Adrenomedullin (AM) is a novel vasodialator peptide that has been recently isolated from human pheochromocytoma cells by monitoring the elevated activity of platelet cyclic adenosine monophosphatase [4]. AM is present in several normal tissues, such as adrenal medulla, lungs, kidneys and cardiac atrium, and has a potent and long-lasting hypotensive effect [5]. In 2000, Cases *et al.* [6] reported that plasma AM levels and nitrate levels were increased in HD patients, but only AM levels were higher in sustained hypotensive than in normotensive (NT) and hypertensive HD patients. Based on these results, they suggested the involvement of AM in the pathophysiology of SH in HD patients. Although several studies supported the association between AM and SH, there is no report to describe the association between AM and EH, which is more frequent among the dialysis population.

Ghrelin is a novel growth hormone (GH)-releasing peptide, originally isolated from the rat stomach, which was identified as an endogenous ligand for an orphan receptor termed GH secretagogue receptor [7]. Human ghrelin is a 28-amino acid peptide with a fatty acid chain modification on the N-terminal third amino acid. The hydroxygen atom of the hydroxyl group of the N-terminal third amino acid serine residue is replaced by a hydrophobic moiety, $C_7H_{15}CO$; in other words, the hydroxyl group of Ser^3 is octanoylated [7]. The n-octanoyl group at this position of the ghrelin molecule seems to be essential for some of the hormone's activity, including GH release and appetite. Non-acylated (desoctanoyl or desacyl) ghrelin circulates in far greater amounts than the acylated form and does not displace ghrelin from its hypothalamic and pituitary binding site [7].

Ghrelin is synthesized in several organs, such as intestine and kidney, as well as stomach [8, 9]. Recent studies reported that peripheral administration of ghrelin or GH secretagogue causes not only GH release from the pituitary gland, but also improvement in cardiac function, increase in food intake, fat accumulation and decrease in blood pressure [10, 11]. Furthermore, Wiley and Davenport investigated the vasodilator function of ghrelin by using human internal mammary artery *in vitro*, and concluded that ghrelin was an effective, endothelin-independent vasodilator of the long-lasting constrictor endothelin-1 in human arteries producing responses similar to those of AM [12]. From this point of view, it is speculated that accumulated ghrelin, as well as AM, before dialysis may cause hypotensive status in HD patients through its vasodilator effect. However, to date there is no study to clarify the association between plasma ghrelin concentration and SH and/or EH.

Based on the above background, we hypothesized that increased production of AM and ghrelin might be the underlying mechanism of SH and EH in HD patients. This study was designed to assess the possible role of AM and ghrelin in the pathogenesis of SH and EH in hemodialyzed patients.

Methods

Study participants

The study subjects were 76 patients with renal diseases on maintenance HD (23 SH, 30 EH and 23 NT patients). SH was defined as SBP less than 100 mmHg at predialysis in at least 80% of blood pressure measurements in the previous three months [1]. EH was defined as decreases of more than 25 mmHg during HD and/or as hypotension requiring medication during HD [13]. NT was defined as SBP less than 145 mmHg and DBP less than 90 mmHg at predialysis at least 80% of blood pressure measurements in the previous three months [1]. The causes of renal disease were chronic glomerulonephritis (n = 61), diabetic nephropathy (n = 12), Crohn's disease (n = 1) and undefined (n = 2). None of the patients were anephric, had evidence of cardiac disease, such as myocardial infarction, or suffered from chronic obstructive pulmonary disease or hepatic dysfunction. None of the patients received antihypertensive treatment and vasodilatory drugs. Before the study, ethical approval was obtained from the special committee of Nagasaki University School of Medicine (project registration no. 15052224). Blood samples from patients were collected at three hospitals in Nagasaki city (see Acknowledgment). In all cases, a signed informed consent was obtained before the study.

Measurement of plasma AM and ghrelin concentrations

Blood samples were collected in tubes with 2 mg/ml of ethylenediaminetetraacetic acid (EDTA)-2Na and 500 KIU/ml aprotinin just before the dialysis. After collection, the samples were promptly centrifuged at 4°C. Plasma total AM was measured by immunoradiometric assay using a specific kit for each form

(adrenomedullin RIA Shionogi, adrenomedullin mature RIA Shionogi; Cosmic Corporation, Tokyo, Japan).

Plasma total ghrelin was measured by our specific radioimmunoassay (RIA) system as described previously [9]. Since the active form of ghrelin is unstable in non-acidified normal plasma, the total amount of ghrelin was used in this study.

We defined "suppressed blood pressure ratio" in patients with EH as [(systolic blood pressure at predialysis) – (minimum systolic blood pressure at the episode of hypotension during HD)/systolic blood pressure at predialysis] × 100 (%), and determined its correlation with plasma levels of AM and ghrelin.

Statistical analysis

Statistical analysis was performed using software package SPSS 9.0 for Windows (Chicago, IL). Data are expressed as means ± SD. One-way ANOVA was used for statistical comparisons between SH and NT groups, and EH and NT groups. Pearson correlation analysis was performed for correlation between variables, and a p value less than 0.05 was accepted as statistically significant.

Results

Table 1 summarizes the hemodynamic and laboratory values for the three groups of HD patients. Systolic, diastolic, and mean blood pressures were significantly different between SH and NT patients, and EH and NT patients ($p<0.001$ and $p<0.001$, respectively). On the other hand, there was no significant difference between SH and EH patients. Duration of HD was significantly longer in SH than in NT patients ($p<0.001$). There was no significant difference of hematocrit (Ht) in SH (34.5 ± 8.1%) and in EH patients (31.8 ± 3.2%), compared with NT patients (30.9 ± 2.4%).

When data of all patients were analyzed, plasma levels of AM correlated with those of ghrelin ($r = 0.29$, $p = 0.003$, Fig. 1). Plasma levels of AM in HD patients were significantly higher in SH (34.3 ± 8.3 fmol/ml, $p<0.01$) than in NT patients (27.6 ± 5.2 fmol/ml, Table 1), 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

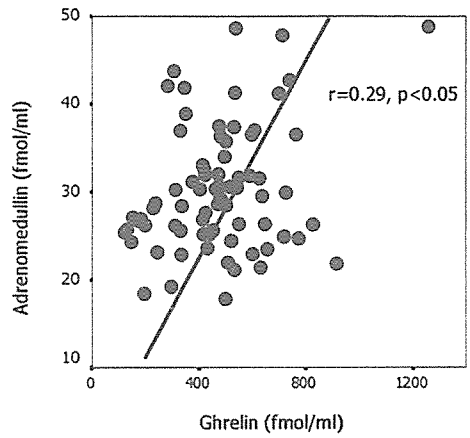

Fig. 1. Relationship between ghrelin and AM using data of all HD patients ($r = 0.29$, $p<0.05$).

Table 1. Demographic, hemodynamic, and laboratory values of the three groups of HD patients.

	SH patients	EH patients	Normotensive patients
Number of patients	23	30	23
Males (%)	30.4	53.1	69.5
Age (years)	57.3 ± 12	56.7 ± 14.7	64.4 ± 13.8
Duration of HD (years)	18.7 ± 11.1[b]	10.6 ± 8.3	8.0 ± 5.4
Systolic BP (mmHg)	87.1 ± 11.6[b]	104.1 ± 11.5[b]	119.1 ± 16.6
Mean BP (mmHg)	62.1 ± 8.5[b]	79.4 ± 12.2[b]	94.1 ± 8.7
Diastolic BP (mmHg)	49.5 ± 7.9[b]	64.0 ± 13.7[b]	71.5 ± 8.9
Interdialysis weight gain (g)	1218.3 ± 1102.7	2690.7 ± 1221.5	2269.7 ± 993.4
Hematocrit (%)	34.5 ± 8.1	31.8 ± 3.2	30.9 ± 2.4
Plasma AM (fmol/ml)	34.3 ± 8.3[a]	30.8 ± 6.1	27.6 ± 5.2
Plasma total ghrelin (fmol/ml)	548.1 ± 426.5	544.6 ± 174.3	400.0 ± 219.7

Data are mean ± SD.
[a]$p<0.01$, [b]$p<0.001$, *vs* normotensive patients.
HD, hemodialysis; BP, blood pressure.

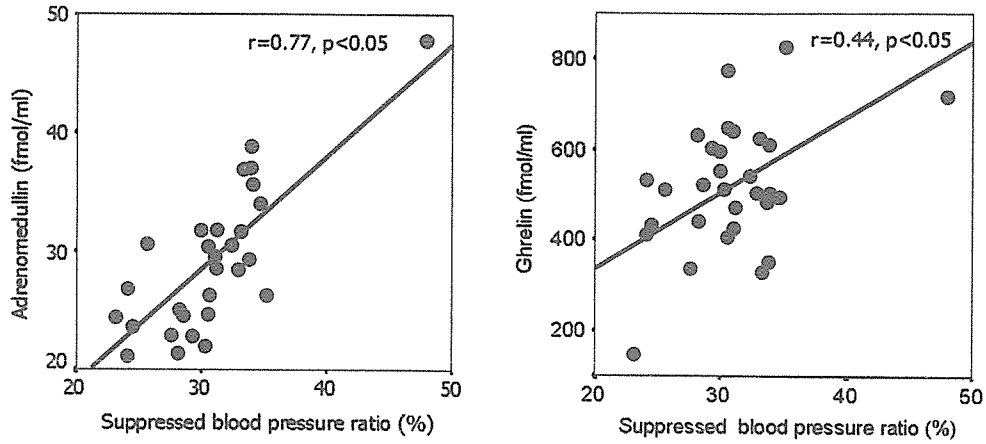

Fig. 2. Relationships between AM and depressed mean blood pressure ($r = 0.77$, $p<0.05$, left), and ghrelin ($r = 0.44$, $p<0.05$, right) and "suppressed blood pressure ratio" in EH patients.

