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Drug Insight: the functions of ghrelin and its potential as a multitherapeutic hormone

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SUMMARY

The endogenous ligand for the growth-hormone (GH) secretagogue receptor was purified from stomach and named ghrelin. It has potent GH-releasing activity and stimulates appetite by acting on the hypothalamic arcuate nucleus, a region known to control food intake. Ghrelin thus plays important roles in maintaining GH release and energy homeostasis in vertebrates. Ghrelin, moreover, stimulates gastric motility and acid secretion, shows positive cardiovascular effects, and has direct actions on bone formation. The diverse functions of ghrelin raise the possibility of its clinical application for GH deficiency, eating disorders, gastrointestinal disease, cardiovascular disease, osteoporosis and aging.

KEYWORDS acyl modification, appetite, ghrelin, growth hormone

REVIEW CRITERIA

We searched in PubMed for original articles focusing on ghrelin that were published between 1999 and 2005. We also searched for original articles on GHS (growth-hormone secretagogue) published between 1980 and 2005. All papers identified were English-language full-text papers.

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INTRODUCTION

The name 'ghrelin' is based on 'ghre', a word root in Proto-Indo-European languages for 'grow', in reference to its ability to stimulate growth-hormone (GH) release. Ghrelin was originally purified from stomach and exerts potent appetite-stimulating activities as well as its GH-releasing effects.[1,2] The rat and human ghrelin precursors are both composed of 117 amino acids. In these precursors, the 28-amino-acid active ghrelin sequence immediately follows the signal peptide (Figure 1). Ghrelin is a peptide hormone, in which the serine at position 3 is n-octanoylated; this modification is essential for its neuroendocrine and orexigenic activities. The enzyme that catalyzes the acyl modification of ghrelin has not yet been identified. The universal incorporation of n-octanoic acid in ghrelin of mammals, fish, birds, and amphibians, however, suggests that this putative enzyme is rather specific in its choice of medium-chain fatty-acid substrates.

In all vertebrate species, ghrelin is mainly produced in the stomach. Ghrelin-containing cells in the stomach are more abundant in the fundus than in the pylorus.[3] The X/A-like cells in the oxyntic mucosa of the stomach contain round, compact, electron-dense granules that are filled with ghrelin. Ghrelin-producing cells are also found in the duodenum, jejunum, ileum, colon, pancreas, kidney and placenta. Ghrelin is, moreover, found, at very low levels, in the brain;[4] it has been found in the hypothalamic arcuate nucleus, an important region for controlling appetite. In addition, a recent study has reported the presence of ghrelin in previously uncharacterized hypothalamic neurons adjacent to the third ventricle between the dorsal, ventral, paraventricular, and arcuate hypothalamic nuclei.[4,5] These ghrelin-containing neurons send efferent fibers to neurons that contain neuropeptide Y (NPY) and agouti-related protein (AgRP)—NPY/AgRP neurons—and might stimulate the release of these OREXIGENIC PEPTIDES.

Analysis using reverse transcriptase polymerase chain reaction demonstrates ghrelin-receptor mRNA expression in many peripheral organs, including heart, lung, liver, kidney, pancreas, stomach, small and large intestines, adipose tissue and immune cells, indicating that ghrelin has multiple functions in these tissues. In fact, ghrelin not only affects GH release and appetite stimulation but also has cardiovascular effects, increases gastric movement and secretion of gastric acid, suppresses sympathetic nerves and regulates glucose metabolism.

Here, we will provide an overview of the physiological functions of ghrelin and will discuss its possible clinical applications.

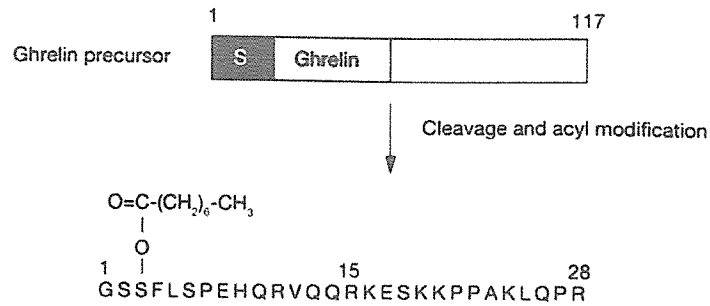

Figure 1 From the ghrelin precursor to an active peptide. Human ghrelin mRNA is translated into a 117-amino-acid ghrelin precursor (preproghrelin). Protease cleavage and acyl modification of the ghrelin precursor result in the production of the 28-amino-acid mature ghrelin peptide. The acyl modification, mainly n-octanoyl modification, is essential for the activity of ghrelin. S, signal peptide.

PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL FUNCTIONS OF GHRELIN

Growth-hormone-releasing activity

Box 1 summarizes ghrelin's effects, one of the most important resulting from its interaction with the ghrelin receptor (the GH secretagogue, or GHS, receptor) in the pituitary; this leads to an increase in the intracellular Ca^{2+} concentration via inositol triphosphate and stimulates GH release.[6] The ghrelin receptor is distinct from the GHRH (GH-releasing hormone) receptor. Ghrelin stimulates GH release both *in vitro* and *in vivo* in a dose-dependent manner.[1] Intravenous injection of ghrelin induces potent GH release in both mammalian and non-mammalian vertebrates. When injected intravenously into rats, the maximal stimulation effected by ghrelin is two or three times greater than that of GHRH.

Ghrelin stimulates GH release from cultured primary pituitary cells, which indicates that ghrelin can act directly on the pituitary (Figure 2).[1] The involvement of the hypothalamus in ghrelin-mediated stimulation of GH release has also been strongly suggested. Patients with organic lesions in the hypothalamic region show insufficiency of GH release even when stimulated by ghrelin.[7] When using primary pituitary cells, ghrelin treatment only increases GH release by two to three times above the basal level,[1] which is lower than the level of induction seen when ghrelin is administered to rats *in vivo*.

These facts suggest that other factors are involved *in vivo* in order for the maximal level of GH release to be achieved by ghrelin administration. One possibility is transmission via the vagus nerve and the nucleus of the solitary tract (Figure 2).[8,9] When the vagus nerve is cut, GH release after ghrelin injection is dramatically decreased, indicating that the vagus nerve is needed for the maximal stimulatory effects of ghrelin. Another possible explanation for the relatively poor response of primary pituitary cells is the lack of GHRH in such cells. Coadministration of ghrelin and GHRH has

Box 1 Effects of ghrelin.

Effects on hormone secretion
- Increased growth-hormone release
- Weakly increased adrenocorticotropic-hormone release
- Weakly increased cortisol release
- Weakly increased prolactin release
- Variable effect on insulin release

Anabolic effects
- Increased appetite
- Increased adiposity
- Increased blood glucose levels

Effects on gastric function
- Increased gastric-acid secretion
- Increased gastric movement
- Increased turnover of gastric and intestinal mucosa

Effects on cardiovascular function
- Increased cardiac output
- Decreased blood pressure

Effect on bone formation
- Proliferation and differentiation of osteoblasts
- Increased BMD

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GLOSSARY

OREXIGENIC PEPTIDES
Peptides that stimulate appetite and increase food intake, for example ghrelin, neuropeptide Y, agouti-related peptide, and orexin

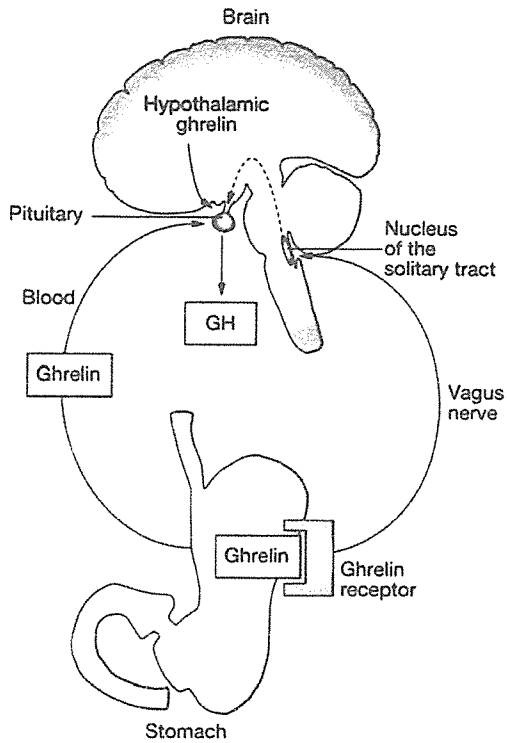

Figure 2 Regulation of growth-hormone release from the pituitary by ghrelin. Ghrelin-stimulated growth-hormone release is controlled by three pathways: one is a direct effect of circulating ghrelin on pituitary cells, the second is an indirect effect through the vagus nerve, and the third is a direct effect by ghrelin from the hypothalamus. GH, growth hormone.

a synergistic effect on GH secretion; that is, coadministration results in more GH release than does either GHRH or ghrelin alone.[10] The synergistic effect on GH release is also observed by coadministration of GHSs, synthetic ghrelin agonists, and GHRH. This finding implies that GHRH is necessary for ghrelin to be maximally effective in inducing GH release.

