

Long-term effects of ghrelin and ghrelin receptor agonists on energy balance in rats

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Strassburg S, Anker SD, Castaneda TR, Burget L, Perez-Tilve D, Pfluger PT, Nogueiras R, Halem H, Dong JZ, Culler MD, Datta R, Tschöp MH. Long-term effects of ghrelin and ghrelin receptor agonists on energy balance in rats. *Am J Physiol Endocrinol Metab* 295: E78–E84, 2008. First published May 6, 2008; doi:10.1152/ajpendo.00040.2008.—Ghrelin, an endogenous ligand of the growth hormone secretagogue receptor (GHS-R), is the only circulating agent to powerfully promote a positive energy balance. Such action is mediated predominantly by central nervous system pathways controlling food intake, energy expenditure, and nutrient partitioning. The ghrelin pathway may therefore offer therapeutic potential for the treatment of catabolic states. However, the potency of the endogenous hormone ghrelin is limited due to a short half-life and the fragility of its bioactivity ensuring acylation at serine 3. Therefore, we tested the metabolic effects of two recently generated GHS-R agonists, BIM-28125 and BIM-28131, compared with ghrelin. All agents were administered continuously for 1 mo in doses of 50 and 500 nmol·kg⁻¹·day⁻¹ using implanted subcutaneous minipumps in rats. High-dose treatment with single agonists or ghrelin increased body weight gain by promoting fat mass, whereas BIM-28131 was the only one also increasing lean mass significantly. Food intake increased during treatment with BIM-28131 or ghrelin, whereas no effects on energy expenditure were detected. With the lower dose, only BIM-28131 had a significant effect on body weight. This also held true when the compound was administered by subcutaneous injection three times/day. No symptoms or signs of undesired effects were observed in any of the studies or treated groups. These results characterize BIM-28131 as a promising GHS-R agonist with an attractive action profile for the treatment of catabolic disease states such as cachexia.

body weight; food intake; cachexia

CATABOLIC STATES SUCH AS CACHEXIA occur in advanced stages of most chronic diseases, including AIDS, cancer, cystic fibrosis, chronic obstructive lung disease, and chronic heart or kidney failure, as well as in Alzheimer's disease and frail elderly patients in general, worsening the prognosis of these individuals (2, 24, 28, 30, 39, 43, 56). Catabolism is characterized by a negative energy balance and results in weight loss, leading to a state of pathologically low levels of lean and fat mass called cachexia. There are only few available therapeutic options, and those are of limited potency. Since they mostly aim to improve the underlying disease, they are targeting only particular forms

of cachexia. The pathways targeted by the stomach gastric peptide ghrelin, which is also known as the "hunger hormone," may offer a powerful general treatment option for patients suffering from cachexia independent of its cause.

In 1999, ghrelin was found to be an endogenous ligand for the growth hormone secretagogue receptor (GHS-R), which had been cloned three years earlier (20, 22). Ghrelin is a 28-residue peptide that is octanoylated in its active form and secreted mainly by gastric endocrine cells (19, 23, 55). As a ligand of the GHS-R, ghrelin is a modulator of growth hormone secretion. The GHS-R1a is the specific isoform mediating ghrelin's actions. GHS-R1a activates the phospholipase C-signaling pathway, leading to increased inositol phosphate turnover and protein kinase C activation, followed by the release of Ca²⁺ from intracellular stores (55). Interestingly, ghrelin's effects on energy metabolism and body weight have been shown to be independent from its effect on growth hormone secretion (42, 60). Ghrelin stimulates food intake and induces adiposity (34, 35, 53, 59). Due to meal-associated peaks in ghrelin secretion, it has also been proposed to represent a meal initiation (33) or meal preparation (57) signal. Ghrelin's anabolic effects are mediated via a central mechanism involving, among other players, neuropeptide Y (NPY) and agouti-related peptide (AgRP), two appetite-stimulatory peptides in the hypothalamus (8, 18, 21, 41, 61). Hypothalamic neurons producing these two neuropeptides also express the GHS-R (19, 55). Despite controversial reports based on models (3, 10), afferent signaling of the vagus nerve does not seem to play an important role for metabolic actions of ghrelin, as shown in humans (49). Although there are numerous peptides affecting appetite or eating behavior in rodents, ghrelin is the first and only gut peptide with orexigenic properties in humans (7, 38). Therefore, the ghrelin system is uniquely positioned as a drug target for the treatment of anorexia and cachexia, especially if novel optimized ghrelin receptor agonists can be engineered.