(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" significantly correlated with plasma levels of AM ($r = 0.77$, $p<0.001$) and of ghrelin ($r = 0.44$, $p<0.05$, Fig. 2). On the other hand, there was no correlation between "suppressed blood pressure ratio" and Ht.

Discussion

Our study showed that plasma AM levels are significantly increased in patients with SH. In addition, we showed that in patients with EH, plasma AM levels correlated positively with the "suppressed blood pressure ratio". Furthermore, we showed for the first time that the levels of plasma total ghrelin also correlated with the "suppressed blood pressure ratio" in EH patients. These results suggest that as vasodilator peptides, these two hormones may be involved in the pathophysiology of hypotension in HD patients, especially in EH.

Several groups have investigated the mechanism(s) of high levels of vasodilator agents during HD [1, 14–16]. Imai *et al.* [14] conducted a hemodynamic study and reported that while cardiac index, heart rate or stroke volume were similar in hypotensive and normotensive HD patients, total peripheral vascular resistances were lower in the former group. They suggested that increased biosynthesis and/or release of vasodilator agents might be critical in the pathogenesis of hypotension during HD. Plasma atrial natriuretic peptide levels have been reported to be similar in hypotensive and normotensive dialysis patients [15, 16], but the possible role of this molecule in chronic hypotension in uremia is controversial [6]. In addition, it has been shown that plasma levels of adenosine, a strong hypotensive agent, are increased, while the activity of intracellular adenosine deaminase, the enzyme that metabolizes this agent, is reduced in HD patients, but not in predialysis or peritoneal dialysis patients [17]. However, the possible role of this agent in hypotension in dialysis patients has yet to be fully evaluated.

In addition to these peptides, AM was reported to be increased in patients with SH [6, 18]. However, there is little or no information regarding the relationship between AM and EH. In the present study, we found that plasma AM levels are increased in patients with SH. In addition, we showed that AM levels positively correlated with the "suppressed blood pressure ratio" in patients with EH. Thus, the present results suggest that AM may be involved in the pathophysiology of EH and SH in HD patients.

The exact mechanism of the increased production of vasodilators including AM is still unknown. However, it is likely that the inflammatory state of uremia plays some role [19]. The production of both nitric oxide and AM is induced by cytokines, such as hepatocyte growth factor (HGF), which induces endothelial proliferation and nitric oxide-mediated vasodilation, and other studies showed that HGF was increased in hypotensive HD patients [20]. Several studies also suggested the possible roles of microinflammatory state in chronic hypotension of dialysis patients, through the

induction of synthesis of several vasodilator substances [21, 22]. Although further studies are needed, similar mechanism(s) may be associated with the pathophysiology of EH during dialysis.

Besides vasodilator effect, it is suggested that AM may also be associated with circulating blood volume in HD patients [23]. In our current study, there was no relationship between Ht, one of the markers of circulating blood volume, and hypotension. Also there was no relationship between Ht and AM. Further studies will be needed to clarify the contribution of AM to EH and SH through the change of blood volume in HD patients.

Ghrelin, an endogenous peptide recently linked to growth hormone secretagogue receptor [7], is a potent, endothelium-independent vasodilator of human arteries, effectively reversing endothelin-1 (ET-1)-mediated constriction. Ghrelin is present in human plasma at approximately 100 pmol/L [7], a concentration considerably higher than other vasoactive peptides. Yoshimoto *et al.* demonstrated that plasma ghrelin in patients with renal disease is increased in parallel with the severity of renal damage [24]. They also revealed that approximately half of the plasma ghrelin, as well as half of the serum creatinine or blood urea nitrogen, are removed from the blood by a single course HD, and that bilateral nephrectomy in mice causes marked increase in plasma ghrelin concentrations. They concluded that increased plasma ghrelin in renal failure may result from decreased clearance or degradation in the kidney. Although overproduction of ghrelin in organs other than the stomach may contribute to higher plasma concentrations [25, 26], a similar pathophysiological changes may occur in patients in HD. In our current study, we showed a positive correlation between plasma AM and ghrelin concentrations in HD patients, and found a positive correlation between "suppressed blood pressure ratio" and plasma levels of ghrelin in EH patients. These results suggest that ghrelin, in cooperation with AM, may contribute to the development of EH through its vasodilatory effect. On the other hand, we could not reveal the significant difference of total ghrelin levels between SH and NT patients, and EH and NT patients. Although this may merely reflect our small sample size, ghrelin may not play an important role in the occurrence of hypotension in chronic state.

In addition to this effect, ghrelin is associated with changes in body composition in patients on HD. Ayala *et al.* investigated patients with end-stage renal disease and found markedly high plasma ghrelin concentrations in this group, and its level correlated significantly with plasma insulin, body mass index, log serum leptin levels and truncal fat mass [27]. These findings suggest that the kidney is an important site for clearance and/or degradation of ghrelin.

There are several limitations in this study. First, we measured AM and ghrelin only just before, not during and after HD. Observation on the dynamics of AM and ghrelin in a series of HD will be available to clarify the contribution of these hormones, more precisely. Second, we could not measure plasma levels of atrial natriuretic peptide and adenosine, which have been suggested to have possible roles in SH patients [19, 28]. Third, our sample size was relatively small to completely identify the roles of AM and ghrelin in SH and EH patients. Additional sampling may overcome the insufficient statistical significance in our study.

In this study, we observed that duration of HD was significantly longer in SH than in NT patients. It is known that longer duration of HD is associated with autonomic neuropathy, which is one of the major causes of hypotension in HD patients [29]. Besides vasodilator agents, such clinical factors should be also considered to be key factors for the occurrence of hypotension in HD patients. In conclusion, our results suggested the possibility that ghrelin and AM might play important roles as vasodilator local hormones and control of blood pressure during HD. Further studies are clinically important to clarify the implication of these hormones in the clinical control of hypotension during HD.

Acknowledgment

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Fibrotic response to angiotensin II is blunted in the kidney, but not in the heart, in insulin-sensitive long-lived transgenic dwarf rats

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Abstract. Insulin resistance is a characteristic feature of cardiovascular and renal diseases, and angiotensin II (Ang II) has been suggested to induce insulin resistance. The aims of this study were to elucidate the effect of chronic Ang II infusion on vascular reactivity and organ damage in insulin-sensitive rats. We confirmed the following three points. First, there was no significant difference in pressor response to chronic Ang II infusion (600 ng/kg/min) between insulin-sensitive transgenic rats (Tg) and control rats (C). Second, there was no significant difference in cardiac hypertrophy and fibrosis by chronic Ang II infusion between the two groups. However, third, fibrotic response to chronic Ang II infusion evaluated by histopathological scoring in the kidney was significantly decreased in insulin-sensitive transgenic rats (renal fibrosis and nephropathy score: C+Ang II vs Tg+Ang II; 2.5 vs 1.3; $p<0.05$). Furthermore, the expression of TGF-ß, a fibrosis indicator, was also significantly suppressed in the kidneys of the transgenic rats (TGF-ß1/GAPDH ratio: C+Ang II vs Tg+Ang II; 1.15 vs 0.81; $p<0.05$). This result indicates that the growth hormone/insulin-like growth factor-1 axis is critically involved in the development of renal injury and fibrosis, rather than hypertension, cardiac hypertrophy, and cardiac fibrosis induced by chronic Ang II administration.

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Key words: angiotensin II, insulin sensitivity, growth hormone, fibrosis, kidney

Introduction

We have previously reported a long-lived transgenic dwarf rat model in which the growth hormone (GH)/insulin-like growth factor (IGF)-1 axis was selectively suppressed by the overexpression of the antisense GH transgene (tg). This transgenic rat is unique in having a longer lifespan, mainly due to a reduced prevalence of cardiovascular and nephropathic events, smaller body size, and an increased insulin sensitivity (1,2). Recently, GH antagonist transgenic mice demonstrated a marked reduction in circulating IGF-1, blood glucose, and insulin levels, and an increased peripheral insulin sensitivity as measured by an insulin tolerance test and hyperinsulinemic euglycemic clamp analysis, suggesting that the chronic elevation of GH levels plays an important role in insulin resistance (3). Diminished insulin sensitivity is a characteristic feature of various pathological conditions such as hypertension, heart failure, and type 2 diabetes (4,5). In these pathological conditions, angiotensin II (Ang II) has been suggested to induce insulin resistance. Moreover, increased target organ damage in the cardiovascular system and pressor responsiveness to Ang II were found in insulin-resistant hypertensive rats. In the heart, Ang II induces cardiac hypertrophy, perivascular and interstitial fibrosis, and oxidative stress, which have been linked to end-stage heart failure (6,7). Furthermore, renal cell growth and extracellular matrix (ECM) synthesis and degradation were modulated by Ang II, leading to glomerulosclerosis and interstitial fibrosis and consequently to advanced renal failure (8). It has also been shown that ACE inhibitor and the Ang II receptor blocker improve insulin sensitivity and reduce blood pressure in essential hypertension and fructose-induced hyperinsulinemic hypertensive rats (9-11). However, it is not clear whether a blunted response to Ang II is found in insulin-sensitive animal models. Accordingly, our insulin-sensitive rats may have diminished responses to Ang II, which can be linked to their reduced prevalence of cardiovascular and nephropathic complications, and subsequently to their longevity. In the present study, we examined the effect of chronic Ang II infusion on vascular reactivity and organ

damage in long-lived transgenic dwarf rats compared with control rats.

