

PLASMA GHRELIN LEVELS AFTER DIET-INDUCED WEIGHT LOSS OR GASTRIC BYPASS SURGERY

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ABSTRACT

Background Weight loss causes changes in appetite and energy expenditure that promote weight regain. Ghrelin is a hormone that increases food intake in rodents and humans. If circulating ghrelin participates in the adaptive response to weight loss, its levels should rise with dieting. Because ghrelin is produced primarily by the stomach, weight loss after gastric bypass surgery may be accompanied by impaired ghrelin secretion.

Methods We determined the 24-hour plasma ghrelin profiles, body composition, insulin levels, leptin levels, and insulin sensitivity in 13 obese subjects before and after a six-month dietary program for weight loss. The 24-hour ghrelin profiles were also determined in 5 subjects who had lost weight after gastric bypass and 10 normal-weight controls; 5 of the 13 obese subjects who participated in the dietary program were matched to the subjects in the gastric-bypass group and served as obese controls.

Results Plasma ghrelin levels rose sharply shortly before and fell shortly after every meal. A diet-induced weight loss of 17 percent of initial body weight was associated with a 24 percent increase in the area under the curve for the 24-hour ghrelin profile ($P=0.006$). In contrast, despite a 36 percent weight loss after gastric bypass, the area under the curve for the ghrelin profile in the gastric-bypass group was 77 percent lower than in normal-weight controls ($P<0.001$) and 72 percent lower than in matched obese controls ($P=0.01$). The normal, meal-related fluctuations and diurnal rhythm of the ghrelin level were absent after gastric bypass.

Conclusions The increase in the plasma ghrelin level with diet-induced weight loss is consistent with the hypothesis that ghrelin has a role in the long-term regulation of body weight. Gastric bypass is associated with markedly suppressed ghrelin levels, possibly contributing to the weight-reducing effect of the procedure. (N Engl J Med 2002;346:1623-30.)

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OBESITY represents a global epidemic¹ and is a leading cause of illness and death worldwide.^{2,3} Weight reduction achieved by dieting, exercise, or medical therapy often elicits compensatory changes in appetite and energy expenditure^{4,5} that make weight loss of more than 5 to 10 percent unlikely to be sustained.⁶⁻¹⁰ In contrast, gastric bypass surgery, in which most of the stomach and duodenum are bypassed with the use

of a gastrojejunal anastomosis, typically causes substantial, long-term weight loss.¹¹⁻¹³ The operation appears to undermine the normal compensatory physiologic responses to energy deficit. This effect is unlikely to result from gastric restriction alone, and it has been proposed that a disruption of gut-derived factors that regulate eating behavior is involved, although no such factors have been identified.^{14,15}

Ghrelin is a recently discovered¹⁶ orexigenic hormone that is secreted primarily by the stomach and duodenum¹⁷ and has been implicated in both meal-time hunger and the long-term regulation of body weight.¹⁸⁻²⁴ In humans, plasma ghrelin levels rise shortly before and fall shortly after every meal, a pattern that is consistent with a role in the urge to begin eating.²⁴ If circulating ghrelin participates in long-term regulation of body weight, its level should increase with weight loss as part of the compensatory response to an energy deficit. In contrast, gastric bypass may disrupt ghrelin secretion by isolating ghrelin-producing cells from direct contact with ingested nutrients, which normally regulate ghrelin levels,^{18,24} and this effect may contribute to the efficacy of the procedure in reducing weight. To test these hypotheses, we determined the 24-hour plasma ghrelin profiles in subjects before and after diet-induced weight loss, and compared these values with those in subjects who had lost weight after proximal gastric bypass surgery.

METHODS

Study Subjects

Subjects in the dietary-weight-loss group and normal-weight controls were recruited through advertising in local newspapers and on the campus of the University of Washington. All subjects were at least 18 years old and had had stable body weight for at least three months. Criteria for exclusion included chronic medical or psychiatric illness, pregnancy, tobacco use, substance abuse, consumption of more than two alcoholic drinks per day, aerobic exercise for more than 30 minutes three times per week, and previous gastrointestinal surgery. The Human Subjects Review Committee at the University of Washington approved all procedures and

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protocols, and written informed consent was obtained from all subjects before enrollment. Studies were performed at the General Clinical Research Center of the University of Washington.

