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Ghrelin secretion is not reduced by increased fat mass during diet-induced obesity

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Qi X, Reed JT, Wang G, Han S, Englander EW, Greeley GH Jr. Ghrelin secretion is not reduced by increased fat mass during diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* 295: R429–R435, 2008. First published May 21, 2008; doi:10.1152/ajpregu.90329.2008.—Ghrelin is a stomach hormone that stimulates growth hormone (GH) secretion, adiposity, and food intake. Gastric ghrelin production and secretion are regulated by caloric intake; ghrelin secretion increases during fasting, decreases with refeeding, and is reduced by diet-induced obesity. The aim of the present study was to test the hypotheses that *1*) an increase in body adiposity will play an inhibitory role in the reduction of gastric ghrelin synthesis and secretion during chronic ingestion of a high-fat (HF) diet and *2*) chronic ingestion of an HF diet will suppress the rise in circulating ghrelin levels in response to acute fasting. Adult male Sprague-Dawley rats were fed a standard AIN-76A (~5–12% of calories from fat) or an HF (~45% of calories from fat) diet. The effect of increased adiposity on gastric ghrelin homeostasis was assessed by comparison of stomach ghrelin production and plasma ghrelin levels in obese and nonobese rats fed the HF diet. HF diet-fed, nonobese rats were generated by administration of triiodothyronine to lower body fat accumulation. Our findings indicate that an increased fat mass per se does not exert an inhibitory effect on ghrelin homeostasis during ingestion of the HF diet. Additionally, the magnitude of change in plasma ghrelin in response to fasting was not blunted, indicating that a presumed, endogenous signal for activation of ingestive behavior remains intact, despite excess stored calories in HF-fed rats.

gut hormones; regulation

THE STOMACH HORMONE ghrelin is a 28-amino acid peptide that was discovered in extracts of the rat stomach as the endogenous ligand for the growth hormone (GH) secretagogue (GHS) receptor (GHS-R) (31, 46). The search for ghrelin resulted directly from the development of synthetic substances called GHSs and the subsequent discovery of a natural receptor, GHS-R, for these artificial ligands (8, 9, 21, 47). Systemic administration of ghrelin or GHSs evokes GH secretion from the pituitary by activation of GHS-R. GHSs are synthesized on the basis of the structure of enkephalins and do not resemble the hypothalamic hormone GH-releasing hormone, nor do they cause GH secretion by activation of GH-releasing hormone receptor.

Ghrelin exerts a variety of important metabolic actions. It stimulates GH secretion, food intake, body growth, and adiposity (17, 31, 42, 49, 53) and also influences gastric acid secretion and exerts a prokinetic action on the stomach (38). Ghrelin is the first endogenous peptide to be isolated with an *n*-octanol modification at Ser^3 (31). The acyl and desacyl forms of ghrelin have been shown to exert biological activity (4, 6, 7, 10, 31). Although ghrelin is expressed in the pancreas, intestine, kidney, hypothalamus, heart, and placenta (51), the stomach epithelium is the primary site for synthesis of ghrelin immunoreactivity measured in the general circulation. Ghrelin is produced in gastric enteroendocrine X/A cells (14). Our laboratory and others have shown that gastrectomy in rats and humans will diminish plasma ghrelin levels ~85% (5, 22).

Regulation of ghrelin secretion from the stomach is unique compared with other gastrointestinal hormones. In contrast to other gut hormones, plasma ghrelin levels increase in response to fasting and decrease on refeeding (22). Furthermore, plasma ghrelin levels are reduced by chronic intake of high-calorie diets and obesity in humans (50). In rodents, prolonged exposure to high-fat (HF) diets will result in a positive energy balance, obesity, and a reduction of stomach production and secretion of ghrelin (33); however, little is known about the extent to which the increased adiposity itself exerts an inhibitory influence on stomach ghrelin production and secretion.

The purpose of the present study, therefore, was to investigate the role of the increased body adiposity on ghrelin production and secretion during ingestion of an HF diet. To diminish the influence of the increased adiposity during the HF diet, we treated HF-fed rats with triiodothyronine (T_3) to "clamp" their body weights and adiposity at levels comparable to those measured in control rats.

METHODS AND MATERIALS

Animals

All animal experiments were conducted in accordance with mandated standards of humane care and were approved by the Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats were maintained in an air-conditioned and light-regulated room (lights on from 0600 to 1800) and given access to food and water ad libitum.

Chemicals and Peptides

All chemicals were obtained from Sigma Chemical (St. Louis, MO). Synthetic peptides were purchased from Phoenix (Belmont, CA).