Materials and methods

Animal model. The transgenic rats [Jcl:Wistar-TgN (ARGHGEN)1Nts] were kindly provided by Nippon Institute for Biological Science (Oume City, Tokyo, Japan) and the present colony has been established in a barrier facility in the Laboratory Animal Center at Nagasaki University School of Medicine since 1997. The transgene consisted of four copies of the thyroid hormone response element, the rat GH promoter, and antisense cDNA sequences for rat GH. Sixteen-week-old male heterozygous (tg/-) rats and male Wistar control (-/-) rats (Japan Clea Inc., Tokyo, Japan) were used in this study. Because homozygous (tg/tg) rats do not have long lives due to the increased prevalence of leukemia and other neoplastic disease, we used heterozygous (tg/-) rats as a model of long-lived insulin-sensitive rats. The following study was approved by the Animal Rights Committee, Nagasaki University.

Ang II infusion. Ang II (600 ng/kg/min) was continuously administered to heterozygous (tg/-) rats (n=8) and control (-/-) rats (n=8) for 4 weeks via a subcutaneous-implanted osmotic mini-pump (Alzet model 2002, Palo Alto, CA). As a vehicle control, saline was continuously administered to heterozygous (tg/-) rats (n=8) and control (-/-) rats (n=8) for 4 weeks. Ang II (Sigma Aldrich Chemicals Japan, Tokyo, Japan) was dissolved in saline at 4 mg/ml, and acetic acid was added to maintain its stability (final concentration, 0.01 mol/l).

Blood pressure, heart rate, and body weight measurements. Blood pressure (BP) and heart rate (HR) were measured in conscious rats by the tail-cuff method (Model MK-2000, Muromachi Kikai Co, Tokyo, Japan) and body weight (BW) was recorded before and after 4 weeks of Ang II or saline infusion.

Echocardiography. At 4 weeks of treatment, echocardiography was performed under pentobarbital anesthesia (50 mg /kg, ip). A Toshiba Aplio echocardiograph machine (SSA-700A) equipped with a 7.5-MHz phased-array transducer was used. Anterior and posterior wall thickness (PWT) and left ventricle (LV) internal dimensions were measured. Systolic function was assessed by calculating endocardial fractional shortening (FS). Relative wall thickness (RWT) was calculated as 2xPWT/LV internal dimension (12,13).

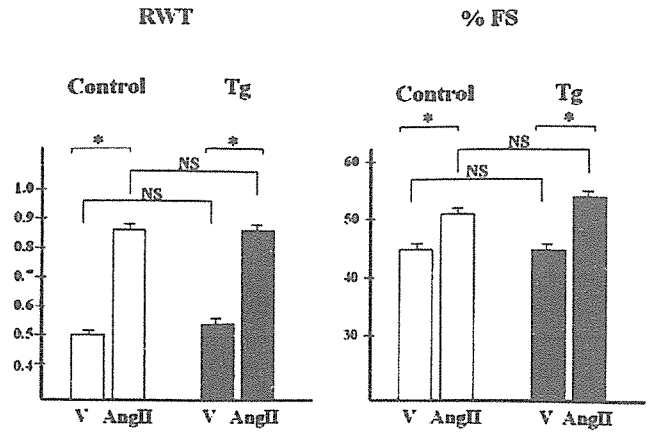

Figure 1. Transthoracic echocardiographic findings in the control and transgenic rats. Open bars, control (-/-) rats; solid bars, transgenic (tg/-) rats; *p<0.05 compared with those in the respective vehicle-infused groups. Ang II, angiotensin II; V, vehicle; FS (%), fractional shortening; and RWT, relative wall thickness.

Blood sampling and organ collection. At the end of the experiment, the blood of the rats was collected in chilled plastic tubes containing 0.5 ml of 3.8% EDTA. Plasma IGF-1 level was measured by radioimmunoassay. The heart and right kidney were dissected in each rat. One part of these specimens was fixed in 10% formalin for histopathological examination and another part was frozen in liquid nitrogen and kept at -80˚C until homogenized.

Histological analysis. The paraffin-embedded sections were prepared and stained either with hematoxylin and eosin or Masson's trichrome for light microscopy analysis. Fibrosis in the heart was graded from 0-4 as determined by perivascular

Table I. Animal characteristics and serum IGF-1 after 4 weeks of Ang II infusion.

	C+V (n=8)	C+Ang II (n=8)	Tg +V (n=8)	Tg+Ang II (n=8)
Systolic blood pressure (mmHg)	124.4±2.7	223.4±12.9[a]	133.0±3.7	218.7±5.2[b]
Heart rate (bpm)	468.4±14.7	462.3±8.9	411.6±5.7	454.5±21.7
Body weight (g)	461.1±7.3	340.0±7.1[a]	336.6±9.4[c]	272.8±5.4[b,d]
Heart weight/body weight (mg/g)	2.40±0.06	3.31±0.14[a]	2.82±0.07	3.54±0.08[b]
Kidney weight/body weight (mg/g)	2.84±0.18	3.55±0.14[a]	2.80±0.08	3.51±0.06[b]
Serum IGF-1 (ng/ml)	790±26	484±60[a]	487±19[c]	346±41

Data are expressed as mean ± SEM. C, control group; V, vehicle-infused group; Ang II, Angiotensin II-infused group; and Tg, transgenic group. [a]P<0.05, C+V groups vs C+Ang II groups; [b]P<0.05, Tg+V groups vs Tg+Ang II groups; [c]P<0.05, C+V groups vs Tg+V groups; and [d]P<0.05, C+Ang II groups vs Tg+Ang II groups.

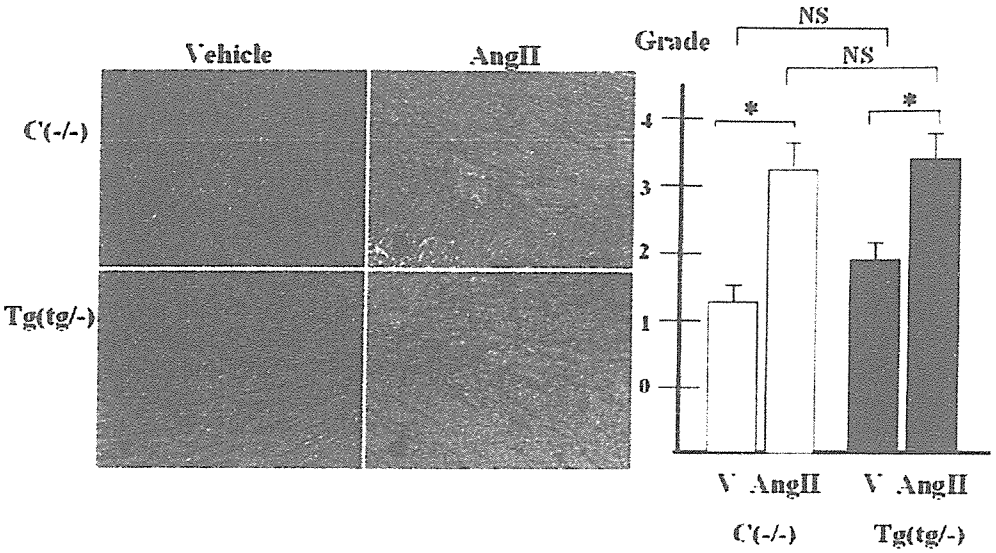

Figure 2. Light microscopy photomicrographs showing representative images of ventricle stained with Masson's trichrome corresponding to the control rat: vehicle infusion (a) and Ang II infusion (b); and the Tg (tg/-) rat: vehicle infusion (c) and Ang II infusion (d). Bar is 100 μm. Grading of cardiac fibrosis was defined as follows: grade 0, no lesion; grade 1, perivascular fibrosis; grade 2, perivascular fibrosis + myocardial fibrosis, minimal; grade 3, perivascular fibrosis + myocardial fibrosis, mild; and grade 4, perivascular fibrosis + myocardial fibrosis, moderate. *p<0.05 compared with those in the respective vehicle-infused groups.

