The physiological significance and potential clinical applications of ghrelin

TAKASHI AKAMIZU*,**, KENJI KANGAWA*,***

*Ghrelin Research Project, Department of Experimental Therapeutics, Translational Research Center, Kyoto University Hospital, Kyoto University School of Medicine, Kyoto 606-8507, Japan, **The First Department of Medicine, Wakayama Medical University, Wakayama 641-8509, Japan, and ***Department of Biochemistry, National Cerebral and Cardiovascular Center Research Institute, Osaka 565-8565, Japan

Short title: RESEARCH ON GHRELIN

Key words: cachexia, anorexia nervosa, GH deficiency, clinical trial, ghrelin, gastrectomy

Correspondence to: Dr. Takashi AKAMIZU, Ghrelin Research Project, Department of Experimental Therapeutics, Translational Research Center, Kyoto University Hospital, 54 Shogoin-Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan

Phone: +81-75-751-4723, Fax: +81-75-751-4731, E-mail: akamizu@kuhp.kyoto-u.ac.jp

Abstract

Ghrelin, a natural ligand for the growth hormone (GH)-secretagogue receptor (GHS-R), is now known to play a role in a number of different physiological processes. For example, ghrelin increases GH secretion, feeding, and body weight when administered centrally or peripherally. These unique effects of ghrelin should be invaluable for the development of novel treatments and disease diagnostic techniques. Clinical trials have already been performed to assess the utility of ghrelin for the treatment of several disorders including anorexia, cachexia, and GH-related disorders. This review summarizes the recent advances in this area of research.

1. Introduction

Ghrelin is a peptide hormone that was discovered in 1999 as an endogenous ligand for the growth hormone (GH)–secretagogue receptor (GHS-R) [1]. Ghrelin is a 28-amino-acid peptide and possesses a unique fatty acid modification, *n*-octanoylation, at Ser 3. There are two circulating forms of ghrelin, acylated and unacylated (desacyl), and the acylated form is essential for ghrelin's biological activity through GHS-R. Recently, however, desacyl ghrelin was reported to influence both cell proliferation and adipogenesis through another unknown receptor [2-5]. Ghrelin is produced primarily in the stomach and circulates in the blood at a considerable plasma concentration. Expression of ghrelin is also detectable in the hypothalamus, intestine, pituitary, placenta, and other tissues [1, 6-8]. Ghrelin is now known to play a role in a number of different physiological processes; for example, ghrelin increases GH secretion and feeding, and decreases insulin secretion [1, 9-19].

These unique effects of ghrelin and growth hormone secretagogues (GHS) should be invaluable for the development of novel treatments and disease diagnostic techniques [20-22]. Clinical trials have already been performed to assess the utility of ghrelin for the treatment of various disorders including anorexia [23-26], cachexia [27-29], malnutrition [30], GH-related disorders [31], and postgastrectomy/esophagectomy [32, 33]. Because many excellent reviews concerning basic and clinical research on ghrelin have already been published, we will summarize and discuss recent clinical trials of ghrelin in this work.

2. Physiological actions of ghrelin

2. 1. Orexigenic action

Ghrelin has a well-established role in stimulating appetite and increasing food intake [34, 35]. Peripheral administration of ghrelin stimulates GH secretion and appetite in both animals and humans [10, 18]; it is the only hormone known to have this effect. Ghrelin increases c-fos expression in the arcuate nucleus, and also activates hypothalamic neuropeptide Y (NPY)/Y1 receptors and agouti-related peptide (AgRP) pathways [36-38]. In addition, ghrelin induces food intake via the orexin pathway [39]. These functions are mediated at least in part by vagal nerve pathways [40]. Repeated administration of ghrelin resulted in significant weight gain in rats [41] and patients with chronic obstructive pulmonary disease (COPD) [28].

2. 2. Stimulation of GH secretion

Ghrelin strongly stimulates GH secretion in humans [12, 16, 17, 42], several-fold more potently than GHRH under similar conditions. Furthermore, ghrelin and growth hormone releasing hormone (GHRH) synergistically increase GH release [17]. Ghrelin might also play a role in GH release in a non-acute setting [43, 44]. GH regulates IGF-I levels, promotes anabolism, and increases muscle strength [45, 46]. While GH enhances lipolysis, IGF-1 stimulates protein synthesis, myoblast differentiation, and muscle growth.

2. 3. Anti-inflammatory action

Evidence that ghrelin exerts anti-inflammatory actions has been accumulating. Ghrelin suppresses the production of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α both *in vitro* [47, 48] and *in vivo* [49-51]. In clinical trials, daily administration of ghrelin for three weeks decreased inflammatory cytokine levels and neutrophil density in sputum from patients with chronic respiratory infections [52]. In contrast, ghrelin induces the anti-inflammatory cytokine IL-10 [49, 53].

Ghrelin inhibits the activation of NF-κB, a transcription factor known to control the production of multiple pro-inflammatory cytokines during inflammatory insults [48, 50, 53]. Although the molecular mechanisms and cellular targets mediating ghrelin inhibition of NF-κB activation remain to be determined, the vagus nerve may play an important role in the ghrelin-mediated inhibition of pro-inflammatory cytokine release [50, 54]. Cachexia and muscular wasting occur via protein degradation by the ubiquitin–proteasome pathway [55]. Two muscle-specific ubiquitin ligases, muscle RING-finger protein-1 (MuRF1) and atrogin-1/muscle atrophy F-box (MAFbx), are up-regulated under catabolic conditions. NF-κB activation may regulate skeletal muscle proteasome expression and protein degradation. The elevation in MuRF1 and MAFbx expression seen in skeletal muscle after thermal injury, arthritis, and dexamethasone administration were normalized, attenuated, and prevented, respectively, by ghrelin or GHS administration [56-58]. IGF-1 prevents the expression of MuRF1 and MAFbx by inhibiting Forkhead box O (FOXO) transcription factors via stimulation of the phosphatidylinositol-3-kinase (PI3K)/Akt pathway. The IGF-1 receptor triggers activation of several intracellular kinases, including phosphatidylinositol-3-kinase (PI3K) [59]. Thus, the effects of ghrelin on NF-κB activation and IGF-1 synthesis are favorable for minimizing inflammatory responses and sarcopenia in patients with cachexia.