Protocol for Diet-Induced Weight Loss and Stabilization

Through the Nutrition Research Unit of the research center, 13 obese subjects (Table 1) received a low-fat, high-protein, liquid-formula diet of 1000 kcal per day, supplemented with daily multivitamins and minerals, for three months. Subjects then underwent a gradual transition over the course of two weeks to a solid diet containing 30 percent fat, 15 percent protein, and 55 percent carbohydrates — a macronutrient composition approximating that of the average diet in the United States.²⁵ The total number of calories was adjusted to stabilize weight, and subjects were taught to continue this process at home in order to maintain a stable, reduced weight for three months. Throughout the six months of the program of weight loss and stabilization, subjects met with research dietitians two to three times per week and were weighed on a single scale.

At the beginning and end of the study, subjects were admitted to the research center after fasting overnight, and intravenous catheters were placed in both arms. Blood was withdrawn for measurement of insulin and leptin, followed by a tolbutamide-modified frequently sampled intravenous glucose-tolerance test to determine insulin sensitivity with the use of the minimal model of glucose kinetics.²⁶ Blood was collected in EDTA tubes every 30 minutes between 8 a.m. and 9 p.m., then hourly until 8 the following morning (24 hours in all). Samples were stored at 4°C during the collection period, after which plasma was stored at -80°C. Breakfast, lunch, and dinner (based on the solid diet described above) were served at 8 a.m., noon, and 5:30 p.m., respectively. At the beginning and end of the study, the percentage of body fat was measured by underwater weighing,²⁷ the volumes of subcutaneous and visceral fat were assessed by computed tomography,²⁸ and the adipocyte volume was determined by applying Goldrick's equation to the measured diameters of 400 adipocytes aspirated from the posterior superior iliac-crest region.²⁹

Gastric-Bypass Study

The 24-hour plasma ghrelin profiles were determined as described above in subjects who had undergone a proximal Roux-en-Y gastric bypass 9 to 31 months previously (mean [\pm SE], 1.4 \pm 0.4 years) (Table 1). These profiles were compared with the 24-hour ghrelin profiles from two control groups without previous gastrointestinal surgery: normal-weight subjects and a group of matched obese subjects who had recently lost weight by dieting and were matched to the subjects in the gastric-bypass group according to final body-mass index (the weight in kilograms divided by the square of the height in meters), age, and sex. The latter group was a subgroup of the dietary-weight-loss group and was included to control for the effects of obesity, age, and sex on ghrelin levels.

Subjects in all three groups had stable weight at the time of 24-hour sampling, as defined by a change of no more than 5 percent in body weight during the preceding three months. We selected normal-weight controls whose weight was at its lifetime maximum and who had maintained this weight for at least three months; during the two weeks preceding blood sampling, their caloric intake was adjusted twice weekly to maintain stability of weight. During this period, the average body-mass index of the group varied by only 0.4 \pm 0.3 percent.

Hormone Assays

Plasma immunoreactive ghrelin was measured in duplicate with a radioimmunoassay involving an iodine-125-labeled bioactive ghrelin tracer and a rabbit polyclonal antibody against full-length, octanoylated human ghrelin that recognizes the acylated and desacyl forms of the hormone (Phoenix Pharmaceuticals). The lower and upper limits of detection were 80 and 2500 pg per milliliter

TABLE 1. CHARACTERISTICS OF THE SUBJECTS.*

VARIABLE	DIETARY-WEIGHT-LOSS GROUP (N=13)	GASTRIC-BYPASS GROUP (N=5)	NORMAL-WEIGHT CONTROLS (N=10)	MATCHED OBESE CONTROLS (N=5)
Age (yr)	42.9 \pm 2.4	43.6 \pm 4.8	48.0 \pm 4.1	44.8 \pm 4.7
Sex (no.)				
Female	8	3	9	3
Male	5	2	1	2
Body-mass index†				
Initial	35.6 \pm 1.6	68.0 \pm 7.8	27.4 \pm 0.9	48.2 \pm 5.2
Final	29.4 \pm 1.5	43.5 \pm 6.0	27.3 \pm 0.9	40.0 \pm 3.9
Loss (%)	17.4 \pm 1.5	36.0 \pm 4.8	0.4 \pm 0.3	17.0 \pm 3.0

*Plus-minus values are means \pm SE.

