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A twin study of differences in the response of plasma ghrelin to a milkshake preload in restrained eaters

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Abstract

Background—Genetic, physiological, and psychological factors can affect food intake, but twin studies can distinguish inherited from environmental contributors. We examined the influence of attempted cognitive control of eating (“restrained eating”) on levels of appetite-regulating hormones.

Methods—Sixteen female, monozygotic twin pairs, discordant for Restraint Scale score (i.e., one twin a restrained eater with score > 15 whereas the co-twin was unrestrained), were selected from the University of Washington Twin Registry. Serial plasma ghrelin concentrations were monitored

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during meals and a preload study paradigm involving intake of a milkshake followed by an *ad libitum* ice cream “taste test.”

Results—Body weight, body mass index, resting energy expenditure, and fasting leptin levels were very similar between restrained and unrestrained twins. In a preload study, twins ate similar amounts of ice cream shortly after drinking identical milkshakes (mean $\pm$ SD; restrained 239 ± 158 vs. unrestrained 228 ± 132 kilocalories; $P = 0.83$). However, ghrelin concentrations during the preload study were significantly higher ($P = 0.03$) in restrained twins than in their unrestrained co-twins. Regardless of restraint status, ghrelin levels prior to the preload study were prospectively and positively associated with ice cream intake ($P = 0.001$).

Conclusions—Compared to their unrestrained co-twins, restrained twins had higher endogenous ghrelin levels during a preload study, but ate similar amounts. This finding is consistent with exertion of cognitive control relative to the state of physiologic appetite stimulation. Moreover, these findings in twins suggest that higher ghrelin levels result from restrained eating behavior and not from genetic predisposition.

Keywords

restrained eating; ghrelin; appetite; genetic

1. Introduction

Twin studies are a useful tool for controlling common confounding factors that affect food intake, including age, sex and genetics. If reared together, twins are also typically extraordinarily well-matched for shared family background and childhood exposures, allowing for further control of unmeasured family environment factors. In a co-twin control design, the characteristics of a twin with the phenotype of interest – in this case restrained eating – are compared to the characteristics of the unaffected co-twin.

Restrained eating is a construct that describes an individual's attempt to manage body weight by cognitively controlling food intake (1). Despite attempted restraint, evidence is mixed as to whether restrained eaters maintain a hypocaloric state (2, 3). Their weight fluctuates, and their body mass index (BMI) and maximum lifetime weights are often higher than those observed in unrestrained eaters (4). Paradoxically, some restrained eaters also display a propensity to overeat or “disinhibit” eating (2). The classic description of this phenomenon showed that restrained eaters increased their ice cream intake after a high-calorie milkshake preload (5). Disinhibition also can occur among restrained eaters who falsely believe that they have just eaten a high-calorie food (6), or in the presence of stress (7) or anxiety (8). Unraveling the relationship of restraint to disinhibited eating is critical to contextualize studies that prospectively associate eating restraint with weight gain in adult women (9) and with the onset of obesity in girls (10). Although cognitive and psychological triggers probably contribute to disinhibited eating (11), they do not fully explain the findings (12). Recently, attention has focused on potential physiologic links between these behaviors.

Studies suggest a hormonal milieu consistent with appetite stimulation in restrained eaters, including low leptin (13, 14) and high ghrelin levels (15). Both hormones act in key brain

areas known to regulate energy homeostasis and exhibit compensatory responses to weight loss (16, 17). Plasma ghrelin levels also increase before mealtimes (17) and are suppressed after eating in proportion to the number of calories consumed (18), but the extent to which endogenous plasma levels and/or dynamic changes in ghrelin contribute to the physiological drive to eat is less well understood. Thus, both low leptin and elevated ghrelin levels are potential, but as yet unconfirmed, contributors to the paradoxical overeating observed in restrained eaters. We therefore hypothesized that ghrelin levels and *ad libitum* caloric intake would be consistently higher among restrained-eating monozygotic (MZ) twins in the context of a preload study paradigm designed to trigger disinhibited eating as well as during a typical meal, accompanied by lower leptin levels.