Therefore, we tested the hypothesis that ghrelin receptor agonists BIM-28125 and BIM-28131 were equally or more potent than ghrelin in generating a positive energy balance and increasing body weight.

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MATERIALS AND METHODS

Animals. Three sets of experiments were performed in male Sprague-Dawley rats weighting 316 ± 2 (osmotic minipumps at 50 $\text{nmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$), 297 ± 2 (osmotic minipumps at 500 $\text{nmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$), and 305 ± 1 g (injections vs. miniosmotic pumps at 50 $\text{nmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) at the beginning of the experiments. Animals were housed individually at the Laboratory Animal Facility of the Genome Research Institute, University of Cincinnati. Rats had ad libitum access to pelleted chow and water at all times during the study. They were maintained on a 12:12-h light-dark cycle, with lights on at 0500 (0700 in the injection vs. pump study). At the end of the experiments, rats were killed by decapitation and blood plasma samples were taken. Plasma was stored at -80°C until assayed. All procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Compounds. BIM-28131, BIM-28125, and human ghrelin were provided by IPSEN (Milford, MA) (11, 12). BIM-28131 and BIM-28125 are the peptide analogs that bind selectively to ghrelin receptor (GHS-1a) with a greater affinity [inhibitory constant (K_i): 0.42 ± 0.063 and 0.48 ± 0.16 nM, respectively] than natural ghrelin (K_i : 1.12 ± 0.17 nM). BIM-28131 and BIM-28125 are also more potent (EC_{50} : 0.71 ± 0.09 and 0.98 ± 0.08 nM, respectively) in activating GHS-1a receptor than native ghrelin (EC_{50} : 4.2 ± 1.2 nM), as assessed in vitro by calcium mobilization. BIM-28131 and BIM-28125 have greater enzymatic stability in plasma than native ghrelin ($t_{1/2}$ rat plasma was 24 vs. 1.9 h for ghrelin) and, when injected intravenously, are observed to have a 10-fold greater circulating half-life compared with native ghrelin.

Pump implantation. The compounds BIM-28131, BIM-28125, and ghrelin were administered at two different doses: 50 and 500 $\text{nmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. Those compounds were dissolved in the vehicle solution, which contained 0.9% sodium chloride, 2% heat-inactivated rat serum, and 5% Tween 80. Osmotic minipumps (Alzet, Cupertino, CA) were filled in the evening before surgery and primed in a water bath overnight at 37°C . For the surgery, rats were anesthetized with ketamine-xylazine. Buprenorphine was administered for analgesia. The rats were randomized and the minipumps implanted subcutaneously in the upper back, pumping at a rate of 0.5 $\mu\text{l/h}$ for 14 days. Pumps were removed and immediately replaced on day 15.

Body composition. Body composition was measured with an NMR instrument (Echo Medical Systems, Houston, TX) specifically developed and established for the measurement of body composition in rats. Measurements of body composition were performed before the pump implantation and once weekly in the following weeks. In study 3, measurements were performed at the beginning and at the end of the study.

Energy expenditure, locomotor activity, and food intake. Food and water intake, O_2 uptake, and CO_2 production, as well as locomotor activity, were measured continuously in a 32-cage combined indirect calorimeter (Technical and Scientific Equipment, Bad Homburg, Germany). Animals were adapted to this environment before the actual measuring periods of 24 h each. The respiratory quotient and energy expenditure were calculated from the O_2 uptake and CO_2 production and put in analyzed relative to individual body weights. Measurements with this system were performed before the first pump implantation, 2 days after pump implantation for evaluation of acute effects, and after 28 days of treatment to investigate chronic effects.

Comparison of injections vs. pumps. In a separate study, low-dose BIM-28131 vs. vehicle was given in pumps vs. three times/day injections over a period of 14 days. The four groups were assigned randomly, and implantation of pumps was done as described above, but no exchange of the pumps was necessary. The compound was dissolved in the vehicle solution as described above, and the injection solution was diluted in such a way as to achieve the same final dose per animal per day. From the master injection solution, aliquots for each single injection were stored at -80°C and thawed immediately

before each injection. Subcutaneous injections were given at 2, 6, and 10 h past lights on. Body weight and food intake were measured daily.

