Ghrelin Enhances Appetite and Increases Food Intake In Humans

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Abstract   Ghrelin is a recently identified endogenous ligand for the growth hormone secretagogue receptor. It is synthesized predominantly in the stomach and found in the circulation of healthy humans. Ghrelin has been shown to promote increased food intake, weight gain and adiposity in rodents. The effect of ghrelin on appetite and food intake in man has not been determined. We investigated the effects of intravenous ghrelin (5.0 pmol/kg/min) or saline infusion on appetite and food intake in a randomised double-blind cross-over study in nine healthy volunteers. There was a clear-cut increase in energy consumed by every individual from a free-choice buffet (mean increase $28 \pm 3.9\%$, $p<0.001$) during ghrelin compared with saline infusion. Visual analogue scores for appetite were greater during ghrelin compared to saline infusion. Ghrelin had no effect on gastric emptying as assessed by the paracetamol absorption test. Ghrelin is the first circulating hormone demonstrated to stimulate food intake in man. Endogenous ghrelin is a potentially important new regulator of the complex systems controlling food intake and body weight.

Introduction

Obesity is a modern epidemic, which still has no effective medical treatment. A very small percentage increase in energy intake over a long period of time can result in obesity (1). In order to understand the pathophysiology of obesity it is necessary to investigate the physiology of normal body weight regulation. Energy intake and body weight are tightly regulated at a remarkably consistent "set-point" by control systems in the hypothalamus (2). These hypothalamic circuits receive feedback from peripheral signals. The adipocyte-derived hormone leptin signals the state of fat stores to the brain, inhibiting food intake and further fat accumulation. Surprisingly no circulating stimulus to feeding and adiposity has been characterized (2).

Ghrelin, like previous synthetic agonists of its receptor, is a growth hormone (GH) secretagogue (3;4). In addition to this action, recent studies in rodents suggest that ghrelin may provide a peripheral signal to the brain to stimulate food intake and adiposity (5;6). We have previously shown that, in rats, ghrelin stimulates food intake following systemic and intracerebroventricular administration (5). In rodents, acute ghrelin administration has been shown to increase respiratory quotient (RQ) suggesting a switch towards glycolysis and away from fatty acid oxidation for energy expenditure, favouring fat deposition (6). Chronic ghrelin administration is also reported to cause weight gain and adiposity in rodents (6).

We aimed to investigate the effect of ghrelin on food intake and energy expenditure in man. Ghrelin is a gastric hormone and has been reported to stimulate gastric motility in rodents (7). To investigate whether ghrelin might have any direct effects on the gastrointestinal tract in man, the effect of ghrelin on gastric emptying was also investigated.

Research Design and Methods

Materials: Human ghrelin was obtained from Penninsula Laboratories, St Helens, Merseyside, UK. The Limulus

Amoebocyte Lysate assay test for pyrogen was negative and the peptide was sterile on culture. Saline (0.9%) was purchased from Bayer, Haywards Heath, UK.

Subjects: Nine non-obese Caucasian volunteers (five male and four female) were recruited, aged 21-32 years (mean $\pm$ SEM, 25 ± 1.1), body mass index (BMI) 19.8-26.8 (23.2 ± 0.7) kg/m^2. Ethical approval was obtained from the local Research Ethics Committee (project registration no. 2000/5941) and the study was performed in accordance with the principles of the Declaration of Helsinki. Subjects gave informed written consent, took no regular medication except oral contraceptive, had no allergies and had normal physical examination and electrocardiogram. The Dutch Eating Behaviour Questionnaire (8) was used to exclude subjects with abnormal eating behaviour determined by high scores for restrained eating. Blood tests confirmed normal renal and liver function, full blood count and fasting glucose. Volunteers filled out a food diary for three days prior to the cross-over infusions, and were required to maintain similar eating habits before each infusion. All subjects were fasted and drank only water from 9pm on the evening prior to each study. Subjects were advised to avoid alcohol and strenuous exercise for 24 hours before study.