2. Study Design and Methods

2.1 Participants and recruitment

Study participants were female MZ twin pairs enrolled in the University of Washington Twin Registry (19). In August 2006, a written health survey, including the Restraint Scale (see below), was mailed to all twins then enrolled in the Registry ($n = 4407$), with a 55% response rate (20). Responding twin pairs who were MZ based on questionnaire responses (19), met age criteria (18-65 years), had been raised together in the same household until at least age 15, and were discordant for restrained eating (as defined below) were invited to participate. To distract attention from our interest in eating restraint, we provided a cover letter explaining that the study would investigate genetic and hormonal influences on taste perception. All Registry and data collection procedures were approved by the University of Washington Institutional Review Board, and written informed consent was obtained from all participants. Exclusionary criteria were major medical illness, pregnancy, current enrollment in a weight-loss program, use of weight-loss medication, allergies to research protocol foods, inability to taste, current smoking, current consumption of more than two alcoholic drinks per day, recreational drug use, lifetime history of an eating disorder or weight-loss surgery, participation in intense daily aerobic exercise, and self-reported weight and height at the time of screening that indicated a BMI under 20 kg/m^2 or over 30 kg/m^2 . Nineteen percent of individuals screened were ineligible.

2.2 Eating behavior measures

The revised Restraint Scale (21) is a 10-item self-report questionnaire that identifies individuals who have a high degree of concern with weight and dieting but disinhibit their eating in certain situations (22). We defined restrained eating as a score of at least 15 on the Restraint Scale (23). For a twin pair to be considered discordant, the restrained twin also had to score at least 3 points higher than her co-twin. The internal reliability, test-retest reliability, and predictive validity of the Restraint Scale are well established (24), although scores may be overestimated in obese populations (25). We therefore limited our sample to non-obese twins. The Three-Factor Eating Questionnaire Revised-18 item (TFEQ-R18) was administered during the study visit to measure uncontrolled and emotional eating (26).

2.3 Preload Study

The preload study is an objective test of overeating among restrained eaters as identified by the Restraint Scale. It is significant because it elicits the classic example of disinhibited eating of high-calorie foods following consumption of a high-calorie preload (5). Details of the procedure are provided below.

2.4 Procedures

Twins spent one full day and night at the University of Washington Clinical Research Center. They began fasting at 9:30 PM on the night before the study and arrived at the study site the next day at 7:00 AM. Urine β HCG tests ruled out pregnancy. Investigators, research staff, and participants remained blinded to restraint status throughout the study.

2.4.1 Meals and preload study paradigm—Twins were separated during all periods of food intake. We surreptitiously measured the caloric and macronutrient content of all foods consumed during study procedures by weighing foods before and after administration (ProNutra, Viocare Technologies, Princeton, NJ).

At 8:00 AM, participants received an isocaloric standardized beverage described as a “low-calorie breakfast drink” to consume completely in 10 minutes. The purpose of the overnight fast and beverage administration was to standardize feeding status at the onset of the study and to assess ghrelin response during an isocaloric meal. The beverage consisted of vanilla-flavored instant breakfast and Genisoy protein powder mixed in a combination of whole milk, half and half, and water (303 kcals, 18% protein, 38% fat, 44% carbohydrate). All twins received the same caloric content, which was calculated on the basis of a 2000-calorie diet.

At 12:00 noon, we conducted a preload study in which twins had 10 minutes to drink a standardized beverage with nearly identical caloric and macronutrient content to that of the breakfast beverage (313 kcals, 19% protein, 38% fat, 43% carbohydrate) but ingredients that were altered to provide a richer, chocolaty flavor and mouth feel. It was served frozen and designated a “milkshake.” After rating the milkshake’s taste, participants were then presented with an *ad libitum* “taste test” of three types of ice cream served in quantities sufficient to mask the amounts consumed (5).

