The Journal of Nutrition
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

Impaired Ghrelin Response after High-Fat Meals Is Associated with Decreased Satiety in Obese and Lean Chinese Young Adults[1,2]

Nianhong Yang,[3,4]* Xiaoli Liu,[5] Eric L. Ding,[4] Mingjia Xu,[3] Songwen Wu,[3] Liegang Liu,[3] Xiufa Sun,[3] and Frank B. Hu[3,4]*

[3]Department of Nutrition and Food Hygiene, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, PR China; [4]Department of Nutrition, Harvard School of Public Health, Boston, MA 02115; and [5]Shenzhen Chronic Disease Hospital, Shenzhen 518020, PR China

Abstract

Ghrelin and peptide tyrosine tyrosine (PYY) are known to affect appetite and body weight, but the acute effects of fat-rich and carbohydrate-rich meals on plasma ghrelin, PYY response, and appetite remain unclear. We hypothesized that obese individuals had impaired postprandial ghrelin and PYY response based on macronutrient content of meals, affecting appetite and energy intake. We conducted a randomized crossover trail comparing fasting ghrelin and PYY concentrations, postprandial ghrelin and PYY responses, and subjective appetite in 15 obese and 12 lean Chinese young adults after they consumed isocaloric high-carbohydrate [HC; 88% energy carbohydrate, 4% energy fat, 8% energy protein] and high-fat (HF; 25% energy carbohydrate, 71% energy fat, 4% energy protein) meals. Ghrelin concentrations over time differed between HC and HF meals ($P < 0.01$) via repeated measures of ANOVA, with lower postprandial ghrelin suppression after HF meals, especially among obese participants. PYY response differed between meals among lean participants, with a delayed and higher postprandial PYY peak after the HF meal ($P < 0.01$); however, PYY response did not differ among obese participants. The incremental area under the curve of PYY was higher in lean than in obese participants after the HF meal ($P < 0.01$). These results suggest that impaired ghrelin response after HF meals may contribute to reduced satiety and overeating, especially among obese individuals. Whether an attenuated response of PYY in obese participants after a HF meal bears any physiological consequences warrants further study. J. Nutr. 139: 1286–1291, 2009.

Introduction

Ghrelin and peptide tyrosine tyrosine (PYY)[6] are 2 gastrointestinal (GI) tract-derived hormones known to affect appetite through the activation of various neurons in the hypothalamus. Ghrelin is primarily secreted (mostly preprandially) by endocrine cells in the stomach (1). The first known GI-brain peptide hormone with orexigenic, appetite-stimulating effects, ghrelin appears to play a pivotal role in the regulation of food intake. Ghrelin concentrations in plasma rise gradually before a meal and decrease immediately after a meal (2–5). In addition, exogenous administration of ghrelin induces profound stimulation of food intake in both rodents and humans (6–9). These effects suggest that ghrelin plays a role in feelings of hunger and in meal initiation. Given the inverse association between BMI and fasting plasma ghrelin concentrations (2,4,10,11), ghrelin is thought to be involved in not only meal initiation but also body weight control.

PYY is a 36-amino acid peptide derived from L-cells primarily located in the distal intestine. Released in proportion to energy intake, PYY enzymatically cleaved by dipeptidyl peptidase-IV to yield the major circulating form, $PYY_{3–36}$ (12,13). As part of the neuropeptide Y (NPY) family, $PYY_{1–36}$ binds to and activates the Y1, Y2, and Y5 NPY receptor subtypes and $PYY_{3–36}$ preferentially binds to the inhibitory presynaptic Y2 receptor, which is highly expressed in NPY neurons in the appetite regulatory center in the arcuate nucleus (14). Data show that exogenous infusion of $PYY_{3–36}$ decreases food intake in both rodents and humans (13,15–21). Although $PYY_{1–36}$ was once reported to increase appetite in rodents (22), it is unlikely to be important in regulating energy intake in humans and dogs (19,23). In humans, lower circulating PYY concentrations have been reported in obese individuals (17), but whether they play a role in the etiology of obesity remains controversial (24,25).

Reports suggest that the secretions of ghrelin and PYY are modulated by food ingestion, but the relative effects of different

[1] Supported by the National Natural Science Foundation of China (NSFC 30671765), Danone Institute Nutrition Foundation, China (DIC 2004-09), and Harvard Asian Center's Faculty Research Grant.

[2] Author disclosures: N. Yang, X. Liu, E. L. Ding, M. Xu, S. Wu, L. Liu, X. Sun, and F. B.Hu, no conflicts of interest.

