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Chronic Administration of Ghrelin Improves Left Ventricular Dysfunction and Attenuates Development of Cardiac Cachexia in Rats With Heart Failure

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Background—Ghrelin is a novel growth hormone (GH)–releasing peptide that may also induce vasodilation and stimulate feeding through GH-independent mechanisms. We investigated whether ghrelin improves left ventricular (LV) dysfunction and attenuates cardiac cachexia in rats with chronic heart failure (CHF).

Methods and Results—Ligation of the left coronary artery or sham operation was performed; 4 weeks after surgery, rat ghrelin (100 μg/kg SC BID) or saline was administered for 3 weeks. Echocardiography and cardiac catheterization were performed. Serum GH and insulin-like growth factor-1 were significantly higher in both CHF and sham rats treated with ghrelin than in those given placebo ($P<0.05$ for both). CHF rats given placebo showed an impaired increase in body weight compared with sham rats given placebo ($P<0.05$). CHF rats treated with ghrelin, however, showed a significantly greater increase in body weight than those given placebo ($+10\%$ versus $+3\%$, $P<0.05$). They showed significantly higher cardiac output (315 ± 49 versus 266 ± 31 mL $\cdot$ min^{-1} $\cdot$ kg^{-1}, $P<0.05$) and LV dP/dt$_{max}$ (5738 ± 908 versus 4363 ± 973 mm Hg/s, $P<0.05$) than CHF rats given placebo. Ghrelin increased diastolic thickness of the noninfarcted posterior wall, inhibited LV enlargement, and increased LV fractional shortening in CHF rats (from $15\pm3\%$ to $19\pm3\%$, $P<0.05$).

Conclusions—Chronic subcutaneous administration of ghrelin improved LV dysfunction and attenuated the development of LV remodeling and cardiac cachexia in rats with CHF. (***Circulation***. 2001;104:1430-1435.)

Key Words: heart failure ■ hormones ■ growth substances ■ nutrition

Left ventricular (LV) remodeling (dilatation and wall thinning) and cardiac cachexia (body weight loss and muscle wasting) are often observed in patients with end-stage chronic heart failure (CHF).[1,2] Growth hormone (GH) and its mediator, insulin-like growth factor-1 (IGF-1), are essential for skeletal and myocardial growth and metabolic homeostasis.[3,4] Earlier studies have shown that GH supplementation may have beneficial effects on myocardial structure and function in some patients with CHF,[5–7] although neutral results of randomized trials have also been reported.[8,9] Elevated serum GH with normal or low IGF-1 levels was observed in cachectic patients with CHF.[10,11] These findings indicate that the GH/IGF-1 axis may be related to LV dysfunction and the resultant cachexia.

Ghrelin is a novel GH-releasing peptide, isolated from the stomach, that is identified as an endogenous ligand for GH secretagogue receptor (GHS-R).[12] Considering the hemodynamic and anabolic effects of GH/IGF-1, ghrelin may have beneficial effects on LV function and energy metabolism in CHF through GH-dependent mechanisms. Conversely, ghrelin may have direct cardiovascular and metabolic effects through GH-independent mechanisms: GHS-R mRNA is detected not only in the hypothalamus and pituitary but also in the heart and vessels,[13] stimulation of GHS-R has been shown to prevent cardiac damage after ischemia-reperfusion in hypophysectomized rats,[14] intravenous injection of ghrelin decreases arterial pressure and increases cardiac output in healthy humans,[13] and ghrelin causes a positive energy balance by stimulating food intake[15] and inducing adiposity.[16] These findings raise the possibility that administration of ghrelin may be beneficial in cachectic CHF.

Thus, the purposes of this study were (1) to investigate whether ghrelin supplementation improves LV myocardial structure and function in rats with CHF and (2) to examine

whether ghrelin attenuates the development of cardiac cachexia.

Methods

Model of CHF

Myocardial infarction was produced in male Wistar rats weighing 200 to 240 g by left coronary ligation as described previously.[17] The control rats underwent a sham operation consisting of thoracotomy and cardiac exposure but without coronary artery ligation. The surviving rats were maintained on standard rat chow. There was a 33% mortality rate within 48 hours after coronary ligation, and the subsequent mortality was 15% for 4 weeks. Four weeks after surgery, 31 infarct rats were randomly divided into 2 groups and given either ghrelin (n=16) or placebo (n=15) for 3 weeks. Similarly, 26 sham-operated rats were randomized to receive ghrelin (n=13) or placebo (n=13).