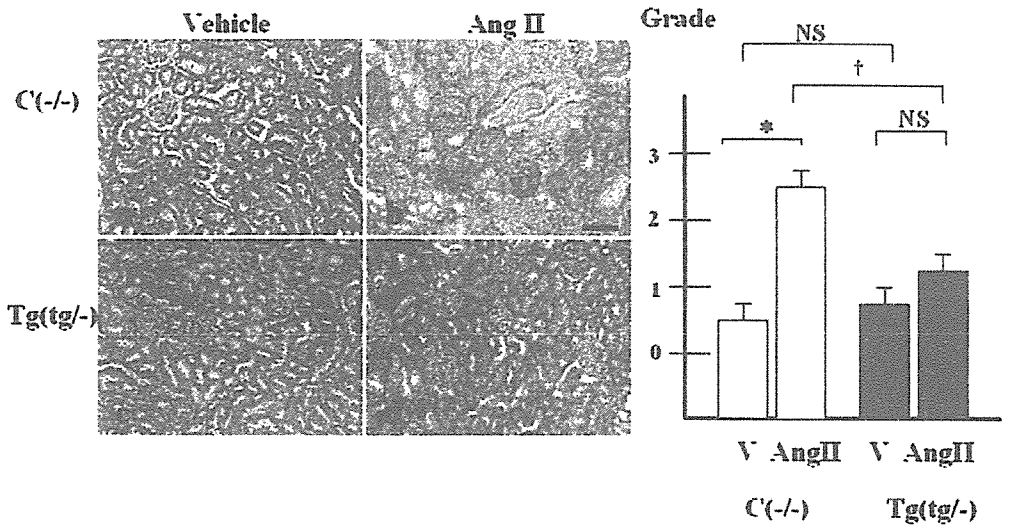

Figure 3. Light microscopy photomicrographs showing representative images of kidney stained with Masson's trichrome corresponding to control rat: vehicle infusion (a) and Ang II infusion (b); and the Tg (tg/-) rat: vehicle infusion (c) and Ang II infusion (d). Bar is 100 μm. Grading of renal involvement was defined as follows: grade 0, no lesion; grade 1, interstitial fibrosis, mild; grade 2, interstitial fibrosis, mild + nephropathy, mild and grade 3, interstitial fibrosis, moderate + nephropathy, moderate. *p<0.05 compared with those in the respective vehicle-infused groups. †p<0.05 compared with those in the respective Ang II-infused groups.

fibrosis and myocardial fibrosis. Renal involvement was graded from 0-3 as determined by interstitial fibrosis and nephropathy. The severity of nephropathy was scored using the method of Maeda and colleagues with some modifications (14,15).

Semi-competitive RT-PCR. Total RNA was isolated from the kidney and ventricle using Trizol reagent (Invitrogen Japan, Tokyo, Japan) according to the manufacturer's instructions. Reverse transcription followed by polymerase chain reaction (RT-PCR) was performed using a Qiagen OneStep RT-PCR kit (Qiagen Japan, Tokyo, Japan) according to the manufacturer's protocol. Primers used were as follows: TGF-ß1, 5'-AAG AAC TGC TGT GTG CGG-3' and 5'-GCA CTT GCA GGA GCG CAC AA-3' (296 bp); and GAPDH, 5'-AGA TCC ACA ACG GAT ACA TT-3' and 5'-TCC CTC AAG ATT GTC AGC AA-3' (309 bp) (16,17). Quantitative analysis was performed by NIH imaging.

Statistical analysis. Data were represented as the mean ± SEM. One-way analysis of variance (ANOVA) followed by the Scheffe's F-test were performed for statistical comparisons. Differences were considered statistically significant when $p<0.05$.

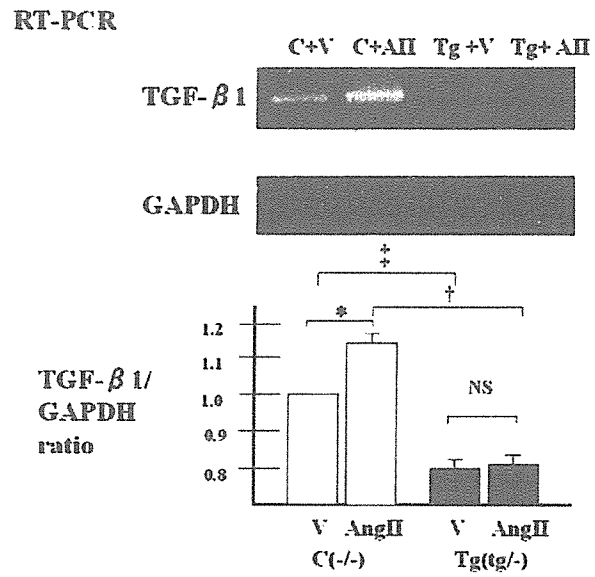

Figure 4. RT-PCR analysis of renal TGF-ß1 expression in control + vehicle, control + Ang II, Tg (tg/-)+vehicle and Tg (tg/-) + Ang II. *p<0.05 compared with those in the respective vehicle-infused groups. †p<0.05 compared with those in the respective Ang II-infused groups. ‡p<0.05 compared vehicle-infused control groups with those in the respective transgenic groups.

Results

Animal characteristics and IGF-1 determination. Baseline BP and HR readings were similar in heterozygous (tg/-) (124.9±2.7 mmHg, 436.8±8.3 bpm) and control (-/-) (118.5±4.5 mmHg, 448.3±10.1 bpm) rats. BP, HR, BW, heart weight, kidney weight, and serum levels of IGF-1 after 4 weeks of Ang II or vehicle infusion to transgenic and control rats are shown in Table I. Ang II infusion increased BP in both the control and transgenic groups to the same extent. Body weight was lighter in the transgenic rats than in the control rats, while rats that received Ang II infusion showed lighter BW than those that received vehicle infusion. To note, transgenic rats had a higher heart weight/BW ratio than control rats during vehicle infusion (statistically insignificant, p=0.06), although Ang II infusion increased the heart weight/BW ratio to the same extent in transgenic and control rats. The kidney weight/BW ratio also increased by Ang II infusion in both transgenic and control rats. Similar kidney weight/BW ratios were observed between transgenic and control rats either with Ang II or vehicle infusion. Ang II reduced serum IGF-1 level in both transgenic and control rats.

Echocardiography. As shown in Fig. 1, RWT and %FS in the Ang II-infused transgenic and control rats (C+Ang II and Tg+Ang II) were significantly higher than those in the respective vehicle-infused rats (C+V and Tg+V). Both control and transgenic rats showed a marked increase in RWT and %FS after Ang II infusion.

Histological analysis. Fig. 2 indicates that there was no difference in cardiac fibrosis between Ang II-infused transgenic and control rats (C+Ang II and Tg+Ang II) and their respective vehicle-infused rats (C+V and Tg+V) (cardiac fibrosis score C+Ang II vs Tg+Ang II; 3.3 vs 3.5; statistically insignificant). However, as shown in Fig. 3, the severity of renal involvement was remarkably higher in Ang II-infused control rats (C+Ang II) than in those of Ang II-infused transgenic rats (Tg+Ang II) (renal fibrosis and nephropathy score: C+Ang II vs Tg+Ang II; 2.5 vs 1.3; p<0.05).

Expression of TGF-ß1 mRNA in the kidney. To examine whether the fibrotic response in the kidney by Ang II infusion was blunted in transgenic rats, RT-PCR analysis of TGF-ß1, as a marker of fibrosis, was performed. As shown in Fig. 4, the expression of the mRNA level of TGF-ß1 was significantly higher in control rats (C+Ang II) compared to transgenic rats (Tg+Ang II) after Ang II infusion (TGF-ß1/GAPDH ratio: C+Ang II vs Tg+Ang II; 1.15 vs 0.81; p<0.05).

Discussion

In the present study, we first confirmed the effects of the chronic administration of Ang II on pressor response as well as morphological and histological changes in the heart and the kidney in an insulin-sensitive transgenic rat model. Recently, many reports concerning growth hormone (GH)-suppressed rodents have documented longevity, small size, and insulin hypersensitivity. Insulin hypersensitivity was also demonstrated using a glucose tolerance test and glucose clamp test (18,19). It is well recognized that the renin-angiotensin system (RAS) plays an important physiological role in body fluid and sodium homeostasis and blood pressure regulation, whereas RAS activation causes hypertension and other cardiovascular and renal diseases (20). Experimental and clinical studies have demonstrated the benefit of the RAS blockade in the treatment of such cardiovascular and renal diseases (21-24). Bunnag *et al* (25) suggested that the blood pressure response to an acute infusion of Ang II was not significantly different among high fructose-fed rats, high sucrose-fed rats, and control rats. However, Gaboury *et al* (26) confirmed that hypertensive subjects but not normotensive subjects display a striking negative correlation between pressor response to Ang II and insulin resistance from the results of acute Ang II infusion and a glucose clamp test. Although it is not clear whether the response to chronic Ang II infusion is enhanced in insulin-resistant animal models, there are studies showing that the administration of the Ang II receptor blockade reduces or abolishes blood pressure elevation and hyperinsulinemia in insulin-resistant rats (27,28). Thus, we assumed that a blunted response to chronic Ang II would be found in our insulin-sensitive transgenic rats. In the present study, we confirmed the following three points. First, there was no significant difference in pressor response to chronic Ang II infusion between insulin-sensitive transgenic and control rats. Second, there was no significant difference in cardiac hypertrophy and fibrosis by chronic Ang II infusion between the two groups. However, third, fibrotic response to chronic Ang II infusion in the kidney was significantly decreased in insulin-sensitive transgenic rats. Generally, continuous Ang II infusion has been shown to cause blood pressure elevation,

cardiac hypertrophy, and renal dysfunction in animal models. In this study, Ang II infusion induced marked blood pressure elevation and hypertrophic responses, including increases in heart weight and LV wall thickness in both groups. In contrast, the preventive effect of GH/IGF-1 axis suppression on renal fibrosis and injury induced by Ang II was identified in insulin-sensitive transgenic rats. Furthermore, expression of TGF-ß, a fibrosis indicator, was also significantly suppressed in the kidneys of the transgenic rats. This result indicated that the GH/IGF-1 axis was critically involved in the development of renal injury and fibrosis, rather than hypertension, cardiac hypertrophy, and cardiac fibrosis induced by Ang II. The mechanism of this difference by chronic Ang II infusion observed in the heart and kidneys is not clear at present. Anderson *et al* (29) suggested that insulin, itself, significantly increases TGF-ß and extracellular matrix gene expression in rat mesangial cells. However, Ang II alone has modest effects, while Ang II and insulin have additive effects. They also suggested that enhancement of mitogen-activated protein (MAP) kinase activity and AT1 receptor message level by insulin may contribute to the additive effects of insulin and Ang II in rat mesangial cells. Moreover, Brown-Borg *et al* (30) suggested that the reduced levels of glutathione peroxidase (GPX) proteins, one family of antioxidant proteins, in the heart and skeletal muscle were modest in comparison to those observed in the liver and kidney in Ame dwarf mice by growth hormone administration. The organ damage difference between the heart and kidney may be explained by the organ-specific response under oxidative stress. Further study is required to elucidate the differences in organ damage between the heart and kidneys with regard to signal transduction below the level of insulin/IGF-1 and Ang II receptors. In conclusion, insulin hypersensitivity with GH/IGF-1 axis suppression ameliorates renal fibrosis and injury caused by excessive Ang II. Application of these results clinically may lead to a promising new treatment for hypertension, heart failure and type 2 diabetes.