2.4. Other actions

The role of ghrelin in stimulating gastric emptying and acid secretion is well-established [60]. This effect may ameliorate gastrointestinal symptoms in patients with anorexia–cachexia syndrome. Ghrelin also increases endogenous nitric oxide (NO) release [61, 62], which may influence its orexigenic and anti-inflammatory actions [63, 64].

3. Potential clinical applications of ghrelin

3.1. Appetite-related disorders

3.1.1. Anorexia nervosa (AN) and related disorders

Anorexia nervosa (AN) is an eating disorder characterized by chronically decreased caloric intake, resulting in self-induced starvation. Plasma ghrelin levels are elevated in lean patients with anorexia nervosa, consistent with a state of negative energy balance [65-67]. Only a few preliminary studies have been performed to examine the effects of ghrelin in individuals with AN. Miljic *et al*. infused ghrelin (300-min intravenous infusion of 5 pmol/kg/min ghrelin) into nine AN patients with very low body weights, six AN patients who had partially recovered their body weights but who remained amenorrheic, and ten constitutionally thin female subjects [68]. The fifteen AN patients felt significantly less hungry compared with the constitutionally thin subjects, suggesting that AN patients are less sensitive to the orexigenic effects of ghrelin than healthy controls. In another paper, however, six of nine patients with restrictive AN were reported to have been hungry after ghrelin administration (1.0 μg/kg as an intravenous bolus), a similar ratio to that seen in normal subjects (five of seven) [69]. We examined the effects of ghrelin on appetite, food intake, and nutritional parameters in AN patients [26]. Five female patients who met the Diagnostic and Statistical Manual IV (DSM-IV) criteria for restricting-type AN [70] and desired to recover from the disorder participated in this study. The patients were hospitalized for 26 days (6 days pre-treatment, 14 days ghrelin infusion, and 6 days post-treatment). The patients received an intravenous infusion of 3 μg/kg ghrelin twice a day (before breakfast and dinner). Attitudes toward food were evaluated by visual analogue scale (VAS) questionnaires and daily energy intake was calculated by dieticians. Ghrelin infusion improved epigastric discomfort or constipation in four patients, whose hunger scores on VAS also increased significantly after ghrelin administration. Daily energy intake during ghrelin administration increased by 12–36% compared with the pre-treatment period. The change in body weight of the five patients ranged from +1.5 to 2.4 kg. Nutritional parameters such as total protein and triglyceride levels improved. There were no serious adverse effects, including psychological symptoms. All patients who did not gain weight during hospitalization did so after discharge. These findings suggest that ghrelin may have therapeutic potential in AN patients who cannot gain weight because of gastrointestinal dysfunction. Clearly, further studies, including randomized controlled trials, are needed to determine whether ghrelin is useful for the treatment of AN.

Functional dyspepsia (FD) is a disorder characterized by the presence of chronic or recurrent symptoms of upper abdominal pain or discomfort [71]. Although no known specific organic abnormalities are present in FD, abnormalities in gastrointestinal motility and sensitivity are thought to play a role in a substantial subgroup of patients. In addition, some patients suffer from anorexia and body-weight loss. We found that levels of plasma acylated, but not desacyl, ghrelin correlated with a subjective symptom score in FD patients, suggesting that acylated ghrelin may play a role in the pathophysiology of FD [72]. We attempted to evaluate the clinical response to repeated ghrelin administration in patients with anorexia caused by functional disorders, such as FD and ‘other eating disorders’ or ‘unspecified eating disorders’ [24]. The inclusion criteria in this study were subjects who 1) were diagnosed with functional anorexia, including FD or other eating disorders with the exception of anorexia nervosa, 2) were lean (BMI < 22 kg/m^2), and 3) exhibit decreased food intake. Subjects received an intravenous infusion of ghrelin for 30 minutes twice a day (before breakfast and dinner) for two weeks, and we investigated the effects on food intake, appetite, hormones, and metabolic parameters. Six patients with FD were enrolled in this study. Ghrelin administration tended to increase daily food intake in comparison to levels before and after completion of treatment, but this difference, which was the primary endpoint of the study, did not reach statistical significance. Hunger sensation was significantly elevated at the end of drip infusion. No severe adverse effects were observed. These results suggest that ghrelin administration is safe and that this treatment has stimulatory effects on appetite in patients with FD. Further studies remain necessary to confirm the efficacy of ghrelin treatment for anorexia-related disorders.

3.1. 2 Cachexia and related disorders

A number of trials seeking to utilize ghrelin for the treatment of cachexia have recently been performed [73]. These studies have sought to evaluate ghrelin as a treatment for patients with cachexia associated with congestive heart failure (CHF), COPD, cancer, and End-stage renal disease (ESRD). Cachexia manifests as excessive weight loss in the setting of an underlying chronic disease [74], and is typically associated with anorexia as a major cause of weight loss. Weight loss and decreased appetite are the major causes of morbidity and mortality in patients with anorexia–cachexia syndrome. There is an immediate need for effective, well-tolerated treatments to stimulate appetite [75], prompting several trials to explore the application of ghrelin as a treatment for patients with cachexia.

