is essential for GHS-R type 1a (GHS-R1a) activation and the direct endocrine actions of ghrelin [27]. GOAT was cloned in 2008 [31] and consistent with its function, GOAT mRNA is primarily limited to the stomach; the main ghrelin-secreting tissue [31]. Ghrelin concentrations in blood mainly include des-acyl ghrelin (85%–90%) and in lesser amounts acyl ghrelin (10%–15%) and C-terminal proghrelin peptides [32]. Ghrelin is higher in women tan men [33] and declines with age, BMI, hypertension, and other markers of the metabolic syndrome [33]. Appropriate sample collection and storage are necessary to limit ghrelin deacylation before assay. In human blood, it is not easy to accurately determine acyl ghrelin. Recent studies have evaluated the different methods

for the stabilization of acyl ghrelin in human blood collections, in order to improve its stability [34].

Unacylated ghrelin is not biologically inactive, and is capable of sharing antiproliferative effects on human and prostate cancer cell lines with ghrelin, as well as negative inotropic effect on cardiac papillary muscle [35] and the stimulation of bone marrow adipogenesis [36]. Multiple biological effects have been reported for unacylated ghrelin, which does not bind to GHS-R1a. These effects include the differentiation of skeletal muscle, relaxation of vascular smooth muscle, suppression of proinflammatory cytokines, inhibition of the apoptosis of cardiomyocytes, pancreatic beta cells and endothelial cells, antagonism of the stimulation by ghrelin of somatic growth, hepatic gluconeogenesis, and appetite, hypotensive effects, repression of fatty-acid oxidation, and stimulation of adipogenesis [29,37]. Although local acylation could explain certain actions of unacylated ghrelin, other opposite or different effects from those of acylated peptide raise the possibility of non- GHS- R1a mediation. To date no other GHS-R have been cloned to explain this data [38].

Ghrelin plays a role in the neuroendocrine and metabolic response to food intake. The orexigenic effect of ghrelin was first discovered in the animal model [39]. GHS-R1a in the arcuate is involved in the regulation of GH secretion, food intake, and adiposity [40]. Ghrelin levels increase following weight loss achieved either by diet alone or diet and exercise, and are suppressed by overfeeding or treatment of anorexia nervosa [41,42].

Based on the bioinformatic prediction that there was another peptide also derived from the precursor proghrelin exists, a hormone named obestatin was isolated. Treatment of rats with obestatin suppressed food intake, inhibited jejunal contraction, and decreased body-weight gain. Obestatin was thought to bind to the G-protein-coupled receptor GPR39. As a result, two peptide hormones with opposite actions in weight regulation are derived from the same ghrelin gene [43]. This 23-amino-acid fragment of preproghrelin, has not fulfilled its original nomenclature as an antagonist of ghrelin’s orexigenic effects [44], as the initial results obtained with obestatin have not been confirmed by other groups [45], although it could participate in thirst regulation, beta-cell survival, gastroduodenal motility. The physiological relevance of obestatin is unclear at the present time. Obestatin should probably be better termed, ghrelin-associated peptide [37] and does not alter pituitary hormone secretion [46].

2. GHRELIN IN OBESITY, PHYSIOLOGICAL ASPECTS

2.1. Ghrelin Secretion in Obesity

Ghrelin levels increase before meals and at night, and are rapidly suppressed by food intake, particularly by high calorie or high carbohydrate meals. [47,48]. Food intake, especially amino acids, glucose, long-chain fatty acids, euglycemic hyperinsulinemia, somatostatin, obesity, weight gain, hyperthyroidism, and leptin injection suppress ghrelin [37,49-52]. The ingestion of proteins, more than lipids, suppresses plasma acyl ghrelin, whereas carbohydrate initially suppresses and then stimulates circulating ghrelin in humans [53]. Gastrectomy reduces total ghrelin concentrations by 65%–80% [54], confirming that the stomach is its major source.

