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Ghrelin improves body weight loss and skeletal muscle catabolism associated with angiotensin II-induced cachexia in mice

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ABSTRACT

Ghrelin is a gastric peptide that regulates energy homeostasis. Angiotensin II (Ang II) is known to induce body weight loss and skeletal muscle catabolism through the ubiquitin–proteasome pathway. In this study, we investigated the effects of ghrelin on body weight and muscle catabolism in mice treated with Ang II. The continuous subcutaneous administration of Ang II to mice for 6 days resulted in cardiac hypertrophy and significant decreases in body weight gain, food intake, food efficiency, lean mass, and fat mass. In the gastrocnemius muscles of Ang II-treated mice, the levels of insulin-like growth factor 1 (IGF-1) were decreased, and the levels of mRNA expression of catabolic factors were increased. Although the repeated subcutaneous injections of ghrelin (1.0 mg/kg, twice daily for 5 days) did not affect cardiac hypertrophy, they resulted in significant body weight gains and improved food efficiencies and tended to increase both lean and fat mass in Ang II-treated mice. Ghrelin also ameliorated the decreased IGF-1 levels and the increased mRNA expression levels of catabolic factors in the skeletal muscle. IGF-1 mRNA levels in the skeletal muscle significantly decreased 24 h after Ang II infusion, and this was reversed by two subcutaneous injections of ghrelin. In C2C12-derived myocytes, the dexamethasone-induced mRNA expression of atrogin-1 was decreased by IGF-1 but not by ghrelin. In conclusion, we demonstrated that ghrelin improved body weight loss and skeletal muscle catabolism in mice treated with Ang II, possibly through the early restoration of IGF-1 mRNA in the skeletal muscle and the amelioration of nutritional status.

1. Introduction

Chronic heart failure (CHF), which often includes body weight loss and skeletal muscle catabolism, is commonly termed cardiac cachexia and is a predictor of poor outcome [1]. Cachexia is a complex metabolic syndrome that is associated with an underlying illness and that is characterized by a loss of skeletal muscle with or without a loss of fat mass [2]. Several therapies for cachexia are being explored, all of which slightly increase body weight with little impact on muscle function [1].

The mechanisms of cardiac cachexia are poorly understood, but there is recent evidence that angiotensin II (Ang II) plays an important role. The Ang II plasma levels in patients with CHF associated with cachexia were higher than in those without cachexia [3]. Ang II infusions to rats have been shown to induce marked reductions in body weight that were accompanied by decreased levels of circulating and skeletal muscle insulin-like growth factor (IGF-1) [4]. In addition, Ang II infusion to transgenic mice with muscle-specific expression of IGF-1 did not result in decreased body weight or muscle mass [5]. These findings suggested that a down-regulation of IGF-1 signaling in the skeletal muscle could mediate the muscle catabolism that is associated with Ang II. Recent studies using in vitro models of muscle atrophy have indicated that IGF-1 acts through Akt and FoxO to suppress atrogin-1 and muscle RING finger protein 1 (MuRF1) transcription [6]. Atrogin-1 and MuRF1 are ubiquitin ligases, the expressions of which are increased in various muscle atrophy models [7]. Ang II induced an upregulation of atrogin-1 and MuRF1, which was followed by skeletal muscle catabolism [8]. Moreover, Ang II infusion increased circulating IL-6, suggesting that Ang II-induced inflammation contributed to muscle wasting [9]. It has also been reported that Ang II is involved in the wasting conditions that are associated with cancer, chronic renal failure, and sarcopenia [10–12], suggesting that Ang II plays a common role in the skeletal muscle catabolism that is associated with various diseases and aging.

Abbreviations: Ang II, angiotensin II; IGF-1, insulin-like growth factor 1; CHF, chronic heart failure; MuRF1, muscle RING finger protein 1; GHS-R, growth hormone secretagogue receptor; GH, growth hormone; sc, subcutaneous; ip, intraperitoneal; RT-PCR, real-time polymerase chain reactions; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; DEX, dexamethasone; SE, standard errors.