Plasma ghrelin measurements. Ghrelin measurements were performed with an RIA Kit (Phoenix Pharmaceuticals) according to the manufacturer's instructions. The sensitivity of this kit was 4.26 pg/tube, and intra-assay coefficients of variation were $<10\%$.

Statistics. All values are presented as means $\pm$ SE. Comparisons between two groups were made by Student's *t*-test and among multiple groups by ANOVA followed by Fisher's post hoc test; in followup, ANOVA for repeated measures was performed. The *P* values in the figures are Fisher's post hoc values if ANOVA was significant ($P < 0.05$). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Body weight. Both BIM-28125 and BIM-28131, as well as ghrelin, increased body weight gain significantly compared with controls when given in the higher dose (Fig. 1, A–C). However, the overall change in body weight in rats treated with the ghrelin agonist BIM-28131 was significantly higher than that of the ghrelin-treated ones ($P = 0.04$). In the low-dose treatments, only the BIM-28131-treated group had significantly greater weight gain than the control rats. After both the first and second surgeries, a small weight loss or steady weight was observed in all animals, and this weight was reached again after a maximum of 4–5 days following each surgery as expected (data not shown). No edema or other sign for adverse effects was visible in any of the rats.

Body composition. Analysis of the rats' body composition showed that the greater weight gain induced by BIM-28131 was based on both increased gain in fat mass and increased gain in lean mass (Fig. 1, D–F). In the high-dose treatment, the rats treated with either of the ghrelin agonists BIM-28125 and BIM-28131, as well as the rats treated with ghrelin, gained lean mass compared with the controls (42, 49, 46, and 29%, respectively, $P < 0.001$). However, the major weight gain in the high-dose groups was due to an increase in fat mass (BIM-28125, 234%; BIM-28131, 298%; ghrelin, 248%; and vehicle, 52%). Fat mass increase was also significantly higher vs. controls in the low-dose BIM-28131-treated group (BIM-28131, 70% vs. vehicle, 52%, $P = 0.04$), but that effect was not significant in any of the other groups (BIM-28125, 36%; ghrelin, 42%). Lean mass did not change significantly in the low-dose treatment.

Energy balance parameters. Measurements of all energy balance parameters prior to the beginning of the study showed no differences between the groups. High-dose BIM-28131-treated rats had higher food intake than the controls acutely and chronically ($P < 0.001$; Table 1 and Fig. 2). Also, high-dose ghrelin-treated rats had higher food intake after 4 wk of treatment ($P = 0.03$). In both the acute and the chronic measurements, there were no significant effects on water intake, respiratory quotient, and energy expenditure between the groups. Neither treatment with BIM-28131 nor BIM-28125 resulted in any change of locomotor activity.

Injections. Animals treated with BIM-28131 showed a significantly greater increase in body weight than the controls (62 ± 4 g injection BIM-28131 vs. vehicle, 41 ± 2 g pump BIM-28131 vs. vehicle, both $P < 0.001$). There was no difference with regard to means of administration, that is, by three times/day injections or constant release by pumps (Fig. 3). Body composition analysis showed an increase in lean (both