Diet composition influences postprandial ghrelin. Most studies have used mixed or carbohydrate rich test meals, which typically suppress ghrelin levels [55]. After a solid carbohydrate-rich test meal and after an oral glucose load ghrelin levels decreased, while a fat-rich meal also decreased plasma ghrelin; and there was no change in ghrelin after modified sham feeding. Gastric distension achieved by infusion of water into the stomach does not lead to ghrelin reduction [56], although the ingestion of non-nutritive fibre does decrease ghrelin levels. Increasing the calorie content of meals in healthy subjects progressively lowered nadir levels of ghrelin. Obese patients have lower fasting ghrelin levels, and the degree of reduction after the consumption of different test meals was lower than in the normal subjects [57]. In another clinical model, chronic renal failure patients, both total and acylated ghrelin secretion was found to be partially refractory to the acute inhibitory effect of oral feeding [58]. Different studies have found that insulin plays an important role in postprandial ghrelin regulation. Total and acylated ghrelin fell significantly after the ingestion of a mixed meal and acylated ghrelin levels correlated negatively with the postprandial insulin levels. After a carbohydrate- enriched breakfast, the decrease in ghrelin as a percentage was inversely correlated with the increase in insulin and glucose [59, 60]. However, there are conflicting data regarding the role of insulin on postprandial ghrelin regulation [61,62]. Unexpected fasting in humans decreases circulating ghrelin [63,64], while a strong inverse association was found between ghrelin and cortisol in serum [63] in one study, and reciprocal changes in

endogenous ghrelin and growth hormone in healthy women in another study, suggesting that GH could inhibit ghrelin secretion [64].

Due to the well established GH-releasing activity of ghrelin [26,65]; it could be possible that GH regulates ghrelin secretion through a classic feedback system. Here it has been found that administering GH to GH-deficient patients induces a decrease in ghrelin levels [66]. Most (but not all), studies suggest that GH does not participate in the regulation of ghrelin secretion in humans [63,67], and that chronic GH excess does not consistently modify ghrelin production [67]. Acylated ghrelin typically changes in parallel with total ghrelin and in the fed state may rise recurrently before GH pulses [68]. Leptin could participate in the postprandial regulation of ghrelin [59], although most of the studies did not suggest a significant role for leptin in ghrelin regulation [62]. Short-term infusions of peptide YY, oxyntomodulin, and urocortin, all putative appetite suppressing peptides, led to a decrease in plasma ghrelin levels [69]. Low systemic ghrelin has been reported in hyperthyroidism, in male hypogonadism, in the polycystic ovary syndrome, in the presence of Helicobacter pylori-induced gastritis, or after total gastrectomy [69,70].

Circulating total ghrelin levels are increased in anorexia and cachexia but reduced in obesity [51,52] and plasma total ghrelin levels are negatively correlated with body mass index, body fat mass and plasma leptin, insulin and glucose levels [60, 62,71]. Patients with Prader-Willi syndrome have very high systemic total ghrelin levels, hyperphagia and extreme obesity, suggesting that ghrelin plays a role in the pathogenesis of obesity in this disease [41,60]. Postprandial suppression of serum ghrelin is less marked in patients with Prader-Willi syndrome as compared to matched obese and healthy controls [70].

Systemic ghrelin levels decrease in morbidly obese patients after gastric bypass surgery, suggesting that ghrelin may be involved in the mechanisms that lead to weight loss [48]. This observation has been confirmed in some, but not all subsequent studies, [69,72], as it depends, at least in part, on the surgical procedure [72,73]. The suppression of ghrelin levels following gastric bypass surgery may contribute to reduced hunger and the weight reducing effect of the procedure [74]. This suppression of ghrelin may be explained by major loss of gastric mucosa, although the mechanism is not yet understood [74].

The effect of exercise on ghrelin secretion has been studied in adolescents and adults. In adolescents total ghrelin was not affected by exercise; in contrast acylated ghrelin significantly increased after exercise, and this increase was greater in lean rather than in

obese adolescents. Higher acylated ghrelin correlated with an increase in markers of appetite [75]. In adult subjects, after a similar energy deficit imposed by food restriction or exercise, appetite and ad libitum energy intake responded in a compensatory fashion to food restriction, but were not influenced by exercise. Plasma acylated ghrelin concentrations increased, whereas PYY (3-36) decreased, in response to food restriction. Exercise did not induce these compensatory responses, suggesting a mediating role of acylated ghrelin and PYY (3-36) in determining divergent feeding responses to energy deficits imposed by food restriction and exercise [76].

From a clinical practice perspective, it has been found that obese women with higher leptin and lower ghrelin levels at baseline seem to be more resistant to fat mass loss. The leptin to ghrelin ratio could be proposed, if confirmed in future studies, as a predictor of treatment results for obesity [77]. One year after initial weight reduction, levels of the circulating mediators of appetite that encourage weight regain after diet-induced weight loss do not revert to the levels recorded before weight loss [78]. Long-term strategies to counteract this change may be needed to prevent obesity relapse [78,79]. Altered ghrelin levels have also been found in different clinical situations such as advanced liver cirrhosis or renal failure, suggesting that ghrelin could contribute to the altered appetite in these diseases [80-83]. In (Fig. 1a and 1b) we present factors and clinical situations associated with changes in circulating ghrelin levels.