Protocol: Fasted subjects had 2 intravenous cannulae (1 for infusion and 1 for sampling) placed at 08.00h and infusions commenced at 08.30h (0 min). Blood samples were collected into plastic EDTA tubes containing 0.6mg aprotinin (Bayer). Plasma was immediately separated by centrifugation at 4C and stored at -70C until assayed. Basal blood samples were taken at -20 and 0 min. The same subjects had an initial ghrelin infusion during which plasma samples were obtained for GH measurement by Advantage automated chemi-luminescent immunoassay (Nichols Institute Diagnostics). Infusion was commenced at 0.2pmol/kg/min. Every 20 min samples were taken for GH measurement and infusion rate was doubled to a maximum of 25.6 pmol/kg/min. The GH response was used to determine the infusion rate for the subsequent study.

Cross-over study The investigation protocol is summarized in figure 1. Each subject was studied on two occasions separated by at least one week in a double-blinded,

randomised cross-over design. Subjects received an infusion of either ghrelin 5.0pmol/kg/min in normal saline or normal saline alone in random order (5 saline first, 4 ghrelin first). Pilot studies demonstrated that plasma ghrelin infusion achieves steady state within 60 min (data not shown). Infusions commenced in fasted volunteers at 08.30h (0 min) and lasted for 270 min. At 120 min, subjects ate a standard test breakfast consisting of 40g cornflakes, 250ml whole milk, 3.5g sugar (1 sachet) and 100ml fresh orange juice (1550 kJ, 62% carbohydrate, 13% protein and 25% fat). Gastric emptying was estimated using the paracetamol absorption method (9), as paracetamol is not absorbed in the stomach but rapidly enters the blood stream from the small intestine (10). Subjects consumed 1.5g paracetamol with the test breakfast.
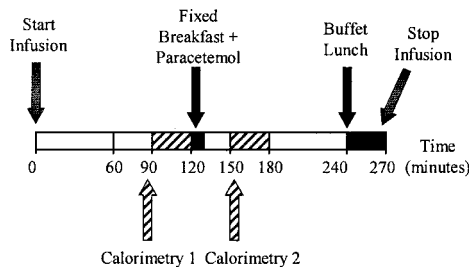

Figure 1. Protocol for investigation of the effect of saline or ghrelin (5.0pmol/kg/min) infusion on appetite, food intake, gastric emptying and energy expenditure

Blood was taken before and at 5, 10, 15, 20, 30 and 40 min after consumption. Plasma paracetamol concentration was determined by enzymatic assay (Olympus Diagnostics, Southall, UK; Reagent OAR 6905) using an Olympus AU600 analyser. Peak paracetamol concentration (C_{max}) and time to peak concentration (T_{max}) were determined for each subject and area under the curve (AUC) was calculated using the trapezoid rule. Subjects reclined at 45° throughout the infusion until the buffet lunch. At 90 and 150 min respectively, fasting and post-prandial respiratory quotient (RQ) and energy expenditure (EE) were measured by indirect calorimetry (Deltatrac II). RQ is the ratio of the volume of CO_2 produced (VCO_2) to the volume of O_2 consumed (VO_2). EE was calculated as $1.32VO_2(1.23RQ +3.81)$ (11). At 240 min, while the infusions continued, a standard free-choice buffet was offered. Subjects were instructed to eat as much as they wanted. The choices consisted of chicken curry, plain boiled rice, fruit-salad, a variety of mini chocolate bars and fruit-flavoured sweets and water as required. Food was designed to be in sufficient excess to satisfy all appetites and subjects were informed that they could take any uneaten food away with them if desired. The meal was completed at a single sitting, all subjects having finished eating within 30 min whilst the infusion continued. Food was weighed before and after the meal and caloric intake calculated. At 270 min the infusion was discontinued. Subjects then completed a food diary for the rest of the day. Visual analogue scales rating hunger and how much subjects felt they could eat (possible scores 0-10cm) were completed pre-infusion (0 min), pre-breakfast (120 min), pre-lunch (240 min) and after lunch (300 min). Positions on the scale were measured in cm and mm by a blinded investigator.