Experiments

Experiment 1. Six groups of rats (n = 8–10 rats/group) were studied. Two groups (AIN + VEH and HF + VEH) were fed a commercial AIN-76A (~5% of calories from fat by weight) or an HF (~45% of calories from fat) diet for 8 wk (BioServ, Frenchtown, NJ) and treated with vehicle (VEH). Two groups (AIN + T_3 and HF + T_3) were fed the AIN-76 and HF diets and treated concurrently with T_3 (100 μg/kg sc, every other day). T_3 was administered to avert body

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fat accumulation in rats fed the HF diet. Thyroid hormones (thyroxine and T_3) are the predominant regulators of basal metabolic rate, and circulating levels of thyroid hormones are directly correlated with energy expenditure and calorie loss (35). T_3 was prepared as described previously (23). Because T_3 has been reported to stimulate food intake (32, 36) and food intake affects stomach ghrelin homeostasis, a group of rats ($HF_{PFHF + VEH} + T_3$) were treated with T_3 and pair fed an HF diet according to the daily caloric intake of rats fed the HF diet but not treated with T_3. We pair fed another group of rats ($HF_{PFAIN + VEH}$ + VEH) the HF diet according to the daily caloric intake of rats fed ad libitum the AIN-76A diet to determine the extent to which a higher percentage of ingested dietary fat is a key signal in reduction of stomach ghrelin production and blood ghrelin levels. Daily food intake was monitored in AIN + VEH and HF + VEH groups throughout the experiment. Food intake was measured in AIN + T_3 and HF + T_3 groups during the last 2 wk of the experiment. At the end of the 8-wk treatment period, body weight was recorded, and the rats were killed. Plasma was harvested for measurement of plasma ghrelin, insulin, leptin, and triglyceride levels. The stomach-fundal mucosa was collected for extraction of total cellular RNA and ghrelin peptide. Total body fat was measured by quantitative nuclear magnetic resonance using an MRI Whole Body Composition Analyzer (Echo Medical Systems, Houston, TX) (37). Plasma triglyceride levels were measured using a commercial kit (Sigma-Aldrich). The compositions (approximate percentage of calories) of the commercial AIN-76A biscuit and HF diets are as follows: 5% fat, 20% protein, and 72% carbohydrate for the AIN-76A biscuit and 48% fat (beef tallow), 16% protein, and 34% carbohydrate for the HF diet. Two percent of the fat calories in the beef tallow-containing diet were derived from safflower oil to supply essential fatty acids. All rats had unrestricted access to water. A preweighed amount of food was provided daily, and food intake was determined daily from the amount of food remaining in the food bowl + waste below the cage. Pair-fed rats were given an additional 10% of food to accommodate for loss due to spillage.

Experiment 2. Two groups of adult male Sprague-Dawley rats (n = 10 rats/group) were fed the AIN-76A (~12% of calories from fat) or HF (~40–45% of calories from fat) diet for 5 wk. All rats had unrestricted access to water. Plasma was collected from ad libitum-fed and fasted (~24 h) groups of AIN-76A- and HF-fed rats for measurement of ghrelin levels by immunoassay. Food intake was determined for rats fed the AIN-76A and HF diets for 2 wk as described for *experiment 1*.

Ghrelin, Insulin, and Leptin Assays

For *experiment 1*, plasma acyl-ghrelin was measured using a commercial ELISA kit that detects acyl-ghrelin specifically (ALPCO, Salem, NH). The sensitivity for this assay is 3.9 pg/ml. An anticoagulant (EDTA) and a protease inhibitor (*p*-hydroxymercuribenzoic acid, 1 mM in the final sample volume) were added at the time of blood collection. After separation of plasma, 100 μl of 1 N HCl was immediately added per milliliter of collected plasma, and the sample was centrifuged briefly. Supernatants were saved for assay. Blood and plasma samples were kept on ice until frozen. A double-antibody radioimmunoassay procedure was used to measure plasma ghrelin in *experiment 2* and stomach tissue ghrelin in *experiment 1* as described in detail previously by our laboratory (33). The ghrelin antiserum was produced in rabbits by immunization with a synthetic carboxy-terminal fragment of rat ghrelin. This ghrelin antibody recognizes acyl- and desacyl-ghrelin equally; hence, ghrelin levels reflect total ghrelin (acyl- + desacyl-ghrelin). The sensitivity and ID_{50} (50% inhibition of bound radio-iodinated ligand) were 0.01 and 0.2 ng/tube, respectively.