2.2. Ghrelin Actions in Obesity

Ghrelin has a strong GH-releasing activity [65] through the activation of the GHS-R1a. GHS-Rs are concentrated in the hypothalamus-pituitary unit but are also distributed in other tissues. Apart from stimulating GH secretion, ghrelin and many synthetic GHSs: 1) stimulate prolactin and corticotrophin (ACTH) secretion; 2) negatively influence the pituitary-gonadal axis; 3) stimulate appetite and positive energy balance; 4) modulate pancreatic endocrine function and affect glucose levels; 5) have cardiovascular actions; 6) stimulate gastric motility; 7) modulate cell survival and cachexia; 8) modulate adipogenesis; 9) stimulate bone formation; 10) modulate immune function [29,37,84]. In (Fig. 2) we present the principal actions of ghrelin.

2.2.1. Pituitary Endocrine Actions

The most important pituitary actions are on GH secretion. Ghrelin is able to selectively stimulate GH secretion from both rat pituitary cells in culture and rat *in vivo* [26,27]. Ghrelin has a direct effect on pituitary somatotroph secretion *in vitro* and acts synergistically with GHRH to stimulate GH secretion [26,65]. Ghrelin may have direct hypothalamic effects to further induce GH secretion. In high doses, ghrelin may also stimulate prolactin, corticotrophin, and cortisol secretion [29]. The effect of ghrelin has been compared with that of hexarelin (a synthetic GHS) and GHRH. The GH response to ghrelin was clearly higher than that recorded after GHRH and even significantly higher than after hexarelin. Active ghrelin is a consistently effective agonist of GH secretion in multiple species [37,65,85].

Ghrelin acts as an amplifier of GHRH, the primary regulator of GH secretion. Ghrelin or synthetic GHS achieves synergy with GHRH on GH secretion [37,65,86]. Synergistic stimulation of GH release is absent in pituitary cells *in vitro* and in patients with lesions that separate the hypothalamus and pituitary gland [86,87], thus defining a critical role for joint hypothalamus-pituitary effects. Nonetheless, the precise mechanisms responsible for this synergy are not well established. Proposed mechanisms include ghrelin mediated opposition to somatostatin and/or stimulation of an unknown synergy factor [37]. The additive interaction between ghrelin and GHRH at maximal doses indicates that these peptides act via different mechanisms [65].

Considering the importance of ghrelin on GH secretion and the control of weight homeostasis, we have studied GH secretion after the administration of ghrelin in obesity. The GH response to ghrelin or GHRH plus ghrelin is modestly diminished in obese patients, while in contrast the response to GHRH is markedly decreased in obese patients when compared to normal subjects [65]. The GH response to ghrelin was about 8 times greater than that of GHRH in obese patients. In marked contrast, in normal subjects the GH response to ghrelin was only twice the response to GHRH.

GHSs may well be considered analogues of ghrelin [27], although the knowledge accumulated with these compounds cannot be automatically transferred to ghrelin. Our study in obese subjects is in agreement with the evidence that ghrelin releases more GH than GHRH and nonnatural GHSs [65]. The response of obese patients to the natural GHS ghrelin was greater than the response to the nonnatural GHS GHRP- 6 both on its own

and in combination with GHRH [88]. When considered on a molar basis, the present results suggest that ghrelin is more potent in releasing GH than synthetic GHSs.

The massive GH discharge that followed the administration of GHRH plus ghrelin was not observed after any stimulus in obesity, clearly indicating that the impaired GH secretion is a functional and potentially reversible state and suggesting that decreased ghrelin secretion could be responsible for the GH hyposecretion. However, the persistence of a decreased response in obese subjects after ghrelin alone or combined with GHRH suggests the existence of another defect involved in the altered GH secretion of obesity [89]. The pathophysiological mechanism responsible for the hyposecretion of obesity is probably multifactorial [90-92].

