Ghrelin treatment reverses the reduction in weight gain and body fat in gastrectomised mice

Charlotta Dornonville de la Cour¹, Andreas Lindqvist², Emil Egecioglu³, YC Loraine Tung⁴, Vikas Surve¹, Claes Ohlsson⁵, John-Olov Jansson⁵, Charlotte Erlanson-Albertsson², Suzanne L Dickson³,⁴ and Rolf Håkanson¹

¹ Department of Pharmacology, Institute of Physiological Sciences, University of Lund, Lund, Sweden
² Department of Cell and Molecular Biology, University of Lund, Lund, Sweden
³ Department of Physiology, Gothenburg University, Gothenburg, Sweden
⁴ Department of Physiology, University of Cambridge, Cambridge, UK
⁵ Research Center for Endocrinology & Metabolism, Sahlgrenska University Hospital, Gothenburg, Sweden

Keywords: gastrectomy, ghrelin, body weight, food intake, body fat

Abbreviations: Gx, gastrectomy; GH, growth hormone; GHS, growth hormone secretagogue; sc, subcutaneous.

Correspondence to:
Professor Rolf Håkanson
Department of Pharmacology, Institute of Physiological Sciences
University of Lund, BMC F13
S-221-84 Lund, Sweden
Email: rolf.hakanson@farm.lu.se
ABSTRACT

Background & Aims: The gastric hormone ghrelin has been reported to stimulate food intake, increase weight gain and cause obesity, but its precise physiological role remains unclear. We investigated the long-term effects of gastrectomy-evoked ghrelin deficiency and of daily ghrelin injections on daily food intake, body weight, fat mass, lean body mass and bone mass in mice.

Methods: Ghrelin was given by subcutaneous injections (12 nmol/mouse once daily) for 8 weeks to young female mice subjected to gastrectomy or sham operation one week before.

Results: Gastrectomy reduced the plasma concentrations of total ghrelin (octanoylated and des-octanoylated) and active (octanoylated) ghrelin by ~80%. Immediately after injection of ghrelin, the plasma concentration was supraphysiological and remained elevated still 16 h later. Daily food intake was not affected by either gastrectomy or ghrelin treatment. The effect of ghrelin on meal initiation was not studied. At the end-point of the study, the mean body weight was 15% lower in gastrectomized mice than in sham-operated mice (p<0.001); daily ghrelin injections for 8 weeks partially prevented this weight loss. In sham-operated mice, ghrelin was without effect on body weight. The weight of fat was reduced in gastrectomized mice (~30%, p<0.01). This effect was reversed by ghrelin, enhancing the weight of fat also in sham-operated mice (+20%, p<0.05). Gastrectomy reduced the lean body mass (~10%, p<0.01) and bone mass (~20%, p<0.001) compared to sham-operated mice. Ghrelin replacement prevented the gastrectomy-induced decrease in lean body mass but did not affect bone. In sham-operated mice, ghrelin affected neither of these two parameters.

Conclusions: Ghrelin replacement partially reversed the gastrectomy-induced reduction in body weight, lean body mass and body fat but not in bone mass. In sham-operated mice, ghrelin only increased fat mass. Our results suggest that ghrelin is mainly concerned with the control of fat metabolism and that ghrelin replacement therapy may alleviate the weight loss associated with gastrectomy.
INTRODUCTION

The peptide hormone ghrelin is produced by A-like cells in the acid-producing part of the stomach but small amounts occur also further down in the digestive tract and in the pancreas [1-4]. In addition, there are reports of ghrelin-immunoreactive neurons in the hypothalamus [5-7]. Ghrelin was discovered by virtue of its ability to stimulate Ca^{2+} entry in a cell line expressing the growth hormone secretagogue (GHS) receptor. Subsequently, ghrelin was shown to release growth hormone (GH) from somatotrophs in the adenohypophysis [5]. Indeed, a ghrelin challenge is known to raise the serum concentration of GH [5, 8-10].