Appetite regulation

The recent identification of appetite-regulating humoral factors reveals regulatory mechanisms not only in the central nervous system, but also in the peripheral nervous system, mediated by factors secreted from peripheral tissues (Figure 3).[11] Leptin, produced in adipose tissues, is an appetite-suppressing factor that transmits satiety signals to the brain. Hunger signals from peripheral tissues, however, had remained unidentified until ghrelin's discovery. Ghrelin is produced primarily in gastrointestinal organs in response to hunger and starvation, and circulates in the blood, serving as a peripheral signal telling the central nervous system to stimulate feeding. When ghrelin is injected into the cerebral ventricles of rats, their food intake is potently stimulated.[12–14] Not only intracerebroventricular (ICV) injection, but also intravenous and subcutaneous injection of ghrelin have, moreover, been shown to increase food intake.

In the brain, the hypothalamic arcuate nucleus is the main site of ghrelin's appetite-stimulating activity (Figure 3). At least part of the orexigenic effect of ghrelin is mediated by upregulation of the genes encoding potent appetite stimulants, such as NPY and AgRP, since ICV injection of ghrelin induces expression of c-Fos (a marker of neural activity) in NPY/AgRP neurons and increases the amount of NPY and AgRP mRNA in the arcuate nucleus.[12] In contrast, the appetite-stimulating effects of ghrelin are blocked by ICV injection of an NPY-receptor-1 antagonist, an AgRP inhibitor, anti-NPY IgG, or anti-AgRP IgG. Immunohistochemical analysis indicates that fibers of ghrelin-expressing neurons directly contact NPY/AgRP neurons.[5] These results indicate that ghrelin exerts its appetite-enhancing activity by stimulating NPY/AgRP neurons in the hypothalamus to promote the production and secretion of NPY and AgRP. Intravenous injection of ghrelin also stimulates the same neurons in the hypothalamus. Studies of mice with knockouts of NPY, AgRP or both confirm these results.[15] Although deletion of either NPY or AgRP causes a modest or no effect on the orexigenic action of ghrelin, the double-knockout mice completely lack a response to the actions of ghrelin.

Peripherally injected ghrelin activates hypothalamic neurons and stimulates food intake.[8,16,17] In general, peptides injected peripherally do not pass the blood–brain barrier. Indeed, the rate at which peripheral ghrelin passes the barrier has been shown to be very low. Thus, peripheral ghrelin can activate the appropriate hypothalamic regions via an indirect pathway. The detection of ghrelin-receptor mRNA in vagal afferent neurons in the rat nodose ganglion suggests that ghrelin signals from the stomach are transmitted to the brain via the vagus nerve.[8,18] The observation that ICV administration of ghrelin induces

expression of c-Fos in the dorsomotor nucleus of the vagus and stimulates gastric-acid secretion, moreover, indicates that ghrelin activates the vagus system.[19]

In contrast, vagotomy inhibits the ability of ghrelin to stimulate food intake and GH release.[19,20] A similar effect was also observed when capsaicin, a specific afferent neurotoxin, was applied to vagus-nerve fibers to induce sensory denervation. On the other hand, fasting-induced elevation of plasma ghrelin levels is completely abolished by subdiaphragmatic vagotomy or atropine treatment.[9] These results indicate that the response to ghrelin during fasting is partly transmitted through vagal afferents.

Other physiological functions

Effects of ghrelin on the cardiovascular and gastrointestinal system, as well as on bone and glucose metabolism, have been reported. Intravenous injection of ghrelin dose-dependently increases gastric acid secretion and stimulates gastric motility.[21] The maximum response of gastric acid secretion by ghrelin is almost as high as that elicited by histamine.

The expression of mRNA encoding both ghrelin and its receptor has been observed in the cardiovascular system, and intravenous injection of ghrelin induces a reduction in blood pressure without changing the heart rate.[22,23]

Ghrelin and its receptor have been identified in osteoblasts; ghrelin directly promotes osteoblast proliferation and differentiation *in vitro* and increases BMD *in vivo*.[24] These actions of ghrelin on bone formation are not entirely caused by activation of the GH–IGF-I (insulin-like growth factor 1) axis; ghrelin also acts directly, since it promotes bone formation even in GH-deficient spontaneous dwarf rats.

The role of ghrelin in insulin secretion is under debate. Ghrelin has been shown to inhibit insulin secretion in some experiments and to stimulate insulin release in others.[25–28] This discrepancy might be due to experimental design, in particular the chosen glucose level, since plasma insulin levels are affected by blood glucose level and make it difficult to assess the exact contribution of ghrelin.

Life without ghrelin

The stomach is the major source of circulating ghrelin. Total gastrectomy, as is performed in the treatment of gastric cancer or severe gastric ulcers, has been shown to reduce the plasma concentration of ghrelin to 30–50% of that of a normal person when measured 30 min after the operation.[29] This concentration gradually increases over a few months to approximately 70% of the level before the operation. This indicates that gastric ghrelin production accounts for about 50–70% of circulating ghrelin, but that this percentage is subject to compensatory production, possibly by the intestines and pancreas. The appetite loss that is frequently observed after gastrectomy could be in part due to the decrease in ghrelin concentration.

A ghrelin-knockout mouse strain has been produced, and its phenotype has been examined.[30,31] These mice show normal size,

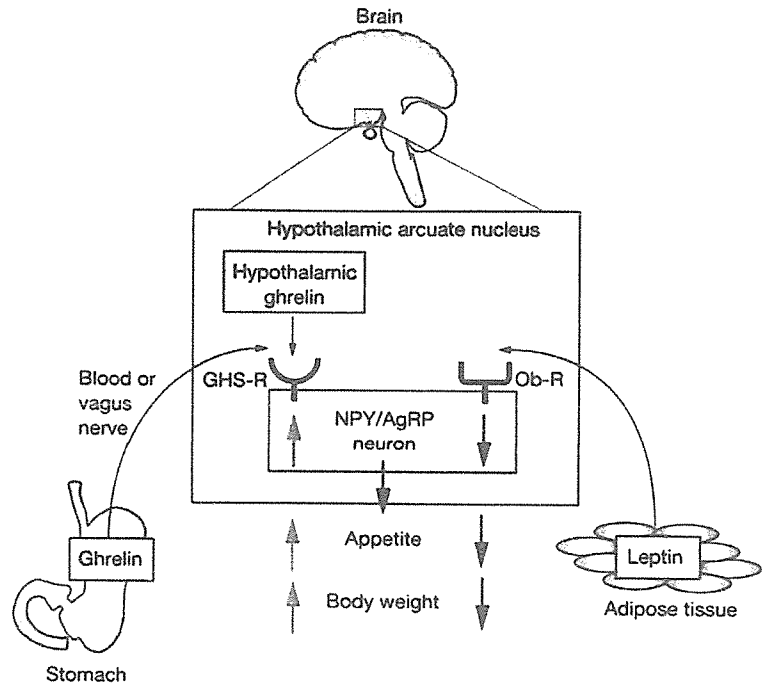

Figure 3 Hypothalamic appetite regulation by ghrelin and leptin. The arcuate nucleus of the hypothalamus is the main target of ghrelin and leptin. Ghrelin is a peripheral orexigenic signal secreted from the stomach, whereas leptin is a peripheral anorexigenic signal secreted from adipose tissue. In the arcuate nucleus, ghrelin stimulates neurons expressing neuropeptide Y and agouti-related peptide, and leptin suppresses them. Neuropeptide Y released in response to ghrelin stimulates appetite and increases body weight. Appetite regulation in the hypothalamus is, therefore, controlled through the neuropeptide Y signaling pathway. The effects of leptin are opposite to those of ghrelin; leptin decreases neuropeptide Y release to suppress appetite and body weight.
AgRP, agouti-related peptide; GHS-R, growth-hormone-secretagogue receptor (ghrelin receptor); NPY, neuropeptide Y; Ob-R, leptin receptor.

GLOSSARY

RESPIRATORY QUOTIENT
The ratio of carbon dioxide produced to oxygen consumed; it decreases if lipid, and increases if carbohydrate, is the main metabolic substrate

HYPERPHAGIA
A condition in which appetite is stimulated and food intake is increased

growth rate, food intake, body composition, reproduction, and gross behavior, without any pathologic changes. Since survival is more acutely threatened by starvation than by obesity, it might be expected that orexigenic-peptide-null mouse strains show no change in food intake and body weight.

The ghrelin-null mice did, however, show a significant reduction in RESPIRATORY QUOTIENT, which means that they preferably utilize fat rather than carbohydrate as an energy substrate; they also show a trend for lower body fat mass when fed with a high-fat diet.[31] These results indicate that ghrelin is not a critically required orexigenic factor, but might function in nutrient-sensing and switching of metabolic substrates. During the dark period of the day–night cycle, moreover, heat production in ghrelin-knockout mice (15 to 25 weeks old) was higher than that of the wild-type littermates, indicating that ghrelin regulates storage or consumption of energy.[32]

Regulation of ghrelin production and secretion

The most important factor for the regulation of ghrelin secretion is the energy state of the body. The plasma ghrelin concentration is increased when fasting and decreases after food intake.[33,34] It is not clear what elements are involved in the regulation of ghrelin secretion. Blood glucose level might be critical: oral or intravenous administration of glucose decreases the plasma ghrelin concentration.

The plasma ghrelin concentration is low in obese people and high in lean people.[35,36] Related to this fact, the plasma ghrelin level is highly increased in patients with anorexia nervosa (AN) and returns to normal levels upon weight gain and recovery from the disease.[37–39] The ghrelin concentration is also increased in patients with bulimia nervosa. As detailed below, patients who have had a gastric bypass show a decrease of ghrelin levels and lose weight.[40,41] Changes in ghrelin concentration associated with food intake are diminished in these patients. The plasma ghrelin concentration also decreases in patients with short-bowel syndrome, probably because of the loss of ghrelin-producing tissues.