†The body-mass index is the weight in kilograms divided by the square of the height in meters.

(24 and 740 pmol per liter), respectively, and in 41 assays, the coefficient of variation was 6.9 percent within assays and 12.8 percent between assays. Plasma insulin was measured in duplicate with a double-antibody radioimmunoassay,³⁰ and leptin was measured in duplicate with a commercial radioimmunoassay kit (Linco Research).

Statistical Analysis

Hormone levels are expressed as means \pm SE. Values for the area under the curve for the 24-hour ghrelin profile were calculated with the use of the trapezoidal rule. End points were compared with the use of two-tailed, paired Student's *t*-tests, and exact binomial methods were used where indicated. Correlations were determined by univariate linear regression.

RESULTS

Effect of Diet-Induced Weight Loss on Plasma Ghrelin Levels

Thirteen obese subjects with stable weight underwent diet-induced weight loss for three months, followed by three months of stabilization at the reduced weight. At the end of this period, subjects had lost a mean (\pm SE) of 17.4 \pm 1.5 percent of their initial body weight ($P<0.001$) (Table 2). A total of 84 percent of the lost weight came from fat and was derived relatively evenly from intraabdominal and subcutaneous adipose tissue. Weight loss was associated with significant reductions in adipocyte volume, leptin levels, insulin levels, and blood pressure, as well as with increased insulin sensitivity and improved lipid profiles (Table 2).

The 24-hour plasma ghrelin profiles determined at the beginning and end of the study are shown in Figure 1. The temporal pattern of the levels of circulating ghrelin was similar before and after weight loss. Levels rose progressively for one to two hours

TABLE 2. EFFECTS OF DIET-INDUCED WEIGHT LOSS.*

VARIABLE	BEFORE WEIGHT LOSS	AFTER WEIGHT LOSS	PERCENT CHANGE†	P VALUE‡
Body weight (kg)	99.8±5.6	82.5±5.2	-17.4±1.5	<0.001
Body-mass index	35.6±1.6	29.4±1.5	-17.4±1.5	<0.001
Underwater weighing				
Fat (% of body weight)	42.2±1.9	33.2±2.3	-22.0±3.0	<0.001
Total fat mass (kg)	42.3±3.3	27.7±2.8	-35.3±3.3	<0.001
Nonfat (% of body weight)	57.8±1.9	66.8±2.3	15.5±1.8	<0.001
Total nonfat mass (kg)	57.5±3.5	54.8±3.5	-4.8±1.3	0.003
Abdominal CT				
Intraabdominal fat (cm ²)	143±16	76±13	-46.6±5.4	<0.001
Subcutaneous fat (cm ²)	494±48	317±43	-35.8±5.0	<0.001
Total abdominal area (cm ²)	940±57	689±53	-26.9±2.8	<0.001
Fat-cell volume (μg of triglycerides/cell)	0.73±0.03	0.52±0.03	-28.8±4.2	<0.001
Fasting plasma leptin level (ng/ml)	26.8±4.4	16.7±3.5	-37.7±9.8	0.003
Fasting plasma insulin level (μU/ml)	15.6±2.6	10.9±1.8	-30.1±7.1	0.004
Insulin-sensitivity index§	2.05±0.49	3.04±0.72	48.3±19.2	0.01
Cholesterol level (mg/dl)				
Total	195±8	173±9	-11.3±3.6	0.01
LDL	130±6	110±8	-15.4±4.5	0.005
HDL	44±4	44±3	0±4.1	0.93
Triglycerides	111±24	101±22	-9.0±12.1	0.92
Diastolic blood pressure (mm Hg)	80±3	72±3	-10.0±4.5	0.03

*Plus-minus values are means ±SE. To convert values for leptin to nanomoles per milliliter, multiply by 0.08; to convert values for insulin to picomoles per liter, multiply by 6; to convert values for total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol to millimoles per liter, multiply by 0.026; to convert values for triglycerides to millimoles per liter, multiply by 0.011. CT denotes computed tomography.