According to a number of reports, administration of pharmacological doses of ghrelin to intact animals increases food intake, induces weight gain and causes obesity [11-16]. The orexigenic and body fat-promoting properties of ghrelin and GHS are thought to be independent of GH and mediated by the hypothalamic neuropeptide Y and agouti-related protein systems [11,13,15,17-19]. As noted above, the effects of pharmacological ghrelin treatment are well documented but the role of endogenous ghrelin is by comparison poorly understood. It has been reported recently that knockout of either the ghrelin gene or the ghrelin receptor gene exerts no or minor effects on body growth and body composition [20-22]. However, the lack of phenotypical changes in knockout mice may reflect compensatory mechanisms, manifested already during embryonic development, a stage characterized by great plasticity. This problem may be avoided by depleting animals of ghrelin during adult life.

In humans, gastrectomy (Gx) results in a loss of body weight of about 10% within the first six months after surgery, mainly due to reduced body fat [23]. In addition, Gx patients often complain of fatigue and loss of appetite and several reports describe impaired bone quality (osteopenia/osteomalacia) [24-28]. At present there is no satisfactory treatment for any of these symptoms. In rats, Gx is associated with bone loss (compared to controls), while body weight is affected marginally, if at all [29-31]. On the whole, the effects of Gx on food intake and body composition (other than bone) have been poorly documented in rodents.

In rats and humans, more than 80% of circulating ghrelin is lost following surgical removal of the glandular stomach or the acid-producing part of the stomach [2,32] and we predict a similar reduction in Gx mice. The postulated adipogenic and anabolic effects of ghrelin prompted us to investigate whether the effects of Gx can be reversed by long-term ghrelin treatment in mice.
METHODS

Chemicals
In all, 55 mg octanoylated ghrelin-28 was made available for the study through collection of ghrelin from three different sources. During the first four weeks of the study, we used rat ghrelin kindly donated by Professor Chizuka Yanaihara at the Yanaihara Institute, Shizuoka, Japan. The following two weeks the mice were treated with rat ghrelin from Innovagen, Lund, Sweden (custom synthesis), and subsequently (the two last weeks) they received human ghrelin kindly provided by Dr. Matthias Tschöp (University of Cincinnati, Cincinnati, OH, USA). Each batch of ghrelin was tested for purity by HPLC and for bioactivity by measuring the effects of a single subcutaneous injection of 10 µg (3 nmoles) ghrelin on gastric emptying and/or rise in plasma GH concentration. Ghrelin was dissolved in 0.9% saline (sterile); fresh solutions were prepared daily.

Animals
Female NMRI mice (age 8-9 weeks) were obtained from B&K, Sollentuna, Sweden. They were kept in groups of two or three in plastic cages in a temperature-controlled environment (21°C), on a 12 h light/dark cycle, with free access to standard food pellets (Lactamin, Vadstena, Sweden) and tap water. They were allowed to acclimatize for a week before surgery. At the time of surgery the body weight was approximately 33 g. Body weights were determined every week thereafter. The experiments were approved by the local Animal Welfare Committee, Lund, Sweden.

Selection of the ghrelin dose
In a pilot study, six plus six intact mice received 12 or 24 nmol of ghrelin in 0.9% saline by a subcutaneous bolus injection in the neck (corresponding to 400 and 800 nmol kg⁻¹, respectively). Small blood samples (25µl) were drawn repeatedly by retro-orbital venepuncture before (time 0) and 15, 60, 120, 240 and 300 min after injection of ghrelin. The ghrelin doses tested were selected based on the report of Tschöp et al. [12] (2.4 µmol kg⁻¹). After demonstrating that both 12 and 24 nmol of ghrelin generated blood levels in excess of physiological concentrations for more than 16 h after injection, we settled for a dose of 12 nmoles. The purpose of the study was to replace ghrelin that was lost upon Gx by using doses that generated near-physiological plasma concentrations; there was no intent to induce sustained supraphysiological plasma concentrations of ghrelin. The dose of 12 nmoles was found to satisfy these requirements.

Surgery
Mice were randomly subjected to Gx (26 mice) or to sham operation (22 mice). They were anesthetized with an intraperitoneal injection of a mixture of fluanisone/fentanyl/midazolam (15/0.5/7.5 mg kg⁻¹). Gastric surgery was performed through a midline abdominal incision with clean but not sterile instruments. No antibiotics were used. Gx was carried out by resection of the stomach, followed by anastomozing the esophagus with the duodenum end to end. The procedure of Gx includes bilateral subdiaphragmatic vagotomy. Two of the 26 Gx mice died during the first week after surgery, i.e. before ghrelin treatment started. Sham operation consisted of a midline abdominal incision and gentle manipulation of the stomach. There was no mortality in this group. The mice were allowed to recover from surgery for about a week before starting treatment with ghrelin or vehicle.