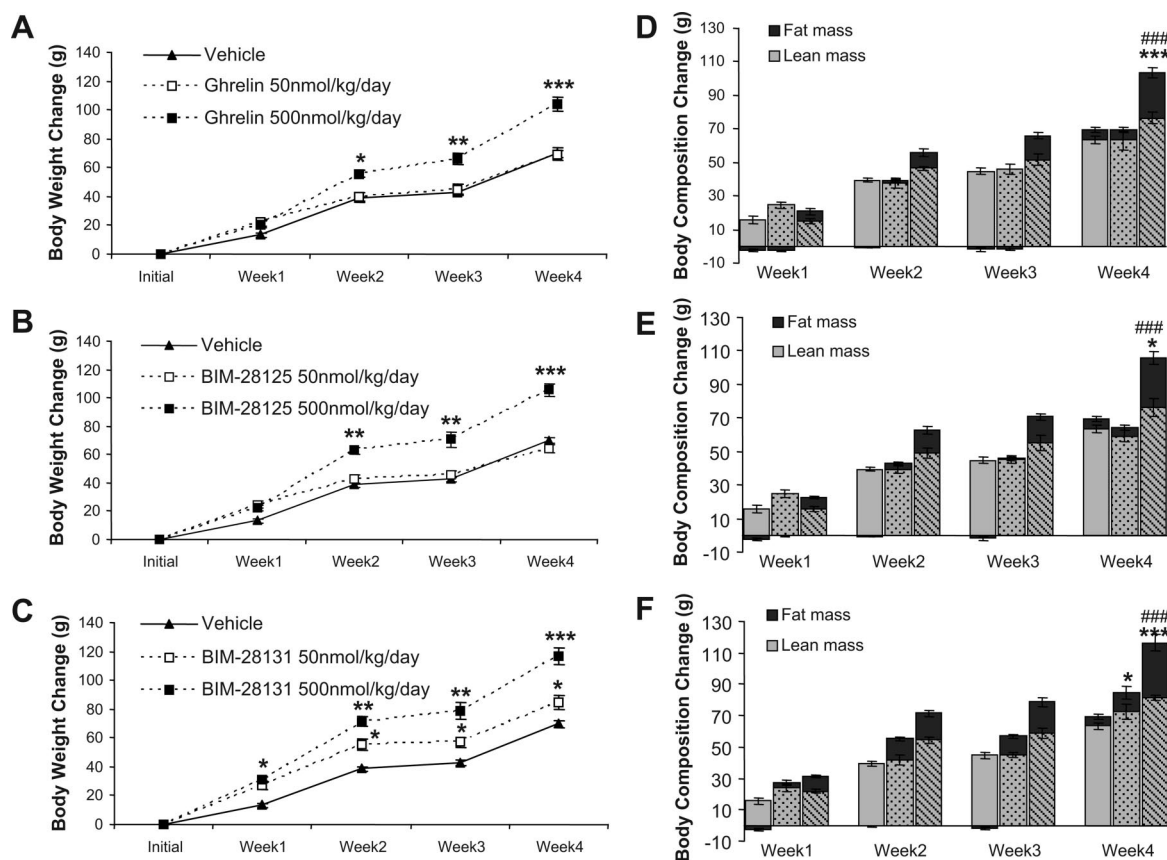


Fig. 1. Effects of chronic administration of BIM-28125, BIM-28131, and ghrelin vs. vehicle on body weight and body composition. Compounds were administered subcutaneously in 2 different dosages (low dose $50 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ and high dose $500 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) with osmotic minipumps. Vehicle $n = 16$, ghrelin low and high dose $n = 8$; BIM-28125 low dose $n = 8$, high dose $n = 5$; BIM-28131 low dose $n = 8$, high dose $n = 7$. A and D: ghrelin in low dose and high dose vs. vehicle. B and E: growth hormone receptor secretagogue receptor (GHS-R) agonist BIM-28125 in low dose and high dose vs. vehicle. C and F: GHS-R agonist BIM-28131 in low and high dose. A–C: body weight. $***P < 0.001$; $**P < 0.01$ vs. vehicle. D–F: fat mass. Solid gray bars show vehicle group, dotted gray bars show low-dose-treated group, and hatched gray bars show high-dose-treated group. $***P < 0.001$; $*P < 0.05$ vs. vehicle, lean mass; $###P < 0.001$ vs. vehicle. The P values in the figures are Fisher's post hoc values if ANOVA was significant ($P < 0.05$).

$P < 0.01$) and fat mass (both $P < 0.001$), with the majority in fat mass increase in both the pump and injection BIM-28131 groups. The 14-day cumulative food intake was increased compared with controls in both the pump and injection BIM-28131 groups (both $P < 0.01$).

Plasma ghrelin levels. Total ghrelin levels increased in the groups treated with low- and high-dose ghrelin and in rats receiving treatment with low- and high-dose BIM-28125 but not in rats treated with BIM-28131 (Fig. 4A). BIM-28125 does not have specific binding; however, it shows nonspecific displacement of the tracer, which does not depend on its concentration (Fig. 4B).