3.1. 2 .1. CHF-associated cachexia

Ghrelin induces a positive energy balance state through both GH-dependent and -independent mechanisms and has protective cardiovascular effects [76]. GH treatment may be especially useful in a subgroup of patients with cardiac cachexia [77]. Ghrelin stimulates food intake, induces adiposity, regulates the central nervous system to decrease sympathetic nerve outflow, and inhibits apoptosis of cardiomyocytes and endothelial cells in a GH-independent manner. Nagaya *et al*. investigated the effects of ghrelin on cardiac cachexia in 10 patients with CHF [27] (Table 1). Daily administration of ghrelin for three weeks increased both food intake and body weight. This study also demonstrated improvements in patient exercise capacity, muscle wasting, and left ventricular function. Ghrelin treatment also resulted in significantly decreased plasma norepinephrine levels. Although this study was neither randomized nor placebo-controlled, the eight CHF patients who did not receive ghrelin (control group) were followed to rule out any time-course effects during hospitalization. None of the aforementioned parameters changed in patients with CHF who did not receive ghrelin therapy. Further studies will be necessary to identify the pathways involved in this use of ghrelin and to determine the best therapeutic strategies for ghrelin use to combat the wasting process found in cardiac cachexia patients [77]. Clinical trials are currently attempting to reproduce these data in a double-blind, placebo-controlled fashion.

3.1. 2 .2. COPD-associated cachexia

Patients with COPD often exhibit some degree of cachexia [78], which is an independent risk factor for mortality in COPD; GH treatment increases muscle mass in such patients. COPD and CHF are both associated with multiple pathophysiological disturbances, including anemia and neurohormonal activation [79]. In COPD patients, ghrelin exhibits anti-inflammatory effects. Chronic respiratory infections, characterized by neutrophil-dominant airway inflammation, lead to end-stage cachexia [80]. The cytotoxicity of accumulated neutrophils against bronchial and alveolar epithelial cells induces a deterioration of pulmonary function in COPD, resulting in excess energy expenditure and weight loss in patients. Intravenous ghrelin treatment for three weeks reduced neutrophil counts in sputum samples as well as the volume of sputum, suggesting that ghrelin suppressed excess neutrophil influx [52].

An open-label pilot study examined the ability of ghrelin to improve cachexia and functional capacity in patients with COPD; ghrelin was administered intravenously for three weeks to seven cachectic patients with COPD [28]. Repeated ghrelin administration significantly increased food intake, body weight, lean body mass, and peripheral and respiratory muscle strength. Ghrelin treatment ameliorated exaggerated sympathetic nerve activity, as indicated by marked decreases in plasma norepinephrine levels. Subsequently, another placebo-controlled trial demonstrated that ghrelin increased both appetite and body weight with an apparent dose-dependent trend towards improved physical performance (chair stand score) [81]. A larger clinical trial is currently being conducted to confirm these data in a double-blind, placebo-controlled fashion. Comparisons of this treatment to current standard medications will be required [79].

3.1. 2 .3. Cancer cachexia

Anorexia is frequently encountered in cancer patients, and is one of the major causes of malnutrition and cachexia in this patient population. Ghrelin administration resulted in significant increases in weight and food intake in rodent models of cancer-associated cachexia [82-84]. DeBoer *et al.* determined that weight gain resulted from a reversal in the loss of lean body mass, a critical component of cachexia [82].

Several randomized, double-blind placebo-controlled trials have demonstrated the efficacy and safety of ghrelin or GHS in patients with cancer-associated cachexia [23, 25, 85]. Nearry *et al*. performed a randomized, placebo-controlled, cross-over clinical trial to determine whether ghrelin could stimulate appetite in seven cancer patients with severe anorexia [23]. Ghrelin infusion resulted in a marked increase in energy intake in comparison to saline-treated controls; all patients in the study demonstrated increased food consumption. The meal appreciation score was also higher in ghrelin-treated individuals. Strasser *et al.* detailed a randomized, double-crossover, phase 1/2 study in 21 patients with advanced cancer [25]. They infused a low or high dose of ghrelin or placebo before lunch daily for four days in each course. Nutritional intake and eating-related symptoms did not differ between the ghrelin- and placebo-treated groups. More patients, however, preferred ghrelin to placebo at the middle and end of the study, although this finding was not dose-dependent. In contrast to the results of Neary *et al.*, this study did not demonstrate any increases in food intake. As the patient characteristics and study designs were very different in the two studies, further investigation is required.

An important concern regarding the use of ghrelin in cancer-associated cachexia is that ghrelin may increase the levels of growth factors, such as GH and IGF-1, that stimulate tumor growth. Additionally, ghrelin itself may have mitogenic potential. As far as we know, no *in vivo* data has examined the differences in tumor growth following ghrelin or GHS treatment. Long-term, large-scale clinical trials are required to determine whether ghrelin treatment promotes tumor growth.