At 12:30 PM, participants received a lunch that consisted of a turkey sandwich, tortilla chips, an apple, milk, carrots, and shortbread cookies (1,032 calories, 16% protein, 34% fat, 50% carbohydrate). At 5:30 PM, a dinner was served consisting of teriyaki chicken, rice, peas, salad, roll, margarine, milk, and a chocolate cookie (1,101 calories, 17% protein, 40% fat, and 43% carbohydrate). A snack was provided at 9:00 PM, consisting of crackers, cheese, grapes, and chocolates (551 calories, 11% protein, 45% fat and 45% carbohydrate). The content of all meals was standardized and subjects consumed the prepared foods *ad libitum*.

2.4.2 Blood sampling—An intravenous catheter was placed, and fasting baseline blood samples were obtained at 8:00 AM, then every 30 minutes thereafter until 3:30 PM, and again at 5:30 PM (before dinner) and 8:30 PM (after dinner). Overnight, a sample was

drawn at the predicted ghrelin peak of 1:00 AM (17). Fasting samples were drawn again at 8:00 AM on the morning of Day 2. Samples were drawn into EDTA tubes and centrifuged at 4° C for 10 minutes at 2000 rpm. Plasma was aliquoted and stored at -80° C.

2.5 Body composition and metabolic rate

Twins changed into hospital gowns for measurement of weight (in duplicate to the nearest tenth of a kg) and height. During the morning of Day 1, dual energy x-ray absorptiometry was used to measure total lean and fat body mass as well as percentages of fat and lean tissue. At 6:00 AM on Day 2, twins underwent indirect calorimetry to determine resting energy expenditure (REE).

2.6 Hormone assays

Total circulating ghrelin levels were measured in duplicate by RIA (Phoenix Pharmaceuticals, Belmont, CA). Plasma leptin and glucose levels were measured by ELISA (Diagnostic Systems Laboratory, Webster, TX) and the hexokinase method, respectively. Control samples from common stock were used to assess intra- and inter-assay variability. All samples from a twin pair were batched together in the same assay.

2.7 Statistical analysis

Descriptive statistics are presented as means and SD, unless otherwise indicated. Percentage fall in ghrelin was calculated as [(post-meal nadir)-(pre-meal peak)/pre-meal peak]*100. Group differences were compared by paired t-tests. Intraclass correlations were calculated to evaluate within-pair similarities. Pearson's correlation coefficients were calculated to describe associations (when twins were stratified by restraint status or within-pair differences were being compared). Spearman's rank correlation coefficients were used instead when analyses indicated significant departures from normal distributions. Generalized estimation equation (GEE) regression models with a robust sandwich variance estimate were used to test associations, resulting in variance estimates that account for clustering by twin pair. For analyses of ghrelin, GEE models accounted for multi-level clustering of data by twin pair and repeated ghrelin measurements, and models included time as a covariate. We assumed an independent working correlation structure for GEE analyses. Therefore, models testing differences in ghrelin concentration during the preload study incorporated the pre- (noon) and post-preload study (12:30 PM) values, and the analysis of the breakfast meal was performed similarly (8:00 and 8:30 AM). Unstandardized coefficients and 95% confidence intervals are presented with *P* values. A model that included restraint status, ghrelin concentration at all 21 time points, and an interaction term for *restraint status * time* was used to test for overall differences in ghrelin level by restraint. An alpha less than 0.05 was considered statistically significant. Analyses were performed by using STATA 9.0 (Statacorp, College Station, TX) and Graphpad Prism 6.00 (GraphPad Software, La Jolla, CA).

3. Results

Sixteen twin pairs completed the study. Average BMI was 23.5 ± 3.1 kg/m² (range 17.9-30.6). Mean age was 31.5 ± 13.6 years. BMI, body weight, lifetime maximum weight,

REE, and body composition were well matched between unrestrained and restrained twins (Table 1). Leptin concentrations did not differ significantly between twins at baseline (unrestrained 15.2 ± 7.6 vs. restrained 14.5 ± 8.0 ng/ml; $P = 0.78$), at 1:00 AM, or on 8:00 AM on Day 2 (data not shown). Mean fasting glucose was 88.8 ± 6.7 mg/dl among unrestrained twins and 87.2 ± 6.6 mg/dl among restrained twins.

