

RAPID COMMUNICATION

Circulating ghrelin concentrations are lowered by intravenous glucose or hyperinsulinemic euglycemic conditions in rodents

K C McCowen, J A Maykel, B R Bistran and P R Ling

Beth Israel Deaconess Medical Center, Brookline Avenue, Boston, Massachusetts 02215, USA
(Requests for offprints should be addressed to P R Ling; Email: pling@caregroup.harvard.edu)

Abstract

Ghrelin is a peptide secreted mainly by gastric parietal cells that may play a role in appetite regulation. Circulating ghrelin is abruptly lowered by food intake, but factors involved in ghrelin regulation remain unclear. The aim of this study was to determine whether intravenous glucose infusion lowers ghrelin, and to determine whether glucose, insulin or some measure of insulin action best predicts the effect of feeding on ghrelin. Rats were infused over 3 h with either A. saline (controls); B. dextrose to steady state blood glucose ~ 16.7 mM, or C. insulin 7.5 mU/kg.min, plus dextrose as needed to clamp to euglycemic basal concentrations. During 3 h of infusion, group B had significantly greater ($P < 0.01$) glucose, 17.4 ± 0.3 mM, than groups A (6.6 ± 0.3) or C (6.1 ± 0.2). Groups B and C had hyperinsulinemia at the

end of the 3 h infusion (894 ± 246 , 804 ± 156 pM) compared with saline-infused (222 ± 24 pM, $P < 0.01$). Ghrelin concentrations were reduced ($P < 0.01$) in both hyperinsulinemic groups (B = 85 ± 2 ; C = 103 ± 0.6 pM) versus controls (163 ± 9). Ghrelin was strongly correlated with insulin ($r = -0.68$), glucose infusion rate ($r = -0.75$) and free fatty acids ($r = 0.67$), when all 3 groups were combined, although only the 2 latter variables were independent predictors of ghrelin. In conclusion, neither a rise in blood glucose nor presence of nutrient in the stomach is required for the effect of feeding on ghrelin. The data suggest that whole body insulin responsiveness plays either a direct or indirect role in meal-related ghrelin inhibition.

Journal of Endocrinology (2002) **175**, R7–R11

Introduction

Ghrelin is a novel growth hormone-releasing acylated peptide secreted by gastric parietal cells that was found serendipitously to possess profound appetite stimulating effects (Kojima *et al.* 1999, Arvat *et al.* 2000). Exogenous administration of recombinant ghrelin to rodents has been shown to increase food intake and gastric emptying rate, and also causes weight gain and reduces fat oxidation (Tschop *et al.* 2000, Asakawa *et al.* 2001).

Ghrelin acts at hypothalamic neurons to activate orexigenic neurotransmitters including neuropeptide Y, and may act centrally to signal nutrient sufficiency. Circulating ghrelin is lowered by food intake, but exact nutritional factors that control ghrelin release remain to be clarified (Ariyasu *et al.* 2001). Gastric parietal cells are anatomically positioned to sense nutrient intake. Distention of the stomach with water did not affect ghrelin concentrations, suggesting that chemical as well as physical factors are

important in its regulation (Tschop *et al.* 2000). Prior to meals, ghrelin rises, consistent with ghrelin functioning as an initiator of food intake (Cummings *et al.* 2001).

In cross-sectional studies, fasting plasma ghrelin and insulin were inversely correlated (Tschop *et al.* 2001, Shiiya *et al.* 2002, Ravussin *et al.* 2001), although this was confounded by more robust relationships between ghrelin and BMI (Tschop *et al.* 2001, Shiiya *et al.* 2002) or ghrelin and percent body fat (Tschop *et al.* 2001, Ravussin *et al.* 2001). The literature contains conflicting reports about the effect of acute increases in insulin on ghrelin concentrations. In humans, ghrelin was reduced acutely following intravenous dextrose, but insulin was not reported (Shiiya *et al.* 2002). If postprandial reductions in ghrelin contribute to satiety, intravenous feeding might not reduce ghrelin, since parenteral nutrition does not inhibit appetite (Reifen *et al.* 1999, Stratton & Elia 1999). Here we address the question whether intravenous glucose and/or insulin infusions lower ghrelin.