DISCUSSION

In rodents and humans, the role of ghrelin has been studied in catabolic states such as anorexia nervosa, cardiac cachexia, and cancer cachexia models, and increased ghrelin levels were found in cachexic states (15, 17, 32, 36, 44). Ghrelin has also been found to be increased in chronic liver disease and liver cirrhosis patients (48). In contrast, ghrelin was not found to be increased in patients with hepatocellular carcinoma, but the respective study did not distinguish between cachexic and noncachexic patients (48). Interestingly, in patients with hepatocellular carcinoma, an inverse correlation between the tumor

marker α -fetoprotein and ghrelin levels was found and proposed to possibly be involved in the mechanisms leading to loss of appetite, one of the hallmarks and typical symptoms in cancer patients along with fever and weight loss.

First results from rodent studies testing ghrelin as an anti-cachexia agent were promising. For example, in a cardiac cachexia animal model, chronic subcutaneous administration of ghrelin improved heart function and attenuated the development of cardiac cachexia (31). Although blood ghrelin levels increased with the progression of cachexia in a mouse cancer cachexia model with melanoma cells, intraperitoneal treatment with $6 \text{ nmol} \cdot \text{mouse}^{-1} \cdot \text{day}^{-1}$ ghrelin suppressed weight loss and increased food intake in this model (17). Preliminary results also demonstrated that treatment with ghrelin can increase food intake in anorectic rats with prostate cancer, although the effect was only modest (58). In addition to ghrelin, a ghrelin receptor agonist, BIM-28131, has also been tested in a rat model of cancer cachexia (12), demonstrating improved lean body mass retention and increases in gene expression of agouti-related peptide and NPY, two orexigenic neuropeptides expressed in the arcuate nucleus of the hypothalamus. However, in that study the compounds were administered for 5 days, and there are no data about long-term studies.

Table 1. *Energy balance parameters*

	Veh HD (n = 8)	Ghr HD (n = 7)	C25 HD (n = 5)	C31 HD (n = 8)
Acute effects (24 h)				
Food intake, g/day	25±1	26±1	25±1	27±1
Water intake, g/day	29±1	30±1	28±1	31±1
Respiratory quotient	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1
Energy expenditure, kcal·kg ⁻¹ ·h ⁻¹	4.8±1.6	5.0±1.3	6.7±1.0	7.3±1.0
Ambulatory, BB/min	194±26	175±27	194±8	150±25
Fine, BB/min	111±11	103±12	114±7	100±8
Rearing, BB/min	69±8	76±9	43±7	78±21
Chronic effects (2 wk)				
Food intake, g/day	26±1	29±1*	28±1	31±1***
Water intake, g/day	30±1	33±1	30±1	34±2
Respiratory quotient	1.0±0.03	1.1±0.04	1.1±0.04	1.1±0.04
Energy expenditure, kcal·kg ⁻¹ ·h ⁻¹	6.8±0.1	6.3±0.2	6.3±0.2	6.4±0.2

Values are means ± SE. Veh, vehicle; HD, high dose; Ghr, ghrelin; C25, BIM-28125; C31, BIM-28131; BB, beam brakes. Effects of HD Ghr, C25, and C31 on food and water intake, respiratory quotient, energy expenditure, and activity. ****P* < 0.001 vs. vehicle; **P* < 0.05 vs. vehicle.

Our chronic study confirms that ghrelin and ghrelin receptor agonists carry substantial potential for the development of new treatment options for cachexia. This is the first time that a dose-dependent effect of ghrelin on body weight has been studied over a treatment period longer than 14 days in rodents. The powerful effects on body weight and body composition observed with the higher dose of ghrelin during that time are unique and have not been reported for any other hormone or circulating factor. We also report here that continuous administration of the new ghrelin receptor agonists BIM-28125 and BIM-28131 over 4 wk leads to substantially elevated body weight gain compared with controls. BIM-28131 was clearly superior regarding its potency to increase body weight gain when compared with ghrelin as the positive control. Indeed, we are not aware of any other pharmaceutical agent that specifically increases body weight that powerfully. This conclusion is particularly well supported by the fact that, with the 10-fold lower dose, body weight gain was increased in the BIM-28131 group but not in the ghrelin or BIM-28125 group compared with vehicle-infused controls. However, the effect of BIM-28131 at the lower dose was significantly smaller than the effect observed with the higher dose, suggesting a nonartificial,