Human ghrelin radioimmunassay (RIA) Ghrelin immunoreactivity was measured using an antibody raised in a rabbit immunised with synthetic human ghrelin conjugated to bovine serum albumin (BSA) by gluteraldehyde and used at a final dilution of 1:40,000. The antibody cross-reacted 100% with human ghrelin and human des-octanoyl[3] ghrelin but did not cross-react with rat ghrelin or any other known gastrointestinal or pancreatic peptide or hormone. [125]I labelled ghrelin was prepared using Bolton & Hunter reagent (Amersham International, UK) and purified by RP-HPLC. The assay was performed in a total volume of 0.8 ml of 0.06 M phosphate buffer (pH 7.2) containing 0.3% BSA and incubated for 3 days at 4°C before separation of free and antibody-bound label by sheep anti-rabbit antibody (Pharmacia & Upjohn, Sweden). Plasma samples were diluted in assay buffer (1:10 and 1:100) prior to assay of 100μl in duplicate. A two-fold dilution curve of the plasma was parallel to the standard curve for human ghrelin. The assay could detect changes of 25 pmol/l of plasma ghrelin with 95% confidence limit. The inter- and intra-assay coefficients of variation (at 20 fmol addition) were 8.5 and 11.2% respectively.

Statistics Comparison between treatments was by paired t test with p<0.05 considered significant.

Results

The plasma GH response to increasing rates of ghrelin infusion is shown in figure 2. The lowest infusion rate of ghrelin to significantly stimulate GH was 3.2 pmol/kg/min (plasma GH after 20 min at this infusion rate 18.2 ± 5.0 μg/L vs. pre-infusion 2.0 ± 1.1 μg/L; p<0.05). A dose of 5.0 pmol/kg/min, corresponding to approximately half-maximal GH stimulating dose, was chosen to study the effects of ghrelin on energy balance.
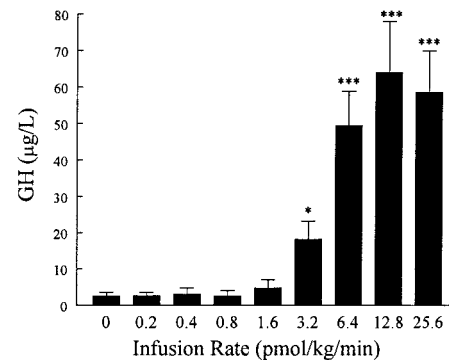

Figure 2. Average GH response to increasing rates of ghrelin infusion, doubling every 20 min from 0.2 to 25.6 pmol/kg/min. * p<0.05, ***p<0.001.

Energy intake from the buffet lunch was increased by 28 ± 3.9% during ghrelin infusion compared with saline infusion (figure 3a: 5997 ± 413 kJ vs. saline 4713 ± 344 kJ; p<0.001). Every individual consumed more calories (range: 8.9-40.7% increase) during ghrelin infusion (figure 3b). There was no change in the proportion of calories obtained from carbohydrate, protein or fat (data not shown). Energy intake for the rest of the day, estimated by analysis of food diaries, showed no evidence of compensatory under-eating following ghrelin-induced hyperphagia. Indeed, on ghrelin infusion days, there was a non-significant increase in average energy intake for the rest of the day (6362 ± 1411 kJ vs. saline 5355 ± 762 kJ; p=0.5) and total 24 hours (ghrelin 12708 ± 1838 kJ vs. saline 10396 ± 971 kJ; p=0.2).
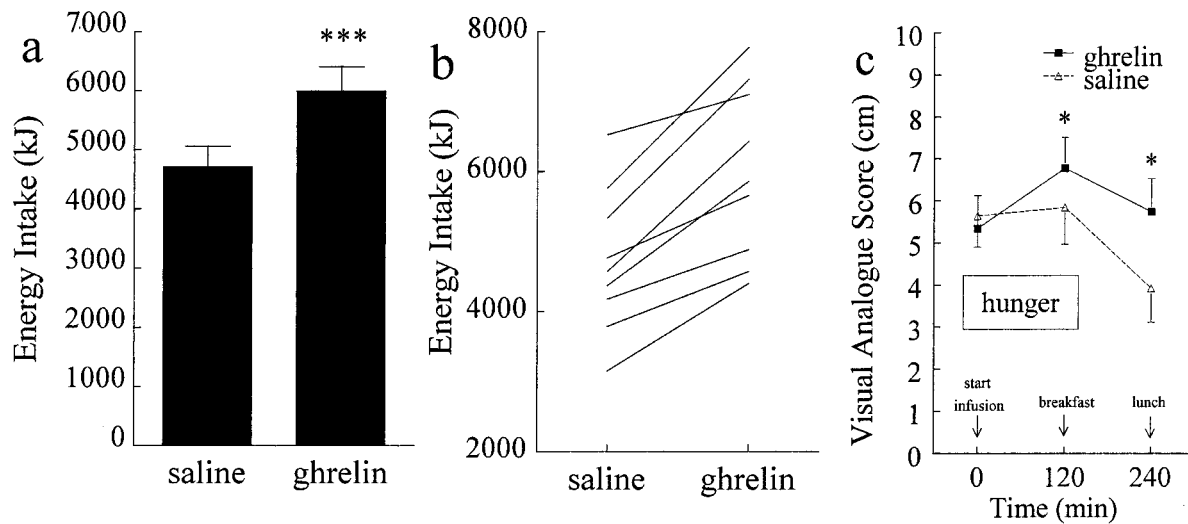
Figure 3 a) mean energy intake from free choice buffet, b) individual changes in energy intake from free choice buffet and c) mean visual analogue scores for hunger during saline or ghrelin infusion. * p <0.05; *** p < 0.001