Methods

Male Sprague Dawley rats (180–200 g) from Taconic Farms (Germantown NY) were maintained on chow with access to water for 5 days before the study. Animal protocols were in compliance with NIH guidelines and approved by the hospital animal committee. A silicone catheter (ID 0.025 in, OD 0.047 in, Helix Medical Inc., Carpinteria, CA) was placed in the internal jugular vein under intraperitoneal anesthesia (xylazine 13 mg/kg and ketamine 87 mg/kg) and tunneled to the interscapular region, then exteriorized and sutured to a swivel (Instech Laboratories, Plymouth Meeting, PA). The animals recovered for 3 days in individual cages.

Rats (not fasted, but 4 h after removal of food from cages) were assigned to one of 3 treatment groups: A. saline-infused control, $n=8$. B. hyperglycemic group infused with 20% dextrose for 3 h to goal blood glucose 16.7 mM, $n=7$. C. hyperinsulinemic-euglycemic clamp (7.5 mU insulin/min per kg body weight, and variable rate 20% dextrose) to reach insulin concentrations similar to group B and glucose similar to group A ($n=11$). Rates of dextrose and insulin infusions were based on our previously published work (Ling *et al.* 1997). Rats had capillary blood sampling from the tail vein every 10 min, measured using a reflectance meter (Glucometer Elite, Bayer, Mishawaka, IN). After 3 h, blood samples were drawn to assay insulin, free fatty acids (FFA) and ghrelin.

Insulin was measured using a commercial RIA kit (ICN, Costa Mesa, CA, USA). Ghrelin was measured using a commercial RIA, with ^{125}I -labeled bioactive ghrelin as tracer and polyclonal rabbit antibodies raised against full-length rat ghrelin (Phoenix Pharmaceuticals, Belmont, CA, USA). This assay measures total ghrelin. FFA were measured using a commercial colorimetric assay kit (Wako Chemicals, Richmond, VA, USA).

Groups were compared for differences using 1-way ANOVA, with Fisher Least Squares difference as a post-hoc test. Correlation was performed using multiple linear regression (SigmaStat 2.0, SSPS Inc., Chicago, IL, USA), by combining data from the 3 groups. Data are presented as mean (S.E.M.). Significance is defined by the 95% CI.

Results

Baseline glucose concentrations were similar in the 3 groups, 5.7 (± 0.6), 5.6 (± 0.7) and 6.3 (± 1.2) mM, respectively and remained unchanged in control (A) and hyperinsulinemic euglycemic (C) groups for the duration of the experiment.

Hyperglycemia was rapidly induced in group B, and maintained for 3 h. Average blood glucose values over the 3 h are shown in Fig. 1A. Average glucose infusion rates required to maintain these blood glucoses, a measure of whole body insulin sensitivity, are shown in Fig. 1B.