Stomach ghrelin peptide was extracted from stomach tissue specimens as reported earlier (33). For *experiment 2*, plasma ghrelin was extracted from rat plasma (1.2 ml/sample) by use of C_{18} Sep Pak cartridges (Waters, Milford, MA). Samples were assayed in duplicate. Plasma insulin was measured as described previously (24). The sensitivity and ID_{50} for this insulin assay were 0.04 and 4.0 ng/ml, respectively. A double-antibody radioimmunoassay procedure was used to measure plasma leptin. The sensitivity and ID_{50} for this leptin assay were 1.0 and 6.0 ng/tube, respectively. All assays used specific antibodies that do not recognize structurally unrelated pancreatic or gastrointestinal peptides. For all assays, the intra- and interassay coefficients of variation were ~3–5.0 and ~8–12%, respectively.

RNA Purification and Northern Blot Analysis

Fresh tissues were homogenized immediately in 4 M guanidinium isothiocyanate containing 25 mM sodium citrate (pH 7.0), 0.5% sodium *N*-lauroylsarcosine, and 0.1 M β-mercaptoethanol (33). Extracts were frozen at −80°C until purification by ultracentrifugation over a cesium chloride cushion (2 ml, 5.7 M). RNA samples were separated on a 1% agarose gel (30 μg/lane) in a 20 mM MOPS running buffer system (33) and then transferred to a nylon membrane and subjected to Northern hybridization. ^{32}P-labeled riboprobes prepared from Strip-EZ RNA kits (catalog no. 1366, Ambion, Austin, TX) were used for Northern hybridizations. Ribosomal 18S was used to normalize for variations in RNA loading and transfer. Expression levels of ghrelin or the 18S genes were quantitated by phosphoimaging.

Statistics

Values are means ± SE. Data were evaluated using an ANOVA followed by Newman-Keuls test where appropriate. Statview software (Cary, NC) was used for data analysis.

RESULTS

Experiment 1

The purpose of *experiment 1* was to examine the role of body adiposity in the reduction of stomach ghrelin production and plasma ghrelin levels that occur in response to a long-term HF diet and to determine the extent to which a higher percentage of ingested dietary fat is a signal to reducing stomach ghrelin production and blood ghrelin levels. An HF diet, i.e., diet-induced obesity, will suppress ghrelin synthesis and secretion in rodents, and obesity will lower circulating ghrelin levels in humans (33, 50). To isolate the influence of increased body fat on stomach ghrelin production and secretion, T_3 treatment was given to rats fed an HF diet to prevent body fat accumulation. Because T_3 administration has been reported to stimulate food intake (32, 36) and food intake influences stomach ghrelin homeostasis, a group of rats ($HF_{PFHF+VEH} + T_3$) were T_3 treated and pair fed an HF diet according to the daily caloric intake of rats fed the HF diet but not given T_3. Another group of rats ($HF_{PFAIN +VEH}$ + VEH) were pair fed the HF diet according to the daily caloric intake of rats fed ad libitum the AIN-76A diet to determine the extent to which a higher percentage of ingested dietary fat is a signal to reducing stomach ghrelin production and blood ghrelin levels.

The body weights of rats given the HF diet plus vehicle injections (HF + VEH), but not the body weights of HF + T_3 rats, were significantly higher ($P < 0.05$) compared with rats given the AIN-76A diet plus vehicle injections (AIN + VEH) (Fig. 1). The body weights of AIN + VEH and AIN + T_3 rats did not differ significantly ($P > 0.05$). The body weights of rats ($HF_{PFAIN+VEH}$ + VEH) pair fed the HF diet according to the daily caloric intake of rats fed ad libitum the AIN-76A diet were significantly higher when compared with rats given the AIN-76A diet (AIN + VEH). The body weights of rats ($HF_{PFHF+VEH} + T_3$) given T_3 and pair fed an HF diet accord-