3.1. 2 .4. End-stage renal disease (ESRD)

ESRD is a chronic condition frequently associated with nutritional dysfunction [86]. This type of malnutrition is highly resistant to intervention and is a major predictor of morbidity and mortality for patients on either peritoneal dialysis (PD) or hemodialysis. Wynne *et al.* sought to determine whether a single injection of ghrelin could enhance food intake in patients with evidence of malnutrition receiving maintenance peritoneal dialysis [30]. Nine PD patients exhibiting mild to moderate malnutrition were subcutaneously administered either ghrelin or a saline placebo in a randomized, double-blind, cross-over protocol. Ghrelin administration significantly increased mean absolute energy intake during the study meals and non-significant increases in energy intake were observed over the first 24 h without a subsequent rebound. This research group subsequently sought to analyze the efficacy of repeated ghrelin administration in malnourished dialysis patients [87] by performing a double-blind randomized cross-over study of a week of daily subcutaneous ghrelin injections in a group of 12 malnourished dialysis patients. Ghrelin administration significantly increased appetite, with increases in energy intake noted at the first study meal. Persistence of this effect throughout the week was confirmed by food diaries and final study meals, indicating that daily ghrelin treatment resulted in a sustained positive change in energy balance in malnourished dialysis patients. In support of this data, experiments using a nephrectomized rat model of renal cachexia demonstrated that daily treatment for two weeks with ghrelin or two GHS agents (BIM-28125 and BIM-28131) resulted in increased food intake, improved lean body mass accrual, and decreased circulating inflammatory cytokines [88]. Long-term studies are needed to demonstrate efficacy in improving appetite, weight gain, lean body mass, and quality of life.

3.2. GH deficiency-related disorders

Strong stimulation of GH secretion by ghrelin has been well documented in humans [12-16, 21, 42]. As with GHS, ghrelin may be useful for the diagnosis and treatment of short stature and GH deficiency. Elderly individuals may be particularly suitable candidates for ghrelin treatment, as ageing is associated with progressive decreases in GH secretion, appetite, and energy intake [89-92]. This reduced GH secretion is called "somatopause" and may be a cause of age-related metabolic and physiologic changes including reduced lean body mass and expansion of adipose mass. Sarcopenia is associated with functional decline and death. Altered blood lipid profiles also favor the development of vascular diseases that may increase overall mortality. The age-related reduction in energy intake has been termed "the anorexia of aging" and predisposes to the development of under-nutrition, which has been implicated in the development and progression of chronic diseases commonly affecting the elderly, as well as in increasing mortality. Growth hormone therapy increases IGF-I levels, promotes anabolism, and increases muscle strength in healthy elderly individuals, as well as in selected patient groups [93-95]. Therefore, ghrelin and GHS may also have therapeutic potential to assist in the recovery of frail patients who require nutritional support and conventional rehabilitation [96]. We evaluated the effects of ghrelin administration on physical performance and body composition in patients undergoing elective total hip replacement (THR) as treatment for osteoarthritis (OA) in a randomized, double-blind, placebo-controlled, phase II study [31]. Thirty-two patients were assigned to two groups of 16 subjects each; the ghrelin group received intravenous injections of 2 μg/kg ghrelin twice daily for three weeks beginning one week before surgery, while the placebo group received vehicle alone. While ghrelin significantly increased lean body mass after the three-week injection period, it did not affect muscle strength or walking ability. Significant decreases in fat mass and GH responses to ghrelin injection were also observed. No severe adverse effects occurred in response to ghrelin treatment. Despite increased lean tissue reserves, ghrelin administration using this study protocol did not provide any favorable effect on physical performance in patients with OA undergoing THR. Further studies are necessary to examine the efficacy of ghrelin treatment in such patients.

We found that plasma levels of acylated ghrelin in healthy elderly female subjects tended to be low and were correlated positively with IGF-1 levels, suggesting that negative feedback mechanism does not function properly in elderly subjects [97]. Further, acylated ghrelin concentrations in elderly females correlated with both systolic blood pressure and the frequency of bowel movements. These findings suggest that, in elderly females, acylated ghrelin may play a role in the regulation of the GH/IGF-1 axis, blood pressure, and bowel movements.

3.3. Post-gastrectomy and -esophagectomy

Body weight loss is common and is a serious outcome in patients who have undergone total gastrectomy and esophagectomy. Such weight loss correlates with decline in postoperative quality of life and is the most reliable indicator of malnutrition, which impairs immune function, susceptibility to infection, and survival [32, 33]. Plasma ghrelin levels decreased after total gastrectomy and esophagectomy [65, 98, 99]. Moreover, a significant correlation between ghrelin concentration and postoperative weight loss suggested a role for loss of ghrelin. To examine this, Adachi *et al.* evaluated the efficacy of ghrelin in 21 patients undergoing total gastrectomy [32]. Food intake and appetite were significantly higher in the ghrelin group (3 μg/kg, twice daily for 10 days after starting oral food intake following surgery) compared with the placebo group, and BW loss was significantly lower in the ghrelin group than in the placebo group. Fat mass, lean body mass, and basal metabolic rate decreased significantly in the placebo group; however, the reductions in lean body mass and basal metabolic rate were not significant in the ghrelin group, although that of fat mass was significant. Thus, short-term administration of synthetic ghrelin successfully lessened postoperative body weight loss and improved appetite and food intake after total gastrectomy. Subsequently, the same research group performed a similar study in 20 patients who underwent esophagectomy [33]. Again, they found that administration of ghrelin after esophagectomy increased oral food intake and attenuated weight loss together with maintenance of lean body weight. Thus, ghrelin administration may be useful in minimizing the side effects of these operations.

3.4. Other disorders

Reflecting the wide expression patterns of both ghrelin and its receptor, this peptide is now known to play a role in a number of different physiological processes including cellular proliferation and differentiation, pancreatic exocrine and endocrine function, glucose metabolism, sleep and behavior, immune regulation, and cardiovascular function. For example, as discussed above, repeated administration of ghrelin in patients with CHF significantly improved left ventricular function as well as food intake. A large number of studies have been performed by investigators worldwide to elucidate the various activities of ghrelin. We believe that some of these may lend support to the development of clinical applications of ghrelin to disorders other than those described above in the future.