Administration of Ghrelin

Rat ghrelin was obtained from the Peptide Institute Inc. Four weeks after surgery, rat ghrelin (100 μg/kg BID for 3 weeks) or saline was injected subcutaneously in both CHF rats and sham-operated rats.

Echocardiographic Studies

Echocardiographic studies were performed before and after 3-week supplementation with ghrelin. 2D targeted M-mode tracings were obtained at the level of the papillary muscles. Anterior and posterior end-diastolic and end-systolic wall thickness, LV end-diastolic and end-systolic dimensions, and LV fractional shortening were measured by the American Society for Echocardiology leading-edge method from 3 consecutive cardiac cycles. LV meridional wall stress was estimated as 0.334×LV pressure×[LV dimension/(1+PWT/LV dimension)], where PWT is posterior wall thickness.[18] LV pressure was recorded within 12 hours of the final echocardiogram.

Hemodynamic Studies

Hemodynamic studies were performed after 3 weeks of treatment with ghrelin or placebo. After anesthesia with pentobarbital, polyethylene catheters were inserted into the right femoral artery and the right ventricle (RV). Hemodynamic variables were measured with a pressure transducer connected to a polygraph. A 1.5F micromanometer-tipped catheter was advanced into the LV and then replaced with a thermomicroprobe for measurement of cardiac output.

After completion of these measurements, blood was drawn from the femoral artery for measurements of GH and IGF-1. Infarct size was determined as a percentage of LV area, as reported previously.[17] Only rats with infarct size >25% of the LV were enrolled in this study as a model of CHF. The right gastrocnemius muscle was weighed, and its protein content was determined by the Lowry method to evaluate the ratios of muscle to tibial length and muscle to body weight in 4 groups (n=5 each).

Histological Examination

LV myocardium was fixed in 10% formalin. Three cross sections in noninfarcted myocardium, cut from apex to base, were obtained from individual rats for comparison among 4 groups (n=5 each). They were embedded in paraffin and stained with hematoxylin-eosin for assessment of myocardial hypertrophy and with Masson's trichrome for measurement of interstitial fibrosis. Muscle fiber diameter was evaluated by direct measurements at ×400 magnification only in cross sections that included a nuclear profile.[19] A total of 40 cells per section were evaluated. After 3 sections per animal and 20 fields per section were scanned and computerized with a digital image analyzer (Win Roof, Mitani Co), volume collagen fraction was calculated as the sum of all connective tissue areas divided by the total area of the image.

Hormone Assays

Serum GH was measured with a radioimmunoassay kit (Biotrak RIA, Amersham). Serum IGF-1 was determined with an enzyme immunoassay kit (Active Rat IGF-1 EIA, DSL Inc).

Acute Hemodynamic Effects of Ghrelin

Acute hemodynamic responses to intravenous injection of ghrelin (10 μg/kg) or placebo were examined in sham rats, CHF rats, and spontaneous dwarf rats, a GH-deficient rat model that carries a disrupted GH gene[20] (n=5 each).

Isolated Myocyte Contractile Function

To investigate whether ghrelin has direct inotropic effects on cardiac myocytes, adult cardiac myocytes (n=6) were isolated from the LV of normal rats by collagenase digestion.[21] Cardiac myocytes were also isolated from the LV noninfarcted region of CHF rats that received 3-week treatment with ghrelin (n=40) or placebo (n=38) to examine the chronic effects of ghrelin treatment on myocyte contractile function. Myocyte contraction was monitored every 4 ms by a high-speed CCD camera (Fastcam-net, Photoron) and measured with NIH Image software (version 1.62, NIH). Myocytes were stimulated at 0.5 Hz with 7-ms pulses.

