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Postprandial Plasma Ghrelin Is Suppressed Proportional to Meal Calorie Content in Normal-Weight But Not Obese Subjects

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Circulating levels of the gastric hormone ghrelin rise before and decrease after a meal. In normal-weight subjects, postprandial suppression of ghrelin is proportional to calories consumed. Obese individuals have lower fasting ghrelin levels; however, it is unclear whether the obese show normal postprandial suppression. This study aimed to compare postprandial ghrelin responses in normal-weight and obese subjects, using mixed macronutrient meals with varied fat and calorie content. Postprandial ghrelin response was measured in normal-weight insulin-sensitive subjects and obese insulin-resistant subjects, after six test meals with different fat and calorie content (250–3000 kcal). Increasing the calorie content of meals in normal-weight subjects progressively lowered nadir levels of ghrelin. The obese had lower fasting ghrelin levels, and the reduction after the consumption of all test meals was less than the normal-weight subjects. The lowest postprandial levels in the obese were no different to the nadir in normal-weight volunteers after 1000-, 2000-, and 3000-kcal meals. Thus, circulating ghrelin levels decreased in normal-weight subjects after mixed meals. Obese subjects demonstrated a much reduced ghrelin postprandial suppression. This reduced suppression may influence satiety, thus reinforcing obesity. (*J Clin Endocrinol Metab* 90: 1068–1071, 2005)

GHRELIN IS A 28-amino acid peptide produced in the stomach (1) and is the endogenous ligand for the GH secretagogue receptor (1). Central and peripheral administration leads to increased food intake in rodents and humans (2–4). Ghrelin has also been postulated to increase appetite while acting as a meal initiator (2–6).

Circulating plasma ghrelin levels increase before a meal and decrease after the consumption of nutrients (6). Gastric distension, as achieved by infusion of water into the stomach, does not lead to ghrelin reduction (2, 7); however, ingestion of nonnutritive fiber does decrease ghrelin levels (8). Although obese patients with Prader-Willi syndrome, which is characterized by hyperphagia and obesity, have elevated ghrelin levels (9), the concentrations of fasting ghrelin in the majority of obese subjects are lower than normal-weight volunteers (9, 10). Insulin resistance has recently been postulated to play a role in determining this lower plasma ghrelin level in the obese (11). It is currently unclear whether postprandial plasma ghrelin levels are normally suppressed in the obese. Some authors have suggested that ghrelin is not suppressed postprandially in the obese (12); however, others have shown that an attenuated reduction occurs (13, 14).

In humans, postprandial suppression of ghrelin is proportional to the calories consumed, although the suppression did not correlate with the interval between meals (15). In diet-induced obese rats, plasma ghrelin at the onset of the dark phase was 29% lower compared with diet-resistant rats, but plasma ghrelin levels were equivalent 6 h later, suggesting less suppression in obese rats (16). Most studies have used mixed or carbohydrate-rich test meals, which classically suppress ghrelin levels and measurements of hunger (13–15). In contrast, some authors have found that protein- and fat-rich meals may increase ghrelin levels (17, 18), while hunger is still suppressed similarly to carbohydrate-rich meals. Thus, ghrelin suppression may depend on the macronutrient content of meals (17).

The aim of this study was to compare postprandial ghrelin responses in normal-weight and obese subjects given mixed-macronutrient meals of 250, 500, 1000, 2000, and 3000 kcal, with calorie content adjusted by fat content.

Subjects and Methods

Experimental subjects

All studies were performed according to the principles of the Declaration of Helsinki and approved by the local research and ethics committee at the Hammersmith Hospital. Written informed consent was obtained from all subjects. Exclusion criteria included chronic medical or psychiatric illness, pregnancy, substance abuse, more than two alcoholic drinks per day, and aerobic exercise for more than 30 min three times per week.

We evaluated the postprandial ghrelin response to a series of six standard meals (Table 1) in 20 obese (age, mean ± SEM, 29.0 ± 2.0 yr) and 20 lean subjects (28.6 ± 1.6 yr) who were all at a stable body mass index of 40.3 ± 1.1 kg/m^2 and 21.7 ± 0.4 kg/m^2, respectively. Each group consisted of 14 females and six males. Insulin resistance was assessed by determining the homeostasis model assessment insulin resistance index, which is calculated as follows: fasting glucose (mM) × fasting insulin (mU/liter)/22.5 (19). All the obese subjects were insulin resistant as indicated by a homeostasis model assessment insulin resistance index of 4.2 ± 0.4 compared with 0.9 ± 0.1 in the normal-weight subjects. Fasting insulin levels were 17.0 ± 1.7 mU/liter in the obese subjects and 4.4 ± 0.3 mU/liter in the normal-weight subjects.

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Abbreviation: AUC, Area under the curve.