Exogenous treatment with somatostatin or its analogues, such as octreotide, as well as infusion of urocortin-1, a potent anorexigenic peptide, suppresses plasma ghrelin concentrations.[42,43] Exogenous GH decreases gastric expression of ghrelin mRNA and plasma ghrelin concentration, but does not affect gastric ghrelin stores.[44] These results suggest that pituitary GH exhibits a feedback regulation on ghrelin production by the stomach.

Feeding disorders and ghrelin

AN is a syndrome often seen in young women and is characterized by a combination of weight loss, amenorrhea, and behavioral changes. Some of the changes are reversible with weight gain. As described above, plasma ghrelin levels in AN patients are high and return to control levels after weight gain by renutrition. AN patients often show markedly elevated GH levels, which might be due to high circulating levels of ghrelin. Such high ghrelin concentrations increase adrenocorticotropic hormone, prolactin, and cortisol levels in humans (Box 1),[45] which might explain the amenorrhea and behavioral changes observed in patients with AN.

Prader-Willi syndrome (PWS) is a complex genetic disorder linked to region q11–q13 on chromosome 15 and is characterized by mild mental retardation, HYPERPHAGIA, short stature, muscular hypotonia, and distinctive behavioral features. The excessive appetite in PWS causes progressive severe obesity, which in turn leads to an increase of cardiovascular morbidity and mortality. It has been suggested that the genetic alteration leads to dysfunction of several hypothalamic areas, including appetite regulatory regions. In patients with PWS, increased numbers of ghrelin-expressing cells, an increased density of ghrelin in these cells and a high plasma ghrelin concentration are observed.[46,47] The mean plasma concentration of ghrelin is threefold to fourfold higher in PWS than in the general population. Thus, ghrelin might be responsible, at least in part, for the hyperphagia seen in PWS. Elucidation of the precise mechanism by which ghrelin gene expression is regulated could reveal the genetic cause of hyperphagia in PWS.

Gastric bypass and ghrelin

To treat severe obesity, gastric bypass operations are often performed. The purpose of this procedure is to reduce the space for food in the gastric cavity and hence reduce total caloric intake. In the US, a total of 40,000 people are estimated to have been treated with a gastric bypass in 2000, and 75,000 in 2001. The exact mechanism of action of this operation is, however, unknown.

Recent research has revealed that ghrelin might contribute to the body-weight reduction that occurs following gastric bypass. Patients receiving such a procedure were examined for ghrelin levels after successful weight loss.[40,41] Total ghrelin secretion was found to be reduced by up to 77% compared with normal-weight control groups and by up to 72% compared with matched obese groups. The normal meal-related fluctuations and diurnal rhythm of ghrelin level were, furthermore, absent in these patients. Thus, the mean plasma ghrelin concentration decreased significantly after gastric bypass surgery, which may have been responsible for their lack of hyperphagia and contributed to their weight loss.

CLINICAL APPLICATIONS OF GHRELIN

The diverse functions of ghrelin raise the possibility of its clinical applications; these are shown in Box 2 and detailed below.

Box 2 Possible clinical applications of ghrelin.

Growth-hormone deficiencies
Diagnosis of pituitary function
Pediatric and adult growth-hormone deficiency

Eating disorders
Anorexia nervosa
Bulimia nervosa

Gastrointestinal disease
Gastric ileus
Gastric ulcer
Inflammatory bowel diseases (Crohn's disease, ulcerative colitis)

Cardiovascular diseases
Heart failure
Dilated cardiomyopathy

Osteoporosis

Aging

Catabolic states or chronic wasting syndromes
Cachexia (cancer, cardiac cachexia)
AIDS
Postoperative patients

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Diagnosis and treatment of growth-hormone deficiency

Because of its potent GH-releasing activity and specificity, ghrelin can be applied to the diagnosis and treatment of GH deficiency. To diagnose GH deficiency, the most common stimulus used is insulin-induced hypoglycemia, in which blood glucose levels decrease to less than 2.2 mM (40 mg/dl). This test can evaluate both GH and adrenocorticotropic-hormone release in patients with pituitary disease. The hypoglycemic action of insulin can, however, cause side effects; insulin-induced hypoglycemia is contraindicated in patients with diabetes, ischemic heart disease, cerebrovascular disease, or epilepsy, and in elderly patients. At present, intravenous injection of ghrelin into humans does not show any side effects, suggesting that ghrelin might be useful for diagnosing GH deficiency in these patients. By its synergistic effect, combined administration of ghrelin and GHRH might be more beneficial than a single test.

In general, GH secretion declines markedly with age so that GH production after middle age is less than 15% of that during puberty. This GH deficiency is paralleled by an age-related decline in muscle mass. Adult GH deficiency can, therefore, be ameliorated by ghrelin treatment. In addition, coadministration of ghrelin and GHRH has a synergistic effect on GH secretion, and their combined administration is the most potent inducer of GH release yet identified.[10]

Weight loss and eating disorders

At present, it seems that ghrelin is a peripheral orexigenic signal that is effective upon its intravenous injection. Thus, ghrelin may be useful as an orexigenic agent for the treatment of weight loss in eating disorders such as AN and bulimia nervosa.[48] Injection of ghrelin can stimulate appetite and improve the nutritional state of these patients; however, the plasma ghrelin concentration in patients with AN is very high. This indicates that sensitivity to ghrelin is severely disturbed in these individuals.[49]

In contrast, blocking or neutralizing ghrelin's action might be a reasonable approach to reversing a chronic obese state. Appetite is, however, regulated by numerous factors that might interact with and compensate for each other; therefore, a ghrelin antagonist might only have a limited effect on obesity. Indeed, ghrelin-null mice showed no obvious abnormalities in feeding behavior.[30,31]

Gastrointestinal diseases

Ghrelin stimulates gastric motility, which makes it a candidate for the treatment of post-operative

GLOSSARY

GASTRIC ILEUS
A condition in which gastric movement is stopped and normal gastric functions are disturbed; caused by injury, operation or inflammation

GASTRIC ILEUS.[50,51] Ghrelin administration has been shown to have a strong prokinetic effect, accelerating gastric emptying and the small-intestinal transit of liquid meals, and reversing delayed gastric evacuation, thereby counteracting gastric ileus.

Central and intraperitoneal administration of ghrelin reduce ethanol-induced gastric ulcers in a dose-dependent manner.[52] This effect is prevented by N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthesis, and by capsaicin, indicating that the gastroprotective effect of ghrelin is mediated by NO and requires capsaicin-sensitive sensory nerve activity.

Cardiac diseases

Ghrelin has positive cardiovascular effects, and its receptor is present in blood vessels and the cardiac ventricles. *In vitro*, ghrelin inhibits apoptosis of cardiomyocytes and endothelial cells.[53] Intravenous administration of human ghrelin decreases mean arterial pressure without changing heart rate.[54] Infusion of ghrelin, moreover, decreases systemic vascular resistance and increases cardiac output in patients with heart failure. Ghrelin, furthermore, increases the diastolic thickness of the noninfarcted posterior wall, inhibits left-ventricular (LV) enlargement, and increases LV fractional shortening in rats with chronic heart failure. Ghrelin therefore improves LV dysfunction and attenuates the development of LV remodeling and cardiac cachexia, which suggests that ghrelin has cardiovascular protective effects and regulates energy metabolism through GH-dependent and GH-independent mechanisms. Ghrelin might thus be a new therapeutic agent for the treatment of severe chronic heart failure.

Other potential applications

Other potential clinical applications of ghrelin are in osteoporosis,[55–57] aging, and catabolic states including those seen in postoperative patients as well as in AIDS-associated and cancer-associated wasting syndromes.[58,59] For example, in HIV-lipodystrophy patients, GH and ghrelin levels are both reduced.[60] The reduced ghrelin level might in part cause decreased GH levels. Ghrelin could therefore be useful to treat HIV lipodystrophy by its GH-releasing activity as well as its orexigenic effects.

CONCLUSIONS

Now, 6 years have passed since the discovery of ghrelin, a peptide hormone from the stomach with potent GH-releasing and appetite-stimulating activities. Attempts to find clinical uses for ghrelin are now in progress. Experiments using GHS-receptor-null mice have, furthermore, confirmed synthetic GHSs as ghrelin-receptor agonists; these agents can possibly be used as oral ghrelin mimetics.[61]

Basically, ghrelin is a peptide hormone that helps to supply cells with nutrition and energy and regulates their metabolic activities. The target diseases of ghrelin will be not only GH deficiency but also feeding disorders and weight loss due to various causes. Ghrelin could, moreover, be used in older people to improve 'quality of life' by prevention and treatment of osteoporosis and improvement of muscle strength, through both direct actions of ghrelin and indirect actions mediated by GH.

KEY POINTS

- **Ghrelin is a hormone secreted mainly from the stomach into the circulation**
- **Ghrelin stimulates growth-hormone release**
- **Ghrelin stimulates appetite and increases food intake**
- **Ghrelin is useful for treatment of weight loss and eating disorders**
- **Gastric bypass decreases ghrelin levels and leads to weight loss**
- **Ghrelin improves cardiovascular function**

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Competing interests

The authors declared they have no competing interests.