Statistical Analysis

Numerical values were expressed as mean±SD. Comparisons of parameters among the 4 groups were made with 1-way ANOVA, followed by the Newman-Keuls test. Changes in parameters during treatment were analyzed with 2-way ANOVA for repeated measures, followed by the Newman-Keuls test. The effects of ghrelin on myocyte motion were analyzed by paired Student's t test. Comparisons of parameters between 2 groups were made by unpaired Student's t test. A value of $P<0.05$ was considered significant.

Results

Effects of Ghrelin on Somatotropic Function and Body Weight

Moderate to large infarcts were observed in both CHF groups, and the mean infarct size was similar in the two (Table 1). Serum GH and IGF-1 were significantly higher in both CHF and sham rats treated with ghrelin than in those given placebo (Figure 1). CHF rats given placebo showed an impaired increase in body weight compared with sham rats given placebo (Figure 2). CHF rats treated with ghrelin, however, showed a significantly greater increase in body weight than those given placebo. Tibial length was significantly increased in rats treated with ghrelin compared with those given placebo (Table 1). LV weight/tibial length was significantly higher in sham rats treated with ghrelin than in those given placebo. RV weight/tibial length was significantly lower in CHF rats treated with ghrelin than in those given placebo. The ratios of gastrocnemius muscle weight to tibial length and muscle protein content to tibial length were significantly lower in CHF rats given placebo than in sham rats given placebo, suggesting the presence of cachexia in this model of CHF. Both parameters, however, were significantly higher in CHF rats treated with ghrelin than in those given placebo.

Effects of Ghrelin on Hemodynamics

Heart rate, mean arterial pressure, RV systolic pressure, and mean right atrial pressure tended to be lower in CHF rats treated with ghrelin than those given placebo, although these changes did not reach statistical significance (Table 2). Cardiac output, stroke volume, and LV dP/dt$_{max}$ were signif-

TABLE 1. Characterization of the Animals

	Sham-Placebo	Sham-Ghrelin	CHF-Placebo	CHF-Ghrelin
Number	13	13	15	16
Infarct size, %	...	...	37±6	36±7
Body weight, g				
Baseline	285±12	291±17	241±24*	245±23*
After treatment	315±13‡	338±16†‡	249±33*	270±32*†‡
Tibial length, mm	40.9±1.3	42.6±1.4†	40.3±1.9	42.3±2.0†
LV wt/body wt, g/kg	1.80±0.10	1.94±0.09	2.23±0.15	2.28±0.25
LV wt/tibial length, mg/mm	13.9±1.0	15.4±1.2†	13.7±1.5	14.5±1.6
RV wt/body wt, g/kg	0.54±0.05	0.56±0.06	1.47±0.32*	0.96±0.31*†
RV wt/tibial length, mg/mm	4.2±0.4	4.5±0.6	8.9±1.5*	6.0±1.5*†
Gastrocnemius muscle wt/tibial length, mg/mm	41±1	43±2	28±5*	35±4*†
Gastrocnemius muscle protein/tibial length, mg/mm	6.8±0.2	7.3±0.3	4.5±0.8*	5.7±0.4*†

Sham-Placebo indicates sham rats given placebo; Sham-Ghrelin, sham rats treated with ghrelin; CHF-Placebo, CHF rats given placebo; and CHF-Ghrelin, CHF rats treated with ghrelin. Gastrocnemius muscle wt/tibial length and gastrocnemius muscle protein/tibial length were calculated from the data of each group (n=5). Data are mean±SD.

*$P<0.05$ vs respective sham group; †$P<0.05$ vs respective placebo group; ‡$P<0.05$ vs baseline.

icantly higher in CHF rats treated with ghrelin than in those given placebo (Figure 3), whereas LV end-diastolic pressure and LV dP/dt$_{min}$ were significantly lower in CHF rats given ghrelin (Table 2). Systemic vascular resistance was significantly lower in CHF rats treated with ghrelin than in those given placebo (Figure 3).

Effects of Ghrelin on LV Geometry and Function
Ghrelin significantly increased diastolic thickness of the noninfarcted posterior wall in both CHF and sham rats (Figure 4). LV diastolic dimension tended to decrease in CHF rats treated with ghrelin, although it tended to increase in those given placebo. Thus, LV diastolic dimension was significantly smaller in CHF rats treated with ghrelin than in those given placebo. As a result, LV wall stresses in systole and diastole were significantly lower in CHF rats treated with ghrelin than those given placebo (Table 3). LV fractional shortening increased significantly in CHF rats treated with ghrelin, although it tended to decrease in CHF rats given placebo (Figure 4).