Analysis of visual analogue scales (VAS) confirmed no difference between occasions in the basal state (0 min) in hunger (pre-ghrelin 5.3 ± 0.8cm vs. pre-saline 5.6 ± 0.7cm; p=0.6) or how much subjects felt they could eat (pre-ghrelin 5.5 ± 0.7cm vs. pre-saline 5.8 ± 0.7cm; p=0.4). Ghrelin infusion then resulted in increased hunger scores compared to saline infusion (figure 3c) both before fixed breakfast (120 min: 16 ± 10% increase; p<0.05) and before lunch (240 min: 46 ± 20% increase; p<0.05). The separate parameter of "how much could you eat right now" was also increased by 32 ± 14% (p<0.05) at the pre-lunch time-point (240 min) during ghrelin infusion. Despite every individual eating more during ghrelin infusion, at 1 hour after lunch (300min) there was no evidence of reduced hunger (VAS: ghrelin 0.8 ± 0.2 vs. saline 0.9 ± 0.3cm; p=0.6) or quantity subjects felt they could eat (VAS: ghrelin 1.1 ± 0.2 vs. saline 0.9 ± 0.2cm; p=0.8).

No change in gastric emptying was apparent during ghrelin infusion as estimated by the paracetamol absorption test (figure 4: T_{max} 27.5 ± 4.5 vs. saline 20.6 ± 4.4 min, C_{max} 86.3 ± 10.3 vs saline 87.5 ± 8.4μmol/L, AUC 2100 ± 301 vs saline 2350 ± 282.1 μmolmin/L; p=NS). Ghrelin infusion did not alter fasting RQ (0.81 ± 0.03 vs. saline 0.82 ± 0.03), post-prandial RQ (0.88 ± 0.07 vs. saline 0.91 ± 0.06) or fasting total energy expenditure (4664 ± 348 kJ/day vs. saline-treated 5091 ± 427 kJ/day). The average post-prandial energy expenditure was lower during ghrelin infusion, but this difference was not significant (ghrelin-treated 4341 ± 239 kJ/day vs saline-treated 5506 ± 590 kJ/day; p=0.09). Steady-state plasma ghrelin concentration during ghrelin infusion was between 2 and 3 fold that during saline infusion. (90 min 2.6 ± 0.1 fold; 6.02 ± 0.46 vs 2.31 ± 0.28 nmol/L; 240 min 2.4 ± 0.1 fold, 5.88 ± 0.26 vs 2.49 ± 0.52 nmol/L).
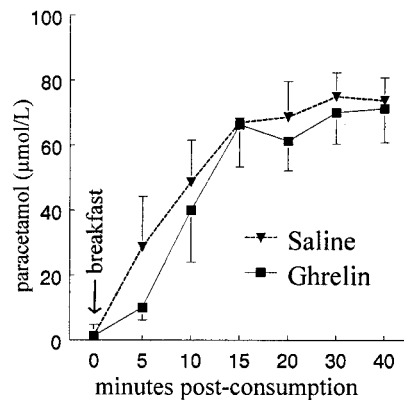
Discussion