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Impact of *Helicobacter pylori* Infection on Gastric and Plasma Ghrelin Dynamics in Humans

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OBJECTIVES: There are contradictory reports on the relationship between *Helicobacter pylori* and circulating ghrelin. We sought to clarify the influence of *H. pylori* infection on gastric and plasma ghrelin dynamics in humans.

METHODS: Using endoscopic biopsies from the corpus of 56 *H. pylori*-infected patients and 25 uninfected subjects, ghrelin mRNA expression levels and gastric ghrelin peptide contents were measured by real-time polymerase chain reaction and radioimmunoassay, respectively. We also measured plasma ghrelin concentrations and analyzed the numbers of ghrelin immunoreactive cells in the fundic gland area. Fifty-one patients with *H. pylori* infection were treated with a 7-day triple therapy consisting of lansoprazole, clarithromycin, and amoxicillin.

RESULTS: The gastric ghrelin mRNA expression level of *H. pylori*-positive patients (1.64 ± 1.27 in arbitrary units) was significantly lower than in *H. pylori*-negative subjects (4.87 ± 4.1, $p < 0.0001$). A similar trend was noted for ghrelin peptide contents (31.2 ± 27.5 *vs* 81.2 ± 64.1 ng/mg protein, respectively, $p < 0.0001$). There was no significant difference in the number of ghrelin immunoreactive cells/mm^2 in terms of *H. plyori* status. Plasma ghrelin concentrations in *H. pylori*-infected patients (144.6 ± 7.8.8 fmol/ml) were significantly lower than in uninfected subjects (196.1 ± 97.2, $p < 0.05$) and increased following cure of the infection. Plasma ghrelin levels correlated positively with the expression levels of ghrelin mRNA ($r = 0.583$, $p < 0.0001$) and peptide products ($r = 0.574$, $p < 0.0001$). There was a significant stepwise decrease in gastric ghrelin mRNA expression ($p < 0.05$), peptide contents ($p < 0.01$) and density of ghrelin immunoreactive cells ($p < 0.05$) with progression of histological severity of glandular atrophy in the corpus. The histological severity of chronic inflammation also negatively influenced the ghrelin mRNA expression ($p < 0.001$) and peptide production ($p < 0.005$).

CONCLUSIONS: *H. pylori* infection has a negative impact on gastric and plasma ghrelin dynamics. Chronic inflammatory and atrophic changes associated with the infection may affect gastric ghrelin biosynthesis and contribute to the low circulating levels.

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INTRODUCTION

Ghrelin is a 28-amino acid peptide recently identified in the stomach as an endogenous ligand for growth hormone secretagogue receptor (1). It potently stimulates growth hormone release but is also implicated in many other homeostatic mechanisms (2, 3). Ghrelin influences appetite, energy balance, gastric motility, and acid secretion (2, 3). This hormone is produced by X/A-like cells in the gastric oxyntic glands (4). Ghrelin messenger ribonucleic acid (mRNA) is most highly expressed in the stomach compared with other tissues (5). Plasma ghrelin levels decrease by as much as 65% after gastrectomy (5), and this is consistent with the findings of decreased plasma levels after gastric bypass surgery (6). Collectively, the stomach is the main source of circulating ghrelin (1–6).

The etiologic concept of gastritis changed dramatically following the discovery of *Helicobacter pylori*. Most cases of chronic gastritis are due to *H. pylori* infection (7). Long persistent gastric mucosal inflammation induced by this organism results in progressive atrophy with loss of pyloric and

oxyntic glands (8). In view of close juxtaposition of the endocrine and parietal cell compartments within oxyntic glands (4), it is tempting to speculate that the inflammatory and atrophic events associated with *H. pylori* infection negatively affect ghrelin production in the stomach and its release into the circulation (9). There are, however, contradictory reports in humans on the relationship between *H. pylori* and ghrelin: a Turkish study reported lack of effect of *H. pylori* on plasma ghrelin levels (10), whereas a British study demonstrated an increase of circulating ghrelin following cure of *H. pylori* (11).

To determine the influence of *H. pylori* infection on gastric ghrelin biosynthesis in humans, we assessed the expression levels of ghrelin mRNA in *H. pylori*-infected and -uninfected subjects by quantitative real-time polymerase chain reaction (PCR) procedure, together with morphometric analysis of ghrelin immunoreactive cells in the fundic gland area. The study was also designed to examine the peptide contents in the plasma and stomach by radioimmunoassay (RIA).

MATERIALS AND METHODS

Patients and Sampling

Consecutive outpatients who underwent upper gastrointestinal endoscopy for dyspepsia between October 2002 and December 2004 were recruited. The study was approved by the Nagasaki University Ethics Committee. All samples were obtained with written informed consent of the patients prior to their inclusion, in accordance with the Helsinki Declaration.

Exclusion criteria were: age <18 or >80 yr, pregnancy, body mass index (BMI) >30 kg/m^2, diabetes mellitus, cachectic state including cancer, systemic infection, thyroid and liver diseases, renal impairment, use of medications effective against *H. pylori* during the preceding 3 months, alcoholic abuse, drug addiction, and chronic corticosteroid or nonsteroidal antiinflammatory drug use. None had undergone gastrointestinal surgery.

On the day of endoscopy, blood samples were taken between 8 and 10 AM after an overnight fast, transferred into chilled tubes containing ethylenediaminetetraacetic acid-2Na and aprotinin, stored on ice during collection, centrifuged, plasma separated, and stored at −80°C until assay. Plasma ghrelin concentrations were measured in-house in duplicate by RIA, as described previously (4). This assay system employs a rabbit polyclonal antibody raised against the C-terminal fragment [13–28] of human ghrelin, and can measure both the acylated and des-acyl forms. The intraassay coefficient of variation (CV) was 2.8% and interassay CV was 3.1% (4). The minimum detection level was 10 fmol/tube. We treated 51 *H. pylori*-positive patients with 7-day triple therapy consisting of lansoprazole, amoxicillin, and clarithromycin (12). Four weeks after cessation of the treatment, fasting plasma ghrelin levels were also measured.

During endoscopy, three biopsy specimens were obtained from the middle portion of the corpus along the greater curvature. Two samples were snap-frozen in an ethanol-dry ice mixture and then stored at −80°C until use; one was used for quantitative analysis of ghrelin mRNA and its peptide contents. The other sample was fixed in 10% formalin and embedded in paraffin for histopathological and immunohistological assessment.

For detection of *H. pylori* infection, two additional biopsies were endoscopically taken from the antrum within 2 cm of the pyloric ring and the corpus along the greater curvature. One sample was used for the rapid urease test (Helicocheck, Otsuka Pharmaceutical Co., Tokushima, Japan) and the other for Giemsa staining.

Real-Time Polymerase Chain Reaction

Total RNA from the biopsy samples was extracted using a commercial kit according to the instructions provided by the supplier (Isogen, Nippon Gene Co., Toyama, Japan). One microgram of total RNA was reverse transcribed into complementary deoxyribonucleic acid (cDNA) in a volume of 25 μL with MuLV reverse transcriptase and random hexamers (both from PE Applied Biosystems, Warrington, UK).

Real-time PCR measurement of human ghrelin cDNA was performed in the ABI PRISM 7700 sequence detector (PE Applied Biosystems) with TaqMan assay (PE Applied Biosystems). The primers and probe sequences were synthesized (PE Applied Biosystems) as follows: ghrelin forward primer, 5′-GCCCGGAAGATGGAGGTCAA-3′; reverse primer, 5′-AGGGCCTGGCTGTGCTGCT-3′; and probe, 5′-AGTCCGGTTAACGCCCCCTTTG-3′, labeled with the reporter dye 6-carboxyfluorescein at the 5′ end and quencher dye 6-carboxytetramethylrhodamine at the 3′ end. PCR was performed in a total volume of 50 μL of each amplification mixture containing 1 μL of each reverse transcribed product, 25 μL of twofold Universal Master Mix (PE Applied Biosystems), 200 nM ghrelin forward and reverse primers, and 100 nM fluorogenic probe. Thermal cycling was initiated at 50°C for 2 min, then a first denaturation step at 95°C for 10 min, and followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

The tubulin alpha 3 cDNA (internal control) was quantified in the same machinery using SYBR Green PCR Core reagents kit (PE Applied Biosystems). The primers used were: forward, 5′-AGATCATTGACCTCGTGTTGGA-3′ and reverse, 5′-ACCAGTTCCCCCACCAAAG-3′. PCR was performed in a total volume of 25 μL of each amplification mixture containing 1 μL of each RT product, 3 μL of 25 mM $MgCl_2$, 2.5 μL of 10-fold SYBR Green buffer, 2 μL of dNTP Mix (5 mM adenosine, deoxycytosine and deoxyguanosine triphosphate, and 2.5 mM denoxyuridine triphosphate), 0.625 U AmpliTaq Gold polymerase, 0.125 U AmpErase, and 100 nM tubulin alpha 3 forward and reverse primers. Thermal cycling was initiated at 50°C for 2 min, followed by a first denaturation step at 95°C for 10 min, and continued with 40 cycles of 95°C for 15 s and 59°C for 1 min. The relative

expression level of ghrelin mRNA was expressed as the ratio of ghrelin/tubulin alpha 3 in arbitrary units (13).

Biopsy samples were also taken from the antrum and the incisura angularis of 17 subjects, and subjected to the aforementioned real time PCR analysis. The relative ghrelin mRNA expression was also assessed following cure of *H. pylori* infection.