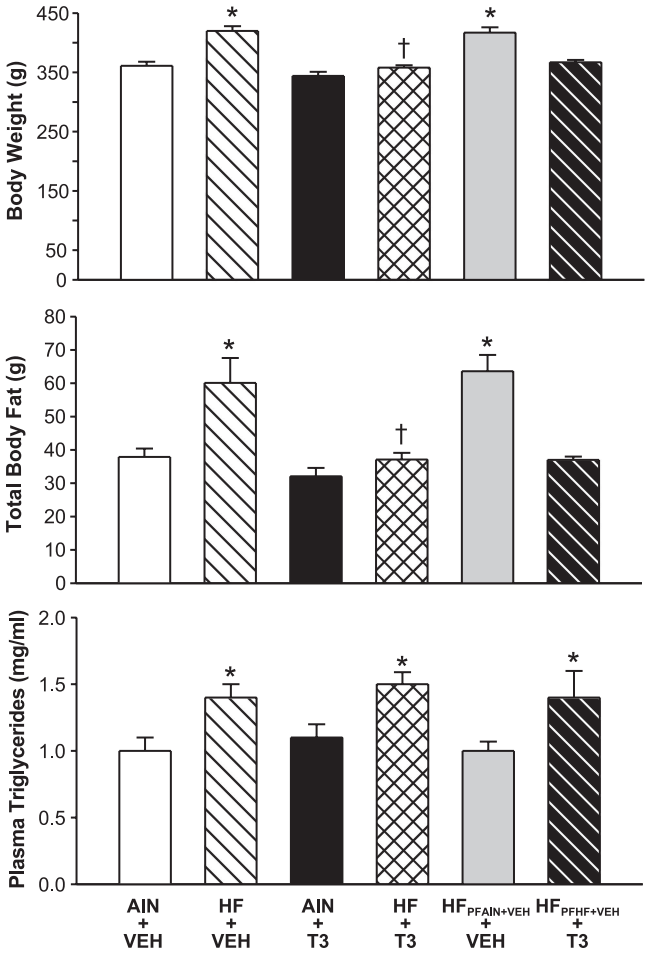

Fig. 1. Influence of a high-fat (HF) diet in combination either with vehicle (VEH) or triiodothyronine (T_3) treatments on body weight, total body fat, and plasma triglyceride levels in rats. Groups of rats pair fed (PF) according to the daily caloric intakes of AIN-76A diet (AIN) + VEH and HF + VEH are included ($HF_{PFAIN+VEH}$ + VEH; $HF_{PFHF+VEH}$ + T_3); the latter group was given T_3. Control rats were fed an AIN-76A diet. Rats were fed AIN-76A (5% fat) or HF (40–45% fat) diets for 8 wk. Body weights, total body fat, and plasma triglyceride levels were measured as described in the text. $*P < 0.05$ vs. AIN + VEH; $+P < 0.05$ vs. HF + VEH. n = 8–10 rats/group.

ing to the daily caloric intake of HF rats but not given T_3 (HF + VEH) did not differ significantly compared with rats given the AIN-76A diet.

Body composition analyses indicated that the body fat contents of HF + VEH but not HF + T_3 rats were significantly greater than the body fat contents of AIN + VEH rats (Fig. 1). The body fat contents of HF + T_3 rats were reduced significantly compared with HF + VEH rats. The body fat contents of AIN + T_3 and AIN + VEH rats did not differ significantly. The body fat contents of $HF_{PFAIN+VEH}$ + VEH rats were increased significantly compared with AIN rats and did not differ significantly compared with HF + VEH rats. The body fat contents of $HF_{PFHF+VEH}$ + T_3 rats were similar to those of AIN + VEH rats and significantly lower compared with HF + VEH rats.

With one exception, plasma triglyceride levels were significantly higher in rats fed the HF diet (HF + VEH; HF + T_3; $HF_{PFHF+VEH}$ + T_3) compared with rats fed the AIN-76A diet. In rats given $HF_{PFAIN+VEH}$ + VEH, plasma triglyceride levels did not differ compared with AIN + VEH rats.

Plasma ghrelin levels were reduced significantly in rats given HF + VEH or HF + T_3 compared with rats given AIN + VEH or AIN + T_3 (Fig. 2). Compared with AIN + VEH rats, plasma ghrelin levels were reduced significantly in rats ($HF_{PFAIN+VEH}$) pair fed the HF diet according to the daily caloric intake of rats fed ad libitum the AIN-76A diet (AIN + VEH). Compared with AIN + VEH rats, plasma ghrelin levels were reduced significantly in rats ($HF_{PFHF+VEH}$ + T_3) that were T_3 treated and pair fed an HF diet according to the daily caloric intake of HF + VEH rats.

In addition, stomach ghrelin mRNA levels were decreased significantly in rats given HF + VEH or HF + T_3 compared with rats given AIN + VEH or AIN + T_3. In rats given $HF_{PFAIN+VEH}$ + VEH or $HF_{PFHF+VEH}$ + T_3, stomach ghrelin mRNA levels were decreased significantly compared with rats given AIN + VEH or AIN + T_3.

Stomach ghrelin peptide levels in HF + VEH and HF + T_3 rats were reduced significantly compared with stomach ghrelin peptide levels in AIN + VEH or AIN + T_3 rats. Stomach ghrelin peptide levels of rats ($HF_{PFAIN+VEH}$ + VEH) pair fed the HF diet according to the daily caloric intake of rats fed ad libitum the AIN-76A diet were not altered significantly compared with AIN-fed rats. Stomach ghrelin peptide levels in $HF_{PFHF+VEH}$ + T_3 rats were reduced significantly compared with AIN + VEH rats.

Plasma leptin levels in HF + VEH rats were significantly higher compared with plasma leptin levels in AIN + VEH, AIN + T_3, HF + T_3, or $HF_{PFHF+VEH}$ + T_3 rats. In HF + T_3 and $HF_{PFHF+VEH}$ + T_3 rats, plasma leptin levels were reduced significantly compared with HF + VEH rats. Plasma leptin levels in $HF_{PFAIN+VEH}$ + VEH rats were increased significantly compared with AIN-76A-fed rats.