TABLE 1. Macronutrient content of the standard test meals

	CHD (g)	Fat (g)	Protein (g)
500-ml meal			
250 kcal	42.3	10.2	16
500 kcal	52	26.5	18
1000 kcal	63.3	75.3	17.1
900-ml meal			
1000 kcal	99	52.9	32.6
2000 kcal	107.5	161.7	29.5
3000 kcal	94.5	274.9	24.7

CHD, Carbohydrate.

Materials and methods

The subjects were randomly allocated to two subgroups. Each subgroup consisted of 10 lean and 10 obese subjects each. Each subject visited the hospital on three occasions after a 12-h overnight fast and received, in random order and in a blinded fashion, either a 500-ml liquid meal (250, 500, and 1000 kcal) or a 900-ml meal (1000, 2000, and 3000 kcal). Subjects were blinded as to the calorie content of each meal. Table 1 shows the macronutrient content of the test meals. The 1000-kcal meals given as 500 or 900 ml allowed us to investigate whether there was a major volume effect on ghrelin response. All meals had a similar taste and viscosity. Venous blood was collected 30 min before the meal and then every 30 min thereafter for 3 h after each meal. Blood samples were centrifuged, and plasma was immediately separated and stored at −20 C until analysis.

Hormone assays

All samples were assayed simultaneously and in duplicate to eliminate the effect of interassay variation. Ghrelin-like immunoreactivity was measured with a specific and sensitive RIA. The assay measures both octanoyl and des octanoyl ghrelin and did not cross-react with any known gastrointestinal or pancreatic peptide hormones. The antisera (SC-10368) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and was used at a final dilution of 1:50,000. The ^{125}I ghrelin was prepared using Bolton & Hunter reagent (Amersham Biosciences, Chalfont St Giles, Buckinghamshire, UK) and purified by reverse phase-HPLC using a linear gradient from 10–40% acetonitrile and 0.05% trifluoroacetic acid over 90 min. The specific activity of ghrelin label was 48 Bq/fmol. Fifty microliters of unextracted plasma were assayed. The assay was performed in a total volume of 0.7 ml of 0.06 M phosphate buffer (pH 7.2) containing 0.3% BSA and incubated for 3 d at 4 C before separation of free from antibody-bound ghrelin label by charcoal absorption. The assay detected changes of 25 pM plasma ghrelin with 95% confidence limit, with an intraassay coefficient of variation of 5.5%.

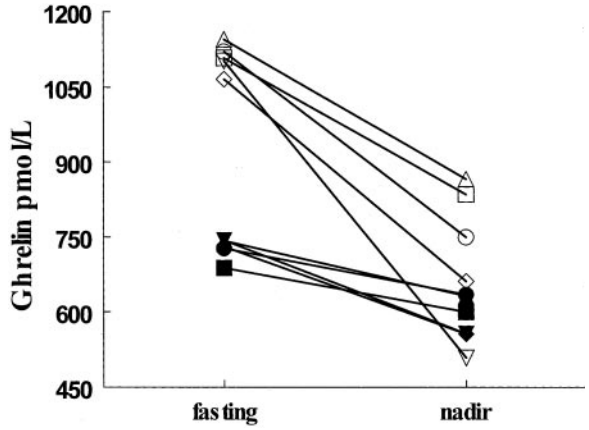

FIG. 2. Fasting and nadir plasma ghrelin concentrations in normal-weight (*open symbols*) and obese (*filled symbols*) subjects after 250 kcal (*square*), 500 kcal (*triangle*), 1000 kcal (*circle*), 2000 kcal (*diamond*), and 3000 kcal (*inverse triangle*).

Statistical analysis

Hormone levels are expressed as means ± SEM. Fasting and nadir levels were compared with the use of two-tailed, paired Student's *t* tests or ANOVA. Correlations were determined by univariate linear regression (GraphPad Prizm, San Diego, CA). The area under the curve (AUC) was calculated using the trapezoid rule.

Results

Fasting plasma ghrelin levels were significantly lower in the obese group (735.4 ± 89.6 pM) compared with the normal-weight group (1108.9 ± 93.3 pM; $P < 0.001$). Ghrelin levels in both the normal-weight and obese groups reached a nadir between 60 and 150 min post prandially ($P = 0.3$), with the median in both groups being 90 min. In normal-weight subjects, meals of higher calorie content led to lower nadir ghrelin levels ($R^2 = 0.94$, $P < 0.001$; Figs. 1 and 2). The reduction in ghrelin levels in the obese subjects after the consumption of the six test meals ($R^2 = 0.63$, $P = 0.06$) was less than the normal-weight subjects ($P = 0.001$; Figs. 2 and 3). One-way ANOVA comparing nadir ghrelin levels showed significant difference in the normal-weight subjects