†Data are the means (±SE) of the percent changes in values in individual subjects.

‡For all end points except triglycerides, values before weight loss were compared with values after weight loss by paired, two-tailed Student's t-tests. Because values for triglycerides were not normally distributed, they were compared by the Mann-Whitney rank-sum test.

§The insulin-sensitivity index was determined by the minimal model of glucose kinetics²⁶ and calculated as the increase of the fractional turnover of glucose per unit increase in insulin concentration (in microunits per minute), multiplied by 10⁻⁴.

before each meal and fell to trough levels within one to two hours after the subjects began eating. The ghrelin level increased from premeal troughs by a mean of 20 percent before breakfast, 45 percent before lunch, and 51 percent before dinner. Between-meal ghrelin values rose gradually throughout the day in a diurnal pattern, with a nadir between 9 a.m. and 10 a.m. and a peak between midnight and 2 a.m., as in normal-weight subjects.²⁴

After weight loss, the mean plasma ghrelin level increased at every time point throughout the 24-hour period (Fig. 1), and the mean (±SE) area under the curve in the ghrelin profile increased by 24 percent (9365±1127 pg-days per milliliter [2772±334 pmol-days per liter] before weight loss, as compared with 11,585±1449 pg-days per milliliter [3429±429 pmol-days per liter] after weight loss, P=0.006). For each meal, the maximally suppressed postprandial mean ghrelin level after weight loss was only slightly lower than the peak preprandial level before weight loss.

Individual values for the area under the curve increased with weight loss in 12 of the 13 subjects (P=0.003 by the exact binomial test). Among these 12, there was a positive correlation between the percentage decrease in either body weight or body-mass index and the percentage increase in the area under the curve (R=0.67, P=0.01). There were similar trends toward positive correlation between the magnitude of the increase in the area under the curve for the ghrelin profile and the magnitude of the decrease in all other measures of adiposity shown in Table 2.

Plasma Ghrelin Levels after Gastric Bypass Surgery

The 24-hour plasma ghrelin profile was determined for subjects in three groups with stable weight (Table 1): obese subjects who had undergone a proximal Roux-en-Y gastric bypass; normal-weight controls; and matched obese controls who had recently lost weight by dieting and were matched to the subjects in the gastric-bypass group according to the final body-mass