Study design
The study comprised 24 Gx mice and 22 sham-operated mice. 12 Gx mice and 12 sham-operated mice received a subcutaneous dose of ghrelin (12 nmoles) daily for 8 weeks. 12 Gx mice and 10 sham-operated mice received daily injections of saline for 8 weeks. On the first
14 days injections were given in the morning (8-9 a.m.), subsequent injections were made in the afternoon (5-6 p.m.). The daily food intake in these animals was calculated as the difference between the amount of food given and the amount of food that remained after 24 h. The cumulative body weight gain was based on weekly determinations of the body weights and expressed as the body weight minus the weight at the start of the ghrelin treatment for each mouse. After 8 weeks of treatment with ghrelin or saline, the mice were killed by decapitation (16-18 h after the last injection). Blood was collected from the neck and plasma prepared (see below). White adipose tissue (WAT) (mesenteric, retroperitoneal, parametrial and inguinal) was dissected out and weighed. Lean tissue and bone mineral density (BMD) was assessed (whole mouse) by dual energy X-ray absorptiometry (DEXA; PIXImus, Lunar Corporation, Madison, MI, USA). Femurs were collected and their lengths were measured after which they were incinerated for 24 h at 600°C. The ash weight was determined.

**Measurement of circulating ghrelin and insulin-like growth factor-1 (IGF-1)**

The concentration of circulating ghrelin was determined in plasma from animals at sacrifice (end-point analysis). Blood samples were collected and plasma prepared according to the respective manufacturer’s protocol. *Total ghrelin*: Immunoreactive ghrelin was measured in 5-20 µl plasma using a commercial radioimmunoassay (RIA) kit (Phoenix Pharmaceuticals, Belmont, CA, USA) with an antiserum raised against acylated human ghrelin; ^125^I-labeled ghrelin-28 was used as tracer and rat ghrelin-28 as standard. The antiserum recognizes both octanoylated and des-octanoylated ghrelin-28 but does not recognize des-Gln\textsuperscript{14} ghrelin. Plasma concentrations were expressed as pmol equivalents of rat ghrelin-28 per liter. The detection limit for total ghrelin is 12 fmol l\textsuperscript{-1}. The intra- and inter assay variation was 3% and 8%, respectively. *Active ghrelin*: Active (octanoylated) ghrelin was determined in 50 µl plasma using an enzyme linked immunosorbent assay (ELISA) kit (LINCO Research, St. Charles, MO, USA). The antiserum does not recognize des-octanoylated ghrelin. The detection limit for active ghrelin is 1 pmol l\textsuperscript{-1}. The intra- and inter assay variation was 3% and 4% respectively. Plasma IGF-1 was measured in 10 µl plasma with a RIA kit from Medipznost (Reutlingen, Germany). The sensitivity of this assay is 0.02 nmol l\textsuperscript{-1}.

**Statistical analysis**

Values are expressed as means ± S.E.M. Differences were analyzed statistically by Student’s unpaired t-test or by analysis of variance (ANOVA) followed by Tukey-Kramer’s test whenever appropriate (continuous measurements). A p-value of <0.05 was considered statistically significant. The half-life of ghrelin in the circulation was calculated based on the curve showing the plasma concentration following a single ghrelin injection (Fig 1) (GraphPad Prism version 3.0, GraphPad Software, San Diego, CA, USA). The correlation between total and active ghrelin was calculated by Pearsons’s linear regression test.
RESULTS

Plasma concentrations of ghrelin
A single subcutaneous injection of either 12 or 24 nmol of ghrelin to intact mice promptly raised the circulating concentration of total (active octanoylated and inactive des-octanoylated) ghrelin with a peak value after 15-60 min (Fig 1A). Five h after injection of either dose, the circulating total ghrelin concentration was markedly decreased compared to the peak value but was still higher than the ghrelin concentration before injection (12 nmol dose: 0.6±0.07 nmol l⁻¹ versus 2.1±0.4 nmol l⁻¹ and 24 nmol dose: 0.8±0.09 nmol l⁻¹ versus 7.1±2.5 nmol l⁻¹). The plasma concentration of active (octanoylated) ghrelin showed a similar time course (Fig 1B); Five h after injection of 12 nmol ghrelin it was still much higher than before injection (19.5±1.6 pmol l⁻¹ versus 570±149 pmol l⁻¹). The half-lives of total and active ghrelin were calculated from the data in Fig 1A and B and found to be 85 and 105 min, respectively.