4. Conclusions

More than ten years have passed since the discovery of ghrelin, and abundant evidence now indicates that it plays a role in a variety of physiological functions. In parallel, clinical trials have proceeded to exploit these activities in the treatment and diagnosis of human disease. There are several characteristic features of the clinical applications of ghrelin: 1) the multiplicity and uniqueness of its function, 2) its unique structure and fatty acid modification, and 3) the paucity of severe adverse effects [100]. These characteristics should allow us to develop novel and unique therapies for a variety of disorders, including many currently intractable and serious diseases. Indeed, research on clinical applications of ghrelin is a challenging and potentially rewarding avenue for the future.

Acknowledgements

Research in the authors' laboratory was supported in-part by funds from the Ministry of Education, Culture, Sports, Science and Technology of Japan; the Ministry of Health, Labour and Welfare of Japan; the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO); the Smoking Research Foundation; and the Tokyo Biochemical Research Foundation and the Foundation for Growth Science.

References

[1] Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999;402: 656-60.

[2] Cassoni P, Papotti M, Ghe C, Catapano F, Sapino A, Graziani A, et al. Identification, characterization, 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 2001;86: 1738-45.

[3] Bedendi I, Alloatti G, Marcantoni A, Malan D, Catapano F, Ghe C, et al. Cardiac effects of ghrelin and its endogenous derivatives des-octanoyl ghrelin and des-Gln(14)-ghrelin. Eur J Pharmacol 2003;476: 87-95.

[4] Thompson NM, Gill DA, Davies R, Loveridge N, Houston PA, Robinson IC, et al. Ghrelin and des-octanoyl ghrelin promote adipogenesis directly in vivo by a mechanism independent of the type 1a growth hormone secretagogue receptor. Endocrinology 2004;145: 234-42.

[5] Broglio F, Gottero C, Prodam F, Gauna C, Muccioli G, Papotti M, et al. Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response

to acylated ghrelin in humans. J Clin Endocrinol Metab 2004;89: 3062-5.

[6] Korbonits M, Bustin SA, Kojima M, Jordan S, Adams EF, Lowe DG, et al. The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumors. J Clin Endocrinol Metab 2001;86: 881-7.

[7] Gualillo O, Caminos J, Blanco M, Garcia-Caballero T, Kojima M, Kangawa K, et al. Ghrelin, a novel placental-derived hormone. Endocrinology 2001;142: 788-94.

[8] Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, et al. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. J Clin Endocrinol Metab 2002;87: 2988.

[9] Tschop M, Smiley DL, Heiman ML Ghrelin induces adiposity in rodents. Nature 2000;407: 908-13.

[10] Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, et al. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 2000;141: 4325-8.

[11] Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. Nature 2001;409: 194-8.

[12] Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, et al. Ghrelin strongly stimulates growth hormone release in humans. J Clin Endocrinol Metab 2000;85: 4908-11.

[13] Arvat E, Di Vito L, Broglio F, Papotti M, Muccioli G, Dieguez C, et al. Preliminary evidence that Ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. J Endocrinol Invest 2000;23: 493-5.

[14] Peino R, Baldelli R, Rodriguez-Garcia J, Rodriguez-Segade S, Kojima M, Kangawa K, et al. Ghrelin-induced growth hormone secretion in humans. Eur J Endocrinol 2000;143: R11-4.

[15] Nagaya N, Kojima M, Uematsu M, Yamagishi M, Hosoda H, Oya H, et al. Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. Am J Physiol Regul Integr Comp Physiol 2001;280: R1483-7.

[16] Arvat E, Maccario M, Di Vito L, Broglio F, Benso A, Gottero C, et al. 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 2001;86: 1169-74.

[17] Hataya Y, Akamizu T, Takaya K, Kanamoto N, Ariyasu H, Saijo M, et al. A

low dose of ghrelin stimulates growth hormone (GH) release synergistically with GH-releasing hormone in humans. J Clin Endocrinol Metab 2001;86: 4552.

[18] Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, et al. Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 2001;86: 5992.

[19] Dezaki K, Sone H, Yada T Ghrelin is a physiological regulator of insulin release in pancreatic islets and glucose homeostasis. Pharmacol Ther 2008;118: 239-49.

[20] Casanueva FF, Dieguez C Growth Hormone Secretagogues: Physiological Role and Clinical Utility. Trends Endocrinol Metab 1999;10: 30-38.

[21] Ghigo E, Arvat E, Camanni F Orally active growth hormone secretagogues: state of the art and clinical perspectives. Ann Med 1998;30: 159-68.

[22] Petersenn S Growth hormone secretagogues and ghrelin: an update on physiology and clinical relevance. Horm Res 2002;58 Suppl 3: 56-61.

[23] Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, et al. Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. J Clin Endocrinol Metab 2004;89: 2832-6.

[24] Akamizu T, Iwakura H, Ariyasu H, Hosoda H, Murayama T, Yokode M, et al.

Repeated administration of ghrelin to patients with functional dyspepsia: its effects on food intake and appetite. Eur J Endocrinol 2008;158: 491-8.

[25] Strasser F, Lutz TA, Maeder MT, Thuerlimann B, Bueche D, Tschop M, et al. Safety, tolerability and pharmacokinetics of intravenous ghrelin for cancer-related anorexia/cachexia: a randomised, placebo-controlled, double-blind, double-crossover study. Br J Cancer 2008;98: 300-8.

[26] Hotta M, Ohwada R, Akamizu T, Shibasaki T, Takano K, Kangawa K Ghrelin increases hunger and food intake in patients with restricting-type anorexia nervosa: a pilot study. Endocr J 2009;56: 1119-28.

[27] Nagaya N, Moriya J, Yasumura Y, Uematsu M, Ono F, Shimizu W, et al. Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. Circulation 2004;110: 3674-9.