3.1 Caloric intake during the preload study and meals

The milkshake preload did not induce excessive *ad libitum* ice cream intake in restrained eaters, nor were there differences in average caloric intake at meals (Table 2) or total daily caloric intake (unrestrained 2579 ± 567 kcals vs. restrained 2682 ± 359 kcals, $P = 0.49$). All participants consumed more than their estimated daily energy needs (unrestrained: $150\% \pm 36$; restrained $156\% \pm 34$). Among all twins, those who ate more ice cream tended to also eat more at lunch ($B=0.46$ kcals; 95% confidence interval $-0.05, 0.97$; $P = 0.08$). Total intake at lunch and dinner were also positively associated ($B=0.25$ kcals; 95% confidence interval $0.01, 0.50$; $P = 0.04$).

3.2 The effect of restrained eating on ghrelin concentrations

Figure 1 shows total ghrelin concentrations by restraint status across time. During the breakfast beverage, ghrelin levels among restrained twins tended to be higher, but not significantly so ($B = 44.5$ pg/ml; 95% confidence interval $-7.0, 96.0$; $P = 0.09$). Total ghrelin concentrations among restrained twins were significantly higher during the preload study (Figure 1; $B = 61.1$ pg/ml; 95% confidence interval $5.5, 116.7$; $P = 0.03$). In a multivariate analysis, ghrelin concentrations during the preload study remained significantly higher among restrained twins after adjusting for BMI and ice cream caloric intake ($B=52.1$ pg/ml; 95% confidence interval $4.3, 99.8$; $P = 0.03$). In a model that tested for group differences in ghrelin over the entire study period, we found borderline evidence for an interaction between eating restraint and time (P for interaction $= 0.058$), but no overall effect of restrained eating on ghrelin levels ($B = 38.9$ pg/ml; 95% confidence interval $-9.9, 87.7$; $P = 0.12$). In other words, the impact of restrained eating on ghrelin levels may have varied over the study day such that no overall increase in ghrelin levels was found.

Examining each pair as a unit, Figures 2A and 2C demonstrate pairwise ghrelin levels before (noon) and after (12:30 PM) the preload study, respectively. Each point represents a single pair. Points closer to the line represent pairs in which the twins' ghrelin levels were similar, and points in which the restrained twin had a higher ghrelin level appear above the line. Figures 2A and 2C show that, despite significant intraclass correlations in ghrelin concentrations between twins, the restrained eating members of the pair consistently had higher ghrelin levels. Figure 2B demonstrates that ice cream intake was also significantly correlated among twins. In 10 of the 16 pairs, the restrained twin ate more ice cream than the unrestrained twin. Figure 2D demonstrates that lunch intake was variable within pairs and in relation to restraint status. In sum, the significant intraclass correlation coefficients demonstrate a genetic component to circulating ghrelin levels, whereas higher levels among restrained twins show the additional, non-genetic, influence of restrained eating behavior.

3.3 Relationship of ghrelin levels to food intake

Noon ghrelin levels were prospectively and positively associated with *ad libitum* ice cream caloric intake (Figure 3; $B = 0.43$ kcals; 95% confidence interval 0.17, 0.69; $P = 0.001$) such that for every 100 pg/ml increase in ghrelin concentration an additional 43 kcals of ice cream were consumed. Noon ghrelin similarly predicted total lunchtime caloric intake ($B = 0.62$ kcals; 95% confidence interval 0.15, 1.08; $P = 0.009$). There was no evidence for an interaction ($P = 0.30$), demonstrating that the association of higher ghrelin levels with more ice cream intake was present regardless of restraint status (unrestrained $r = 0.47$ vs. restrained $r = 0.53$). In contrast, pre-dinner ghrelin concentration was not associated with dinner caloric intake ($B = -0.46$; 95% confidence interval -1.04, 0.12; $P = 0.12$). Finally, total lunchtime caloric intake was negatively associated with post-meal percentage change in ghrelin levels, such that every additional 6.0 kcals (95% confidence interval = -8.1, -3.9; $P < 0.001$) ingested at lunch corresponded to a 1% fall in ghrelin.