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Treatment of Cachexia With Ghrelin in Patients With COPD*

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Study objectives: **Ghrelin is a novel growth hormone (GH)-releasing peptide that also induces a positive energy balance by decreasing fat utility and stimulating feeding through GH-independent mechanisms. We investigated whether ghrelin improves cachexia and functional capacity in patients with COPD.**

Methods: **This is an open-label pilot study. Human ghrelin (2 μg/kg bid) was IV administered to seven cachectic patients with COPD for 3 weeks. Food intake, body composition, muscle strength, exercise capacity, pulmonary function, and sympathetic nerve activity were examined before and after ghrelin therapy.**

Results: **A single administration of ghrelin markedly increased serum GH (21-fold). Three-week treatment with ghrelin resulted in a significant increase in mean (± SEM) body weight (49.3 ± 3.6 to 50.3 ± 3.8 kg; $p < 0.05$). Food intake was significantly increased during ghrelin therapy. Ghrelin increased lean body mass and peripheral and respiratory muscle strength. Ghrelin significantly increased Karnofsky performance status score and the distance walked in 6 min (370 ± 30 to 432 ± 35 m; $p < 0.05$), although it did not significantly alter pulmonary function. Ghrelin attenuated the exaggerated sympathetic nerve activity, as indicated by a marked decrease in plasma norepinephrine level (889 ± 123 to 597 ± 116 pg/mL; $p < 0.05$).**

Conclusions: **These preliminary results suggest that repeated administration of ghrelin improves body composition, muscle wasting, functional capacity, and sympathetic augmentation in cachectic patients with COPD.** ***(CHEST 2005; 128:1187–1193)***

Key words: cachexia; chronic obstructive; exercise capacity; ghrelin; nutrition

Abbreviations: GH = growth hormone; IGF = insulin-like growth factor

Cachexia, which is a catabolic state characterized by weight loss and muscle wasting, occurs frequently in patients with COPD and is a strong independent risk factor for mortality.[1–4] Cachexia also impacts not only the respiratory musculature, but also the peripheral skeletal muscle function, which impairs the quality of life in patients with COPD. However, there have been no promising drugs to improve pulmonary cachexia.

Ghrelin is a novel growth hormone (GH)-releasing peptide that was isolated from the stomach and has been identified as an endogenous ligand for the GH secretagogue receptor.[5] Therefore, ghrelin may induce beneficial effects on muscle strength and energy metabolism via a GH-dependent mechanism.

For editorial comment see page 1084

On the other hand, ghrelin induces a positive energy balance and weight gain by decreasing fat utility[6] and stimulating food intake[7] through GH-independent mechanisms. Interestingly, ghrelin has been shown to act directly on the CNS to decrease sympathetic nerve activity,[8,9] which may attenuate the exaggerated energy expenditure in patients with COPD. An

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experimental study has shown that repeated administration of ghrelin improves cachexia in rats with heart failure.[10] These findings raise the possibility that ghrelin administration may also improve pulmonary cachexia.

Thus, the purpose of this study was to investigate the effects of repeated administration of ghrelin on body composition, peripheral and respiratory muscle strength, and functional capacity in cachectic patients with COPD. This is an open-label pilot study.

Materials and Methods

Study Patients

We studied seven cachectic patients with COPD (five men and two women; mean age, 78 years; range, 76 to 80 years). COPD was diagnosed on the Global Initiative for Chronic Obstructive Lung Disease criteria. Cachexia was defined as those patients with documented nonedematous and nonintentional weight loss of $> 7.5\%$ of their previous normal weight over a period of at least 6 months.[4] All of the patients were clinically stable at the time of evaluation and had no evidence of other primary cachectic states, such as cancer, thyroid disease, heart failure, or severe liver disease. The study was approved by the ethical committee of the National Cardiovascular Center, and all of the patients gave written informed consent.

Preparation of Synthetic Human Ghrelin

Synthetic human ghrelin was dissolved in distilled water with 4% D-mannitol and sterilized by passage through a 0.22-μm filter (Millex; Millipore Co., Bedford, MA). Ghrelin was stored in 2-mL volumes, each containing 200 μg ghrelin. The chemical nature and content of the human ghrelin in vials were verified by high-performance liquid chromatography and radioimmunoassay. All of the vials were stored frozen at −80°C from the time of dispensing until the time of preparation for the administration.

Study Protocol

Human ghrelin (2 μg/kg, 10 mL solution) was administered IV for > 60 min at a constant rate. The infusion was repeated bid (before breakfast and before dinner) for 3 weeks. The GH responses to ghrelin were assessed upon the initial administration. The body height, body weight, Karnofsky performance status, peripheral and respiratory muscle strength, and dietary intake of the patients were assessed at baseline and after the 3-week treatment with ghrelin. Dual radiograph absorptiometry, 6-min walk test, spirometry, and blood sampling were also performed on the patients before and after ghrelin therapy. Long-term medication, including β-agonists (n = 5), anticholinergics (n = 5), xanthines (n = 4), and inhaled steroids (n = 2) was kept constant during this study protocol.

Performance Status

Karnofsky performance status, a measure of functional ability, was assessed by the investigator based on the observation and subjective feedback from the patient, as reported previously.[11]

Dietary Intake

Food intake for 3 consecutive days was assessed before ghrelin administration and during the last week of ghrelin therapy. The food intake was semiquantitatively assessed by staff nurses using a calorie count, based on a 10-point scale method (0 = null intake to 10 = full intake, 1,800 kilocalories), which was averaged for 3 days.

Body Composition

Patient body height was determined to the nearest 0.5 cm, with subjects standing barefoot. Body weight was assessed with a beam scale to the nearest 0.1 kg, with subjects standing barefoot and in light clothing. Dual radiograph absorptiometry (DPX-L; Lunar Radiation; Madison, WI) was performed to assess lean body mass, fat mass, and bone mineral content of the patients.

Peripheral and Respiratory Muscle Strength

Peripheral muscle strength was measured by the maximal voluntary handgrip maneuver. The patients performed four maneuvers on each side with at least a 1-min interval between each of the maneuvers. The average of the best values on the left and right sides was reported. Respiratory muscle strength was examined during maximal voluntary efforts against occluded airways (Vitaropov KH-101; Chest Scientific Instruments Ltd; Westerham, United Kingdom), as reported previously.[12] The maximal inspiratory pressure and maximal expiratory pressure were measured from functional residual capacity. The patients performed four maneuvers, and the highest value was reported.

Pulmonary Function Testing

All of the patients with COPD underwent pulmonary function testing before and after receiving ghrelin therapy. Their lung volumes were measured by the helium gas dilution method, and forced expiratory flow rates were measured by a mass flow anemometer (FUDAC 70; Fukuda Denshi; Tokyo, Japan). The carbon monoxide transfer factor was measured by the single-breath method. Pulmonary function values were expressed as the percentage of predicted values.[13] Arterial blood gases were measured at rest by a blood gas analyzer (ABL 720; Radiometer; Copenhagen, Denmark).

6-Min Walk Test

The 6-min walk test was performed in all of the patients according to a standardized protocol.[14] The subjects were instructed to walk at their own pace but to cover as much ground as possible in 6 min. They tolerated 6-min walk tests without any adverse effects.

Blood Sampling and Assay

Blood samples were taken from the antecubital vein after 30-min bed rest in the morning following an overnight fast. Serum GH and insulin-like growth factor (IGF)-1 were measured by immunoradiometric assay (Ab Bead HGH Eiken; Eiken Chemical Co, Ltd; Tokyo, Japan and Somatomedin CII Bayer, Bayer Medical Ltd; Tokyo, Japan). Plasma norepinephrine was measured by high-performance liquid chromatography (HLC8030; Tosoh Co; Tokyo, Japan). Serum cortisol and insulin were measured by enzyme immunoassay (AIA-PACK CORT, AIA-PACK IRI; Tosoh Co). Serum tumor necrosis factor α and interleukin 6 were measured by enzyme immunoassay (Quantikine HS, R and D Systems Inc; Minneapolis, MN and TFB kit, TFB Co, Ltd; Tokyo, Japan).

Statistical Analysis

Numerical values were expressed as mean (± SEM) unless otherwise indicated. Changes in the parameters during treatment were analyzed with paired Student *t* test. A p value of < 0.05 was considered significant.

Results

The administration of ghrelin transiently caused a slight feeling of being warm and sleepy in three patients. One patient felt slightly thirsty during ghrelin infusion. Other than these minor complaints, all of the subjects tolerated the 3-week administration of ghrelin without incident.