[28] Nagaya N, Itoh T, Murakami S, Oya H, Uematsu M, Miyatake K, et al. Treatment of cachexia with ghrelin in patients with COPD. Chest 2005;128: 1187-93.

[29] Garcia JM, Cata JP, Dougherty PM, Smith RG Ghrelin prevents cisplatin-induced mechanical hyperalgesia and cachexia. Endocrinology 2008;149: 455-

60.

[30] Wynne K, Giannitsopoulou K, Small CJ, Patterson M, Frost G, Ghatei MA, et al. Subcutaneous ghrelin enhances acute food intake in malnourished patients who receive maintenance peritoneal dialysis: a randomized, placebo-controlled trial. J Am Soc Nephrol 2005;16: 2111-8.

[31] Akamizu T, Iwakura H, Ariyasu H, Murayama T, Sumi E, Teramukai S, et al. Effects of ghrelin treatment on patients undergoing total hip replacement for osteoarthritis: different outcomes from studies in patients with cardiac and pulmonary cachexia. J Am Geriatr Soc 2008;56: 2363-5.

[32] Adachi S, Takiguchi S, Okada K, Yamamoto K, Yamasaki M, Miyata H, et al. Effects of ghrelin administration after total gastrectomy: a prospective, randomized, placebo-controlled phase II study. Gastroenterology 2010;138: 1312-20.

[33] Yamamoto K, Takiguchi S, Miyata H, Adachi S, Hiura Y, Yamasaki M, et al. Randomized phase II study of clinical effects of ghrelin after esophagectomy with gastric tube reconstruction. Surgery 2010:

[34] Cummings DE Ghrelin and the short- and long-term regulation of appetite and body weight. Physiol Behav 2006;89: 71-84.

[35] Wren AM, Bloom SR Gut hormones and appetite control. Gastroenterology 2007;132: 2116-30.

[36] Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, et al. Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. Endocrinology 2004;145: 2607-12.

[37] Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. Diabetes 2001;50: 2438-43.

[38] Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, et al. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes 2001;50: 227-32.

[39] Toshinai K, Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, et al. Ghrelin-induced food intake is mediated via the orexin pathway. Endocrinology 2003;144: 1506-12.

[40] Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, et al. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and

growth hormone secretion in rats. Gastroenterology 2002;123: 1120-8.

[41] Wren AM, Small CJ, Abbott CR, Dhillo WS, Seal LJ, Cohen MA, et al. Ghrelin causes hyperphagia and obesity in rats. Diabetes 2001;50: 2540-7.

[42] Akamizu T, Takaya K, Irako T, Hosoda H, Teramukai S, Matsuyama A, et al. Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. Eur J Endocrinol 2004;150: 447-55.

[43] Nass R, Pezzoli SS, Oliveri MC, Patrie JT, Harrell FE, Jr., Clasey JL, et al. Effects of an oral ghrelin mimetic on body composition and clinical outcomes in healthy older adults: a randomized trial. Ann Intern Med 2008;149: 601-11.

[44] Nass R, Farhy LS, Liu J, Prudom CE, Johnson ML, Veldhuis P, et al. Evidence for acyl-ghrelin modulation of growth hormone release in the fed state. J Clin Endocrinol Metab 2008;93: 1988-94.

[45] Gibney J, Healy ML, Sonksen PH The growth hormone/insulin-like growth factor-I axis in exercise and sport. Endocr Rev 2007;28: 603-24.

[46] Velloso CP Regulation of muscle mass by growth hormone and IGF-I. Br J Pharmacol 2008;154: 557-68.

[47] Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, et al. Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine

expression by human monocytes and T cells. J Clin Invest 2004;114: 57-66.

[48] Li WG, Gavrila D, Liu X, Wang L, Gunnlaugsson S, Stoll LL, et al. Ghrelin inhibits proinflammatory responses and nuclear factor-kappaB activation in human endothelial cells. Circulation 2004;109: 2221-6.

[49] Gonzalez-Rey E, Chorny A, Delgado M Therapeutic action of ghrelin in a mouse model of colitis. Gastroenterology 2006;130: 1707-20.

[50] Wu R, Dong W, Zhou M, Zhang F, Marini CP, Ravikumar TS, et al. Ghrelin attenuates sepsis-induced acute lung injury and mortality in rats. Am J Respir Crit Care Med 2007;176: 805-13.

[51] Theil MM, Miyake S, Mizuno M, Tomi C, Croxford JL, Hosoda H, et al. Suppression of experimental autoimmune encephalomyelitis by ghrelin. J Immunol 2009;183: 2859-66.

[52] Kodama T, Ashitani J, Matsumoto N, Kangawa K, Nakazato M Ghrelin treatment suppresses neutrophil-dominant inflammation in airways of patients with chronic respiratory infection. Pulm Pharmacol Ther 2008;21: 774-9.

[53] Waseem T, Duxbury M, Ito H, Ashley SW, Robinson MK Exogenous ghrelin modulates release of pro-inflammatory and anti-inflammatory cytokines in LPS-stimulated macrophages through distinct signaling pathways. Surgery 2008;143:

334-42.

[54] Tracey KJ Physiology and immunology of the cholinergic antiinflammatory pathway. J Clin Invest 2007;117: 289-96.

[55] Jackman RW, Kandarian SC The molecular basis of skeletal muscle atrophy. Am J Physiol Cell Physiol 2004;287: C834-43.

[56] Balasubramaniam A, Joshi R, Su C, Friend LA, Sheriff S, Kagan RJ, et al. Ghrelin inhibits skeletal muscle protein breakdown in rats with thermal injury through normalizing elevated expression of E3 ubiquitin ligases MuRF1 and MAFbx. Am J Physiol Regul Integr Comp Physiol 2009;296: R893-901.