The circulating concentrations of total and active ghrelin were greatly reduced by Gx (measured 8 weeks after the operation) (Fig 1C, 1D). Sixteen h after injection of ghrelin (12 nmol), the plasma concentration (total and active ghrelin) remained elevated compared to after saline injection in both Gx- and sham-operated animals (Fig 1C, 1D). There was a positive correlation between the levels of circulating total and active ghrelin (R=0.54; p<0.008, n=6).

Food intake
The daily food intake, expressed as gram per individual, did not differ between Gx and sham-operated mice, and there was no difference in the daily food intake between mice receiving a daily dose of ghrelin and those receiving saline (Fig. 2A). The daily food intake, expressed as gram per gram body weight, did not differ between the different groups: 9.7±0.6 for Gx mice, 8.8±0.7 for sham-operated mice, 8.9±0.7 in ghrelin-treated Gx mice and 7.9±0.4 in ghrelin-treated sham-operated mice (p<0.05). The cumulative food intake, expressed as gram per individual over time, was similar in sham-operated and Gx mice, and ghrelin treatment did not affect the cumulative food intake in either sham-operated (p=0.28) or Gx (p=0.78) mice (Fig 2B).

Weight gain
The mean body weight was lower in GX mice, 3 weeks after surgery and continuing until 9 weeks after surgery, than in sham-operated mice. Nine weeks after surgery, the body weight of Gx mice was 15% lower than in sham-operated controls (p<0.001; Fig 3). Daily injections of ghrelin to Gx mice raised the body weight (compared to saline-treated Gx mice); the relative weight increase was 8% after 8 weeks of treatment (p<0.001; Fig 4B). Most of the difference in cumulative body weight gain between ghrelin- and saline-treated Gx animals occurred during the first 2 weeks of treatment (Fig. 4D). The cumulative body weight gain in the Gx mice was increased by 8 weeks ghrelin treatment compared to Gx mice receiving saline (5.4±0.7 g versus 1.1±0.8 g, p<0.001) (Fig 4D). In contrast, the body weight and cumulative body weight gain in the sham-operated mice were only significantly affected by ghrelin 2 weeks after initiation of treatment and this difference diminished with time (Fig 4A, C). Ghrelin did not affect the plasma levels of GH-dependent IGF-1 or the femur length in either Gx or sham-operated mice (p>0.05) (Table 1).

Table 1
Lack of effect of ghrelin (versus saline) on femur bone length and plasma IGF-1 concentration in sham-operated and Gx mice.
<table>
<thead>
<tr>
<th></th>
<th>Sham+saline</th>
<th>Sham+ghrelin</th>
<th>P-value</th>
<th>Gx+saline</th>
<th>Gx+ghrelin</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of femur (mm)</td>
<td>17.3±0.2</td>
<td>16.9±0.2</td>
<td>0.30</td>
<td>17.0±0.1</td>
<td>16.9±0.1</td>
<td>0.47</td>
</tr>
<tr>
<td>Plasma IGF-1 (nmol l⁻¹)</td>
<td>53±3.7</td>
<td>54.1±1.5</td>
<td>0.78</td>
<td>52.1±2.2</td>
<td>53.8±2.5</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Length of study 8 weeks. Mean value ± SEM (n=10-12). P-values refer to difference between saline and ghrelin treatments.

**Body composition**

**Fat depots:** Gx reduced the total weight of 4 different fat depots by about 30% (p<0.01) compared to sham operation. Daily administration of ghrelin normalized the total amount of fat in the Gx mice (p=0.95, Gx+ghrelin versus sham+saline) and raised it by 20% (p<0.05) in sham-operated animals (Fig 5A). The effect of ghrelin on individual fat depots was not statistically significantly (Table 2).

Table 2.

Effect of ghrelin (versus saline) on the weight of individual fat depots in sham-operated and Gx mice.