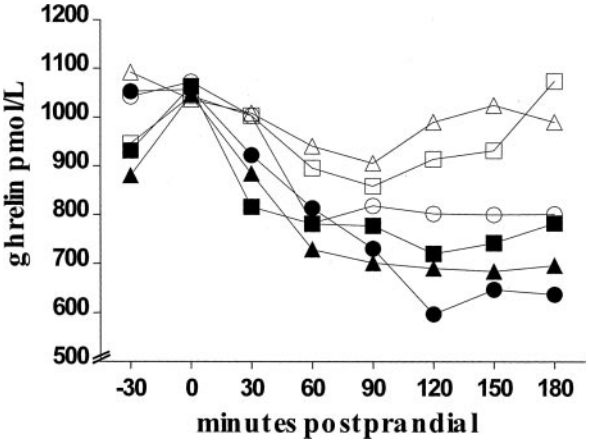

FIG. 1. Pre- and postprandial plasma ghrelin response in normal-weight subjects after 250 kcal (*open square*), 500 kcal (*open triangle*), and 1000 kcal (*open circle*) in 500 ml and 1000 kcal (*filled square*), 2000 kcal (*filled triangle*), and 3000 kcal (*filled circle*) in 900 ml.

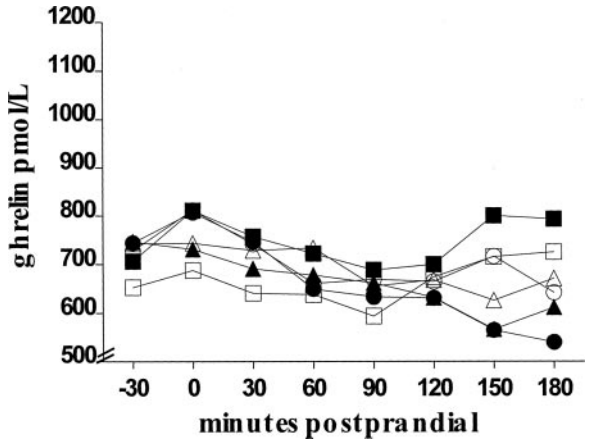

FIG. 3. Pre- and postprandial plasma ghrelin response in obese subjects after 250 kcal (*open square*), 500 kcal (*open triangle*), and 1000 kcal (*open circle*) in 500 ml and 1000 kcal (*filled square*), 2000 kcal (*filled triangle*), and 3000 kcal (*filled circle*) in 900 ml.

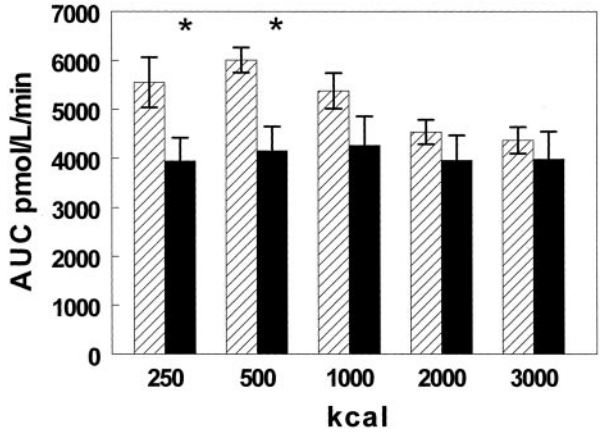

FIG. 4. AUC for normal-weight (*striped bar*) and obese (*black bar*) subjects after 250-, 500-, 1000-, 2000-, and 3000-kcal meals. *, $P < 0.05$.

($P < 0.001$) but not in the obese subjects ($P = 0.737$). The total area AUC was significantly smaller in the obese subjects compared with normal-weight subjects for meals less than 1000 kcal ($P < 0.04$; Fig. 4). For meals of 1000 kcal and more, the lower nadir levels in the normal-weight subjects led to a reduction in AUC; however, this trend was not seen in the obese subjects (Fig. 4). There was no difference between the nadir ghrelin levels in obese and normal-weight subjects after 1000-, 2000-, and 3000-kcal meals ($P = 0.24$). However, nadir ghrelin levels for both groups were significantly lower after all meals (Table 2). In the two meals of 1000 kcal, doubling of the volume did not have an effect on the ghrelin profile (Figs. 1 and 3).

Discussion

Normal-weight subjects have higher fasting ghrelin than obese subjects (10, 14) and show a calorie-dependant suppression of plasma ghrelin (15). Meals consisting of fat have recently been shown to increase ghrelin (17, 18), although other investigators have shown an attenuated suppression of ghrelin after a fat-rich meal compared with a carbohydrate-rich meal (20). We demonstrated that obese individuals do not show a calorie-dependant suppression in postprandial circulating ghrelin, but we confirmed a calorie-dependant suppression in normal-weight volunteers (15), notwithstanding the major macronutrient component of the mixed meal consisting of fat.