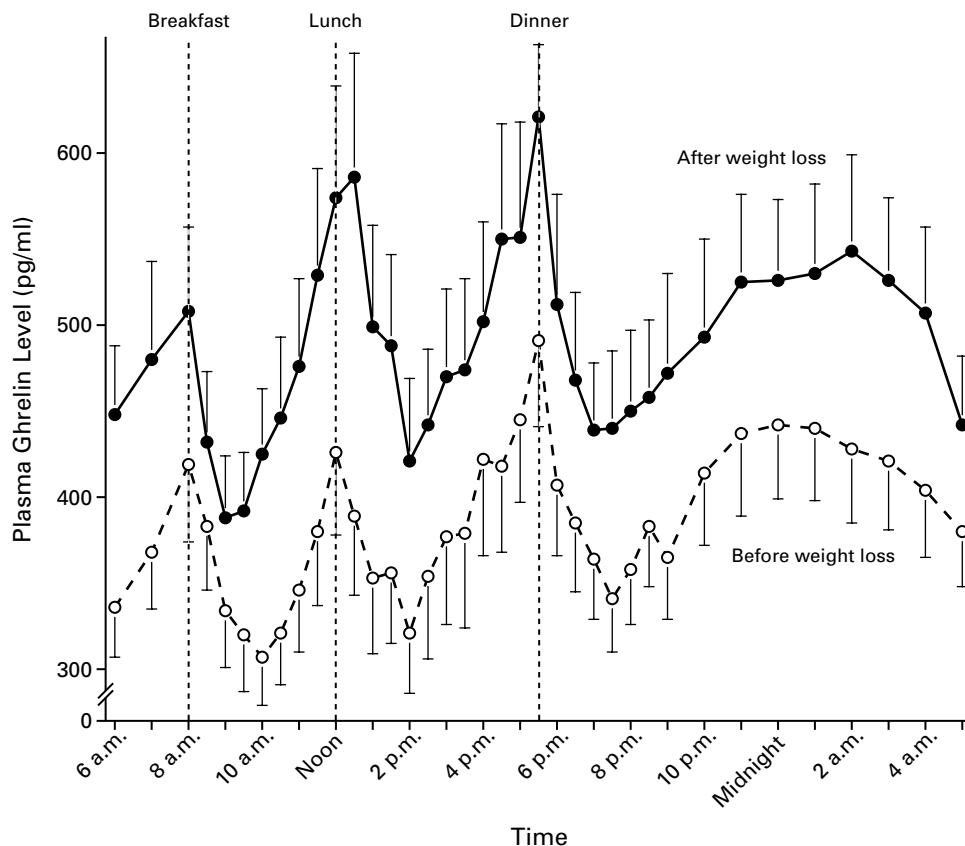


Figure 1. Mean (\pm SE) 24-Hour Plasma Ghrelin Profiles in 13 Obese Subjects before and after Diet-Induced Weight Loss. Breakfast, lunch, and dinner were provided at the times indicated. To convert ghrelin values to picomoles per liter, multiply by 0.296.

index (at the end of the six-month period), age, sex, and fasting plasma leptin level (23.2 ± 9.3 ng per milliliter [1.9 ± 0.7 nmol per milliliter] in the matched obese controls and 21.1 ± 9.8 ng per milliliter [1.7 ± 0.8 nmol per milliliter] in the gastric-bypass group).

Plasma ghrelin levels were markedly lower in the gastric-bypass group than in either control group (Fig. 2). The area under the curve for the 24-hour ghrelin profile of subjects in the gastric-bypass group (3058 ± 718 pg-days per milliliter [905 ± 213 pmol-days per liter]) was 77 percent lower than that in the normal-weight controls ($13,401 \pm 1785$ pg-days per milliliter [3967 ± 528 pmol-days per liter], $P < 0.001$) and 72 percent lower than that in the matched obese controls ($10,803 \pm 3591$ pg-days per milliliter [3198 ± 1063 pmol-days per liter], $P = 0.01$). The ghrelin profile of subjects in the gastric-bypass group showed neither the meal-related oscillations nor the diurnal rhythm that were found in the profiles of both con-

trol groups and the profiles displayed in Figure 1. Instead, ghrelin levels in subjects who underwent gastric bypass remained only slightly above the limit of detection throughout the day. We have previously shown that the temporal pattern of circulating ghrelin is the reciprocal of that of insulin and in phase with that of leptin.²⁴ Subjects in the gastric-bypass group had normal postprandial insulin spikes and an intact diurnal rhythm of leptin levels (Fig. 3), indicating that physiologic changes in the levels of these hormones are not sufficient to cause the normal daily variation in plasma ghrelin after gastric bypass.

DISCUSSION

Our data are consistent with the hypothesis that ghrelin has a role in both mealtime hunger and the long-term regulation of body weight. We have confirmed in obese subjects our previous findings in lean persons²⁴ that plasma ghrelin levels rise shortly be-