<table>
<thead>
<tr>
<th>Fat depot</th>
<th>Sham+saline</th>
<th>Sham+ghrelin</th>
<th>P-value</th>
<th>Gx+saline</th>
<th>Gx+ghrelin</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesenteric</td>
<td>0.5±0.06</td>
<td>0.6±0.07</td>
<td>0.072</td>
<td>0.4±0.04</td>
<td>0.5±0.04</td>
<td>0.093</td>
</tr>
<tr>
<td>Retroperitoneal</td>
<td>0.4±0.08</td>
<td>0.6±0.1</td>
<td>0.40</td>
<td>0.3±0.04</td>
<td>0.4±0.04</td>
<td>0.12</td>
</tr>
<tr>
<td>Parametral</td>
<td>0.6±0.1</td>
<td>0.8±0.2</td>
<td>0.22</td>
<td>0.5±0.07</td>
<td>0.7±0.08</td>
<td>0.36</td>
</tr>
<tr>
<td>Inguinal</td>
<td>0.7±0.2</td>
<td>0.9±0.2</td>
<td>0.02</td>
<td>0.5±0.06</td>
<td>0.8±0.1</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Length of study 8 weeks. Mean value ± SEM (n=10-12). P-values refer to difference between saline and ghrelin treatments.

**Lean body mass:** Gx reduced the amount of lean tissue (-10%, p<0.01) compared to sham operation. Daily administration of ghrelin increased the amount of lean tissue in the Gx mice (8%, p<0.05) but was without effect in sham-operated mice (p>0.05)(Fig 5B)

**Bone mass:** The total BMD and bone ash weight of the femur was lower in Gx mice than in sham-operated mice (-20%, p<0.001). Daily administration of ghrelin failed to affect the BMD and femur ash weight in either Gx- or sham-operated mice (Fig 5C, D).

**DISCUSSION**

Gx decreased body weight, body fat mass, lean body mass and bone mass and was accompanied by greatly reduced circulating concentrations of total and active ghrelin (~80% decrease), raising the possibility that the effects of Gx on body composition are due to hypoghrelinemia. Ghrelin has been put forward as a signal stimulating food intake [11,13-16], body weight gain [10,12-15] and fat deposition [12,14]. In the present study, ghrelin increased body fat but was without effect on daily food intake, weight gain, lean body mass and bone mass in sham-operated mice. In Gx mice, however, daily ghrelin injections, resulting in hyperghrelinemia, partly prevented the reduction in body weight, lean body mass and fat mass without affecting daily food intake and bone mass. The hypothesis originally put forward by Cummings and coworkers [34] that ghrelin deficiency contributes to the loss of fat in patients after gastric bypass surgery, is in line with our results suggesting that ghrelin helps retain body fat (and possibly lean body mass) in mice. The previously reported absence of deficiency symptoms in knockout mice, lacking ghrelin or ghrelin receptors [20-22], could be due to compensatory mechanisms during development. Further studies are needed to clarify the role of ghrelin in the regulation of body composition.
Our results raise the possibility that ghrelin replacement therapy can reverse some of the symptoms associated with Gx in humans. Therapeutic Gx in humans is associated with osteopathy, loss of body weight and body fat as well as fatigue and decreased quality of life [23-28]. At present, there are no generally accepted treatments for the catabolic effects of Gx. In mice, Gx resulted in a reduction in body weight and in the amount of lean tissue, bone and fat, compared to sham operation. Therefore, Gx mice may serve as a model for the clinical symptoms associated with Gx in patients, although Gx mice continued to grow (except during the first two post-operative weeks) and hence cannot be described as catabolic.

The ghrelin-induced weight gain in the Gx mice relative to saline-injected Gx mice was particularly apparent during the first two weeks of treatment, after which time most of the gastrectomy-evoked weight loss had been reversed. Previous studies have shown that the stimulatory effect of ghrelin or ghrelin analogues on body weight in intact mice plateaus after about 2-6 weeks [12, 19], possibly because the animals have reached a new set point for body weight at that time.

The present finding that ghrelin treatment enhanced body weight and body fat mass in Gx mice (that were also vagotomized) is interesting in view of the fact that subdiaphragmatic vagotomy per se has been reported to prevent the meal-initiating effect of ghrelin [11, 35]. Conceivably, vagotomy impairs the ghrelin-stimulated meal initiation but not the long term effects of ghrelin on body fat. In the present study we did not study the effects of ghrelin in animals that were vagotomized only, but the fact that ghrelin was more effective in Gx (and vagotomized) mice than in vagally intact mice, suggests that vagotomy does not prevent ghrelin from stimulating fat deposition.