The physiological importance of ghrelin on GH regulation and growth is unclear. Animal studies that involve genetic ablation suggest that neither ghrelin nor its classic GHS receptor are required for growth [93]. The relevance of these observations to human physiology is uncertain and is being challenged by the report of familial short stature in association with a GHS receptor mutation, leading to decrease binding to the mutant receptor [94]. Significant correlations have been found between the different indices of post-oral glucose GH and ghrelin secretion. These data suggest that ghrelin is a physiological regulator of GH in the post-oral glucose state, and that decreased ghrelin secretion could be one of the mechanisms responsible for altered GH secretion in obesity [95] and the sexual dimorphism on GH secretion [96]. There is evidence suggesting that endogenous ghrelin may be a key regulator of GH secretion in different physiological and pathophysiological situations [97]. It has been recently found that Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. Therefore, an essential function of ghrelin in mice is the elevation of GH levels during severe calorie restriction, thereby preserving blood glucose and preventing death [98], although this has not been confirmed by others [99].

2.2.2. Ghrelin in Appetite Regulation and Weight Homeostasis

The orexigenic effect of ghrelin was first discovered in the animal model [39,100]. Circulating ghrelin levels fall with nutrient ingestion in rodents and humans [39,101]. Cummings et al demonstrated pre-prandial peaks with postprandial suppression of ghrelin levels in human subjects initiating meals voluntarily, suggesting that ghrelin plays a role

in meal initiation [102]. Administration of ghrelin stimulates spontaneous food intake in humans [103]. It has also been hypothesized that ghrelin could play a major role in the endocrine abnormalities that are present in obesity [104].

In addition to its putative role as a short-term signal that regulates meal initiation and satiety, active ghrelin appears to have a role as a long-term signal of nutritional status [105]. Ghrelin administration leads to weight gain in experimental animals by stimulating food intake, decreasing energy expenditure and spontaneous activity, and promoting adipogenesis [39,52,103]. Several lines of evidence suggest a role for ghrelin in the longer-term regulation of energy homeostasis. Firstly, in rodents chronic administration of ghrelin induces hyperphagia and weight gain [39]. Secondly, mice lacking ghrelin signalling either due to deletion of ghrelin (Ghrl-/-) or GHS-R1a (GHSR-null) have lean phenotypes and exhibit a marked resistance to high-fat diet induced obesity [101]. Thirdly, in humans circulating ghrelin levels are inversely correlated to the degree of adiposity, with low levels in obese subjects and high levels in conditions such as anorexia nervosa, malignancy and cachexia associated to chronic heart failure [51,52,55].

Studies in GHSR-null mice have confirmed that GHS- R1a is the receptor mediating the orexigenic effects of ghrelin [29]. Genetic disruption of GHS-R1a blocks orexigenic stimulation by ghrelin, but cachexia does not develop in the transgenic animals, including those with combined ghrelin and GHS-R1a knockout, probably reflecting signal redundancy within nutritional networks [101,106]. Food intake and energy balance regulation is a highly redundant and complex process [107].

Ghrelin enhances appetite by 25%–30% in fasting humans and animals [39,100,103,108]. The orexigenic effect arises by combined activation of neuropeptide Y/agouti related peptide (NPY/AGRP) and orexin A neurons. Ghrelin antagonizes anorexigenic signals, such as leptin, proopiomelanocortin (POMC), and melanocyte- stimulating hormone (MSH) [37,109]. The primary site of action for the peptide appears to be the NPY/AgRP neurons within the arcuate nucleus. Ghrelin activates NPY/AgRP neurons as indicated by enhanced c-fos expression following peripheral administration or electrophysiological studies [100,110]. Furthermore, in mice lacking NPY/AgRP neurons the meal stimulatory effect of ghrelin is abolished [111]. Adenosine monophosphate-activated protein kinase (AMPK) is a regulator of energy homeostasis and acts as a sensor of energy status. Hypothalamic AMPK is implicated in the regulation of food intake [112]. Ghrelin and

adiponectin both have hypothalamic AMPK implications in their orexigenic effects. Rat and mouse hypothalamic AMPK activity is stimulated by both central and peripheral ghrelin administration [74].

Transgenic overexpression of central nervous system and gastric ghrelin causes hyperphagia, increases energy expenditure, and decreases sensitivity to insulin and leptin in mice [113]. In experimental animals, ghrelin is more potent in stimulating appetite when administered centrally rather than systemically [39]. Central ghrelin regulates peripheral lipid metabolism in a growth hormone-independent fashion [114] and modulates hypothalamic lipid metabolism [115]. Recent data suggest that ghrelin neutralization during fasting-refeeding cycle impairs the recuperation of body weight and alters hepatic energy metabolism [116], while other data suggests that diet-induced obesity causes ghrelin resistance by reducing NPY/AgRP responsiveness to plasma ghrelin and suppressing the neuroendocrine ghrelin axis, in an attempt to limit further food intake [117]. Stress-induced eating of calorically dense foods probably contributes to the increased prevalence of obesity in humans experiencing chronic stress or typical depression. It has been found that ghrelin mediates stress-induced food-reward behavior in mice [118].