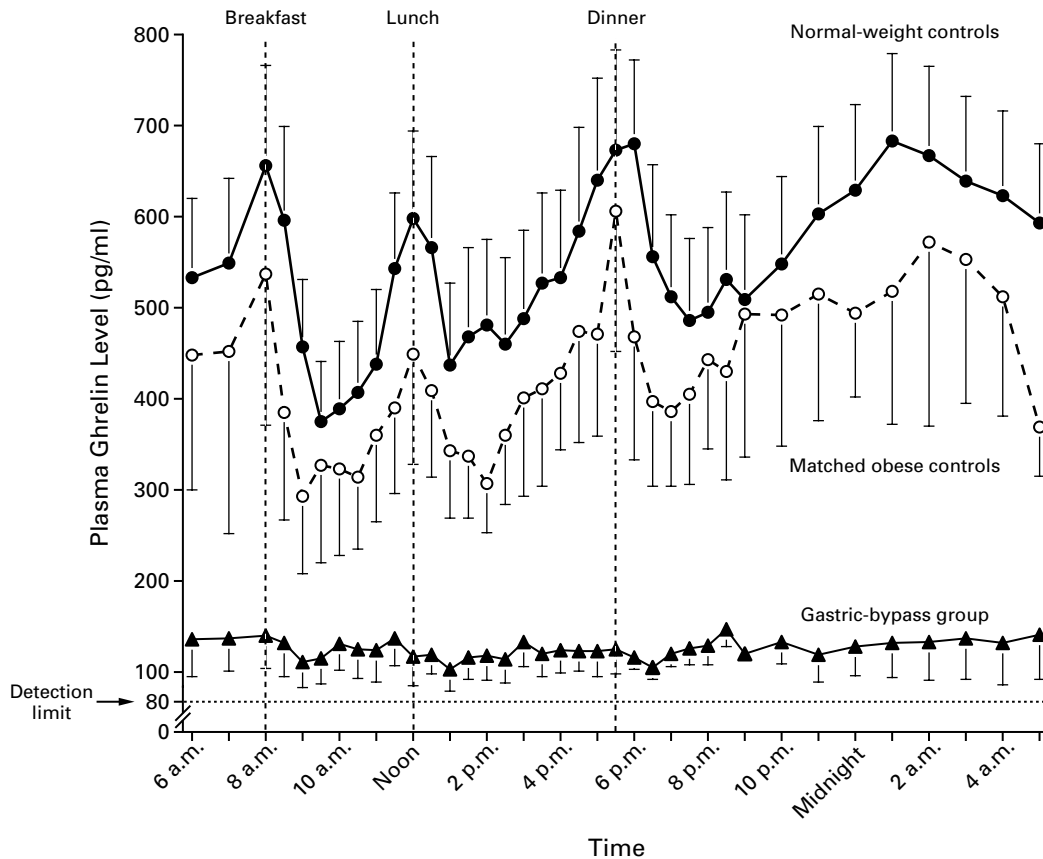


Figure 2. Mean (\pm SE) 24-Hour Plasma Ghrelin Profiles in Subjects Who Underwent Gastric Bypass and in Controls.

The study groups represented are 5 obese subjects who underwent a proximal Roux-en-Y gastric bypass, 10 normal-weight controls, and 5 obese subjects who had recently lost weight by dieting and were matched to the subjects in the gastric-bypass group according to final body-mass index, age, and sex. Breakfast, lunch, and dinner were provided at the times indicated. To convert ghrelin values to picomoles per liter, multiply by 0.296.

fore and fall shortly after every meal. This pattern is consistent with a model according to which ghrelin, a gut hormone with rapid, short-lived orexigenic effects in rodents,¹⁸⁻²¹ has a causal role in mealtime hunger in humans.

Moreover, the levels of circulating ghrelin over a 24-hour period increased after diet-induced weight loss — a finding that is consistent with a role for ghrelin in the long-term regulation of body weight in humans. Several observations from studies in rodents provide further support for such a role. First, continuous administration of ghrelin durably increases body weight.^{18,19,21} Second, in addition to increasing food intake, exogenous ghrelin decreases the metabolic rate²¹ and the catabolism of fat,¹⁸ thereby affecting all aspects of the system of energy regulation in

such a way as to increase body weight. Finally, blockade of ghrelin in the brain leads to a reduction in food intake,¹⁹ suggesting that endogenous ghrelin signaling is required to maintain normal appetite. Our finding that plasma ghrelin levels rise with diet-induced weight loss suggests that increased levels of circulating ghrelin may participate in the adaptive responses that constrain such weight loss.