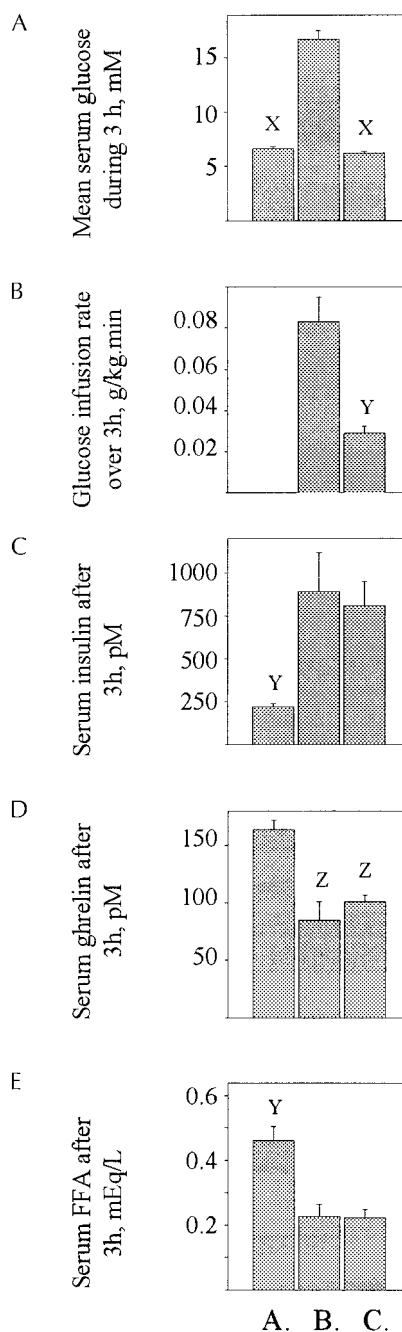


Figure 1 (A) Mean (S.E.M.) blood glucose throughout 3 h of infusion. (B) Glucose infusion rate over 3 h. (C) Serum insulin at termination of 3 h infusion. (D) Serum ghrelin at termination of 3 h infusion. (E) Serum FFA at termination of 3 h infusion. Means with different superscripts are significantly different from one another. $^{\times}P<0.01$ vs group B; $^{\text{y}}P<0.01$ vs other groups; $^{\text{z}}P<0.001$ vs control.

Serum insulin concentrations at the end of 3 h were similar in hyperglycemic and insulin-infused groups, and higher than control, Fig. 1C ($P<0.01$). Ghrelin was

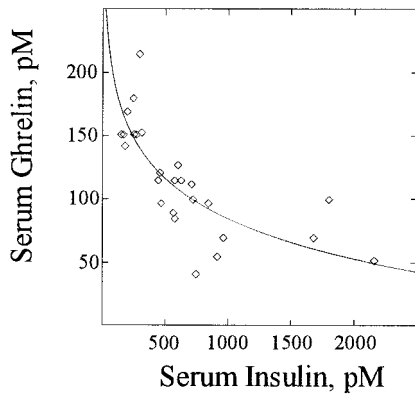


Figure 2 Correlation between ghrelin and insulin at the end of the study. $r = -0.68$, $P < 0.001$, $n = 26$.

lowered similarly in both glucose-infused and insulin-infused, compared with controls, Fig. 1D, $P < 0.001$. Measured as an additional index of insulin effectiveness, FFA concentrations in controls were $0.46 (\pm 0.05)$ and were reduced as expected in both hyperinsulinemic groups to $0.23 (\pm 0.04)$ and $0.22 (\pm 0.03)$ mEq/l respectively, ($P < 0.01$, B or C Vs controls, Fig. 1E).

Strong inverse correlations were present between insulin and ghrelin, Fig. 2, $r = -0.68$, as well as between ghrelin and glucose infusion rate ($r = -0.75$, Fig. 3A), both $P < 0.001$. FFA were directly correlated with ghrelin, $r = 0.67$, $P < 0.001$, Fig. 3B. Average blood glucose did not correlate with ghrelin. Entering significant variables into a stepwise linear regression model revealed that glucose infusion rate and FFA were both independent predictors of ghrelin, the former playing a stronger role.

Discussion

The results from the present study suggest strongly that changes in insulin action – reflected by glucose infusion rate and FFA concentrations – regulate ghrelin. Even when blood glucose was not allowed to rise, increase in insulin during glucose infusion was sufficient to effect a fall in ghrelin. However, the strongest predictors of ghrelin were the glucose infusion rate and FFA, measures of insulin responsiveness during clamped conditions. Ghrelin appeared to plateau with plasma insulin concentrations above physiologic range (Fig. 2B). However, as glucose infusion rate increased, ghrelin was decreased without plateau, suggesting that insulin effectiveness/sensitivity rather than insulin concentrations per se were critical for the effect on ghrelin. Therefore, glucose requirements and FFA, as surrogate markers of insulin effectiveness, predict most of the fall in ghrelin after a rise in insulin.

In humans, the abrupt increase in insulin with food intake is associated with a rapid fall in ghrelin concen-