3. GHRELIN IN OBESITY, PHARMACOLOGICAL ASPECTS

3.1. Pharmacological Uses of Ghrelin and GHSs

Due to its orexigenic and metabolic effects, ghrelin and GHSs are potentially beneficial in antagonizing protein breakdown and weight loss in catabolic conditions such as cancer cachexia, renal, cardiac and pulmonary disease, and age-related frailty. Ghrelin also has potentially useful positive effects on cardiac function and gastric motility. Ghrelin antagonists may be beneficial in increasing insulin sensitivity and potentiate weight loss [119]. In Table 3 we present the potential therapeutic uses of ghrelin and GHSs agonists and antagonists. The ghrelin receptor exhibits high (about 50%) basal constitutive activity [120,121] and responds to inverse agonists, partial agonists, and antagonists [122]. Inverse agonists decrease basal receptor activity [121,123].

Ghrelin and GHSs have therapeutic potential in treating: short stature in patients with preserved GHRH [124]; hyposomatotropism in aging [125] and decreased GH secretion associated with visceral adiposity, including the human immunodeficiency virus (HIV) lipodystrophy syndrome [101,126]. Ghrelin may be useful as orexigenic agent for the treatment of eating disorders such as anorexia nervosa [127]. Administration of ghrelin or GHSs can stimulate appetite and improve nutritional status. However, plasma ghrelin concentrations in anorexia nervosa are high, indicating a situation of ghrelin resistance. These drugs could also be useful in all of the clinical situations associated with cachexia, such as malignancy, advanced cardiac failure, renal failure, postoperative patients and HIV-lipodystrophy syndrome [29,35,69,128,129].

Ghrelin stimulates gastric motility, which makes it a candidate as a prokinetic drug. Administration of ghrelin improves cardiac structure and function, and attenuates the development of cardiac cachexia in rats with heart failure [27]. These results suggest that ghrelin has protective cardiovascular effects. As a result, ghrelin and GHSs may be new therapeutic agents for the treatment of severe chronic heart failure. Other proposed clinical uses of ghrelin and GHSs include the improvement of sleep quality and treatment of fractures, osteoporosis, aging, lupus erythematous, inflammatory bowel disease and central nervous system diseases, among others [29,69,128].

3.2. Pharmacological Uses of Ghrelin and GHSs in Obesity

Obesity results in a secondary reduction in GH secretion [16,88,91]. It is also recognized that adult GH defiency is usually accompanied by an increase in fat accumulation and GH replacement in adult patients with GH deficiency results in a reduction of fat mass and abdominal fat mass in particular [130]. Clinical studies examining the efficacy of GH in obese subjects demonstrated very little or no evidence with respect to weight loss by GH therapy. More evidence exists with respect to the effect in reducing total and abdominal fat mass in obese subjects [130]. Administration of GH has been shown to decrease abdominal visceral fat and total body fat [130]. Studies 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. These results suggest that activation of the GH axis via ghrelin or a ghrelin mimetic in the obese is possible,

although the adipogenic effects of ghrelin make it an unlikely candidate for the treatment of obesity in humans [97].

Obesity can be considered as a low-grade inflammation state. Recent studies have also demonstrated a role for ghrelin in regulating inflammation and inflammatory cytokine expression in humans and rodents as well as T-cell development [131,132]. Ghrelin has been described to be a potent anti-inflammatory mediator both *in vitro* and *in vivo* and a promising therapeutic agent in the treatment of inflammatory diseases [132].

At present, ghrelin is the only peripheral orexigenic factor that is effective when administered intravenously. Both ghrelin and GHSs are being investigated as therapeutic targets in obesity [133]. There are different strategies to neutralize acylated ghrelin biologic activity, such as immunoneutralization, catalytic antibodies, antagonist peptides, antagonist nonpeptides, RNA Spiegelmer, or the administration of des-acyl ghrelin [116,134,135].

Ghrelin receptor antagonists could be used as anti-obesity drugs, by blocking the orexigenic signal from the gastrointestinal tract to the brain. Inverse agonists of the ghrelin receptor, by blocking the constitutive receptor activity, may lower the set-point for hunger between meals [123]. Highly potent GHS-R1a antagonists have been identified [136]. In the rat, some GHS-R1a antagonists decreased food intake and body weight following central or intraperitoneal administration [137]. The GHS-R1a has constitutive activity [137], so inverse GHS-R1a agonists may prove to be more effective in inducing weight loss than GHS-R1a antagonists.