Plasma ghrelin levels in subjects who underwent gastric bypass did not oscillate in relation to meals and were markedly lower than those of both lean controls and matched obese controls, despite massive weight loss. Thus, whereas weight loss achieved by caloric restriction was associated with increased plasma ghrelin levels, that achieved by gastric bypass was associated with abnormally low levels. In addition

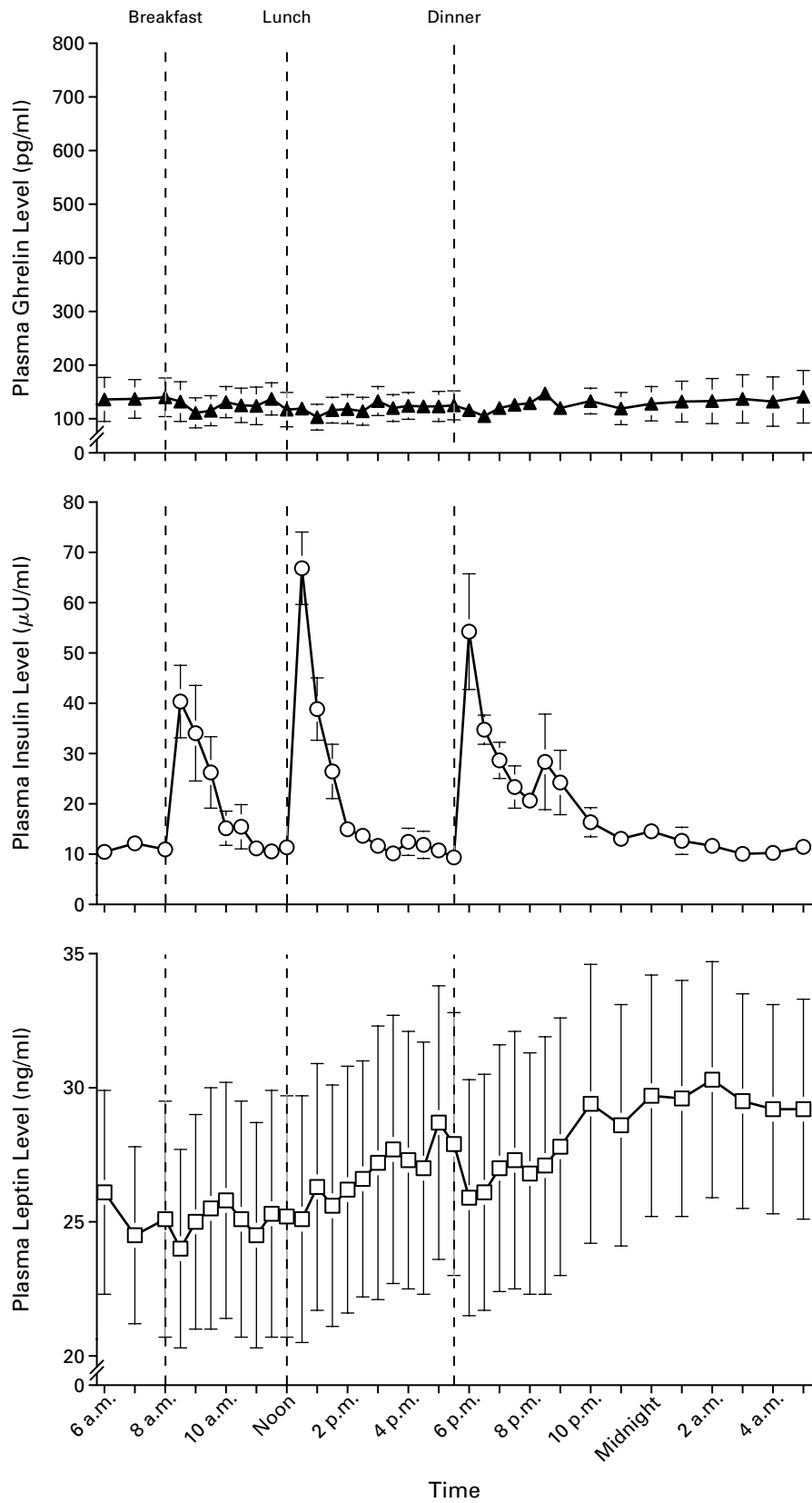


Figure 3. Mean (\pm SE) 24-Hour Profiles of Plasma Ghrelin, Insulin, and Leptin in Five Subjects Who Underwent Gastric Bypass.