Effects of Ghrelin on Somatotropic Function

A single administration of ghrelin markedly increased serum GH level (baseline, 2.0 ± 2.3 ng/mL; peak, 42.1 ± 23.0 ng/mL; $p < 0.001$) [Fig 1]. Ghrelin tended to increase the serum IGF-1 level (92 ± 13 to 103 ± 15 ng/mL; difference was not significant), although it did not reach statistical significance.

Effects of Ghrelin on Food Intake, Body Weight, and Lean Body Mass

The administration of ghrelin stimulated feeding in six of the seven patients. Semiquantitative analysis also demonstrated that treatment with ghrelin increased the food intake in patients with COPD (Fig 2, *left*, *A*). The 3-week administration of ghrelin significantly increased the body weight (49.3 ± 3.6 to 50.3 ± 3.8 kg; $p < 0.05$) [Fig 2, *middle*, *B*] and body mass index (18.6 ± 0.7 to 19.1 ± 0.8 kg; $p < 0.05$). Ghrelin increased the lean body mass (38.2 ± 6.5 to 38.9 ± 6.4 kg; $p < 0.05$) [Fig 2, *right*, *C*], but not the bone mineral content or fat mass.

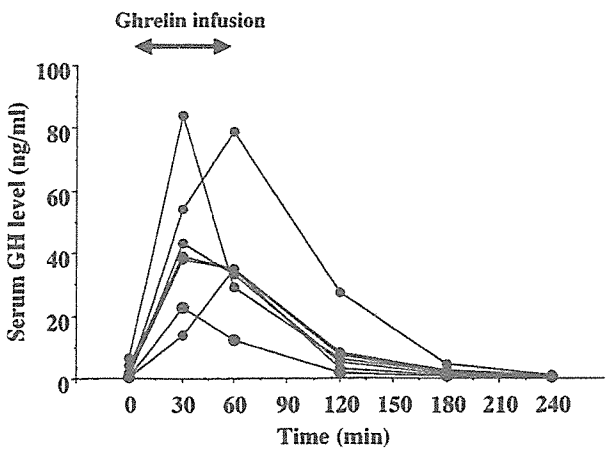

FIGURE 1. Changes in serum GH level after a single administration of ghrelin in patients with COPD.

Effects of Ghrelin on Muscle Strength

Hand-grip strength of the patients was significantly increased by ghrelin therapy (21.5 ± 6.2 to 24.2 ± 6.8 kg; $p < 0.05$) [Fig 3, *left*, *A*]. Furthermore, ghrelin significantly increased respiratory muscle strength, as indicated by increases in the maximal inspiratory pressure (54 ± 18 to 64 ± 23 cm H_2O; $p < 0.05$) [Fig 3, *middle*, *B*] and the maximal expiratory pressure (47 ± 14 to 57 ± 20 cm H_2O; $p = 0.05$) [Fig 3, *right*, *C*].

Effects of Ghrelin on Functional Capacity

Treatment with ghrelin significantly increased the Karnofsky performance status score, a marker for functional capacity (63 ± 8 to 80 ± 12; $p < 0.01$) [Fig 4, *left*, *A*]. Furthermore, ghrelin significantly increased the distance walked in 6 min (370 ± 30 to 432 ± 35 m; $p < 0.05$) [Fig 4, *right*, *B*].

Ghrelin therapy did not significantly alter any pulmonary function parameters on spirometry (Table 1). Neither Pao_2 nor $Paco_2$ changed during the treatment.

Effects of Ghrelin on Sympathetic Nerve Activity and Other Hormone Levels

The plasma norepinephrine level in patients with COPD was significantly higher than the normal value, which was determined from pooled data of 10 age-matched healthy subjects (889 ± 123 vs 193 ± 8 pg/mL; $p < 0.05$). The 3-week administration of ghrelin markedly decreased the plasma norepinephrine level in patients with COPD (889 ± 123 to 597 ± 116 pg/mL; $p < 0.05$) [Fig 5]. Ghrelin did not significantly alter circulating glucose, insulin, cortisol, tumor necrosis factor α, or interleukin 6 (Table 2).

Discussion

This is the first report of the use of ghrelin in patients with COPD, although we have recently reported on the effect of ghrelin in patients with heart failure.[15] In the present study, we demonstrated the following: (1) administration of ghrelin significantly increased the serum GH level in patients with COPD; (2) repeated administration of ghrelin stimulated feeding and increased body weight and lean body mass; (3) treatment with ghrelin increased peripheral and respiratory muscle strength; (4) 3-week administration of ghrelin in-

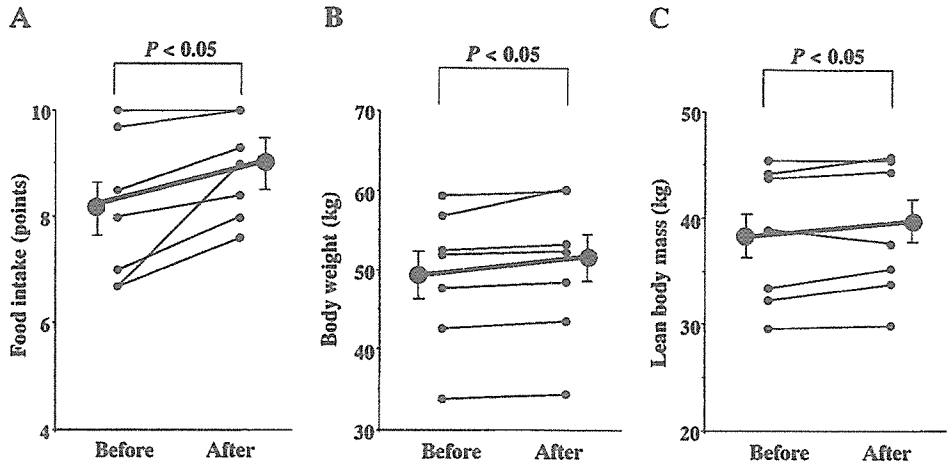

FIGURE 2. Effects of a 3-week administration of ghrelin on food intake (*left*, *A*), body weight (*middle*, *B*), and lean body mass (*right*, *C*).

creased the distance walked in 6 min; and (5) ghrelin therapy resulted in a marked decrease in plasma norepinephrine.

Cachexia, which is a catabolic state characterized by weight loss and muscle wasting, occurs frequently in patients with COPD and is a strong independent risk factor for mortality in such patients.[1–4] We have shown that plasma ghrelin is elevated in cachectic patients with heart failure[16] and those with lung cancer[17] and that the plasma ghrelin level is inversely correlated with the body mass index. Considering the ghrelin-induced positive energy effects,[5–7] the increased ghrelin may represent a compensatory mechanism under catabolic-anabolic imbalance in cachectic patients. These findings raise the possibility that supplementation of ghrelin may improve pulmonary cachexia.

Ghrelin strongly stimulates GH release through a mechanism independent from that of hypothalamic GH-releasing hormone.[5] The GH-releasing effect of ghrelin has been shown to be more potent than that of the GH-releasing hormone.[18] The present study also demonstrated that exogenously administered ghrelin elicits a potent GH release in patients with COPD. Body weight loss and muscle wasting were observed in study patients. However, 3-week administration of ghrelin increased body weight and lean body mass of the patients. Furthermore, ghrelin therapy increased peripheral and respiratory muscle strength. These results suggest that treatment with ghrelin improves body composition and muscle wasting in cachectic patients with COPD. GH and its mediator, IGF-1, both of which are anabolic hormones, are essential for skeletal muscle.[19,20] Thus,

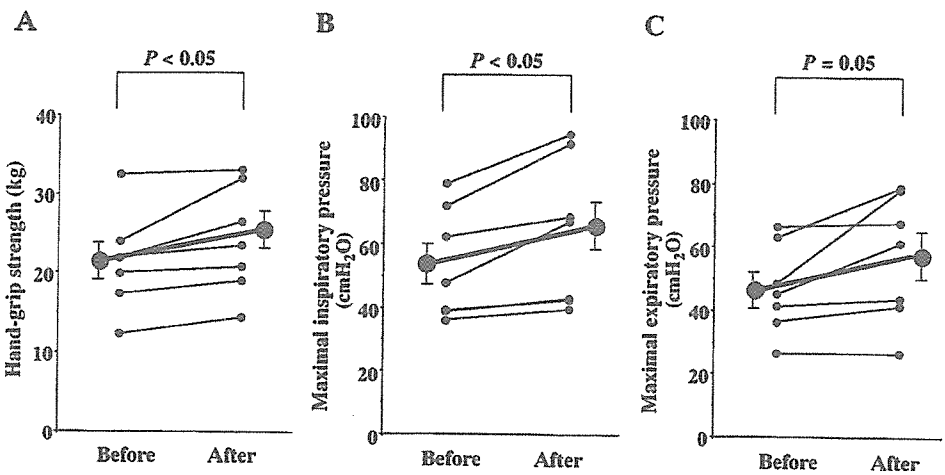

FIGURE 3. Changes in hand-grip strength (*left*, *A*), maximal inspiratory pressure (*middle*, *B*), and maximal expiratory pressure (*right*, *C*) before and after ghrelin therapy.