The simplest interpretation of our data seems to be that endogenous ghrelin contributes to maintain body fat (and indirectly body weight). The low levels of circulating ghrelin in the Gx mice appeared to contribute to the loss of body fat and body weight, as these effects were alleviated by ghrelin treatment. Although ghrelin increased body fat in both Gx and sham-operated mice, ghrelin affected the body weight in Gx mice but not in sham-operated mice. Tschöp et al. [12] observed an effect of ghrelin on both body weight and body fat in intact mice; the ghrelin dose was 6 times higher than in the present study (2.4 µmol kg⁻¹ versus 0.4 µmol kg⁻¹). However, the fact that ghrelin treatment in this study resulted in substantially higher than normal blood levels of ghrelin during a large part of the day would seem to disagree with the interpretation that our treatment protocol was suboptimal. However, we could only partly reverse the Gx-induced decrease in body weight. This could be due to the fact that Gx causes defects other than ghrelin deficiency, such as the loss of the ability to ingest large amounts of food or the loss of other, as yet unidentified, gastric hormones. In any case, the Gx model may be complementary to the ghrelin and ghrelin receptor knock out models [20-22] by providing information about the effects of hypoghrleinemia in adult animals.

The plasma concentration of active (octanoylated) ghrelin was much lower than that of total (octanoylated + des-octanoylated) ghrelin. The ratio of active versus total ghrelin was similar in the different individuals and in the different experimental groups in line with other studies [35]. This finding increases the likelihood that our measurements of active ghrelin reflect the true values, assuming that the concentration of total ghrelin is proportional to that of active ghrelin.

Ghrelin did not stimulate daily food intake (see also [12]) in either intact or Gx mice, which is in line with the view that the postulated stimulatory effect of ghrelin is restricted to the first few hours after injection of the hormone (meal initiation) [13]. Our observation that ghrelin increases body weight and body fat in Gx mice without affecting daily food intake, could suggest that rather than causing hyperphagia ghrelin suppresses energy metabolism, but...
further studies are needed to confirm this. In any case, this is in line with the view of Tschöp et al. [12] that ghrelin exerts long-term effects on energy balance in intact mice by increasing the respiratory quotient, suggestive of an increased utilization of carbohydrates and a decreased utilization of fat as energy source. Interestingly, Wortley et al. [22] recently showed that ghrelin knock out mice had unchanged food intake (but displayed a decreased respiratory quotient on a high fat diet), supporting the notion that long term effects of endogenous ghrelin on body composition are mediated by changes in fuel preference rather than in food intake. In humans, the results of several studies have indicated a negative correlation between circulating ghrelin levels and energy expenditure [36, 37].

Our finding that administration of ghrelin increased body fat mass (despite the well known lipolytic effect of GH) without affecting daily food intake, femur length and plasma IGF-1, suggests that the effect does not reflect an action in the hypothalamus-hypophysis causing GH mobilization. Neither do our results support the view that circulating ghrelin acts on fat (and body weight) via the vagus (since Gx mice responded to ghrelin). Hence, the site of the lipogenic action of ghrelin is not known. Although the AgRP/NPY neurons of the arcuate nucleus [17, 38] represent a possible target for circulating ghrelin, it is perhaps more likely that ghrelin acts by a peripheral mechanism. Indeed, ghrelin has been suggested to exert lipogenic effects directly on adipocytes [39, 40]. Gx does not affect plasma concentrations of glucose, insulin and glucagon in freely fed mice [41]. However, Gx affects pancreatic islet function in that Gx mice display an impaired insulin (and glucagon) response to oral as well as intravenous glucose and a reduced glucose tolerance [41]. Although there were no abnormalities in the serum lipids in the Gx mice [41], it cannot be excluded that the Gx-evoked impairment of pancreatic islet function may contribute to the loss of fat. Within the physiological plasma concentration range ghrelin seems to have modest effects on circulating levels of insulin and glucose [42].