[57] Sheriff S, Joshi R, Friend LA, James JH, Balasubramaniam A Ghrelin receptor agonist, GHRP-2, attenuates burn injury-induced MuRF-1 and MAFbx expression and muscle proteolysis in rats. Peptides 2009;30: 1909-13.

[58] Yamamoto D, Ikeshita N, Matsubara T, Tasaki H, Herningtyas EH, Toda K, et al. GHRP-2, a GHS-R agonist, directly acts on myocytes to attenuate the dexamethasone-induced expressions of muscle-specific ubiquitin ligases, Atrogin-1 and MuRF1. Life Sci 2008;82: 460-6.

[59] Stitt TN, Drujan D, Clarke BA, Panaro F, Timofeyva Y, Kline WO, et al. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced

ubiquitin ligases by inhibiting FOXO transcription factors. Mol Cell 2004;14: 395-403.

[60] Peeters TL Central and peripheral mechanisms by which ghrelin regulates gut motility. J Physiol Pharmacol 2003;54 Suppl 4: 95-103.

[61] Sibilia V, Rindi G, Pagani F, Rapetti D, Locatelli V, Torsello A, et al. Ghrelin protects against ethanol-induced gastric ulcers in rats: studies on the mechanisms of action. Endocrinology 2003;144: 353-9.

[62] Xu X, Jhun BS, Ha CH, Jin ZG Molecular mechanisms of ghrelin-mediated endothelial nitric oxide synthase activation. Endocrinology 2008;149: 4183-92.

[63] Morley JE, Farr SA Cachexia and neuropeptide Y. Nutrition 2008;24: 815-9.

[64] Konturek PC, Brzozowski T, Engel M, Burnat G, Gaca P, Kwiecien S, et al. Ghrelin ameliorates colonic inflammation. Role of nitric oxide and sensory nerves. J Physiol Pharmacol 2009;60: 41-7.

[65] Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, et al. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab 2001;86: 4753-8.

[66] Nakai Y, Hosoda H, Nin K, Ooya C, Hayashi H, Akamizu T, et al. Plasma

levels of active form of ghrelin during oral glucose tolerance test in patients with anorexia nervosa. Eur J Endocrinol 2003;149: R1-3.

[67] Hotta M, Ohwada R, Katakami H, Shibasaki T, Hizuka N, Takano K Plasma levels of intact and degraded ghrelin and their responses to glucose infusion in anorexia nervosa. J Clin Endocrinol Metab 2004;89: 5707-12.

[68] Miljic D, Pekic S, Djurovic M, Doknic M, Milic N, Casanueva FF, et al. Ghrelin has partial or no effect on appetite, growth hormone, prolactin, and cortisol release in patients with anorexia nervosa. J Clin Endocrinol Metab 2006;91: 1491-5.

[69] Broglio F, Gianotti L, Destefanis S, Fassino S, Abbate Daga G, Mondelli V, et al. The endocrine response to acute ghrelin administration is blunted in patients with anorexia nervosa, a ghrelin hypersecretory state. Clin Endocrinol (Oxf) 2004;60: 592-9.

[70] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed.1992; Washington D.C. , Association AP

[71] Talley NJ, Stanghellini V, Heading RC, Koch KL, Malagelada JR, Tytgat GN Functional gastroduodenal disorders. Gut 1999;45 Suppl 2: II37-42.

[72] Shinomiya T, Fukunaga M, Akamizu T, Irako T, Yokode M, Kangawa K, et al.

Plasma acylated ghrelin levels correlate with subjective symptoms of functional dyspepsia in female patients. Scand J Gastroenterol 2005;40: 648-53.

[73] Akamizu T, Kangawa K Therapeutic applications of ghrelin to cachexia utilizing its appetite-stimulating effect. Peptides 2011;32: 2295-300.

[74] Morley JE, Thomas DR, Wilson MM Cachexia: pathophysiology and clinical relevance. Am J Clin Nutr 2006;83: 735-43.

[75] Cummings DE, Foster-Schubert KE, Overduin J Ghrelin and energy balance: focus on current controversies. Curr Drug Targets 2005;6: 153-69.

[76] Nagaya N, Kangawa K Therapeutic potential of ghrelin in the treatment of heart failure. Drugs 2006;66: 439-48.

[77] Akashi YJ, Springer J, Anker SD Cachexia in chronic heart failure: prognostic implications and novel therapeutic approaches. Curr Heart Fail Rep 2005;2: 198-203.

[78] Nagaya N, Kojima M, Kangawa K Ghrelin, a novel growth hormone-releasing peptide, in the treatment of cardiopulmonary-associated cachexia. Intern Med 2006;45: 127-34.

[79] Lainscak M, Andreas S, Scanlon PD, Somers VK, Anker SD Ghrelin and neurohumoral antagonists in the treatment of cachexia associated with

cardiopulmonary disease. Intern Med 2006;45: 837.

[80] Ashitani J, Matsumoto N, Nakazato M Ghrelin and its therapeutic potential for cachectic patients. Peptides 2009;30: 1951-6.

[81] Gertner JM, Oo C 2009 Performance improvement in COPD cachexia with SUN11031 (a synthetic human ghrelin) in a placebo controlled trial [abstract]. The 5th cachexia conference, Barcelona, 2009, p p143

[82] DeBoer MD, Zhu XX, Levasseur P, Meguid MM, Suzuki S, Inui A, et al. Ghrelin treatment causes increased food intake and retention of lean body mass in a rat model of cancer cachexia. Endocrinology 2007;148: 3004-12.