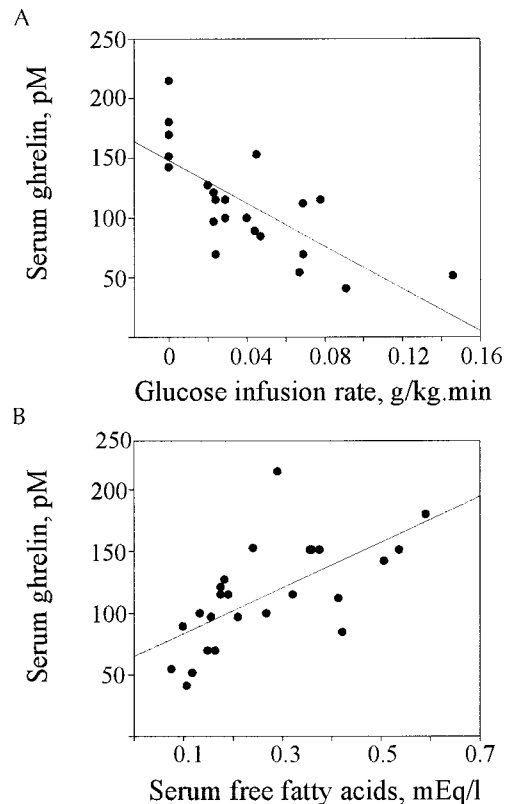


Figure 3 (A) Correlation between glucose infusion rates over 3 h with serum ghrelin at the end of infusion ($r = -0.75$; $P < 0.001$; $n = 26$). (B) Correlation between FFAs and ghrelin at the end of the infusion ($r = 0.67$; $P < 0.001$).

trations. However, in a study that used frequent sampling, insulin concentrations were low and stable in the hours prior to breakfast, while ghrelin rose by $\sim 40\%$ over that time, clearly responding to stimuli other than insulin (Cummings *et al.* 2001). Together, these data suggest that ghrelin may function as an initiator of feeding, and that the resultant rise in insulin leads to suppression of ghrelin, which signals the hypothalamus of satiety. A previous report has demonstrated that insulin administration leads to increases in stomach and plasma ghrelin (Lee *et al.* 2002), which is inconsistent with the results of our study. However, in that study, researchers administered 40 U/kg insulin b.i.d. for 3 days, and recurrent hyperinsulinemia may have different effects from 3 h sustained hyperinsulinemia. More importantly, glucose concentrations are not described; hypoglycemia might potentially alter ghrelin secretion indirectly via counterregulatory responses. Similarly, a bolus injection of insulin (1 U/kg) that produced profound hypoglycemia in rodents was associated with significant increases in stomach mRNA for ghrelin (Toshinai *et al.* 2001), but circulating ghrelin concentrations were not measured. A very recent publication compared oral with intravenous glucose in fasted

humans (Caixas *et al.* 2002). Insulin concentrations remained elevated for ~2 h following oral glucose, and ghrelin fell as expected. IV glucose followed by SC insulin to achieve hyperinsulinemia and euglycemia did not reduce ghrelin, different from our study and leading the authors to suggest that the enteral route of feeding was required for an effect on ghrelin. However, different patient groups in their study received the 2 treatments, and baseline ghrelin concentrations were very different between those given oral and those given intravenous glucose. Alternative considerations for the differences between the studies include more sustained steady state insulin concentrations in our study, as well as the effect of species.

The mechanism whereby insulin might influence ghrelin secretion is unclear. There is little evidence for a direct effect; although rodents clearly possess insulin receptors in the intestine that retain signaling function, there is no evidence either for or against such receptors remaining in adult stomach (Marandi *et al.* 2002). More likely is that insulin could activate neural circuits that influence ghrelin release. Truncal vagotomy in rodents increase basal ghrelin concentrations (Lee *et al.* 2002), suggesting that the vagus nerve exerts tonic inhibitory influences on ghrelin secreting cells, but how insulin might influence such signals has not been studied.

The only uncertainty in virtually all of the available studies of ghrelin regulation including the present work is that total plasma ghrelin is measured, rather than bioactive ghrelin. A recent paper reported that total and bioavailable ghrelin are regulated in parallel, but this needs to be verified in different physiological circumstances (Ariyasu *et al.* 2002).

Recently, the effects of gastric bypass surgery and consequent weight reduction on 24-h ghrelin concentrations were examined and compared with ghrelin in non-operated patients with similar weight loss (Cummings *et al.* 2002). In the former group, ghrelin concentrations were abnormally low at all times, and failed to rise before meals. In contrast, as expected, weight loss through dietary restraint alone resulted in higher ghrelin than at baseline, and appropriate pre-prandial rise. Since gastric bypass prevents ingested nutrients from accessing gastric parietal cells, and since insulin rose after meals in typical fashion in the operated patients, the authors concluded that failure of gastric parietal cells to come in contact with the food resulted in abnormal regulation of ghrelin. These findings suggest that insulin does not have a prominent role in ghrelin regulation, but it is possible that the destructive effects of the surgery contributed to overall substantial reductions in plasma ghrelin, perhaps through gastric parietal cell atrophy.