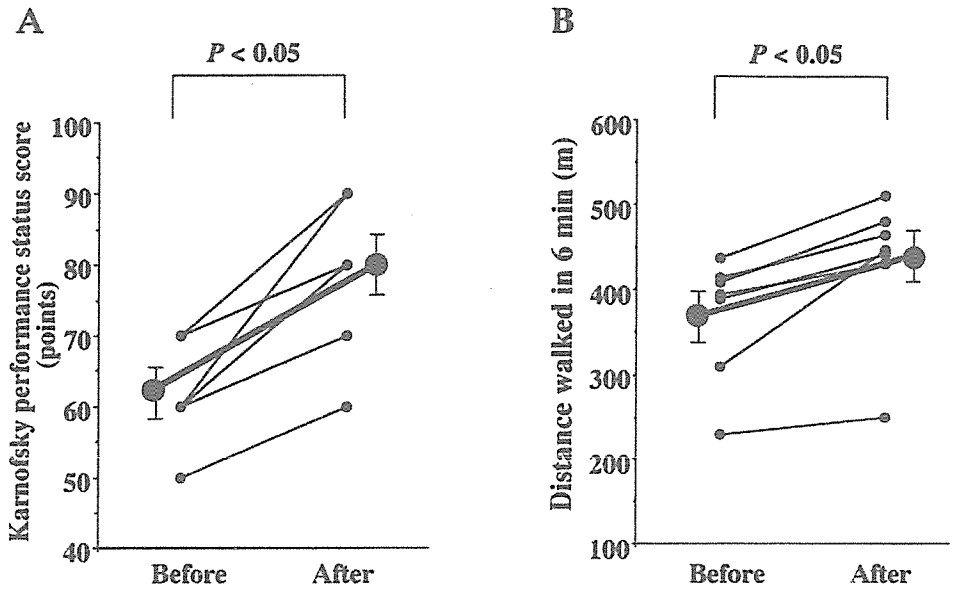

FIGURE 4. Changes in Karnofsky performance status score (*left*, *A*) and the distance walked in 6 min (*right*, *B*) before and after ghrelin therapy.

ghrelin may improve muscle wasting partly through GH-dependent mechanisms. A previous study has shown that administration of ghrelin induces a positive energy balance and weight gain by decreasing fat utilization and increasing carbohydrate utilization through a GH-independent mechanism.[6] In the present study, however, ghrelin did not significantly increase fat mass. The difference may be explained by the difference in the dosage of ghrelin between the two studies.

The present study demonstrated that infusion of ghrelin increased food intake in patients with COPD. Earlier animal studies[7,21,22] have shown that ghrelin elicits oregigenic effects via the activation of neuropeptide Y neurones in the hypothalamic arcuate nucleus. In addition, ghrelin is known to antagonize the action of leptin, an antiorexigenic peptide, through the activation of the hypothalamic NPY/Y1 receptor pathway.[21] Thus, the administered ghrelin may attenuate malnutrition in pulmonary cachexia via its orexigenic property (GH-independent effect).

Increased sympathetic nerve activity leads to excess energy expenditure and impaired energy balance. Thus, norepinephrine is considered to be a catabolic hormone.[23] In the present study, the plasma norepinephrine level was elevated in cachec-

Table 1—*Effects of Ghrelin on Pulmonary Function

Variables	Before Treatment	After Treatment
FEV_1, % predicted	51.5 ± 6.7	55.9 ± 7.5
FEV_1/FVC, %	46.0 ± 6.1	48.6 ± 6.1
VC, % predicted	84.2 ± 3.2	86.6 ± 4.7
RV, % predicted	130.4 ± 9.7	124.2 ± 7.7
TLC, % predicted	102.7 ± 5.5	100.7 ± 5.8
DLCO, % predicted	67.5 ± 10.0	68.9 ± 11.5
PaO_2, mm Hg	69.0 ± 4.2	72.2 ± 4.1
$PaCO_2$, mm Hg	43.7 ± 1.7	42.8 ± 1.2

*Values given as mean ± SEM. VC = vital capacity; RV = residual volume; TLC = total lung capacity; DLCO = diffusing capacity of the lung for carbon monoxide.

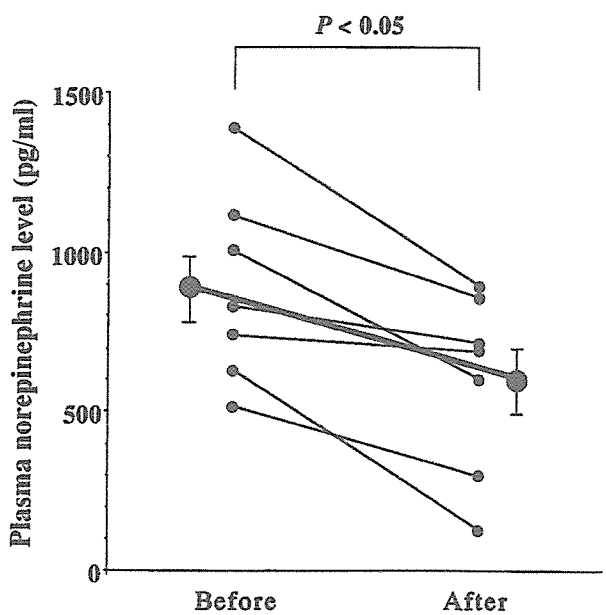

FIGURE 5. Plasma norepinephrine level before and after ghrelin therapy.

Table 2—*Effects of Ghrelin on Circulating Hormone Levels**

Variables	Before Treatment	After Treatment
Fasting glucose, mg/dL	99 ± 5	100 ± 3
Insulin, μU/mL	5.4 ± 1.0	5.1 ± 1.4
Cortisol, μg/dL	15.3 ± 1.5	15.1 ± 2.6
TNF-α, pg/mL	6.2 ± 0.8	5.7 ± 0.4
IL-6, pg/mL	5.3 ± 0.7	4.9 ± 0.6

*Values given as mean ± SEM. TNF = tumor necrosis factor; IL = interleukin.

tic patients with COPD, suggesting the exaggerated sympathetic nerve activity in such patients. Interestingly, 3-week administration of ghrelin resulted in a marked decrease in plasma norepinephrine in patients with COPD. Another study[9] has demonstrated that ghrelin acts directly on the CNS to decrease the sympathetic nerve activity. Thus, ghrelin may attenuate the exaggerated energy expenditure in patients with COPD, possibly through the direct inhibitory effect of ghrelin on sympathetic nerve activity (GH-independent effect).

Three-week administration of ghrelin improved the functional capacity in patients with COPD, as indicated by the marked increases in Karnofsky performance status score and the distance walked in 6 min. A decrease in exercise capacity is attributable not only to an inadequate increase in cardiac output during exercise, which is a central effect, but also to muscle wasting, a peripheral effect.[24] We have shown that infusion of ghrelin increases cardiac output in heart failure.[25] In the present study, ghrelin therapy increased lean body mass and skeletal muscle strength. These results suggest that ghrelin may improve exercise capacity through both the central and peripheral effects.

In the present study, 3-week administration of ghrelin did not significantly influence any pulmonary function parameters in patients with COPD. Nevertheless, the results from this study suggest that ghrelin has anticachectic effects through GH-dependent and independent mechanisms. Although preliminary studies[26,27] documented beneficial effects of GH on cachexia, the results of controlled studies[28,29] have been predominantly negative. However, the present study demonstrated that ghrelin induces GH-independent effects: stimulating feeding and inhibiting sympathetic nerve activity. Thus, ghrelin may have additional therapeutic potential compared with GH supplementation. The major limitation of this pilot trial relates to the small sample size and the lack of a randomized, placebo-controlled group. Nonetheless, all of the changes by ghrelin were consistently in a beneficial direction, suggesting that ghrelin is effective for the treatment of pulmonary cachexia. Based on the results of this study, a double-blind, randomized, placebo-controlled study should be conducted.

In conclusion, our preliminary results suggest that repeated administration of ghrelin improves body composition, peripheral and respiratory muscle wasting, functional capacity, and sympathetic augmentation in patients with COPD. Thus, administration of ghrelin may be a new therapeutic approach for the treatment of pulmonary cachexia.

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LEADING ARTICLE

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Therapeutic Potential of Ghrelin in the Treatment of Heart Failure

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Abstract

Ghrelin is a novel growth hormone (GH)-releasing peptide, isolated from the stomach, which has been identified as an endogenous ligand for the GH secretagogues receptor. The discovery of ghrelin indicates that the release of GH from the pituitary might be regulated, not only by hypothalamic GH-releasing hormone, but also by ghrelin derived from the stomach. Considering the haemodynamic and anabolic effects of GH, ghrelin may have beneficial effects on cardiac function and energy metabolism in heart failure through GH-dependent mechanisms. On the other hand, ghrelin has some GH-independent actions: ghrelin stimulates food intake and induces adiposity. Interestingly, ghrelin acts directly on the CNS to decrease sympathetic nerve activity. It also inhibits apoptosis of cardiomyocytes and endothelial cells. An experimental study has shown that repeated administration of ghrelin improves cardiac structure and function, and attenuates the development of cardiac cachexia in chronic heart failure (CHF). These results suggest that ghrelin has cardiovascular effects and regulates energy metabolism through GH-dependent and -independent mechanisms. Thus, administration of ghrelin may be a new therapeutic strategy for the treatment of severe CHF.

Heart failure is a major public health concern. Currently, there are 5 million Americans with congestive heart failure, with nearly 500 000 new cases every year.[1] In the past 10 years, several large-scale, randomised clinical trials have shown that ACE inhibitors and β-adrenoceptor antagonists (β-blockers) reduce the risk of death in patients with chronic heart failure (CHF).[2-9] Nevertheless, heart failure contributes to >250 000 deaths every year.