dose-dependent effect mediated by the endogenous ghrelin receptor rather than an unspecific phenomenon. The increase in body weight seen in the rats receiving the novel synthetic compounds appeared to be associated with increased food intake. However, it might be possible that the increase in food intake (in 15 days: saline vs. BIM-28131, 390 vs. 465 g, respectively) cannot sufficiently explain the marked weight gain observed in these studies (in 15 days: saline vs. BIM-28131, 348 vs. 370 g, respectively). In this sense, we have observed previously that a combination of increased food intake, decreased energy expenditure (relevant differences could be below the detection limit of indirect calorimetry), and a switch in nutrient partitioning toward lipid storage (50) could be the basis for the powerful generation of a positive energy balance. This fact was demonstrated by the observation that ghrelin infusion stimulated lipogenesis and inhibited lipid oxidation, and those effects occurred independently from ghrelin-induced hyperphagia (50).

We have previously shown in vitro that BIM-28131 is binding the ghrelin receptor with a higher affinity than ghrelin and is more potent in activating it (40). Although it can be assumed that the orexigenic effects of the tested ghrelin agonists may, similar to ghrelin, be mediated via stimulation of hypothalamic NPY/AgRP neurons and midbrain reward circuitry (1), their exact mechanism of action has yet to be proven. Similarly, effects on peripheral metabolism and lipid storage may be mediated via the sympathetic nervous system as shown previously for ghrelin (50).

The discovery of the endogenous ghrelin system is a unique story, since it started with the discovery of synthetic peptides and nonpeptide ligands that were created and evaluated years before it became clear that they would activate the GHS-R and long before the natural ligand, ghrelin, was found (37, 45). Until today, most studies investigating the functions of the ghrelin system have been focusing on its orexigenic effects and have described increased food intake and body weight independent of effects on GH release (16). GHS-R-induced elevated food intake has also been shown to be independent from the leptin system, as demonstrated in lean and obese Zucker rats (5). GHS-R-mediated effects on energy balance appear to be relevant and GH independent in humans, since, for example, seven of 10 children with GH deficiency treated with the GHS-R agonist peptide growth hormone-releasing peptide-2 at

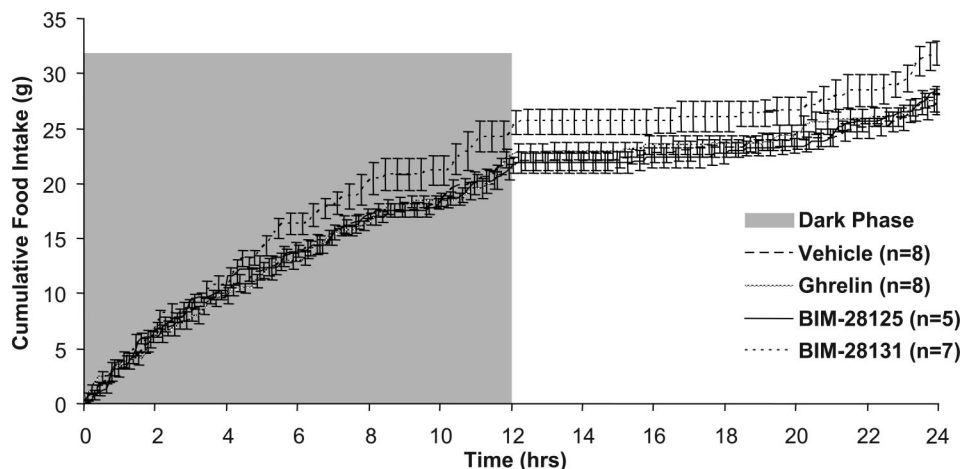


Fig. 2. Cumulative food intake from continuous measurement in special cages over 24 h. BIM-28131-treated rats (high dose) have higher food intake compared with controls. Only every 4th SE is shown.