Measurement of Gastric Ghrelin Peptide Contents

Each biopsy specimen was boiled for 5 min in a 10-fold volume of water to inactivate the intrinsic proteases in accordance with the previous reports (4, 14). The solution was homogenized since acidification of the sample solution preserved the loss of extraction recovery. The supernatant was lyophilized and then subjected to ghrelin RIA. It was also assayed for total protein by a modified Lowry method, and gastric ghrelin content in each sample was expressed in ng/mg protein (15).

Immunohistochemistry

Immunohistochemical staining was performed with the streptavidin-biotin-peroxidase-complex method (Histofine SAB-PO® kit, Nichirei Co., Tokyo, Japan) as described previously (4–16). The following steps were performed at room temperature unless otherwise specified. Paraffin-embedded biopsy specimens were sectioned at 4-μm thickness, deparaffinized and rehydrated. After inhibition of endogenous peroxidase activity for 30 min with methanol containing 0.3% H_2O_2, the sections were reacted for 20 min with 10% normal goat serum to prevent nonspecific binding. They were then incubated overnight with the rabbit polyclonal antighrelin antibody (diluted 1:10,000) at 4°C. On the next day, the sections were washed in 0.01 M phosphate buffered saline (PBS) and incubated for 20 min with 10 mg/mL biotinylated goat antirabbit immunoglobulins. After washing in PBS, the sections were reincubated for 20 min with 100 μg/mL horseradish peroxidase-conjugated streptavidin and stained with 0.02% 3,3′-diaminobenzidine tetrahydrochloride in 0.05 M tris-HCl buffer containing 0.03% H_2O_2. The sections were finally washed in PBS and counterstained with hematoxylin. Control studies were performed with normal rabbit serum or antighrelin antiserum. After taking digital photographs under a light microscope (BX60, Olympus, Tokyo, Japan) with a digital camera (Coolpix950, Nikon, Tokyo, Japan), we counted the numbers of ghrelin immunoreactive cells/mm^2 in the fundic gland area using a computerized image analysis program (Scion Corporation, Frederick, MD) (17).

The numbers of ghrelin immunoreactive cells/mm^2 were also counted before and after cure of the infection in the fundic gland mucosa.

Histopathological Examination

The gastric sections were stained with hematoxylin and eosin. Intestinal metaplasia was defined by the presence of goblet cells in glandular mucosa with Alcian blue (pH 2.5)/periodic acid-Schiff staining. According to the updated Sydney system (7), each histological parameter of activity (neutrophils), chronic inflammation (mononuclear cells), glandular atrophy, and intestinal metaplasia, was graded into none, mild, moderate, or marked.

Table 1. Baseline Characteristics

	H. pylori-Positive (n = 56)	*H. pylori*-Negative (n = 25)
Mean age, yr (range)	54.1 (23–76)	54.0 (20–80)
Male/female	24/32	11/14
Smokers	14 (25.0%)	9 (36.0%)
Alcohol drinkers	18 (32.1%)	9 (36.0%)
Body mass index (kg/m^2) (range)	23.4 (17.9–29.8)	23.7 (18.0–27.3)

Detection of H. Pylori Infection

H. pylori status was assessed by anti-*H. pylori* Immunoglobulin G antibody (HEL-p TEST, AMRAD Co., Melbourne, Australia) using the stored plasma, rapid urease test and Giemsa staining. Patients were defined as *H. pylori*-negative if all test results were negative (18). Eradication of *H. pylori* was considered successful when (13) C-urea breath test was negative (12).

Statistical Analysis

Data were expressed as mean ± standard deviation. Statistical analyses were performed using Fisher's exact, χ^2, Student's *t*-, paired *t*-, Mann-Whitney U, Wilcoxon's sign rank, or Kruskal-Wallis tests. A p value of less than 0.05 was accepted as statistically significant.

RESULTS

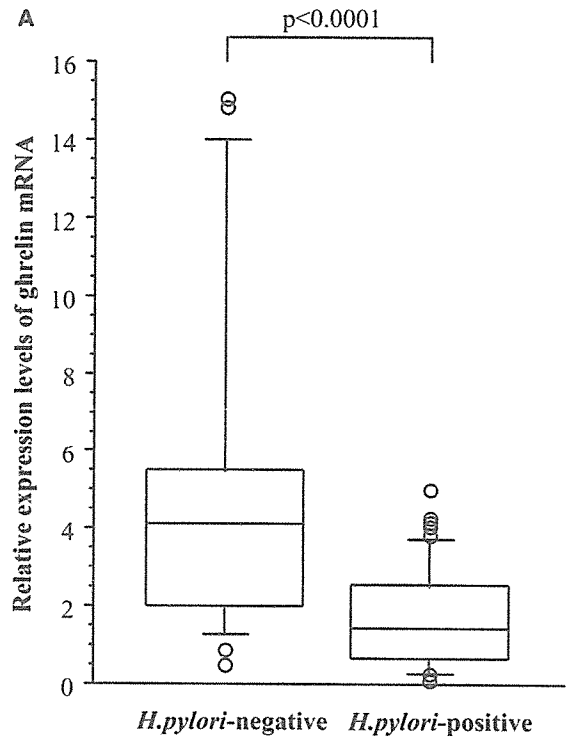
Baseline Characterisitcs

We studied 81 patients (mean age; 54 yr, range: 20–80 yr) consisting of 35 men and 46 women. They included 23 current smokers and 27 alcohol drinkers. Based on the rapid urease test, histopathology, and serology, 56 and 25 subjects were designated as positive and negative for *H. pylori* infection, respectively. Baseline characteristics including age, gender, alcohol intake and smoking habits, and BMI were not different between *H. pylori*-infected and -uninfected subjects (Table 1).

Plasma Ghrelin Concentrations and H. Pylori Infection

Plasma ghrelin concentrations in *H. pylori*-infected patients (144.6 ± 78.8 fmol/mL) were significantly lower than in uninfected subjects (196.1 ± 97.2, $p < 0.05$). Ghrelin circulating levels tended to decrease with increase of BMI ($r = -0.186$), albeit insignificantly. Other parameters including age, gender, current tobacco use, and alcohol intake had no influence on plasma ghrelin levels.

H. pylori infection was cured in 43 out of the 51 patients who received eradication therapy. In patients who showed



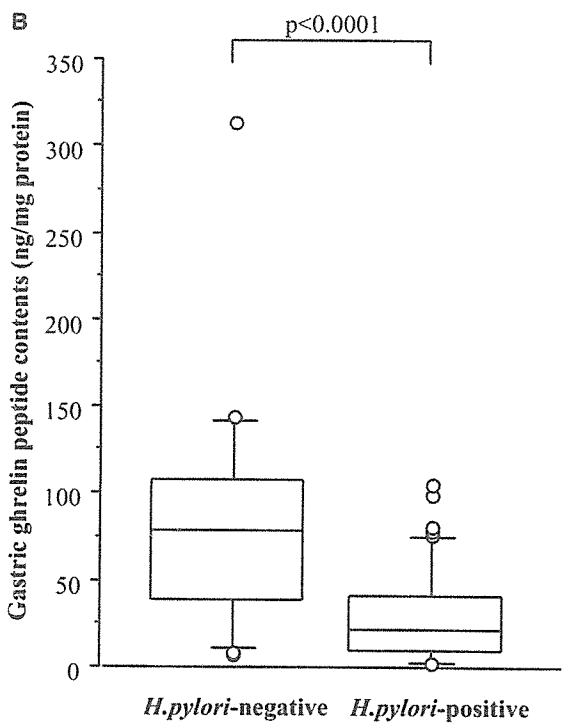


Figure 1. Relative expression levels of ghrelin mRNA, expressed as the ratio of ghrelin/tubulin alpha 3 (internal control) in arbitrary units by real-time polymerase chain reaction in biopsy specimens obtained from the middle corpus along the greater curvature. (*A*) ghrelin peptide contents (ng/mg protein) in gastric biopsy tissues of the fundic mucosa; (*B*) and the numbers of ghrelin immunoreactive cells within the fundic mucosa; (*C*) in terms of *H. pylori* status. Continued.

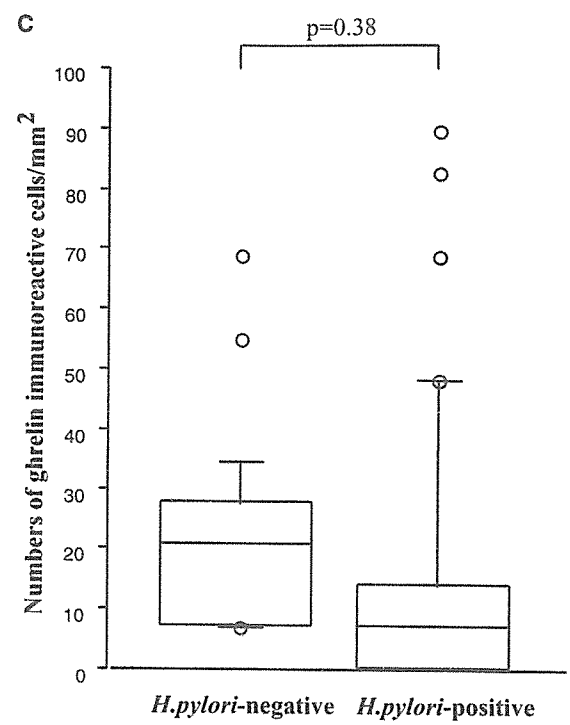


Figure 1. Continued.

successful eradication, there was no significant difference in circulating ghrelin levels between before and at 4 wk after cessation of treatment, and no differences were noted before and after unsuccessful treatment (Table 2). BMI did not change significantly following cure of the infection (23.3 ± 9.7 vs 23.5 ± 9.9 kg/m^2).