Ghrelin is a novel growth hormone (GH)-releasing peptide, isolated from the stomach, which has been identified as an endogenous ligand for the GH secretagogues (GHS) receptor (GHS-R).[10] Human ghrelin is a 28-amino-acid peptide containing an n-octanoyl modification at serine 3 (figure 1a). Ghrelin stimulates GH secretion through a mechanism independent of hypothalamic GH-releasing hormone (GHRH). The biological actions of ghrelin are divided into GH-dependent effects and GH-independent effects (figure 1b). GH and its mediator, insulin-like growth factor (IGF)-1, are anabolic hormones that are involved in several physiological processes, such as the control of muscle mass and function, body composition and regulation of nutrient metabolism.[11,12] In particular, the roles of GH and IGF-1 as modulators of myocardial struc-

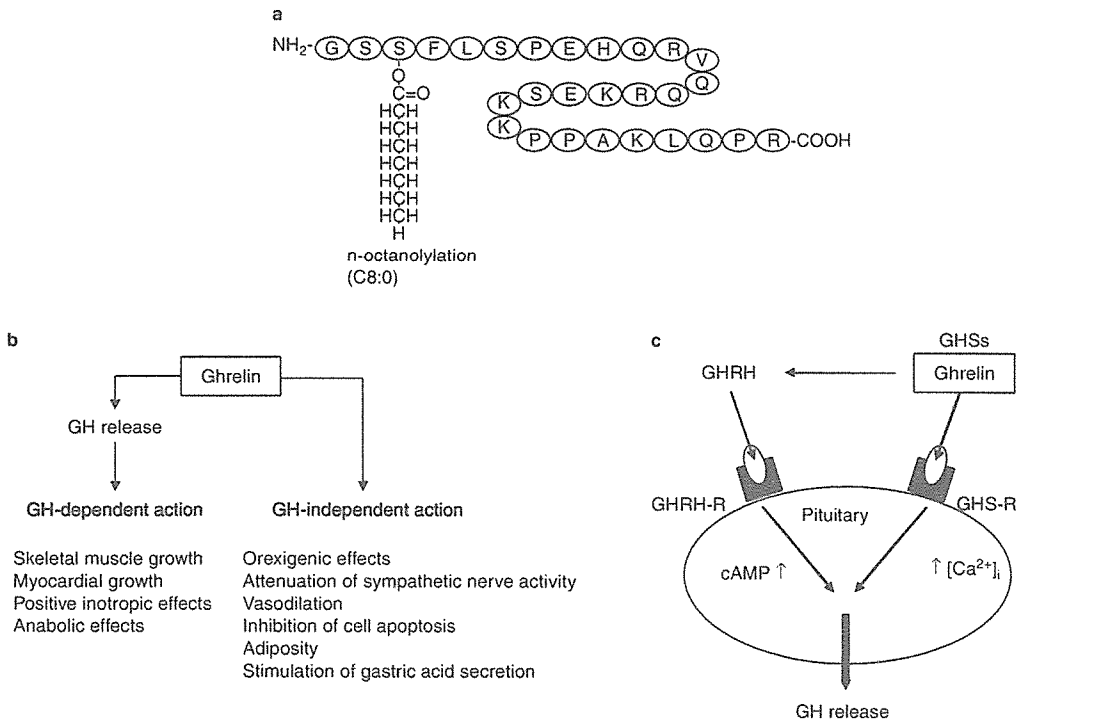

Fig. 1. (a) Structure of human ghrelin. Human ghrelin is a 28-amino-acid peptide containing an n-octanoyl modification. (b) Biological actions of ghrelin: growth hormone (GH)-dependent and -independent mechanisms. (c) Stimulation of GH release by GH-releasing hormone (GHRH) and ghrelin. GHRH acts on the GHRH receptor (GHRH-R) through a cyclic adenosine monophosphate (cAMP)-dependent mechanism, whereas ghrelin and GH secretagogues (GHS) bind to the GHS receptor (GHS-R), followed by the release of Ca^{2+} from intracellular stores. Ghrelin also stimulates GHRH production. ↑ indicates increase.

ture and function are well established.[13] Patients with GH deficiency have abnormalities of cardiac structure and function, such as reduced cardiac mass and impaired diastolic filling. GH supplementation has beneficial effects on myocardial structure and function in some patients with CHF.[14-16] Thus, GH and IGF-1 play a role as anabolic hormones in the regulation of cardiac development and performance. Considering the haemodynamic and anabolic effects of GH/IGF-1, ghrelin may have beneficial effects on left ventricular (LV) function and energy metabolism in CHF through GH-dependent mechanisms. On the other hand, ghrelin may have direct cardiovascular and metabolic effects through GH-independent mechanisms: (i) GHS-R messenger RNA (mRNA) is detected not only in the hypothalamus and pituitary, but also in the heart and blood vessels;[17] (ii) stimulation of the GHS-R has been shown to prevent cardiac damage after ischaemia-reperfusion in hypophysectomised rats;[18] (iii) ghrelin inhibits apoptosis of cardiomyocytes and endothelial cells *in vitro*;[19] (iv) intravenous injection of ghrelin decreases arterial pressure and increases cardiac output in healthy humans;[17] and (v) ghrelin acts directly on the CNS to decrease sympathetic nerve activity.[20,21] These findings raise the possibility that administration of ghrelin may have beneficial haemodynamic effects in patients with CHF.

In patients with end-stage CHF, LV dysfunction as well as cardiac cachexia are observed.[22-24] Cardiac cachexia, which is a catabolic state characterised by weight loss and muscle wasting, is associated with hormonal changes and cytokine activation in patients with CHF.[23-29] Importantly, the presence of cardiac cachexia is a strong independent risk factor

for mortality in patients with CHF.[30] Thus, cardiac cachexia and LV dysfunction are both therapeutic targets in the treatment of CHF.

Interestingly, peripheral and intracerebroventricular administration of ghrelin have been shown to stimulate food intake and increase bodyweight through GH-independent mechanisms.[31] In addition, ghrelin decreases fat utilisation and increases carbohydrate utilisation through a GH-independent mechanism.[32] Taking these results together with the GH-dependent anabolic effects of ghrelin, this peptide may cause a positive energy balance in CHF through GH-dependent and -independent mechanisms. Thus, ghrelin may be used as an anti-cachectic drug. This article summarises the therapeutic potential of ghrelin in the treatment of CHF.

1. Growth Hormone (GH) Secretagogues and the Discovery of Ghrelin

In addition to the physiological stimulation by GHRH, release of GH from the pituitary is stimulated by small synthetic molecules called GHS.[33-35] They act through the GHS-R, a G-protein-coupled receptor,[36] for which the ligand was unknown until the discovery of ghrelin. GHS are synthetic peptidyl and non-peptidyl molecules that have strong, dose-dependent GH-releasing activities *in vivo*. GHS are a heterogenous group but they have the following common characteristics: GHS are synthetic substances, they stimulate GH release from the pituitary, and they act through the GHS-R but not the GHRH receptor. The GHS family includes peptidyl molecules such as SKF 110679 (GH releasing peptide-6) and examorelin (hexarelin), and non-peptidyl molecules such as L 692429 and ibutamoren (MK 0677). In 1995, Merck & Co. discovered ibutamoren, which has good bioavailability and long-lasting effects after oral administration.[37] These GHS compounds have entered clinical trials for therapeutic indications that include idiopathic GH deficiency states, stimulation of anabolic processes in the elderly and supportive therapy in catabolic wasting conditions.

GHRH acts on the GHRH receptor through a cyclic adenosine monophosphate (cAMP)-dependent mechanism (figure 1c). On the other hand, GHS bind to the GHS-R and activate phospholipase C, leading to increased inositol phosphate turnover and protein kinase C activation, followed by the release of Ca^{2+} from intracellular stores. Using GHS-R-expressing cells to monitor intracellular Ca^{2+} concentration, Kojima et al.[10] found that the GHS-R was activated by stomach extracts. Thus, ghrelin, an endogenous ligand specific for the GHS-R, was successfully isolated from the human and rat stomach in December 1999.[10] Human ghrelin is homologous to rat ghrelin apart from two amino acids.

2. Production and Distribution of Ghrelin and its Receptor

Ghrelin is produced mainly in the stomach. To date, four types of endocrine cells (the enterochromaffin-like cells, the D cells, the enterochromaffin cells and X/A-like cells have been identified in the oxyntic mucosa of the stomach.[38] Date et al.[39] have reported that the X/A-like cells) whose hormonal product had not previously been clarified, secrete ghrelin. Ghrelin is not secreted into the gastrointestinal tract but is rather secreted into the blood vessels. Thus, the plasma ghrelin level is relatively high (100–120 fmol/mL).[40] The plasma ghrelin level falls markedly following gastrectomy.[41,42] Ghrelin is also produced in the small and large intestines and is detected in a limited region of the hypothalamic arcuate nucleus that is involved in the regulation of food intake.[10] These results suggest that ghrelin serves as a circulating factor as well as an autocrine/paracrine factor.

Before the discovery of the endogenous ligand, a specific receptor for ghrelin, GHS-R, was discovered in 1996 using a cloning strategy.[36] The GHS-R is a G-protein-coupled receptor with seven transmembrane domains that is present in a variety of tissues including the pituitary and hypothalamus, and is distinct from the GHRH receptor. Interestingly, the GHS-R is detected in the cardiac ventricles and blood vessels, suggesting that ghrelin may cause