Plasma insulin levels in AIN + T_3 rats were significantly lower compared with all other groups. Plasma insulin levels in AIN + VEH, HF + VEH, HF + T_3, $HF_{PFAIN+VEH}$ + VEH, and $HF_{PFHF+VEH}$ + T_3 rats did not differ significantly.

Table 1 shows that daily food intakes of AIN +VEH and HF + VEH rats did not differ significantly. Daily food intakes of rats given AIN + T_3 were significantly higher compared with AIN + VEH rats.

Experiment 2

The aim of *experiment 2* was to determine the extent to which plasma ghrelin levels increase in response to acute fasting in rats fed a long-term HF diet. Control rats were fed the AIN-76A diet (12% fat). Interestingly, in rats fed an HF diet, plasma ghrelin levels increased significantly in response to fasting (Fig. 3). The plasma ghrelin increments in response to fasting were similar in rats fed HF (~1,300 pg) or AIN-76A (~1,400 pg) diets. Food intake was measured for a 2-wk period in rats fed AIN-76A and HF diets. Daily food intake, in terms of grams·day^{-1}·rat^{-1} in rats fed the AIN-76A diet was significantly greater compared with rats fed the HF diet (Table 2). In terms of kcal·day^{-1}·rat^{-1}, the daily food intakes did not differ significantly.

DISCUSSION

The primary aim of these experiments was to investigate the hypotheses that *1*) the increased body adiposity in rats fed an HF diet will exert an inhibitory influence on gastric ghrelin

production and systemic ghrelin levels, *2*) a higher percentage of dietary fat is a signal to reduce stomach ghrelin production and blood ghrelin levels, and *3*) an increased body adiposity will suppress the rise in circulating ghrelin levels in response to acute fasting. In the present studies, T_3 was used to avert body fat accumulation in rats fed the HF diet. It is well known that the thyroid hormones thyroxine and T_3 are the predominant regulators of basal metabolic rate and that circulating levels of thyroid hormones have a direct correlation with energy expenditure and calorie loss (35). T_3 administration may stimulate food intake (32, 36); it is possible, therefore, that stomach ghrelin homeostasis and plasma ghrelin levels are influenced partly by this T_3-induced increase in food intake. Therefore, stomach ghrelin homeostasis and secretion were measured in a group of rats treated with T_3 and pair fed the HF diet according to the daily caloric intake of rats fed the HF diet but not treated with T_3. We pair fed another group of rats the HF diet according to the daily caloric intake of rats fed ad libitum the AIN-76A diet to determine the extent to which a higher percentage of ingested dietary fat plays a role in the reductions of stomach ghrelin production and blood ghrelin levels during ingestion of an HF diet.

Table 1. *Average daily food intake of rats given AIN-76A or HF diets with vehicle or T3 treatments*

Group	Food Intake	
	Weight, g	kCal
AIN + VEH	20.2±1.2	69.8±4.2
AIN + T3	26.2±1.7*	90.6±5.7*
HF + VEH	18.2±1.6	82.1±7.1
HF + T3	19.4±1.2	87.7±5.4

Values are means ± SE; daily food intakes for the last 2 wk of *experiment 1*. VEH, vehicle; T3, triiodothyronine; AIN, AIN-76A diet; HF, high-fat diet. $*P < 0.05$ vs. AIN + VEH.

It is well documented that chronic ingestion of HF diets and the ensuing obesity will reduce stomach ghrelin production and plasma ghrelin levels in rodents; plasma ghrelin levels are also reduced in obese humans (33, 50). The source and identity of the inhibitory signal on ghrelin homeostasis during increased adiposity, however, are not known. One potential source of inhibitory signals is the increased body fat mass that accrues from regular ingestion of excess calories. Possible inhibitory signals secreted by adipose cells include several adipokines, such as leptin, apelin, adiponectin, resistin, and others (48). Studies in humans and rodents show that circulating levels of leptin and ghrelin can be inversely correlated (12, 50): in the isolated perfused rat stomach, leptin infusion will inhibit ghrelin secretion (28). Together, these earlier findings suggest that leptin may participate in the lowering of ghrelin homeostasis during ingestion of an HF diet and obesity. In the present study, plasma ghrelin levels were reduced in two groups of rats (HF + VEH and $HF_{PFAIN + VEH}$ + VEH) with elevated plasma leptin levels; both of these groups were fed the HF diet. However, plasma ghrelin levels were also reduced in rats without elevated plasma leptin levels (HF + T_3 and