Analogs of ghrelin may exhibit partial agonism (for example stimulation of appetite but not GH release, and vice versa), antagonism, and inverse agonism despite similar GHS-R1a binding affinities [122,138]. Theoretically the ideal ghrelin mimetic for obesity treatment should have agonistic effects on GH secretion but an antagonistic effect on appetite. It has been shown that selective ghrelin blocking in diet induced obese mice results in a reduction of bodyweight and body fat mass [116]. Adult ghrelin-deficient mice have lower glucose levels and increased insulin sensitivity under conditions of caloric restriction [139] and are protected from the rapid weight gain induced by early exposure to a high-fat diet [105]. In addition, mice lacking ghrelin receptors resist the development of diet-induced obesity [101], and antagonism of the ghrelin receptor results in a reduction of food intake and weight gain [140]. Therefore, reduced levels of ghrelin

activity in obese individuals, in comparison to non-obese controls, should provide greater protection from further weight gain.

In vitro generated biostable RNAbased compounds that specifically bind ghrelin have been used in animal models to inhibit ghrelin-mediated GHS-R activation [116,141]. Zorrilla *et al*. have developed an anti-ghrelin vaccine, providing a new method to decrease ghrelin activity [134]. Acylated ghrelin induces body weight gain and adiposity by promoting food intake and decreasing energy expenditure [39]. GOAT has been implicated as another potential target for the development of anti-obesity treatment [31]. Chronic treatment of mice on a high-fat diet with GO-CoA-Tat, a peptide-based GOAT inhibitor, prevented weight gains and displayed significantly lower fat mass [142].

Ghrelin receptor antagonists could be useful for the treatment of diabetes mellitus. Esler et al examined the effects of small-molecule GHS-R1a antagonists on insulin secretion, glucose tolerance, and weight loss. Ghrelin suppressed insulin secretion from dispersed rat islets. This effect was blocked by a GHS-R1a antagonist. Consistent with this observation, a single oral dose of a GHS-R1a antagonist improved glucose homeostasis in the rat. Improvement in glucose tolerance was attributed to increased insulin secretion. Daily oral administration of a GHS-R1a antagonist to diet-induced obese mice led to reduced food intake and weight loss due to selective loss of fat mass. Consistent with the hypothesis that ghrelin regulates feeding centrally, the anorexigenic effects of potent GHS-R1a antagonists in mice appeared to correspond with their brain exposure. These observations demonstrate that GHS-R1a antagonists have the potential to improve the diabetic condition by promoting insulin secretion and weight loss [143].

In summary, ghrelin antagonists or partial inverse considered for the treatment of obesity.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Table 1. World Health Organization (WHO) Criteria for Overweight and Obesity [2]. BMI (Body Mass Index).

WHO Criteria for Overweight and Obesity	BMI (kg/m^2)
Normal weight	18,5-24,9
Overweight	25-29,9
Obesity grade 1	30-34,9
Obesity grade 2	35-39,9
Obesity grade 3	≥ 40

Table 2. Main Endocrine and Metabolic Changes in Obesity. SHBG: Sex Hormone Binding Protein; TSH: Tyrotrophin; T3: Triiodothyronine.

Endocrine Gland	Hormonal Alteration
Endocrine Pancreas	Hyperinsulinemia
Adipose tissue	Hyperleptinemia. Decreased Adiponectin
Pituitary	Decreased basal and stimulated GH Decreased response to stimuli of Prolactin
Gonads	Woman: Decreased SHBG. Increased Estrogens and Androgens. Man: Decreased SHBG. Decreased Total and Free Testosterone
Adrenals	Free urinary cortisol increased and normal plasmatic cortisol
Gastrointestinal Hormones	Decreased Ghrelin
Thyroid	Increased TSH and free T3