3.4 Influence of weight differences on ghrelin levels

The mean within-pair difference in body weight (restrained – unrestrained) was -1.1 ± 7.9 kg. Differences in weight between restrained and unrestrained co-twins were negatively correlated with differences in fasting ghrelin concentrations ($r_s = -0.61$, $P = 0.01$) such that when restrained twins weighed less than their unrestrained co-twin they also had higher fasting ghrelin levels. Correlations were not present between within-pair differences in body weight and within-pair differences in ghrelin concentration before the preload study ($r_s = -0.35$, $P = 0.18$), after the preload study ($r_s = -0.13$, $P = 0.63$), or over the entire day (total area under the curve; $r_s = -0.40$, $P = 0.13$).

4. Discussion

Our findings indicate that, in a sample of mostly normal-weight female twins, ghrelin levels in restrained eating twins were higher during a preload study. This was present despite the fact that restrained and unrestrained twins consumed identical meals prior to the preload study and almost identical amounts of milkshake and ice cream in the course of the preload study. Because we studied MZ twins, we can confirm that these differences are independent of age, genetics, and family background. Differences were most pronounced in the context of the preload study, but ghrelin levels in restrained twins were higher at all of the earlier sampling times and regressed toward those of the unrestrained co-twins at later time points in the study. Supporting the conclusion that ghrelin patterns over time did, in fact, differ between restrained and unrestrained twins, a strong trend toward an interaction between restraint status and time in the effect on ghrelin levels was found. The fact that differences in average ghrelin levels diminished over time suggests that, for restrained twins, relatively short-term effects of their behavior before their study participation may have been acting during their first hours at the study site.

Documented influences on ghrelin levels include nutritional factors, such as the macronutrient composition (27) and total caloric content (18) of ingested food, as well as psychological and behavioral factors, such as anticipation of eating (28), knowledge of food composition (29), body weight (30), and weight fluctuations (31). The macronutrient

composition of a meal influences ghrelin suppression after the meal (27), and previous studies have shown that restrained eaters consume more carbohydrates and less fat than do unrestrained eaters (3). However, our study protocol ensured that twins consumed foods with identical macronutrient compositions prior to the preload study, so this nutritional factor cannot explain our findings. Previous research has also found that restrained eaters have higher mean BMI than unrestrained eaters independent of genetic factors (32). In our study, the mean BMI, body weight, and fat mass of restrained twins differed minimally from those of unrestrained twins. However, the small degree to which restrained twins were weight reduced as compared to their co-twin was related to higher ghrelin levels at a fasting baseline blood draw. Although reduced body weight elicits compensatory increases in ghrelin (17), it has not previously been demonstrated that weight differences as small as the ones we observed (less than 2 kg of total fat mass and body weight) can elevate ghrelin levels. Additionally, frequent weight fluctuation, which is a characteristic of restrained eaters identified by the Restraint Scale (25), is associated with elevated ghrelin levels (31). This behavioral factor may also have contributed to our findings. Restrained and unrestrained twins had very similar leptin levels, which closely track body fat mass and are disproportionately reduced during fasting (33). The fact that being weight-reduced as compared to a co-twin – whose weight is tantamount to a counterfactual, genetically determined body weight – is associated with small but detectable increases in ghrelin concentrations is indicative of an unexpectedly fine-tuned mechanism for body weight regulation.

Our findings of elevated ghrelin levels have several other possible contributing factors. Anticipation of eating (28) may have differentially affected the restrained twins in our sample. It is possible that restrained eaters experienced greater anticipation of consuming ice cream as part of the study protocol as restrained eaters are more likely to restrict foods considered forbidden or unhealthy. In addition, psychological components – such as the belief that one food is “high-calorie” or “high-fat” while another is “low-calorie” or “low-fat” – may have influenced ghrelin levels after food consumption, even though the foods in our study were actually isocaloric (29).