Plasma ghrelin concentrations in 10 patients who showed successful eradication were increased from 136.5 ± 40.3 before therapy to 179.9 ± 21.8 fmol/mL at the time point of 6 months ($p < 0.05$), although they did not reach the levels in innately *H. pylori*-negative individuals. In these cured patients, biopsy samples taken at 6 months after eradication showed significant improvement of the histological grades of activity and chronic inflammation of the gastric corpus, but mild improvement of the degree of glandular atrophy.

Relative Expression of Ghrelin mRNA and H. Pylori Infection

The reverse transcriptase-PCR procedures for ghrelin and tubulin alpha 3 yielded 121- and 101-bp specific bands, respectively (data not shown). The relative expression level of ghrelin mRNA in the corporal specimens of *H. pylori*-positive patients was significantly lower than that of *H. pylori*-negative patients (Fig. 1A).

The relative expression levels of ghrelin mRNA tended to increase following cure of the infection (Table 2, $p = 0.09$), albeit insignificantly.

The relative ghrelin expression levels in the corporal samples were significantly higher compared with those in the

Table 2. Changes in Plasma Ghrelin Concentrations and Gastric Expression Levels of Ghrelin mRNA and Density of Ghrelin Immunoreactive Cells Before and 4 wk After Eradication of *H. pylori*

	Successful Eradication			Eradication Failure		
	Before	4 Weeks After Cessation of Therapy	*p*	Before	4 Weeks After Cessation of Therapy	*p*
Plasma ghrelin concentration (fmol/ml)	136.1 ± 72.3	155.5 ± 93.6	NS	140.4 ± 40.0	145.6 ± 92.4	NS
Relative expression of ghrelin mRNA*	1.8 ± 1.6	3.8 ± 4. 8	NS	1.5 ± 1.3	1.4 ± 1.7	NS
Ghrelin immunoreactive cells/mm^2	13.0 ± 12.4	15.8 ± 13.7	NS	13.8 ± 9.8	13.8 ± 7.9	NS

*Data are expressed as the ratio of ghrelin/tubulin alpha 3 in arbitrary units by real-time PCR.
Data are mean ± SD.
NS: Not significant.

angular or antral ones (1.60 ± 1.50, 0.46 ± 0.65, and 0.02 ± 0.02 in arbitrary units, respectively), confirming that ghrelin is primarily produced in the oxyntic gland mucosa. There was also a significant difference in the relative mRNA expression levels between in the antrum and angulus. Again, the relative expression levels of ghrelin mRNA in the corporal mucosa differed significantly in terms of *H. pylori* status not only along the greater curvature (*H. pylori*-negative: 3.34 ± 1.18, *H. pylori*-positive: 1.06 ± 1.14) but also at the angulus (*H. pylori*-negative: 1.16 ± 0.80, *H. pylori*-positive: 0.24 ± 0.43).

Gastric Ghrelin Peptide Contents and H. Pylori Infection

Ghrelin peptide contents in gastric tissues of the corpus in *H. pylori*-positive patients were significantly lower than those of *H. pylori*-negative patients (Fig. 1B).

Ghrelin Immunoreactive Cells and H. Pylori Infection

Ghrelin immunoreactive cells were identified from the neck to the base of oxyntic glands (Fig. 2A). Morphometric analysis revealed that the numbers of ghrelin immunoreactive cells/mm^2 in *H. pylori*-positive subjects tended to lower than in uninfected subjects, although the difference was not significant (Fig. 1C). The *H. pylori*-colonized corpus mucosa with severe glandular atrophy and intestinal metaplasia in the corpus showed extensive lack of ghrelin immunoreactive cells (Fig. 2B).

The number of ghrelin immunoreactive cells/mm^2 tended to be higher in patients with *H. pylori* infection, though the difference was insignificant (Table 2, $p = 0.09$).

Association with Severity of Histopathological Gastritis

There were significant differences in the relative ghrelin mRNA expression levels and gastric ghrelin contents, based on histopathological degree of chronic inflammation and glandular atrophy in the corpus (Fig. 3). As for the numbers of ghrelin immunoreactive cells/mm^2, there was a stepwise reduction with the severity of glandular atrophy (none: 23.2 ± 21.6, mild: 11.0 ± 16.1, moderate: 7.7 ± 16.5, and marked: 0.0 ± 0.0, $p < 0.05$), but no significant correlation with the degree of chronic inflammation (none: 23.9 ± 19.4, mild: 15.6 ± 22.0, moderate: 7.2 ± 11.7, and marked: 20.5 ± 30.0). There were no significant differences in these measured values in terms of the grades of activity and intestinal metaplasia (Table 3).

There was a stepwise decrease in plasma ghrelin concentrations with the progression of chronic inflammation (none: 191.6 ± 97.2, mild: 184.2 ± 95.6, moderate: 124.9 ± 67.1, and marked: 107.9 ± 50.9 fmol/mL, $p < 0.05$), consistent with our recent study (19). Again, the progression of glandular atrophy was associated with parallel falls in circulating

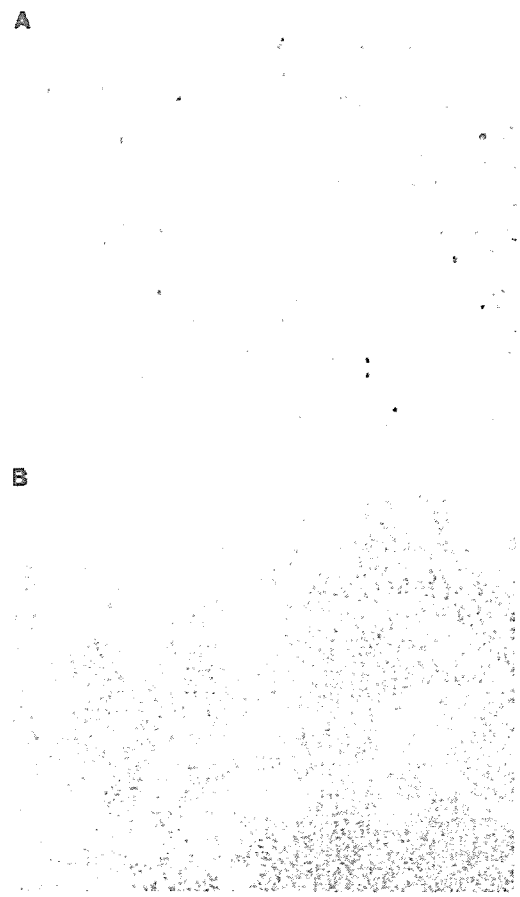


Figure 2. Representative photomicrographs of ghrelin immunoreactive cells in *H. pylori*-negative fundic mucosa (*A*). Note that in the *H. pylori*-infected corporal mucosa of patients with atrophic gastritis, there is little or no immunoreactivity against antighrelin antibody (*B*).