[83] Hanada T, Toshinai K, Kajimura N, Nara-Ashizawa N, Tsukada T, Hayashi Y, et al. Anti-cachectic effect of ghrelin in nude mice bearing human melanoma cells. Biochem Biophys Res Commun 2003;301: 275-9.

[84] Wang W, Andersson M, Iresjo BM, Lonnroth C, Lundholm K Effects of ghrelin on anorexia in tumor-bearing mice with eicosanoid-related cachexia. Int J Oncol 2006;28: 1393-400.

[85] Garcia JM, Graham C, Kumor K, W. P A Phase II, randomized, placebo-controlled, double blind study of the efficacy and safety of RC-1291 for the treatment of cancer-cachexia [abstract]. J Clin Oncol 2007;25: S25.

[86] Bossola M, Tazza L, Giungi S, Luciani G Anorexia in hemodialysis patients: an update. Kidney Int 2006;70: 417-22.

[87] Ashby DR, Ford HE, Wynne KJ, Wren AM, Murphy KG, Busbridge M, et al. Sustained appetite improvement in malnourished dialysis patients by daily ghrelin treatment. Kidney Int 2009;76: 199-206.

[88] Deboer MD, Zhu X, Levasseur PR, Inui A, Hu Z, Han G, et al. Ghrelin treatment of chronic kidney disease: improvements in lean body mass and cytokine profile. Endocrinology 2008;149: 827-35.

[89] Corpas E, Harman SM, Blackman MR Human growth hormone and human aging. Endocr Rev 1993;14: 20-39.

[90] Wurtman JJ, Lieberman H, Tsay R, Nader T, Chew B Calorie and nutrient intakes of elderly and young subjects measured under identical conditions. J Gerontol 1988;43: B174-80.

[91] Morley JE Anorexia of aging: physiologic and pathologic. Am J Clin Nutr 1997;66: 760-73.

[92] Muller EE, Locatelli V, Cocchi D Neuroendocrine control of growth hormone secretion. Physiol Rev 1999;79: 511-607.

[93] Van der Lely AJ, Lamberts SW, Jauch KW, Swierstra BA, Hertlein H, Danielle

De Vries D, et al. Use of human GH in elderly patients with accidental hip fracture. Eur J Endocrinol 2000;143: 585-92.

[94] Weissberger AJ, Anastasiadis AD, Sturgess I, Martin FC, Smith MA, Sonksen PH Recombinant human growth hormone treatment in elderly patients undergoing elective total hip replacement. Clin Endocrinol (Oxf) 2003;58: 99-107.

[95] Yeo AL, Levy D, Martin FC, Sonksen P, Sturgess I, Wheeler MM, et al. Frailty and the biochemical effects of recombinant human growth hormone in women after surgery for hip fracture. Growth Horm IGF Res 2003;13: 361-70.

[96] Bach MA, Rockwood K, Zetterberg C, Thamsborg G, Hebert R, Devogelaer JP, et al. The effects of MK-0677, an oral growth hormone secretagogue, in patients with hip fracture. J Am Geriatr Soc 2004;52: 516-23.

[97] Akamizu T, Murayama T, Teramukai S, Miura K, Bando I, Irako T, et al. Plasma ghrelin levels in healthy elderly volunteers: the levels of acylated ghrelin in elderly females correlate positively with serum IGF-I levels and bowel movement frequency and negatively with systolic blood pressure. J Endocrinol 2006;188: 333-44.

[98] Takachi K, Doki Y, Ishikawa O, Miyashiro I, Sasaki Y, Ohigashi H, et al.

Postoperative ghrelin levels and delayed recovery from body weight loss after distal or total gastrectomy. J Surg Res 2006;130: 1-7.

[99] Doki Y, Takachi K, Ishikawa O, Miyashiro I, Sasaki Y, Ohigashi H, et al. Ghrelin reduction after esophageal substitution and its correlation to postoperative body weight loss in esophageal cancer patients. Surgery 2006;139: 797-805.

[100] Akamizu T, Kangawa K Translational research on the clinical applications of ghrelin. Endocr J 2006;53: 585-91.

Table 1.Clinical studies of ghrelin

Diseases	Reference	Published year	Study design	Number of patients	Ghrelin administration
CHF	[27]	2004	open-label pilot study	10	2 μg/kg b.i.d. for 3 wks, i.v.
COPD	[28]	2005	open-label pilot study	7	2 μg/kg b.i.d. for 3 wks, i.v.
Cancer cachexia	[23]	2004	acute, randomized, placebo-controlled, cross-over study	7	5 pmol/kg/min i.v. for > 180 min
Cancer cachexia	[25]	2008	randomized, placebo-controlled, cross-over study	21	2 or 8μg/kg, i.v. for 4 days, once a day
ESRD	[65]	2005	acute, randomized, placebo-controlled, cross-over study	9	3.6 nmol/kg, s.c.
ESRD	[8]	2009	randomized, placebo-controlled, cross-over study	12	12 μg/kg, s.c. for 1 wk, once a day
AN	[26]	2009	open-label pilot study	5	3 μg/kg b.i.d. for two wks, i.v.
FD	[24]	2008	open-label pilot study	6	3 μg/kg b.i.d. for two wks, i.v.
THR for OA	[31]	2008	randomized, placebo-controlled, double-blind study	32	2 μg/kg b.i.d. for 3 wks, i.v.
Total gastrectomy	[32]	2010	randomized, placebo-controlled, double-blind study	21	3 μg/kg b.i.d. for 10 days, i.v.
Esophagectomy	[33]	2010	randomized, placebo-controlled, double-blind study	20	3 μg/kg b.i.d. for 10 days, i.v.

CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; FD, functional dyspepsia; ESRD, End-stage renal disease; THR, total hip replacement; OA, osteoarthritis