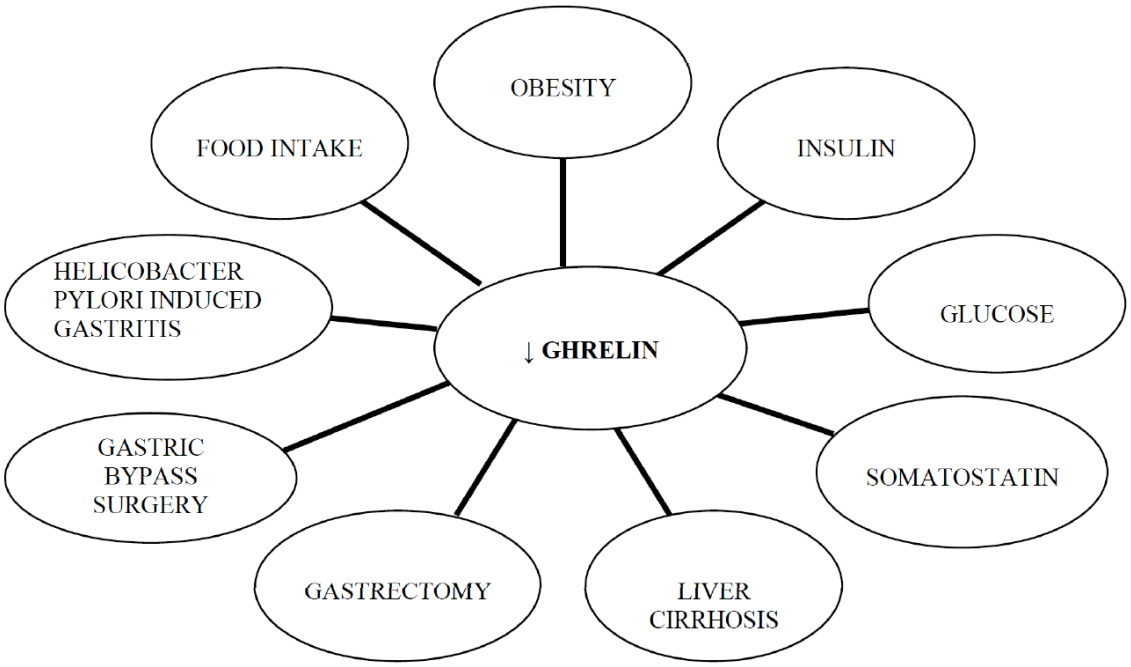

Fig. (1a). Factors and clinical situations associated with decreased circulating ghrelin levels.

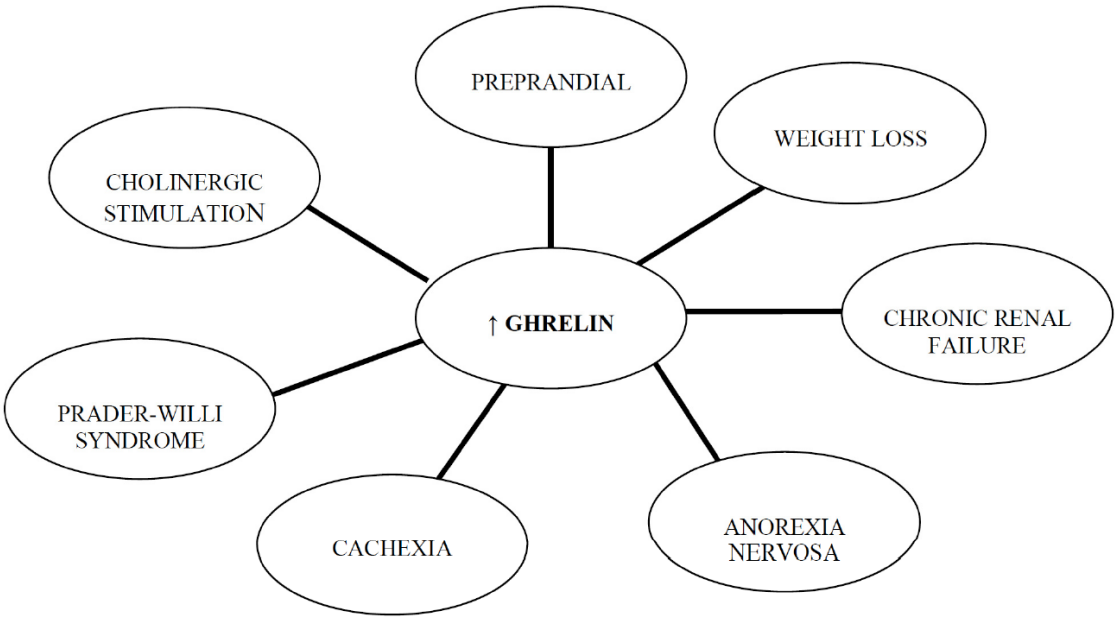

Fig. (1b). Factors and clinical situations associated with increased circulating ghrelin levels.