In sum, the possibility that psychological or behavioral factors may influence ghrelin levels in restrained eaters is perhaps the most intriguing implication of this research. Previous work has shown that suppressing food thoughts (34) or imagining food (35) can influence food intake, and that ghrelin infusion stimulates imagination of favorite foods (36). On the whole, our data support the hypothesis that ghrelin levels are subject to short-term changes caused either by behaviors associated with eating restraint, weight reduction, or by psychological factors such as anticipation of eating.

Because our study was conducted in a genetically informative sample, we can also address the hypothesis that genetic factors produce higher ghrelin levels, such that affected individuals must engage in restraint to control their appetites. Our findings provide no support for this hypothesis, because we observed elevated ghrelin levels among the restrained twins despite controlling for genetic factors by matching restrained twins with their genetically identical unrestrained co-twins. Although the initial predisposition to restrain eating may have a genetic component (20), our findings suggest that once restrained

eating behavior is acquired, endogenous levels of ghrelin are increased independent of genetic influence.

Despite these elevations and the selection for behavioral differences between twins, total ghrelin concentrations remained strongly correlated within twin pairs, providing evidence for a genetic contribution to the inter-individual variability observed in circulating levels. Moreover, ghrelin levels before drinking the preload milkshake were related to subsequent ice cream intake, regardless of restraint status. Although ghrelin levels before dinner did not predict subsequent dinner intake, the study dinner was served in fixed portions, limiting its usefulness for assessing *ad libitum* consumption. In addition, we confirmed that postprandial suppression of ghrelin correlates with the number of calories consumed during the preceding meal (18). In sum, we provide novel evidence regarding the genetic influence on ghrelin concentrations, and supportive evidence that endogenous levels have orexigenic action *in vivo*.

Congruent with findings from some studies in experimental (37) – as opposed to more naturalistic (38) – settings, restrained eaters did not consistently disinhibit their food consumption. Some maintained cognitive control even in the face of greater appetite stimulation by ghrelin. There are several explanations for why some restrained eaters maintained cognitive restraint despite higher ghrelin levels. Laboratory studies suggest that restrained eaters disinhibit eating secondary to a trigger such as anxiety, distraction and the presence of emotion. These triggers were not included in our study. Despite our precautions to separate twins during meals, study participants were accompanied by their twin sisters at all other times, and they undertook intensive physiologic measurements throughout the study visit. These factors may have undermined the usual power of a milkshake to induce disinhibition in restrained eaters. Additionally, the anxiety of study participants may have been reduced due to the familiarity and comfort of their sister being present; thereby minimizing a potential trigger to disinhibition. The potential influence of social norms on caloric intake may also have played a part (39), specifically regarding acceptable levels of food consumption. One notable limitation of this work is that we cannot confirm that our findings apply to acylated ghrelin, the biologically active form, even though total ghrelin levels are highly correlated with acylated ghrelin in peripheral circulation (27). A second limitation is that we did not examine restraint in men or in obese individuals of either sex. Future studies are needed to explore the links between restrained eating behaviors, ghrelin levels, and caloric intake in these populations. A final limitation is that the Restraint Scale is multifactorial, insofar as it identifies restrained eaters who also exhibit disinhibition and overeating. As a result, our findings may not apply to restrained eaters identified by other scales.

Nonetheless, the present findings have important implications for understanding efforts to control body weight. Sustainable weight loss efforts are mostly unsuccessful in the majority of individuals, in large part because energy homeostatic mechanisms, including ghrelin (17), defend body fat mass (40). Prospective studies are warranted to test whether higher ghrelin levels, like those we observed, predict greater subsequent weight gain or disinhibited eating. More research is also needed to address short-term interactions between anticipatory eating,

imagination of food, ghrelin levels, and actual food intake in both restrained and unrestrained eaters.