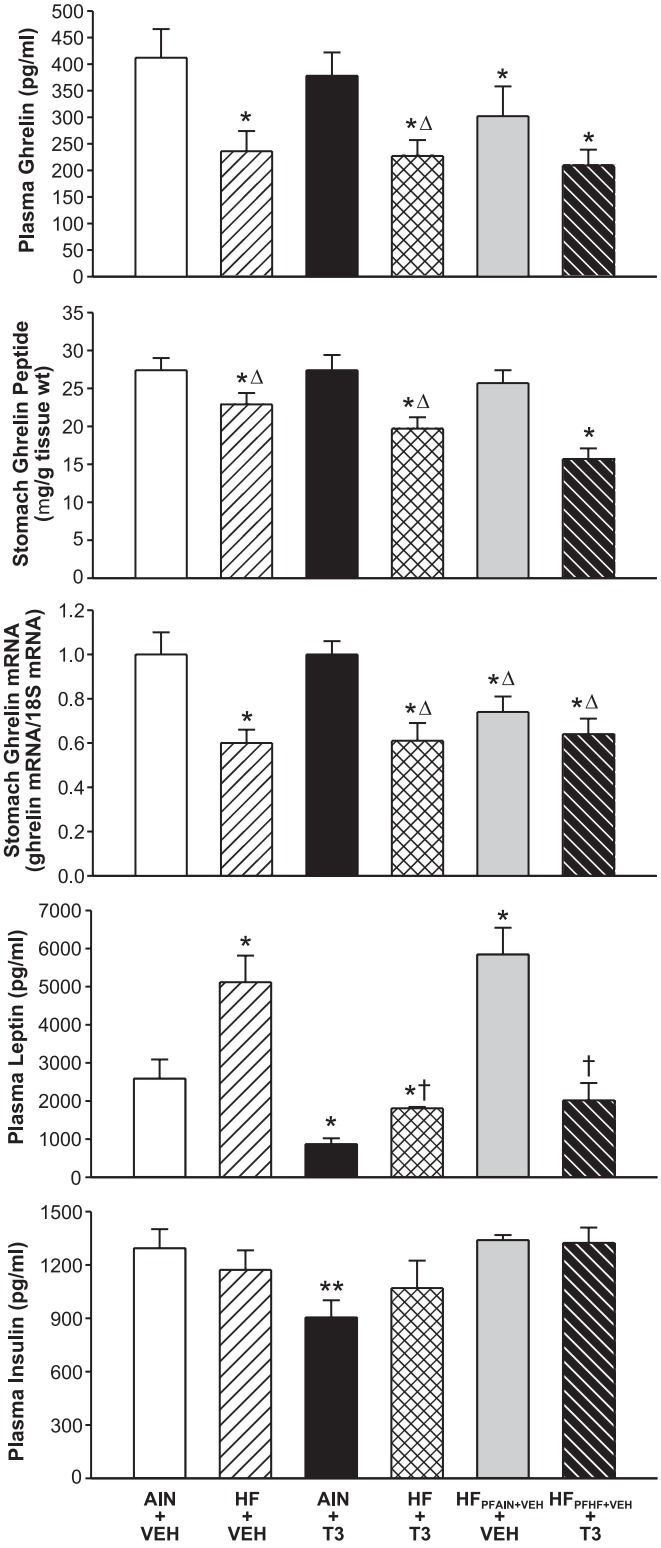

Fig. 2. Role of adipose tissue in suppression of stomach ghrelin production and secretion in obese rats. As described in the text and legend of Fig. 1, rats were fed an AIN-76A or HF diets for 8 wk. Groups of AIN- and HF-fed rats were given vehicle or T_3. Groups of rats pair fed according to the daily caloric intakes of AIN + VEH and HF + VEH are included ($HF_{PFAIN+VEH}$ + VEH; $HF_{PFHF+VEH}$ + T_3); the latter group was given T_3. At the end of the treatment period, plasma was collected for measurements of plasma ghrelin, leptin, and insulin levels, and stomachs were harvested and extracted for measurements of stomach ghrelin mRNA and peptide levels. $*P < 0.05$ vs. AIN + VEH; $\Delta P < 0.05$ vs. AIN + T_3. $\dagger P < 0.05$ vs. HF + VEH; $**P < 0.05$ vs. all other groups. $n = 8$–10 rats/group.