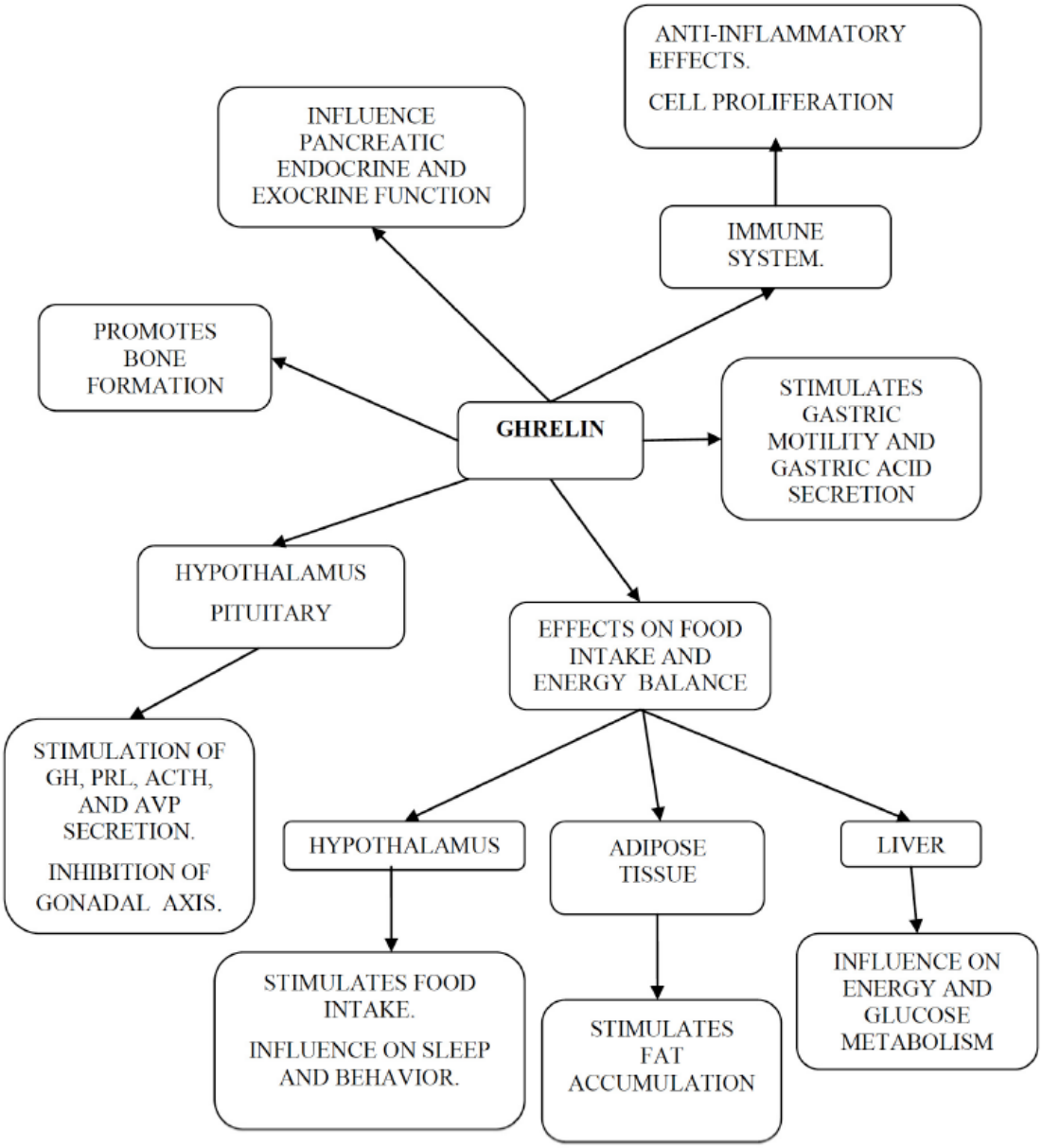

Fig. (2). Principal actions of ghrelin.

Table 3. Potential Therapeutic Uses of Ghrelin and GHSs (Growth Hormone Secretagogues) Agonists and Antagonists.

Ghrelin and GHSs agonists	Ghrelin and GHSs antagonists
Diagnosis of GH deficiency	Obesity
Treatment of GH deficiency	Prader-Willi syndrome
Anorexia nervosa	
Cachexia of malignancy	
Other cachexias	
Chronic heart failure	
Gastrointestinal motility disorders	
Osteoporosis	
Inflammatory diseases	