[6] Abbreviations used: AUC, area under the curve; GI, gastrointestinal; HC, high carbohydrate; HF, high fat; HOMA-IR, homeostasis model assessment of insulin resistance; NPY, neuropeptide Y; PYY, peptide tyrosine tyrosine; VAS, visual analogue scale.

*To whom correspondence should be addressed. E-mail: zynh@mails.tjmu.edu.cn or frank.hu@channing.harvard.edu.

Manuscript received January 14, 2009. Initial review completed February 17, 2009. Revision accepted April 27, 2009.
First published online May 20, 2009; doi:10.3945/jn.109.104406.

macronutrients on ghrelin and PYY release have not been well elucidated. Circulating ghrelin concentrations are known to decrease dose dependently after carbohydrate-rich meals (4,26–28), but some have argued that acute ghrelin response is independent of the energy intake in healthy individuals (29). Whether or not a high-fat (HF) diet suppresses postprandial ghrelin concentrations less effectively is still controversial (30,31). The finding of weaker suppression of ghrelin in obese, insulin-resistant participants suggests that attenuated postprandial ghrelin suppression may contribute to increased food intake in obesity (30). Although postprandial elevation of plasma PYY concentration is proportional to the energy ingested (32), studies also suggest that PYY elevation is affected by the macronutrient composition of a meal. Given the evidence suggesting that fat digestion is necessary for stimulation of PYY in humans (33), fat intake does appear to be the most potent stimulus of PYY and carbohydrates the least potent (34,35). However, it is unknown whether these effects differ between lean and obese individuals.

We hypothesized that obese individuals had impaired postprandial ghrelin and PYY responses based on macronutrient content of meals and that this may contribute to obesity by affecting appetite and food intake. To test this hypothesis, we compared fasting and postprandial concentrations of ghrelin and PYY, and subjective measures of appetite in separate obese and lean participants after isocaloric high-carbohydrate (HC) and HF meals.

Materials and Methods

Participants. Using the criteria from the International Obesity Task Force (36) for Asians, we recruited 15 obese (BMI $\geq$27.5 kg/m^2) and 12 lean (BMI $\geq$18.5 and $\leq$23 kg/m^2) healthy Chinese participants who were graduate school students in Wuhan, Hubei province. Exclusion criteria included chronic medical and psychiatric illness, pregnancy, or substance abuse. None of the participants changed their weight status during the 3 mo prior to the study. All participants gave their informed consent; the study was approved by the ethical committee of Huazhong University of Science and Technology.

Protocol. Each participant attended the laboratory on 2 occasions, 1 wk apart. They received a HC breakfast on the first occasion and a HF breakfast on the second visit. They were asked to completely consume the meal within 30 min. The HC test meal consisted of 100 g of steamed bread and 50 g of honey (1700 kJ) for women and 150 g of steamed bread and 55 g of honey (2200 kJ) for men. The macronutrient composition of the HC meal was comprised of 88% energy from carbohydrate, 4% energy from fat, and 8% energy from protein. The HF test meal consisted of 50 g steamed bread and 40 g butter (1700 kJ) for women and 65 g steamed bread and 50 g butter (2200 kJ) for men. The macronutrient composition of the HF meal was comprised of 25% energy from carbohydrate, 71% energy from fat, and 4% energy from protein.

Participants came to the laboratory at 0800 after fasting (defined as no eating or drinking except for water from 2000 the previous night). Venous blood was collected just prior to each breakfast and at intervals of 30, 60, 120, 180, and 240 min afterward. Blood samples were collected into plastic tubes containing EDTA Na_2 (7.5%, 13 μL in 1 mL blood) and aprotinin (0.5 kU/L blood). All samples were chilled in an ice bath until centrifugation at 3000 $\times$ *g*; 15min at 4°C; plasma was immediately separated and stored at −80°C until analysis. We used visual analogue scales (VAS) (37) to assess hunger before and 240 min after each test breakfast and satiety at 30 min after each meal. Participants provided ratings on a 100-mm VAS with text clues indicating the direction of the most positive and most negative scores.

Anthropometry measurements. For all participants, weight and height were measured by the same observer to the nearest 0.5 kg and 0.5 cm, respectively. BMI was calculated as weight (kg)/height (m)^2. Waist and hip circumferences were measured to a precision of 0.1 cm and the waist: hip ratio was calculated. Triceps, subscapular, and abdominal skinfolds were measured in duplicate on the right side of the body to a precision of 0.2 mm and we used the mean of the 2 measurements.