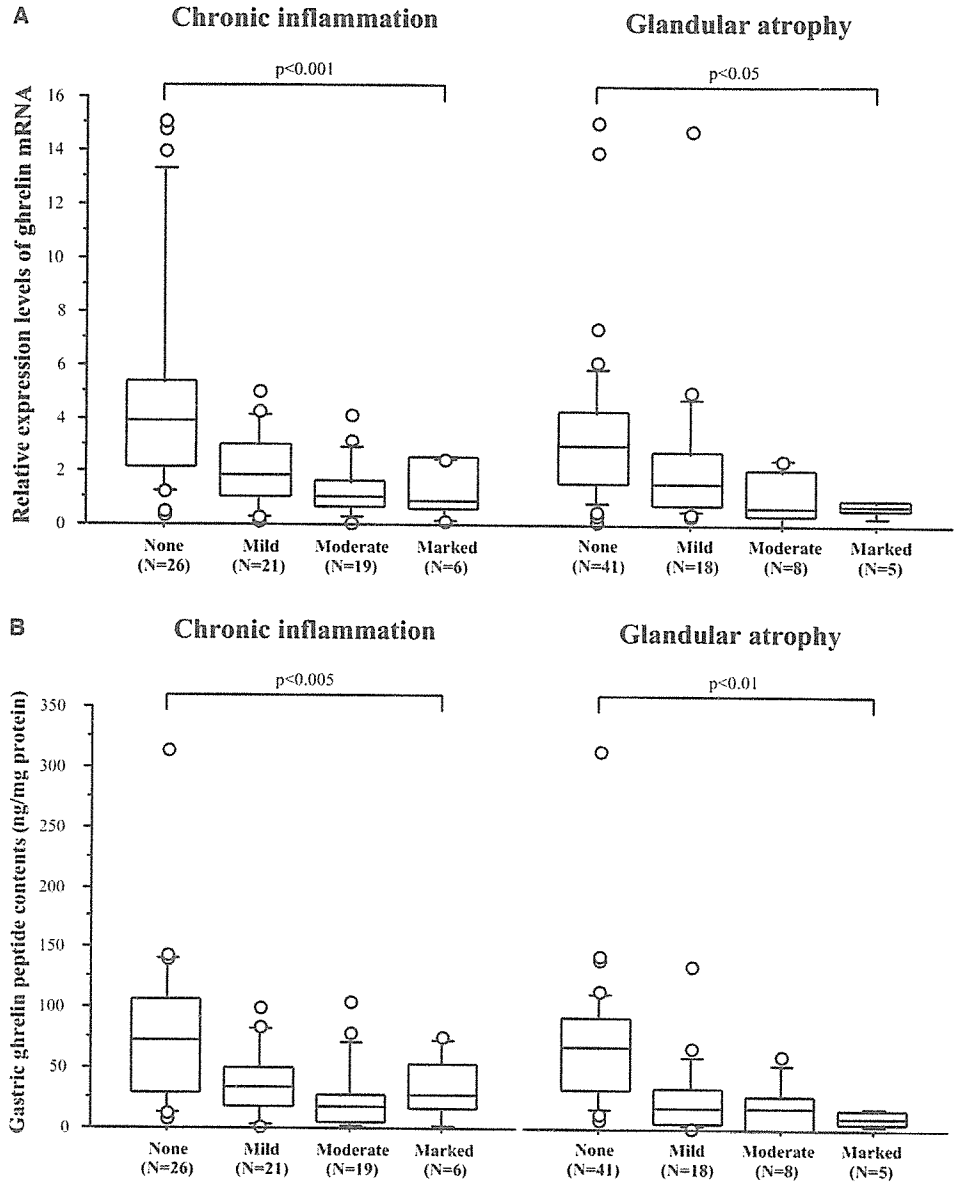


Figure 3. Relationship between histopathological severity of chronic inflammation (*A*) and glandular atrophy (*B*) in the corpus and the relative ghrelin mRNA expression levels and gastric ghrelin peptide contents.

ghrelin levels (none: 189.4 ± 90.8, mild: 169.7 ± 83.9, moderate: 76.5 ± 55.8, and marked: 87.9 ± 15.8 fmol/mL, $p < 0.005$).

Relationship Between Various Parameters Pertaining Plasma and Gastric Ghrelin

There were significantly positive correlations between plasma ghrelin concentrations, the relative mRNA expression levels, and gastric peptide contents (Fig. 4). Furthermore, there was a modest but significant correlation between the ghrelin immunoreactive cell density and ghrelin peptide contents in the fundic gland area ($r = 0.238$, $p < 0.05$).

DISCUSSION

Our results showed significantly lower plasma ghrelin concentrations in *H. pylori*-positive than in *H. pylori*-negative subjects, whereas in *H. pylori*-infected Mongolian gerbils, circulating ghrelin levels were rather increased 17 and 23 wk after the experimental induction of *H. pylori* gastritis (14). On the other hand, the density of ghrelin immunoreactive cells (per 0.1 mm^2) within gastric fundic mucosa of *H. pylori*-infected gerbils was significantly lower than those of uninfected controls (14), inconsistent with our findings in humans. In fact, total gastric contents of ghrelin peptide in *H. pylori*-colonized gerbils were comparable to those of

Table 3. Histological Severity of Activity and Intestinal Metaplasia and Various Gastric Ghrelin-Related Parameters

Histological Degree of Gastritis (Numbers)	*p*	Ghrelin mRNA Expression Level*	*p*	Gastric Ghrelin Contents (ng/mg Protein)	*p*	Ghrelin Immunoreactive Cells (Number/mm^2)	*p*
Activity							
None (50)	NS	3.3 ± 3.4	NS	52.3 ± 54.4	NS	15.6 ± 22.6	NS
Mild (14)		1.9 ± 1.6		48.4 ± 34.1		13.6 ± 20.6	
Moderate (8)		1.1 ± 0.6		25.6 ± 36.4		9.4 ± 16.3	
Marked (0)							
Intestinal metaplasia							
None (65)	NS	3.0 ± 3.1	NS	52.7 ± 50.5	NS	17.9 ± 20.4	NS
Mild (2)		1.2 ± 1.8		8.7 ± 12.3		0.0 ± 0.0	
Moderate (1)		0.49		19.3		48	
Marked (4)		0.6 ± 0.2		9.3 ± 6.8		0.0 ± 0.0	

*Data are expressed as the ratio of ghrelin tubulin alpha 3 in arbitrary units by real-time PCR.
Data are mean ± SD.
NS = not significant.

uninfected controls (14), as the inflamed stomach was enlarged in this model (14, 20). Therefore, the unexpectedly high values of circulating ghrelin in *H. pylori*-infected gerbils are unlikely to be due to augmented ghrelin production in the stomach. It is suggested that there may be a compensatory increase in plasma ghrelin concentrations in response to the decreased density of ghrelin-producing cells at 17 and 23 wk of infection. However, long-term infection by this organism leads to further extension of mucosal atrophy toward the corpus in gerbils (20) as well as humans (9, 16, 19), and the compensatory effect may be nullified in older affected gerbils. One may speculate that extragastric ghrelin is the origin of elevated ghrelin concentrations in the blood, although the total gastric ghrelin content was estimated to be at least 74-fold greater than that of the small intestine in gerbils (14). Similar to humans and other rodents (2–6), the stomach also seems to be main source of circulating ghrelin in gerbils. Alternatively, it is possible that the high plasma ghrelin concentrations are due to enhanced degranulation of ghrelin producing X/A-like cells induced by inflammatory stimuli such as proinflammatory cytokines (21) or free radicals (22) during earlier stages of *H. pylori* colonization, although there might be interspecies differences in the regulation of ghrelin biosynthesis and secretion.

Our results demonstrated that *H. pylori* infection could modulate ghrelin dynamics in human stomach. We found

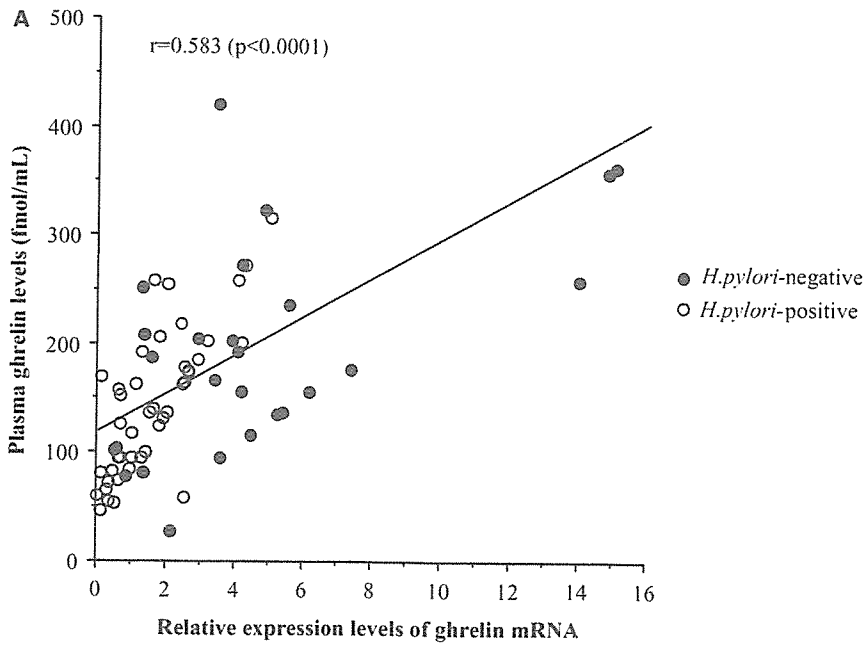


Figure 4. Relationship between plasma ghrelin concentrations and the relative mRNA levels of ghrelin in biopsy specimens taken from the fundic mucosa (*A*); plasma and gastric peptide concentrations (*B*); the relative expression of ghrelin mRNA and its peptide contents (*C*). Continued.