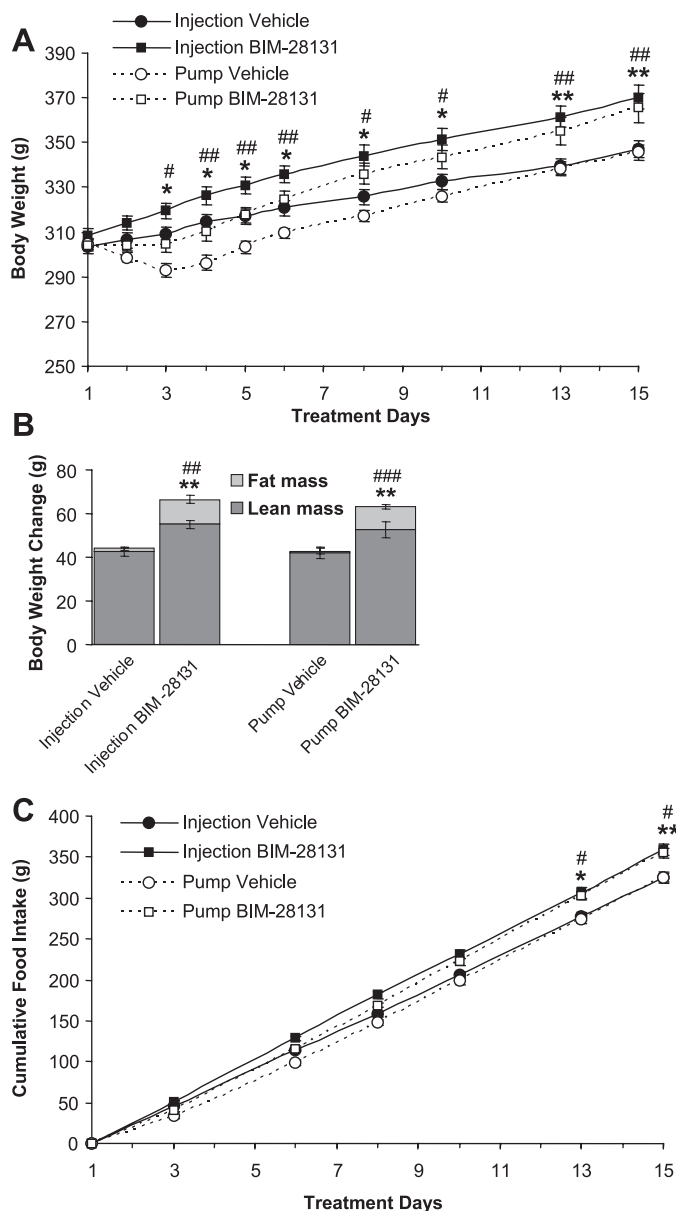


Fig. 3. Body weight (A), body composition (B), and food intake (C) after 2 wk of low-dose treatment ($50 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) with BIM-28131 or vehicle. Comparison of different subcutaneous administration forms: continuous by minipump or 3 times/day injections ($n = 8$ for each group). ** $P < 0.01$; * $P < 0.05$ vs. vehicle; ### $P < 0.001$; ## $P < 0.01$ vs. vehicle. The P values in the figures are Fisher's post hoc values if ANOVA was significant ($P < 0.05$).

a dose of $900 \mu\text{g/kg}$ bid for 1 yr reported an increase in appetite during the first 6 mo (29). The same compound increased food intake by $35.9 \pm 10\%$ in healthy lean males (25). Ipamorelin, another ghrelin receptor agonist, increased body weight in GH knockout mice (26), showing yet again that the main effects of GHS-R activation are independent from the growth hormone axis. However, there are almost no data on longer-term treatments or a more detailed metabolic profiling of such observations, including body composition measurements. The GHS-R agonist LY-444711 (27) represents an exception, since it has been analyzed for energy balance parameters other than food intake and body weight. As seen in rodent studies with other

ligands (26, 52–54), weight gain after treatment with LY-444711 was due to increased fat mass with a decrease or no change in lean mass. In contrast and as an exception, the GHS-R agonist MK-677 increased lean mass, whereas there was no change in fat mass during a 2-mo treatment period in humans (47). However, this study was performed in obese individuals. This may matter, since one hypothesis that is currently being investigated suggests that GHS-R activation may lead to different effects on fat mass and lean mass depending on the body composition status at the start of the treatment period. In cachexia, GHS-R activation may increase both lean and fat mass to reinstall a physiological body composition and body weight. In obese individuals, the GHS-R-induced positive energy balance may lead predominantly to excess fat storage.