Histological Analysis
Muscle fiber diameter of the noninfarcted myocardium was significantly larger in CHF rats treated with ghrelin than

those given placebo (Figure 5). There was no significant difference in collagen volume fraction between CHF rats treated with ghrelin and those given placebo.

Acute Hemodynamic Response to Ghrelin
A single injection of ghrelin significantly decreased mean arterial pressure in both sham-operated rats (-8 mm Hg, $P<0.05$) and CHF rats (-7 mm Hg, $P<0.05$). The hypotensive effect was also observed in GH-deficient rats (-6 mm Hg, $P<0.05$). Ghrelin significantly decreased systemic vascular resistance in sham, CHF, and GH-deficient rats (-12%, -13%, and -10%, $P<0.05$, respectively). There were no significant changes in heart rate, LV end-diastolic pressure, or LV dP/dt$_{max}$ in each group (data not shown). Placebo injection had no effect on these hemodynamic parameters (data not shown).

Isolated Myocyte Contractile Function
Fractional cell shortening relative to baseline value was not significantly altered by each dose of ghrelin (99±3% for 1 pmol/mL, 97±17% for 10 pmol/mL, and 91±15% for 100 pmol/mL), suggesting that ghrelin has no direct inotropic
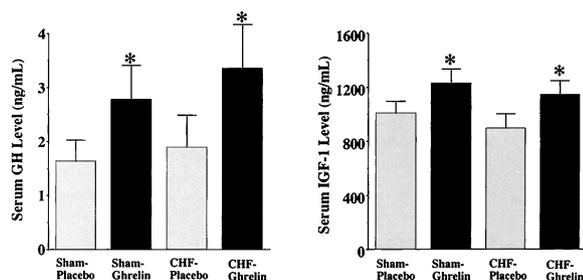

Figure 1. Effects of ghrelin on circulating GH and IGF-1 in CHF and sham rats. Data are mean±SD. *$P<0.05$ vs respective placebo group.
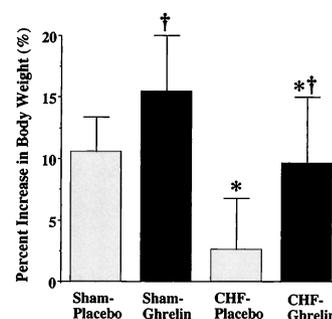

Figure 2. Effects of ghrelin on relative changes in body weight in CHF and sham rats. Data are mean±SD. *$P<0.05$ vs sham group; †$P<0.05$ vs placebo group.

TABLE 2.   Effects of Ghrelin on Hemodynamic Parameters

	Sham-Placebo	Sham-Ghrelin	CHF-Placebo	CHF-Ghrelin
Heart rate, bpm	415±38	397±30	424±30	399±36
Mean arterial pressure, mm Hg	121±8	116±7	105±10*	103±8*
LV systolic pressure, mm Hg	133±9	131±10	111±15*	107±14*
LV end-diastolic pressure, mm Hg	7±1	6±1	16±5*	11±3*†
RV systolic pressure, mm Hg	35±4	36±5	67±12*	57±11*
Right atrial pressure, mm Hg	2±1	2±1	3±2*	2±1
LV dP/dt_{min}, mm Hg/s	−4892±563	−5277±790	−3557±663*	−4527±617*†

Abbreviations as in Table 1. Data are mean±SD.

*$P<0.05$ vs respective sham group; †$P<0.05$ vs respective placebo group.

effects. In contrast, fractional cell shortening in myocytes of CHF rats treated with ghrelin for 3 weeks was significantly greater than that in CHF rats given placebo (19±1% versus 17±1%, $P<0.05$). Shortening velocity in ghrelin-treated CHF rats was significantly increased compared with that in CHF rats given placebo (278±14 versus 213±10 μm/s, $P<0.001$).