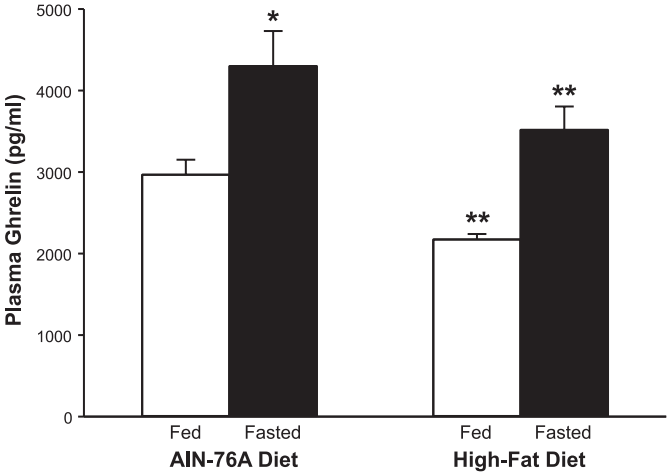

Fig. 3. HF diet does not inhibit the stomach ghrelin secretory response to acute fasting. Rats were fed AIN-76A (12% fat) or HF (40–45% fat) diet for 5 wk. Plasma was collected from ad libitum-fed (fed) and 24-h-fasted (fasted) rats for measurement of plasma ghrelin levels. $*P < 0.05$ vs. fed. $**P < 0.05$ vs. AIN-76A diet-fed.

$HF_{PFHF + VEH} + T_3$). These latter findings indicate that the reduced plasma ghrelin levels in rats fed an HF diet are not dependent on an elevation of circulating leptin levels and, furthermore, suggest that the inhibitory signal during ingestion of an HF diet is most likely not leptin. Alternatively, if the inhibitory signal includes leptin, leptin is a partial component. The lower plasma leptin levels in the HF + T_3 and $HF_{PFHF + VEH} + T_3$ groups are consistent with the reduced body fat content of these groups. In nonobese rats fed the HF diet (HF + T_3 and $HF_{PFHF + VEH} + T_3$), the amount of body adiposity was not significantly different from the amount measured in the lean AIN + VEH group. The influence of other adipokines, such as apelin, adiponectin, and resistin, on gastric ghrelin production and secretion has not been investigated.

Another potential inhibitor of plasma ghrelin levels during ingestion of an HF diet is insulin, since circulating insulin levels can be elevated during ingestion of chronic HF diets and obesity (15, 34, 39), and, in the isolated perfused rat stomach, insulin infusion will inhibit ghrelin secretion (28). Additionally, in humans, plasma ghrelin levels decrease in response to an insulin infusion or a meal, suggesting that insulin is a physiological inhibitor of ghrelin secretion (13, 18). It should be pointed out that other studies indicate that insulin does not affect ghrelin secretion (3). In the present report, plasma insulin levels were unaltered by the various treatment combinations, indicating that alterations in plasma ghrelin levels during ingestion of HF diets are not influenced by plasma insulin.

An elevated fat mass will also result in elevated systemic levels of several cytokines, including TNF-α and IL-6 (20, 27). The effects of exogenous TNF-α and IL-6 on gastric ghrelin production and secretion have not been investigated, although administration of LPS, an activator of IL-6 and TNF-α secretion (29, 30, 43, 44), will lower ghrelin secretion in rats (52).

Our results indicate that the reduction of gastric ghrelin homeostasis during ingestion of an HF diet is not associated primarily with an inhibitory signal from adipose tissue. Reductions of stomach ghrelin homeostasis and plasma ghrelin levels appear to be related to ingestion of the HF diet: it is an enteric signal. In nonobese rats fed the HF diet (HF + T_3 and $HF_{PFHF + VEH} + T_3$), the amount of body adiposity is equal to that measured in the lean AIN + VEH group. Despite normal body adiposity, gastric ghrelin production and secretion remain reduced.

In the present studies, we pair fed a group of rats ($HF_{PFAIN + VEH}$ + VEH) the HF diet according to the daily caloric intake of rats fed ad libitum the AIN-76A diet to determine the extent to which a higher percentage of ingested dietary fat signals reduction of stomach ghrelin production and blood ghrelin levels. Interestingly, plasma ghrelin and stomach ghrelin mRNA levels were reduced in these rats. However, these rats also have elevated body fat contents; therefore, it is impossible to discern the influence of a fat-calorie-dense diet on stomach ghrelin homeostasis and plasma ghrelin levels apart from other nutrition-associated effectors. However, the reduced plasma ghrelin and stomach ghrelin mRNA levels in the $HF_{PFAIN + VEH}$ + VEH group indicate that the inhibitory mechanism does not involve intake of excess calories, since calories were restricted. We did not expect to find increased body weight and body fat content in rats fed a calorie-restricted HF diet. These findings, however, agree with and extend earlier data showing that HF diets produce obesity partly because they produce a metabolically efficient state (41, 45, 54).