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Highlights

- Restrained eaters' ghrelin levels were compared to their unrestrained co-twins
- Restrained twins had higher ghrelin levels during a preload study but ate similarly
- There is a genetic influence on circulating ghrelin levels
- Restrained eating behavior increases ghrelin, independent of this genetic influence

Figure 1.

Mean (SEM) total ghrelin concentrations in unrestrained vs. restrained twins across time. Breakfast beverages were matched between twins and also were isocaloric with the milkshake served during the preload study. Beverages were labeled either “low-calorie breakfast drink” or “milkshake.” Caloric intake during the preload study's *ad libitum* ice cream taste test (which followed ingestion of identical milkshakes) was not significantly different between unrestrained and restrained eating twins. Group differences in ghrelin concentrations during breakfast (NS, $P=0.09$) and during the preload study ($*P=0.03$) were tested by using generalized estimating equations. Abbreviation: pg/ml = picograms per milliliter, *ad lib* = *ad libitum*, NS = not significant.

Figure 2.

Total ghrelin concentrations and caloric intake in twin pairs. Total ghrelin concentration within each pair before (noon; A) and after (1230; C) the preload study. Each data point represents a single pair and points appearing above the midline represent pairs in which the restrained eating twin had a higher ghrelin level. *Ad libitum* ice cream (B) and lunch caloric intake (D) are plotted similarly. Abbreviations: pg/ml = picograms per milliliter, *ad lib* = *ad libitum*, ICC = intraclass correlation, NS = not significant.

Figure 3.

Higher total ghrelin concentrations are associated with greater *ad libitum* ice cream intake. Noon plasma samples were taken just prior to the preload study. Ice cream was offered *ad libitum* as a taste test following consumption of a standardized milkshake. Abbreviations: pg/ml = picograms per milliliter.

Table 1

Characteristics of 16 monozygotic twin pairs discordant for restrained eating

Characteristics	Unrestrained <i>Mean (SD)</i>	Restrained <i>Mean (SD)</i>
Age, <i>years</i>	31.6 (13.9)	31.6 (13.9)
Body mass index, <i>kg/m²</i>	23.9 (3.5)	23.1 (2.8)
Weight, <i>kg</i>	65.2 (9.8)	63.4 (7.6)
Lifetime maximum weight, <i>kg</i>	67.4 (6.2)	68.3 (9.1)
Height, <i>cm</i>	165.4 (6.2)	165.6 (5.9)
REE, ^{1,2} <i>kcal</i> s	1,333 (137)	1,368 (202)
Percentage body fat, %	33.1 (8.9)	32.1 (8.0)
Total fat mass, <i>kg</i>	21.3 (8.6)	19.8 (6.8)
Total lean mass, <i>kg</i>	41.0 (3.3)	40.8 (3.5)
Restraint Scale score	10.8 (2.0)	17.6 (2.2)
TFEQ-R18 Emotional eating scale score ³	5.1 (1.9)	5.6 (2.3)
TFEQ-R18 Uncontrolled eating scale score ³	15.5 (3.5)	17.8 (5.1)

kcal = kilocalories; REE = resting energy expenditure; TFEQ-R18 = Three Factor Eating Questionnaire, Revised 18-item version.

¹ REE was determined by indirect calorimetry

² One twin pair and one restrained individual had missing data.

³ One twin pair had missing data.

Table 2

Food intake in unrestrained and restrained eating twins

	Unrestrained <i>Mean (SD)</i>	Restrained <i>Mean (SD)</i>	<i>P</i> value ¹
Ice cream, ² <i>kcal</i> s	228 (133)	239 (158)	0.83
Lunch, <i>kcal</i> s	498 (254)	556 (141)	0.41
Dinner, <i>kcal</i> s	836 (211)	855 (157)	0.69

*kcal*s = kilocalories; SD = standard deviation.

¹ *P* values were determined by paired t-tests.

² Ice cream was eaten during the preload study after administration of a standardized milkshake preload (303 *kcal*s).