In our studies presented here, although most of the weight gain induced by the tested compounds was due to an increase in fat mass, we observed a significant increase in lean mass in animals receiving the higher dose treatment. Since the compound BIM-28131 showed a promising, significant effect at the lower dose as well, we performed another experiment with this

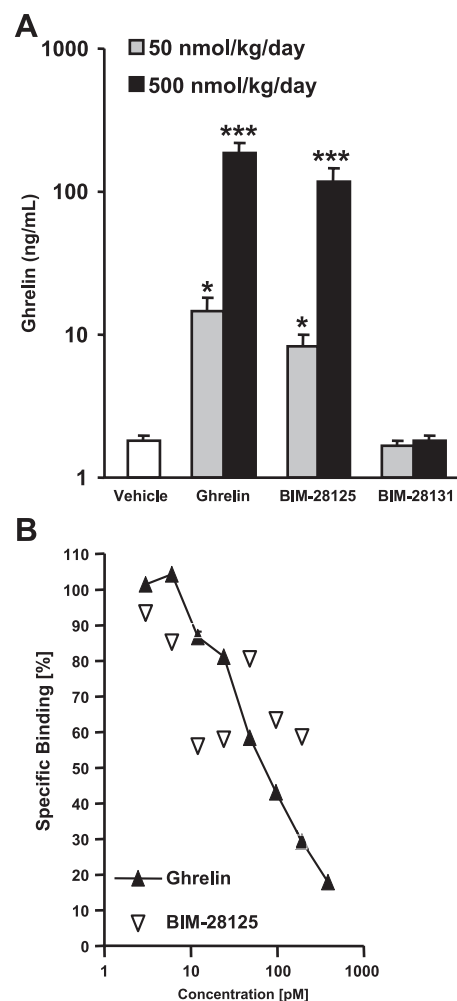


Fig. 4. A: ghrelin levels in blood plasma with low-dose ($50 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) and high-dose ($500 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) subcutaneous minipump treatment with the GHS-R agonists BIM-28125 and BIM-28131, ghrelin, or vehicle. *** $P < 0.001$; * $P < 0.05$ vs. vehicle. B: displacement of the tracer by ghrelin and BIM-28125 at different concentrations.

single compound at the lower dose to repeat that result and test whether chronic infusion was advantageous regarding pharmacodynamics and efficacy. In a low-dose injection vs. pump comparison, we found an increase in both fat and lean mass compared with the controls. These findings principally repeated the respective result from the first set of studies but were even more encouraging due to the significant increase in lean mass even with this lower dose. One explanation for this fact could be the higher statistical power in the second experiment, in which fewer groups were involved. Also, the study was shorter and involved only one pump implantation surgery, and weight loss after the second pump surgery may have reduced the overall weight gain in the first experiments. Animals lost weight after each surgery due to stress and possibly in part also as a result of the diuretic effect of xylazine (6). Comparing repeated injection vs. pump infusion did not reveal any difference between the two forms of administration. Ghrelin itself has a half-life of ~10–30 min (4, 51). Its release is pulsatile in humans and rats and peaks before meals in humans (9). Therefore, a continuous pattern of administration rather than repeated generation of blood concentration peaks could have been less effective. This was not the case, and we made sure that the pattern of food intake over light and dark phases was not changed by automated and continuous measurement of feeding over 24 h (Fig. 2). It is also important to note that BIM-28125 significantly increased plasma ghrelin levels. This effect is explained by the nonspecific displacement of the tracer that does not depend on its concentration, suggesting that BIM-28125 interferes with a meaningful ghrelin RIA measurement.

In progressive cancer cachexia, it has been shown recently that body fat is lost more rapidly than lean mass and that fat mass loss predicts survival (14). Nevertheless, loss of lean mass remains significant, and regaining lean mass is more difficult. Therefore, novel compounds such as BIM-28131 may open up unprecedented treatment options. However, further research is needed. Just to name one example for a possible concern, and in analogy to the very well known phenomenon of leptin resistance in obesity, ghrelin resistance in cachexia could make the development of a ghrelin-based cachexia treatment more difficult if not impossible. Finally, even if positive results for ghrelin and its analogs regarding cachexia treatment would continue to emerge (46), their use requires very cautious attention to safety, especially regarding potential therapy of cancer cachexia, since in vitro findings reveal that ghrelin promotes the proliferation and invasiveness of human pancreatic adenocarcinoma cells (13). The effects of BIM-28131 suggest the opportunity for the development of novel treatment options for catabolic diseases and thus merit further investigation.

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