T_3 treatment has been reported to stimulate food intake in rats (32, 36). Interestingly, our findings show that food intake increased in the AIN + T_3 group compared with the AIN + VEH group, whereas food intake did not increase significantly in the HF + T_3 group compared with the HF + VEH group. To determine the extent to which this possible T_3-enhanced food intake plays a role in lowering stomach ghrelin production and secretion, we fed a group of rats ($HF_{PFHF + VEH} + T_3$) the HF diet and treated them with T_3 but restricted the daily amount of ingested calories to an amount consumed by rats fed the HF diet but not treated with T_3. In the $HF_{PFHF + VEH} + T_3$ group, stomach ghrelin production and secretion were reduced. Furthermore, T_3 treatment of rats fed the HF diet does not enhance food intake. Together, these findings indicate that the reduced stomach ghrelin production and secretion in the HF + T_3 group is not due to an enhanced food intake. Additionally, the persistent reductions in stomach ghrelin production and secretion in the $HF_{PFHF + VEH} + T_3$ group along with a normal body weight and total body fat content further support the idea that increased body fat does not have a role in the reduction of stomach ghrelin production and secretion in rats fed the HF diet. Although speculative, the marginal but insignificant reduction in plasma ghrelin levels of the AIN + T_3 group may be attributed to the increased food intake.

Table 2. *Average daily food intake of rats given AIN-76A or HF diets*

Group	Food Intake	
	Weight, g	kCal
AIN-76A	21.7±0.4	72.6±1.3
HF	15.8±0.4*	71.6±1.7

Daily food intakes were monitored for a 2-wk period in *experiment 2*. $*P < 0.05$ vs. AIN-76A.

Our results imply that the inhibitory signal on gastric ghrelin production and secretion during HF diet consumption is an enteric or pancreatic hormone, ingested nutrients, or both. As expected (11), the present study shows that HF diet consumption results in hyperlipidemia (accumulation of triglycerides in plasma). Because blood-borne nutrients can depress ghrelin secretion, the increased levels of blood lipids may act on the systemic side of ghrelin cells to reduce ghrelin secretion. The identity of a possible inhibitory enteric hormone during consumption of HF diets or obesity is not known, but it is expected to act systemically on ghrelin cells. Interestingly, our laboratory has reported that gastric lavage of soybean trypsin inhibitor, a secretagogue for release of intestinal cholecystokinin (40), will lower ghrelin secretion in fasted rats (22), implying that cholecystokinin might act to inhibit ghrelin secretion. Because physiological changes occur in muscle and liver tissue during consumption of HF diets and obesity (2, 16, 19), a factor(s) from these tissues might conceivably affect ghrelin homeostasis.

Ghrelin is a potent activator of food intake, and its secretion from the stomach is sensitive to caloric intake (22, 33). Our findings show that the acute elevation in secretion of gastric ghrelin in response to fasting in rats fed the HF diet is unaffected by an excess of stored calories, i.e., obesity. Furthermore, the plasma ghrelin increment in rats fed the HF diet was comparable to that measured in rats fed the AIN-76A diet: ~1,400 and ~1,300 pg/ml in AIN-76A- and HF-fed rats, respectively.

Stomach ghrelin is synthesized and circulates as acylated and desacylated variants (1, 26). Levels of desacyl-ghrelin predominate over acyl-ghrelin in the rat stomach, and plasma desacyl-ghrelin levels are ~4- to 10-fold higher than acyl-ghrelin levels (26, 56; unpublished observations). Although both ghrelin variants have multiple biological activities, only acyl-ghrelin stimulates food intake (31).

The enzyme that acylates ghrelin has been described recently (25, 55). Substrate for this enzyme is medium-chain, primarily C_8 and C_{10}, fatty acids. In our experiments, the HF diet consists of long-chain fatty acids. In *experiment 1*, plasma levels of acyl-ghrelin (C_8) are measured. Although speculative, the decreased plasma acyl-ghrelin levels measured in rats fed an HF diet may reflect, in part, the composition of the diet and an inadequate level of enzyme substrate. In contrast, in rats fed the AIN-76A diet, a diet with little fat and a reduced caloric density, the likelihood of β-oxidation and generation of medium-chain fatty acids for ghrelin acylation is greater.

Perspectives and Significance

Ghrelin is an exceptionally intriguing stomach hormone, since data indicate that luminal and systemic nutrient signals influence ghrelin secretion. Exactly how excess caloric intake and obesity exert a negative regulation over stomach ghrelin production and secretion is enigmatic. One very likely source of inhibitory signals is the elevated body fat mass. However, our results clearly indicate that adipose mass does not feed back to inhibit gastric ghrelin homeostasis. This is a primary finding of the present study. Another important finding is that the inhibitory signal is enteric in nature: it may be a gastrointestinal or pancreatic hormone or specific ingested nutrients. It is most likely a combination of multiple inhibitory signals. Future studies should address and elucidate identities of inhibitory signals.

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