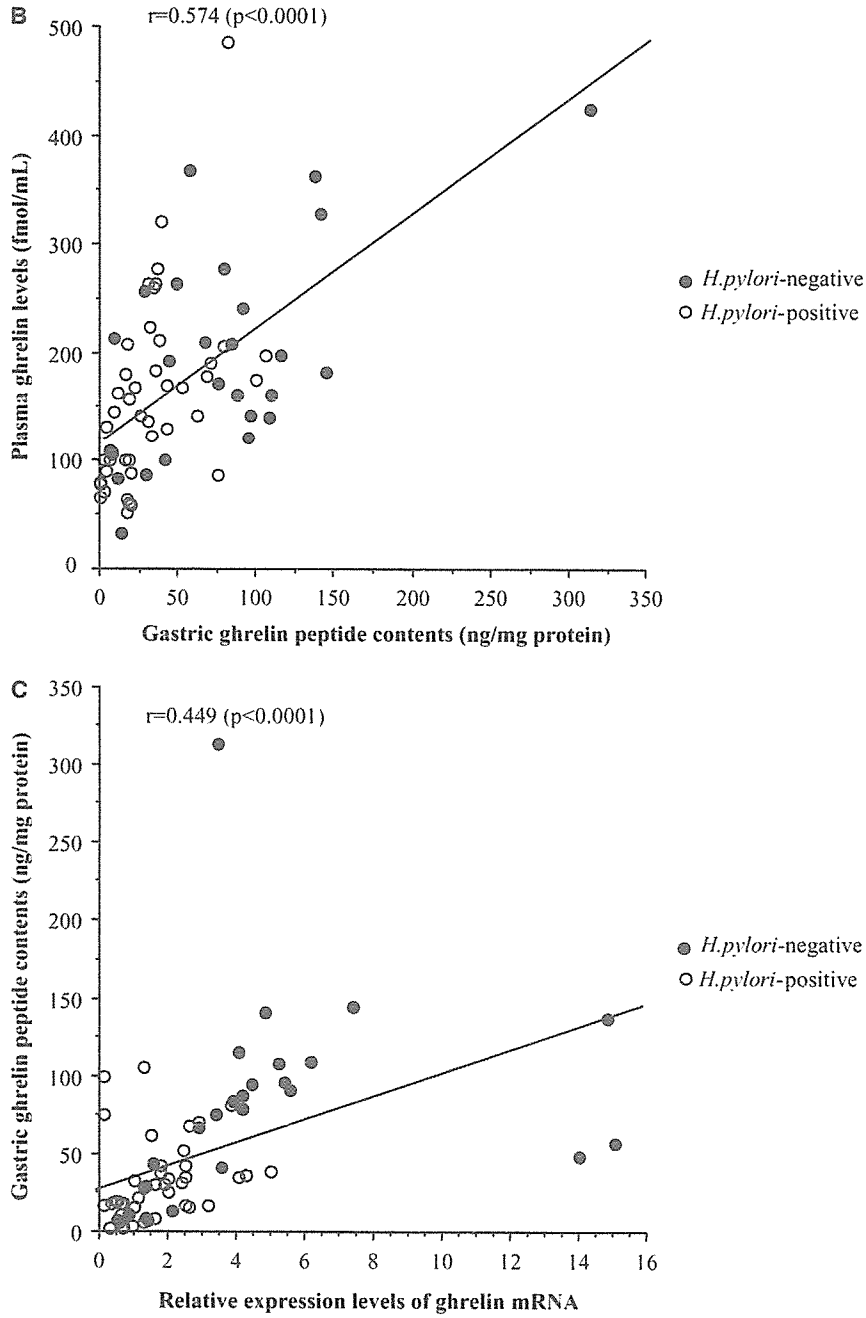


Figure 4. Continued.

significantly low ghrelin mRNA expression in gastric mucosal tissues infected with *H. pylori* at the angulus as well as along the greater curvature. In addition, ghrelin peptide contents in the *H. pylori*-infected mucosa were significantly decreased and correlated positively with the relative mRNA expression levels. Recently, similar findings were reported in chronically infected Mongolian gerbils (14).

Another major finding of this study is that the histological severity of mononuclear cell infiltration and glandular atrophy of the corpus significantly influenced the relative expression levels of ghrelin mRNA, its peptide contents and the density of immunoreactive cells, indicating that gastric ghrelin biosynthesis seems to be affected by chronic mucosal inflammation and/or atrophy in association with *H. pylori* infection. In particular, there were stepwise falls in the relative expression levels of ghrelin mRNA and its peptide contents with worsening of glandular atrophy. Similarly, Suzuki *et al.* (14) noted that *H. pylori*-colonized gerbils with severe glandular atrophy had very low gastric peptide contents. Since gastric ghrelin products correlated with the density of

ghrelin immunoreactive cells, the impaired ghrelin biosynthesis may reflect, at least in part, the loss of ghrelin-producing cells caused by the inflammatory and atrophic events.

There are contradictory reports on the relationship between *H. pylori* and circulating ghrelin levels (10, 11, 19). No significant difference in plasma ghrelin concentrations was noted between *H. pylori*-positive and -negative women of the same age and BMI in the Turkish study (10). The exact reason for this discrepancy is not clear, but the following factors should be considered: (1) differences in study populations of diverse races, nutrient status, and dietary habits; (2) small sample size; (3) inadequate assessment of *H. pylori* status, *i.e.*, only by histology, leading to underestimation of infection in their series; and (4) differences in RIA protocols for ghrelin. Nevertheless, our data showed positive correlations between plasma ghrelin concentrations and the relative expression levels of ghrelin mRNA and its peptide contents in the corpus, indicating that the low circulating ghrelin levels may be due to impairment of gastric ghrelin production/secretion. In line with this, plasma ghrelin levels correlated negatively with increased severity of corporal chronic inflammation and glandular atrophy.

Nwokolo *et al.* (11) reported that 6-h integrated plasma ghrelin (between 8:00 and 13:00) significantly increased no more than 4 wk after cure of *H. pylori*, suggesting that depressed circulating ghrelin in *H. pylori* infection might be, in part, caused by "functional" impairment of ghrelin dynamics. Abnormalities in other gastric neuroendocrine hormones including hypergastrinemia and disturbance in somatostatin-producing cell numbers and its function have been described in *H. pylori*-infected subjects and the levels of these hormones return to normal soon after cure of the infection (23, 24). Apart from the British study (11), we assessed circulating ghrelin levels at only one point after an overnight fast. In the present series, circulating ghrelin levels tended to be increased in patients who showed eradication of the organism, along with the tendency of postcure reduction in gastric ghrelin mRNA expression and density of ghrelin immunoreactive cells, but the differences and changes of BMI were insignificant at 4 wk after cessation of treatment. On the other hand, modest but significant elevations of plasma ghrelin concentrations were noted at 6 months after the cessation of treatment in patients who showed eradication of the organism, along with substantial improvement of histological gastritis. Furthermore, 23 out of the 51 patients treated with the anti-*H. pylori* regimen had extensive glandular atrophy toward the middle corpus along the great curvature. In this regard, while the effect of anti-*H. pylori* therapy on advanced atrophic gastritis is still controversial (25–27), it seems to take more than 12 months after cure of the infection for such gastritis to improve even in those studies that showed the reversibility of atrophy (25, 26). Further studies to compare the much longer-term effect of such treatment and placebo on ghrelin production in a large number of patients and controls are warranted to elucidate the reversibility of ghrelin production.

In conclusion, we demonstrated that *H. pylori* infection has a significant negative impact on gastric and plasma ghrelin dynamics. Inflammatory and atrophic changes associated with the infection may cause impairment of gastric ghrelin biosynthesis, leading to falls in ghrelin blood levels. Further characterization of the implications of low production/release of ghrelin on various physiological functions is warranted.

In this article, the relationship between ghrelin and the infection of the gastric mucosa of *H. pylori*-infected patients with or without triple therapy was assessed. The expression on ghrelin was determined by real-time PCR and plasma ghrelin as well as ghrelin contents in the biopsy specimens were examined. In addition, a group of patients underwent the triple therapy to check the ghrelin status after eradication of this organism. It was found that the expression of mRNA for ghrelin and the plasma ghrelin and its content in the gastric mucosa were all significantly lower in the *H. pylori*-infected *H. pylori*-noninfected individuals, along with the decrease in plasma ghrelin following bacteria eradication. The ghrelin expression was significantly decreased in patients with severe gastritis and atrophy. From these observations, authors conclude that *H. pylori* exerts negative influence on ghrelin expression and release. Overall this is of interest, to clarify the influence of *H. pylori* on ghrelin expression in humans.

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Plasma Concentrations of Adrenomedullin and Ghrelin in Hemodialysis Patients with Sustained and Episodic Hypotension

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Abstract. Sustained and/or episodic hypotension during hemodialysis (HD) is an important clinical issue. Plasma adrenomedullin (AM) is increased in HD patients with sustained hypotension, but little is known about its implications for episodic hypotension. Ghrelin may also contribute to the pathophysiology of hypotension in HD patients. We evaluated plasma levels of AM and total ghrelin in sustained hypotensive (SH; n = 23), episodic hypotensive (EH; n = 30) and normotensive (NT; n = 23) HD patients. In the EH group, the relationship between low blood pressure during HD and circulating levels of AM and ghrelin was also evaluated. Plasma levels of AM were significantly higher in SH (34.3 ± 8.3 fmol/ml, $p<0.01$) than in NT patients (27.6 ± 5.2 fmol/ml), but not in EH patients (30.8 ± 6.1 fmol/ml). There was no significant difference of plasma total ghrelin in SH (548.1 ± 426.5 fmol/ml) and in EH patients (544.6 ± 174.3 fmol/ml), compared with NT patients (400.0 ± 219.7 fmol/ml). On the other hand, in EH patients, the "suppressed blood pressure ratio" during HD significantly correlated with plasma AM ($r = 0.77$, $p<0.001$) and with total ghrelin levels ($r = 0.44$, $p<0.05$). Our results suggest that ghrelin, as well as AM, may play an important role as vasodilator local hormones and regulation of blood pressure during HD, especially the occurrence of EH. Further studies are necessary to clarify the implication of these hormones in the control of hypotension during HD.

Key words: Adrenomedullin, Chronic hypotension, Episodic hypotension, Ghrelin, Hemodialysis, Sustained hypotension
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SIGNIFICANT hypotension is a major cardiovascular complication in patients with end-stage renal disease undergoing hemodialysis (HD). Two types of hypotension are recognizable in the setting of maintenance HD: episodic hypotension (EH) during HD is the most common manifestation of hemodynamic instability, and occurs in around 30–40% of the dialysis population [1]. A second form is sustained hypotension (SH), characterized by a systolic blood pressure (SBP) lower than 100 mmHg, during the interdialysis period and is present in approximately 5–10% of patients [2, 3]. Both groups of patients require a substantial amount of medical and nursing care during and after HD to control hypotension-related symptoms. Although several clinical factors, such as autonomic dysfunction, reduced pressor response to vasopressor agents and cardiac dysfunction, have been shown to be responsible for the occurrence of EH and SH [1], the pathophysiology of chronic hypotension in dialysis patients has yet to be fully clarified.

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