Discussion

Ghrelin is a novel GH-releasing peptide, isolated from the stomach, that acts through a mechanism independent of that of hypothalamic GH-releasing hormone.[12] The GH-releasing effects of ghrelin are thought to be mediated by specific receptors, GHS-R, present mainly in the pituitary. Taken together with our results that administration of ghrelin increased serum GH and IGF-1 in both CHF and sham rats, this molecule may reach and act on the anterior pituitary via the blood circulation.

In the present study, chronic treatment with ghrelin tended to decrease mean arterial pressure and significantly decreased systemic vascular resistance in CHF rats. IGF-1 has been shown to cause vasodilation through a stimulatory effect on nitric oxide synthesis,[22] suggesting that the vasodilator effects of ghrelin may be mediated at least in part via IGF-1. Conversely, we recently found that GHS-R exists in blood vessels and that a single injection of ghrelin induces a hypotensive effect without an increase in IGF-1.[13] In the present study, a decrease in systemic vascular resistance by ghrelin injection was observed not only in CHF rats but also in GH-deficient rats. These findings indicate that ghrelin has GH/IGF-1–independent vasodilatory effects.

This study also demonstrated that long-term administration of ghrelin improved cardiac performance in CHF rats, as indicated by increases in cardiac output, stroke volume, LV dP/dt_{max}, and LV fractional shortening and by increases in fractional cell shortening and shortening velocity of isolated myocytes. Although the GHS-R gene has been shown to be abundantly expressed in the myocardium,[13] the present study could not demonstrate direct inotropic effects of ghrelin on isolated cardiac myocytes. Thus, a decrease in systemic vascular resistance by ghrelin may be responsible for the increased cardiac output. Alternatively, because GH upregulates sarcoplasmic Ca^{2+}-ATP and thereby enhances myocardial contractility,[23] part of the cardiac effects of ghrelin may be mediated by GH. In the present study, treatment with ghrelin increased posterior wall thickness, inhibited the progressive LV enlargement, and thereby reduced the LV wall stress in rats with CHF. Histological analysis also demonstrated that ghrelin induced a cardiac hypertrophic response without development of significant fibrosis. GH and IGF-1 have been shown to enhance physiological compensatory hypertrophy in rats after myocardial infarction, resulting in a decrease in LV wall stress leading to improvement in cardiac function.[24] Thus, ghrelin may also improve cardiac function through GH/IGF-1–dependent mechanisms. Taken together, an improvement in cardiac function by ghrelin may be related not only to its effects on the GH/IGF-1 axis and but also to a result of its vasodilatory effects.
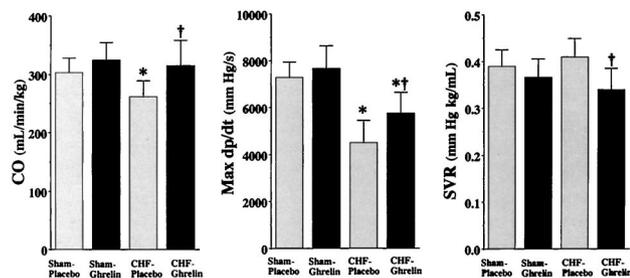
Figure 3. Effects of ghrelin on hemodynamic parameters in CHF and sham rats. CO indicates cardiac output; SVR, systemic vascular resistance. Data are mean±SD. *$P<0.05$ vs sham group; †$P<0.05$ vs placebo group.
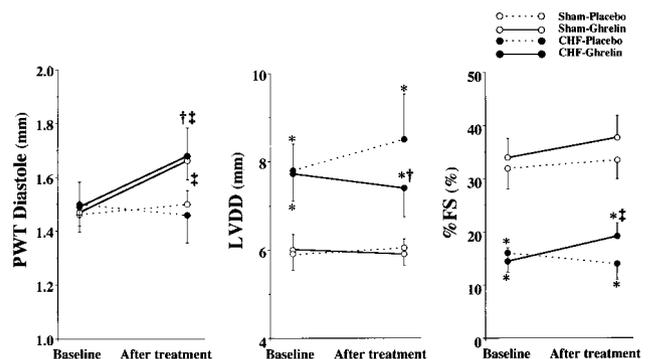
Figure 4. Effects of ghrelin on LV geometry and function determined by echocardiography in CHF and sham rats. PWT indicates posterior wall thickness; LVDD, LV diastolic dimension; and %FS, LV fractional shortening. Data are mean±SD. *$P<0.05$ vs sham group; †$P<0.05$ vs placebo group; ‡$P<0.05$ vs baseline.