In conclusion, results from our study suggest that a fall in ghrelin may occur in response to the action of insulin, without a requirement for blood glucose to increase. While a rise in ghrelin appears to influence appetite

(Cummings *et al.* 2001), we suspect that decrease in ghrelin post prandially is not the main mediator of satiety, since intravenous glucose does not effectively reduce appetite.

Acknowledgement

K C M was supported by a grant from the American Liver Foundation

References

- Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyooka S, Shirakami G, Usui T, Shimatsu A, Doi K, Hosoda H, Kojima M, Kangawa K & Nakao K 2001 Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *Journal of Clinical Endocrinology and Metabolism* **86** 4753–4758.
- Ariyasu H, Takaya K, Hosoda H, Iwakura H, Ebihara K, Mori K, Hosoda K, Akamizu T, Kojima M, Kangawa K & Nakao K 2002 Delayed short term secretory regulation of ghrelin in obese animals: evidenced by a specific RIA for the active form of ghrelin. *Endocrinology* **143** 3341–3350.
- Arvat E, Di Vito L, Broglio F, Papotti M, Muccioli G, Dieguez C, Casanueva FF, Deghenghi R, Camanni F & Ghigo E 2000 Preliminary evidence that Ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. *Journal of Endocrinological Investigation* **23** 493–495.
- Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Nijima A, Fujino MA & Kasuga M 2001 Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* **120** 337–345.
- Caixas A, Bashore C, Nash W, Pi-Sunyer F & Laferrere B 2002 Insulin, unlike food intake, does not suppress ghrelin in human subjects. *Journal of Clinical Endocrinology and Metabolism* **87** 1902–1905.
- Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE & Weigle DS 2001 A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* **50** 1714–1719.
- Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP & Purnell JQ 2002 Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *New England Journal of Medicine* **346** 1623–1630.
- Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H & Kangawa K 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402** 656–660.
- Lee HM, Wang G, Englander EW, Kojima M & Greeley GH Jr 2002 Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. *Endocrinology* **143** 185–190.
- Ling PR, Sierra P, Qu Z & Bistrian BR 1997 Insulin-like growth factor-I improves glucose utilization in tumor necrosis factor-treated rats under hyperinsulinemic-euglycemic conditions. *Metabolism* **46** 1052–1058.
- Marandi S, De Keyser N, Saliez A, Maermoudt AS, Sokal EM, Stilmant C, Rider MH & Buts JP 2002 Insulin signal transduction in rat small intestine: role of MAP kinases in expression of mucosal hydrolases. *American Journal of Physiology. Gastrointestinal and Liver Physiology* **280** G229–G240.
- Ravussin E, Tschop M, Morales S, Bouchard C & Heiman ML 2001 Plasma ghrelin concentration and energy balance: overfeeding and negative energy balance studies in twins. *Journal of Clinical Endocrinology and Metabolism* **86** 4547–4551.

- Reifen R, Khoshoo V & Dinari G 1999 Effect of parenteral nutrition on oral intake. *Journal of Pediatric Endocrinology and Metabolism* **12** 203–205.
- Shiia T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe S, Hosoda H, Kangawa K & Matsukura S 2002 Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *Journal of Clinical Endocrinology and Metabolism* **87** 240–244.
- Stratton RJ & Elia M 1999 The effects of enteral tube feeding and parenteral nutrition on appetite sensations and food intake in health and disease. *Clinical Nutrition* **18** 63–70.
- Toshinai K, Mondal MS, Nakazato M, Date Y, Murakami N, Kojima M, Kangawa K & Matsukura S 2001 Upregulation of Ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochemical and Biophysical Research Communications* **281** 1220–1225.
- Tschop M, Smiley DL & Heiman ML 2000 Ghrelin induces adiposity in rodents *Nature* **407** 908–913.
- Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E & Heiman ML 2001 Circulating ghrelin levels are decreased in human obesity. *Diabetes* **50** 707–709.