Breakfast, lunch, and dinner were provided at the times indicated. To convert values for ghrelin to picomoles per liter, multiply by 0.296. To convert values for insulin to picomoles per liter, multiply by 6. To convert values for leptin to nanomoles per milliliter, multiply by 0.08.

to having altered gastrointestinal anatomy, subjects who underwent gastric bypass differed from the normal-weight controls in that they had lost substantial amounts of body weight and in that they were (still) obese. However, neither of these differences accounts for the suppression of ghrelin in these subjects. Although it has been shown that obesity is associated with mildly decreased levels of circulating ghrelin,³¹ the area under the curve for the 24-hour ghrelin profile of the matched obese controls in our study was 3.5 times that of the subjects in the gastric-bypass group, even though these groups had the same average body-mass index. Nor can the very low ghrelin values in the gastric-bypass group be explained by the fact that the subjects in that group had lost more weight than the matched obese controls, since weight loss should increase the plasma ghrelin level (Fig. 1). Thus, it seems clear that gastric bypass surgery is itself associated with decreased levels of circulating ghrelin.

These findings raise the possibility that suppression of ghrelin is one mechanism by which gastric bypass reduces body weight. Gastric restriction after this operation should cause early satiety, and this effect has been clearly documented.^{13,32} However, if gastric restriction were the only important alteration, patients who had undergone such surgery would be expected to eat more frequent, small meals and to favor calorie-dense foods, especially with continued weight loss, which should elicit compensatory hyperphagia. In contrast, patients who undergo gastric bypass have been shown to feel hungry less often after the operation,³²⁻³⁴ eat fewer meals and snacks per day,³² and voluntarily reduce their intake of calorie-dense foods such as fats, high-calorie carbohydrates, high-calorie beverages, red meat, and ice cream.^{32,34,35} These alterations occur despite the fact that patients report no change in their perception of the deliciousness of high-calorie carbohydrates (i.e., sweets) or in their overall enjoyment of food.³² This finding suggests that other mechanisms beyond gastric restriction contribute to the loss of appetite and body weight caused by gastric bypass.

Our finding of markedly reduced ghrelin levels after gastric bypass suggests that suppression of ghrelin can now be studied as a potential mechanism by which this procedure causes weight loss. This hypothesis offers a plausible explanation for the paradoxical reduction of hunger between meals that occurs after gastric bypass, as well as for the observation that the procedure is more effective than gastroplasty in facilitating long-term weight loss.^{12-14,35-40} These operations produce equivalent gastric restriction,^{35,41} but only gastric bypass isolates ghrelin cells from contact with enteral nutrients.

The mechanism by which gastric bypass leads to a

reduction in ghrelin levels remains to be determined. Our data show that ingested nutrients powerfully regulate the level of circulating ghrelin. Although an empty stomach is associated with an increased ghrelin level in the short term, it is possible that the permanent absence of food in the stomach and duodenum that results from gastric bypass causes a continuous stimulatory signal that ultimately suppresses ghrelin production through the process of "override inhibition." By this mechanism, continuous gonadotropin-releasing hormone signaling initially stimulates but eventually suppresses gonadotropin secretion,⁴² and a similar desensitization occurs with the unabated stimulation of growth hormone by growth-hormone-releasing hormone.⁴³ The possibility that override inhibition occurs in the case of ghrelin is suggested by our data showing a progressive decline in the circulating level during an overnight fast (Fig. 1 and 2).²⁴

In summary, 24-hour plasma ghrelin levels increase in response to diet-induced weight loss, suggesting that ghrelin may play a part in the adaptive response that limits the amount of weight that may be lost by dieting. We also found that ghrelin levels are abnormally low after gastric bypass, raising the possibility that this operation reduces weight in part by suppressing ghrelin production. These data suggest that ghrelin antagonists may someday be considered in the treatment of obesity.

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