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Table 1
Primers and probes for real-time PCR.

Gene		Sequence
Atrogin-1	Forward primer	AGCGACCTCAGCAGTTACTGC
	Reverse primer	CTTCTGGAATCCAGGATGGC
MuRF1	Forward primer	TGTCTGGAGGTCGTTTCCG
	Reverse primer	GTGCCGGTCCATGATCACTT
FoxO1	Forward primer	CTCGAACCAGCTCAAATGCTAGTAC
	Reverse primer	GTGGATACACCAGGGAATGCA
FoxO3a	Forward primer	TCGTCTCTGAACTCCTTGCGT
	Reverse primer	TGGAGTGTCTGGTTGCCGT
IGF-1	Forward primer	AGTGTTGCTTCCGGAGCTGT
	Reverse primer	GGCTGCTTTTGTAGGCTTCAGT
	TaqMan probe	ATCTGAGGAGACTGGAGATGTACTGTGCCC
GAPDH	Forward primer	TGCACCACCAACTGCTTAG
	Reverse primer	GGATGCAGGGATGATGTTC
	Taqman probe	CAGAAGACTGTGGATGGCCCCTC

Ghrelin was identified as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R) in the stomach [13]. While ghrelin has a potent orexigenic effect that is independent of growth hormone (GH) secretion [14], ghrelin has also been shown to have a number of pleiotropic effects on energy metabolism, such as a reduction of fat utilization, the stimulation of adiposity, and the inhibition of sympathetic nerve activity and inflammatory cytokines [15–17]. These observations suggest that ghrelin might improve cachectic conditions. Anabolic effects of ghrelin have been expected based on the activation of the GH–IGF-1 axis. Indeed, recent reports have suggested that ghrelin was able to improve lean body mass in tumor-bearing rats [18] and in a chronic kidney disease model of 5/6 nephrectomy in rats [19] and inhibited muscle protein breakdown in rats with thermal injury [20]. However, it has not been examined how and when ghrelin acts on

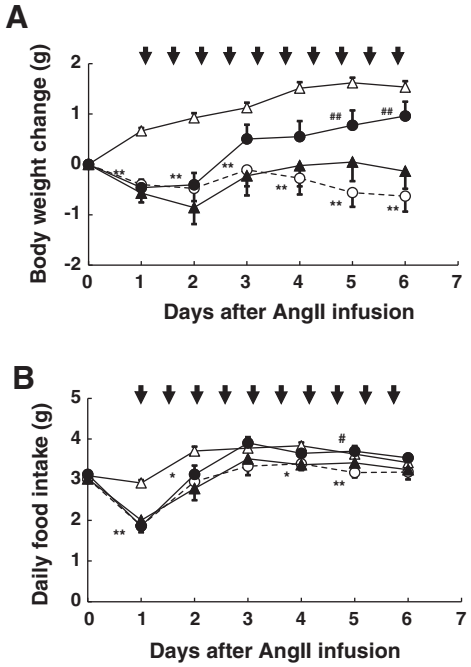

Fig. 1. Effects of ghrelin on body weight gain (A) and daily food intake (B) in mice treated with angiotensin II (Ang II). Ang II (1 μg/kg/min) was administered to mice for 6 days by subcutaneous infusion. Day 0 indicates the first day of Ang II infusion. Ghrelin (0.1 or 1.0 mg/kg) was subcutaneously administered twice daily for 5 days from Day 1 to Day 5. The arrows indicate ghrelin administration. Open triangles: saline + vehicle (control group); open circles: Ang II + vehicle (vehicle group); solid triangles: Ang II + 0.1 mg/kg ghrelin (0.1 mg/kg group); solid circles; Ang II + 1.0 mg/kg ghrelin (1.0 mg/kg group). Data represent the means ± standard error (SE) of 16 mice. $^{*}P<0.05$, $^{**}P<0.01$ vs the control group by *t*-test. #$P<0.05$, ##$P<0.01$ vs the vehicle group by Dunnett's test.