TABLE 3.   Echocardiographic Data

	Sham-Placebo	Sham-Ghrelin	CHF-Placebo	CHF-Ghrelin
AWT diastole, mm				
Baseline	1.5±0.1	1.5±0.1	1.1±0.1*	1.1±0.1*
After treatment	1.5±0.1	1.7±0.1‡	1.1±0.1*	1.2±0.1*
AW thickening, %				
Baseline	65±10	64±8	15±12*	12±10*
After treatment	63±9	66±8	12±9*	8±6*
PWT diastole, mm				
Baseline	1.5±0.1	1.5±0.1	1.5±0.1	1.5±0.1
After treatment	1.5±0.1	1.7±0.1†‡	1.5±0.1	1.7±0.1†‡
PW thickening, %				
Baseline	67±10	67±15	33±8*	35±8*
After treatment	65±10	69±8	33±10*	41±9*
LV diastolic wall stress, kdyne/cm^2, after treatment	11±2	10±2	40±17*	23±8*†
LV systolic wall stress, kdyne/cm^2, after treatment	109±12	92±14	209±35*	162±32*†

AWT indicates anterior wall thickness; AW, anterior wall; PWT, posterior wall thickness; and PW, posterior wall. Data are mean±SD.

*$P<0.05$ vs respective sham group; †$P<0.05$ vs respective placebo group; ‡$P<0.05$ vs baseline.

Cardiac cachexia, which is a catabolic state characterized by weight loss and muscle wasting, occurs frequently in patients with end-stage CHF[2,25–27] and is a strong independent risk factor for mortality in patients with CHF.[27] In the present study, CHF rats given placebo showed an impaired increase in body weight and a significant decrease in the muscle/bone ratio, suggesting the presence of cardiac cachexia. In contrast, CHF rats treated with ghrelin revealed an appropriate increase in body weight and a preserved muscle-to-bone ratio similar to those observed in sham-operated rats. The ratios of gastrocnemius muscle weight to body weight and of protein content to body weight were significantly higher in CHF rats treated with ghrelin than in those given placebo (0.53±0.04% versus 0.46±0.07% and 0.09±0.01% versus 0.07±0.01%, both $P<0.05$), and mean right atrial pressure did not increase significantly in those given ghrelin. These data indicate that the increase in body weight by ghrelin was not due to peripheral fluid retention but rather to muscle weight gain. Tschop et al[16] showed 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. In addition, ghrelin has been shown to elicit a potent, long-lasting stimulation of food intake via activation of neuropeptide Y neurones in the hypothalamic arcuate nucleus.[15] Furthermore, ghrelin enhanced production of GH and IGF-1, both of which cause anabolic effects such as maintenance of skeletal muscle mass and myocardial growth.[3,4] These findings suggest that ghrelin may play an important role in the regulation of metabolic balance and in preventing cardiac cachexia through GH-dependent or -independent effects.

Although many animal studies have documented beneficial effects of GH,[5,6,24] controlled studies in humans have been predominantly negative.[8,9] Nevertheless, ghrelin may have additional therapeutic potential because it has GH-independent effects, such as vasodilatory actions and antica-chectic effects.

Conclusions

Chronic subcutaneous administration of ghrelin improved LV dysfunction and attenuated the development of LV remodeling and cardiac cachexia in rats with CHF. Administration of ghrelin may be a new therapeutic approach to the treatment of CHF.
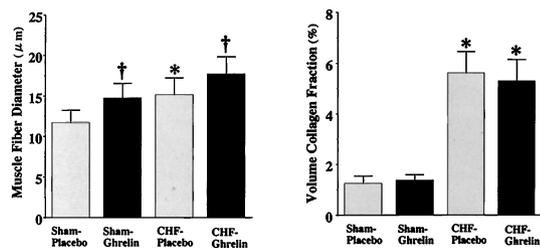
Figure 5. Effects of ghrelin on myocyte growth and collagen density in CHF and sham rats. Data are mean±SD. *$P<0.05$ vs sham group; †$P<0.05$ vs placebo group.

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