After 1000-, 2000-, and 3000-kcal meals in the normal-weight subjects, the nadir levels of ghrelin were similar to that of the obese subjects after 250, 500, 1000, 2000, and 3000 kcal. Doubling of the volume of the meal did not significantly affect the ghrelin profile in either the normal-weight or obese subjects, suggesting that gastric distension was not responsible for the postprandial ghrelin response.

The meals in this study did not consist of single components but, instead, reflected a typical diet consisting of a mixture of carbohydrates, protein, and fat. Meals of two constant volumes were used, and the calories were increased by adding a higher percentage of fat-rich components. Protein content increased between the 500- and 900-ml meals, although it was kept at a similar level within each volume. The 250-kcal meal consisted predominantly of carbohydrates, and the 3000-kcal meal consisted predominantly of fat. Thus, the macronutrient composition of the meals in our study did vary. Ghrelin reduction was significantly correlated with fat and calorie content. The finding that high fat meals elevated ghrelin was not reproduced by this study (17, 18).

Ghrelin concentrations in normal-weight and obese subjects reached their nadir at similar postprandial points. In this study, the calculated energy requirement for the obese subjects was higher than the normal-weight volunteers. Calorie-dependant reductions in plasma ghrelin were previously shown in normal-weight subjects when meal calorie content was calculated to be a fixed percentage of energy requirement (15). The meals selected in our study were of a fixed calorie content, independent of individual energy requirement, and caused a proportional reduction of ghrelin. It is unlikely that the higher energy requirement in the obese confounded our results because even a 3000-kcal meal in the obese suppressed ghrelin less than a 1000-kcal meal suppressed ghrelin in the normal-weight group (Table 2).

Ghrelin has been postulated to increase hunger by increasing neuropeptide Y and agouti-related protein in the hypothalamic arcuate nucleus (5). Obese humans take longer to reach satiety (21), yet they have lower circulating (9, 10) and lower production of ghrelin as suggested by reduced stomach ghrelin mRNA in *db/db* mice (22). The reasons for the reduced plasma and mRNA ghrelin levels are still unclear. Insulin may play a role because obese subjects who remain insulin sensitive have higher fasting ghrelin levels (11). Our study suggests that attenuated ghrelin suppression may act in concert with other gut hormone changes in insulin-resistant obese subjects, resulting in inhibition of satiety.

TABLE 2. Fasting and nadir mean plasma ghrelin (SEM) with changes from baseline after increasingly calorific meals

Calories (kcal)	Normal weight			Obese		
	Fast [pmol/liter (SEM)]	Nadir [pmol/liter (SEM)]	Change from baseline [pmol/liter (%)]	Fast [pmol/liter (SEM)]	Nadir [pmol/liter (SEM)]	Change from baseline [pmol/liter (%)]
500-ml meal						
250	1109 (93)	815 (81)[a]	293 (26)	735 (89)	600 (71)[a]	135 (18)
500	1146 (63)	886 (55)[a]	260 (23)	744 (115)	633 (84)[a]	112 (15)
1000	1122 (82)	750 (66)[a]	371 (33)	770 (116)	615 (85)[a]	155 (20)
900-ml meal						
1000	1075 (38)	706 (63)[a]	369 (34)	771 (122)	635 (117)[a]	124 (18)
2000	1062 (31)	662 (47)[a]	400 (38)	739 (81)	557 (67)[a]	182 (24)
3000	1103 (60)	509 (21)[a]	594 (54)	775 (130)	558 (92)[a]	217 (28)

[a] $P < 0.05$ compared to fasting.

Continuous exogenous administration of ghrelin to rodents leads to weight gain (2, 3). However, high ghrelin levels are not a feature of common obesity (9, 10). Reduction in hunger is associated with reduction in ghrelin levels after consumption of a mixed meal in normal-weight subjects (15). This postprandial ghrelin reduction was much reduced in the obese subjects we studied. A recent report indicated that obese individuals have delayed satiety and required approximately 255 kcal more than lean subjects to reach maximal satiety (21). As little as 100 kcal/d in excess of requirements may be enough to cause obesity and keep individuals trapped in the obese state (23). The reduced ghrelin response or insensitivity to calories ingested may thus contribute to the increased calories consumed by the obese. However, because basal ghrelin levels are already reduced in obese subjects, it is unclear how important this attenuated suppression in circulating ghrelin is.

The ghrelin system represents a target for antiobesity therapy. GH secretagogue receptor antagonists have been shown to reduce body weight gain in rodents (24); however, actual weight loss has not been shown. Ghrelin antagonists may have limited potential as antiobesity agents if endogenous plasma ghrelin levels are already low and are not suppressed after consumption of large mixed meals. The relative unresponsiveness to calories, as evidenced by the reduced ghrelin response, may further cause satiety signals to be reduced and could reinforce obesity.

Acknowledgments

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