Table 2
Effect of ghrelin on body weight gain, cumulative food intake and food efficiency in mice treated with angiotensin II.

	Control	Vehicle	0.1 mg/kg ghrelin	1.0 mg/kg ghrelin
Body weight gain (g)	0.87 ± 0.12	−0.23 ± 0.29**	0.44 ± 0.22	1.42 ± 0.25$^{\#\#}$
Total food intake (g)	21.3 ± 1.0	17.9 ± 3.6	18.3 ± 3.3	19.8 ± 2.6
Food efficiency	0.040 ± 0.005	−0.034 ± 0.035**	0.015 ± 0.016	0.068 ± 0.012$^{\#\#}$

Body weight gain and total food intake were evaluated during ghrelin or vehicle treatment period (Day 1 to Day 6). Food efficiency was calculated by dividing body weight gain with total food intake. Values represent the means ± SE of 16 mice. $^{**}P<0.01$ vs the control group by *t*-test. $^{\#\#}P<0.01$ vs the vehicle group by Dunnett's test.

Ang II-induced muscle catabolism and the expression of anabolic and catabolic factors in the skeletal muscle.

In the present study, we investigated the effects of ghrelin on the body weight and body composition of mice with Ang II-induced cachexia. In addition, we examined the effects of ghrelin on the IGF-1 contents in serum and skeletal muscle and the mRNA expression levels of IGF-1 and catabolic factors such as atrogin-1 and MuRF1 in skeletal muscle. We have further tested whether ghrelin suppresses the mRNA expression levels of atrogin-1 in the skeletal muscle with cultured C2C12 cells, which are myoblasts that have been established from a mouse leg muscle and that are known to differentiate to myocytes [21,22].

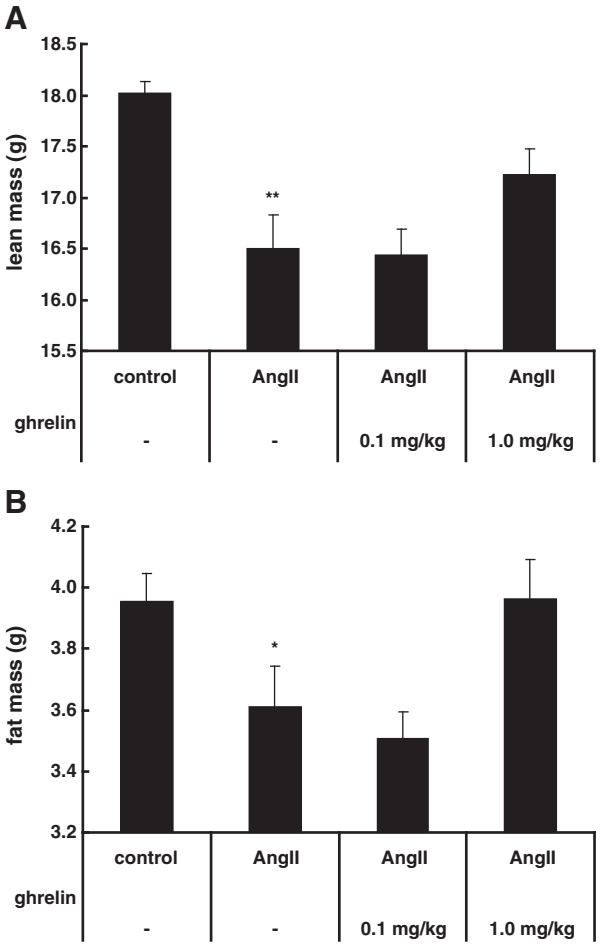

Fig. 2. Effects of ghrelin on body composition in mice treated with Ang II on Day 6. (A) Lean mass. (B) Fat mass. Data represent the means ± SE of 16 mice. $^{*}P<0.05$, $^{**}P<0.01$ vs the control group by *t*-test.