Gx decreased bone mineral density and bone ash weight of the femur compared to sham-operated mice. This is in line with earlier studies of rats, showing that the acid-producing part of the stomach is required to maintain a normal skeleton [29-31,43-47]. It has been suggested that as yet unidentified hormones in the oxyntic mucosa promote the growth and development of trabecular bone by ensuring a proper utilization of circulating Ca$^{2+}$ [43]. Also, GH is thought to be important for normal bone growth [48]. Obviously therefore, ghrelin, being a powerful GH secretagogue, is a candidate for the proposed bone-preserving hormone in the stomach. However, since ghrelin treatment did not affect bone in sham-operated mice and did not reverse the effect of Gx on bone, the present results do not support the idea that ghrelin deficiency can explain the Gx-evoked osteopenia.

In conclusion, the findings suggest that ghrelin treatment can reverse the reduction in body fat and body weight observed in Gx mice. These results are in line with a physiological role of ghrelin in the regulation of body composition (notably fat), raising the possibility that ghrelin replacement therapy may alleviate some of the clinical symptoms of Gx in humans.
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COMPETING INTEREST

John-Olov Jansson is on the board of a company involved in ghrelin research (Gastrotech A/S, Copenhagen, Denmark).

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Legends to figures

Fig 1
Plasma concentrations of total and active (octanoylated) ghrelin. A single subcutaneous injection (arrow) of either 12 or 24 nmol of ghrelin to intact mice promptly raised the plasma concentration of total (A) and active (B) ghrelin (n=6 in each group). The plasma concentrations of total and active ghrelin were low in Gx mice compared to sham-operated mice. The concentration of total ghrelin (C) and active ghrelin (D) remained elevated 16 h after injection of 12 nmol of ghrelin to sham-operated and Gx mice. Means ± S.E.M. (n=10-12), not significant (ns), *p<0.05, **p<0.01, ***p<0.001.

Fig 2
Food intake in sham-operated and Gx mice receiving daily injections of either saline or ghrelin (12 nmol daily sc) for 8 weeks. There was no statistically significant difference in the daily food intake (g/animal) between the different groups at any time point (A), nor did the cumulative food intake (g/animal) during 8 weeks differ between the groups (B). Means ± S.E.M. (N=number of mice).

Fig 3
Body weight at different times before (time 0) and after sham operation or Gx (arrow indicates surgery). After 9 weeks the mean body weight of the Gx mice was 15% lower than that of the sham-operated mice, Means ± S.E.M. (n=10-12). Not significant (ns), **p<0.01, ***p<0.001.

Fig 4
Body weight development in A) sham-operated mice and B) Gx mice, receiving daily injections of saline or ghrelin (12 nmol, sc) for 8 weeks (starting at time 0). Ghrelin increased the body weight in Gx mice (8%) but not in sham-operated mice. Ghrelin increased the cumulative weight gain in Gx mice (D), but not that of sham-operated mice (C). Means ± S.E.M (n=10-12). Vertical bars are invisible at times because S.E.M. is small. Not significant (ns), *p<0.05 **p<0.01, ***p<0.001***p<0.001.

Fig 5
Effects of Gx and/or daily administration of ghrelin (12 nmol sc) for 8 weeks on body composition. A) Gx reduced the amount of fat (-30%); this effect was prevented by ghrelin. Administration of ghrelin increased the amount of fat also in sham-operated mice (20%). B) Gx reduced the lean body mass (-10%), while administration of ghrelin reversed the effect. There was no statistically significant difference in lean body mass between the two groups of sham-operated mice, receiving either saline or ghrelin. C) Gx reduced the bone mineral density (BMD) (-20%); ghrelin treatment was without effect. D) Gx reduced the femur ash weight (-20%). Daily ghrelin administration did not affect the bone of either sham-operated or Gx mice. Means ± S.E.M. (n=10-12). Not significant (ns), *p<0.05, **p<0.01, ***p<0.001.
A

**ghrelin**
- 12 nmol
- 24 nmol

B

ghrelin
- 12 nmol

C

**Gx**
- Sham

D

**saline ghrelin**

**active ghrelin in plasma (pmol l⁻¹)**

**16 h after injection of:**
- saline
- ghrelin

**18 h after injection of:**
- saline
- ghrelin
A

B

N=     10      12                 12       12

0.0    2.5    5.0    7.5    10.0

daily food intake (g)

time (weeks)

Gx+ghrelin □ Gx+saline
Sham+ghrelin □ Sham+saline

0    500    1000

cumulative food intake (g)

ghrelin □ saline

ns  ns  ns
Ghrelin treatment reverses the reduction in weight gain and body fat in gastrectomised mice

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