Ghrelin is the first circulating hormone shown to promote feeding and adiposity following systemic administration in experimental animals (5;6). We now demonstrate that ghrelin stimulates appetite and food intake potently in man. The increase in calories consumed was large (28%, an additional 1284 kJ (306 kcal) in a single meal). In practice much smaller consistent changes in energy intake over longer periods are thought to be involved in clinically significant weight changes. It has been estimated that a 75kg man consumes 900,000kcal a year and that a persistent increase of 1% in energy intake would result in approximately 15kg weight gain over 10 years (1). Ghrelin concentrations in the current study were higher than normal circulating levels, but only just over twice the concentration following an overnight fast. Smaller changes in endogenous ghrelin may contribute to regulation of the smaller daily modulations of food intake that are required to maintain stable body weight.

The possibility that stimulation of appetite was mainly secondary to stimulation of GH is unlikely. A chronic increase in energy intake has been reported in

Figure 4. Plasma paracetamol concentration in response to consumption of a fixed breakfast with 1.5g paracetamol during infusion of saline (inverted triangles, dotted line) or ghrelin (squares, solid line).

children with accelerated growth following GH treatment (12), however this is thought to be secondary to increased metabolic demands (13) and acute changes in food intake have not been reported (14). In the non GH-deficient state there is no consistent evidence for increased energy intake in response to GH (15-17). Ghrelin-stimulated weight gain and adiposity are not attenuated in GH-deficient dwarf rats compared to intact rats (6). Taken together, these observations support the notion that the stimulation of appetite by ghrelin in the present study is independent of GH.

In rodents ghrelin increases respiratory quotient, suggesting reduced fatty acid oxidation and increased glycolysis, which would favour fat deposition (6). No change in either fasting or post-prandial respiratory quotient in man was detected in the present study. A trend towards reduced post-prandial energy expenditure was, however, detected in response to ghrelin. The systemic dose of ghrelin administered in rodents was a bolus of 2.4µmol/kg, which was associated with a rapid peak of plasma ghrelin to approximately 15 fold basal concentration (6). In contrast, in the present study, 5.0pmol/kg/min was administered by infusion for 260 minutes (a total dose of only 1.3nmol/kg over the entire infusion) resulting in an increase in plasma ghrelin of just over 2-fold. The disparity between the current results and those in rodents may reflect a genuine species difference or may reflect the lower dose and slower administration in the current study. Similarly injection of a bolus of ghrelin has been shown to stimulate gastric motility in rodents, but in the current study infusion of ghrelin in man did not appear to alter gastric emptying.

The peripheral hormone leptin regulates hypothalamic networks to inhibit food intake and body weight gain (2). Absence of leptin results in extreme obesity in man and rodents (18). It would be surprising if food intake and body weight maintenance, which are so important to survival, were regulated by a single inhibitory peripheral signal. So far, however, no peripheral stimulator of the hypothalamic control of feeding has been established. In rodents, plasma ghrelin has been demonstrated to increase with fasting and fall in response to re-feeding (6) whilst ghrelin administration causes hyperphagia and obesity (5;6). Systemic ghrelin administration in rats causes neuronal activation in the hypothalamic arcuate nucleus, a key nucleus in body weight regulation (19). We now report that ghrelin stimulates food intake and appetite in man.

A recent report has demonstrated reduced circulating ghrelin in obese compared with lean Caucasians and in obesity-prone Pima Indians (20). This is analogous to the appetite inhibitor leptin, which is increased in obese individuals (2) and raises the possibility that ghrelin and leptin are part of a dynamic feedback system in the regulation of body weight.

In conclusion our data suggest that ghrelin is a potentially important new peripheral signal to the brain to stimulate food intake in man. Further studies are now required to establish the role of ghrelin in the pathogenesis of the energy balance dysregulation associated with obesity and cachexia.

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