*Hormonal assay.*All samples were assayed in duplicate and in a single laboratory analysis to avoid interassay variation. Plasma glucose concentrations were measured by the glucose oxidase method using kits from Nanjing Jiancheng Bioengineering Institute, Nanjing, China, with an intra-assay CV <1.2%. Plasma insulin concentrations were determined by RIA using kits from Chemclin Biotech with an interassay CV <5.8%. Plasma ghrelin and PYY concentrations were determined by RIA using kits from Phoenix Pharmaceuticals. Ghrelin was measured using ^{125}I-labeled bioactive ghrelin as a tracer molecule and a polyclonal antibody raised in rabbits against full-length octanylated human ghrelin, which detects both acylated and des-acylated forms of ghrelin. Intra-assay CV of the assay were <5.4%. There was no cross-reactivity with any relevant molecules (i.e. secretin, vasoactive intestinal peptide, galanin, growth hormone releasing factor, glucagon-like peptide-1, NPY, orexin A, orexin B). PYY was measured using antibody raised in guinea pigs. It recognizes both the $PYY_{1–36}$ and $PYY_{3–36}$ forms of human PYY, with no cross-reactivities with NPY, leptin, glucagon, ghrelin, insulin, or GLP-1. The intra-assay CV were <8.6%.

Statistical analysis. All data are presented as means ± SEM. Student's unpaired *t* test was used for group comparisons at baseline. The time course of each postprandial hormone response was analyzed by 2-way repeated-measures ANOVA with group and type of meal as main effects, followed by a Student Newman-Keuls post hoc test. Differences of postprandial response between meals as well as groups were assessed via time $\times$ meal and time $\times$ group interaction tests. Percent change of ghrelin and PYY at 30 min after ingestion was calculated by [(levels at 30 min-baseline)/baseline)] $\times$100. Area under the curve (AUC) values for ghrelin and PYY were calculated using the trapezoidal method and differences in the AUC values between groups with different meals were analyzed by ANOVA followed by a Student Newman-Keuls post hoc test. Pearson's product-moment correlation was used to evaluate the association between fasting ghrelin, PYY, and insulin concentrations as well as their changes. The homeostasis model assessment insulin resistance index (HOMA-IR) was calculated according to the following equation: fasting insulin (mU/L) $\times$ fasting glucose (mmol/L)/22.5 (38). All statistical analyses were conducted using SAS version 9 (SAS Institute), with α = 0.05.

Results

Descriptive characteristics. Participants in the 2 groups had similar age and height. As expected, body weights, BMI, waist: hip ratios, and skinfold thicknesses were higher in obese participants (**Table 1**). BMI did not differ by gender in either the obese (women, 29.2 ± 0.6 kg/m^2; men, 31.2 ± 0.9 kg/m^2) or lean (women, 19.2 ± 0.2 kg/m^2; men, 21.0 ± 0.4 kg/m^2) group.

Baseline data. In fasting participants, plasma glucose and hormone concentrations did not differ between genders or among study days (Table 1). Obese participants had higher fasting glucose and insulin concentrations and greater insulin resistance as assessed by HOMA-IR. Obese individuals also had lower fasting ghrelin concentrations compared with lean participants, whereas fasting PYY concentrations did not differ.

Postprandial ghrelin and PYY responses. Regardless of obesity status and meal type, mean postprandial ghrelin levels decreased shortly after ingesting a test meal, reaching a nadir 60 min after the meal, and then increasing thereafter (**Fig. 1***A*). However, the decrease in ghrelin concentrations over time was

TABLE 1 Anthropometry and plasma biochemistry of obese and lean participants[1,2]

	Lean	Obese
n	12	15
Age, *y*	25.6 ± 0.5	24.1 ± 0.8
Men/women, *n/n*	7/5	9/6
Height, *cm*	165.2 ± 2.3	165.0 ± 2.1
Weight, *kg*	55.5 ± 2.3	83.1 ± 3.2*
BMI, *kg/m²*	20.5 ± 0.4	30.4 ± 0.6*
Waist, *cm*	72.5 ± 1.3	95.1 ± 2.4*
Hip, *cm*	89.7 ± 1.9	106.8 ± 1.7*
Waist:hip ratio	0.81 ± 0.02	0.89 ± 0.02*
Triceps skinfold, *mm*	21.4 ± 2.3	32.9 ± 2.0*
Subscapular skinfold, *mm*	18.1 ± 1.6	34.5 ± 1.7*
Abdominal skinfold, *mm*	24.7 ± 1.5	44.9 ± 1.3*
Glucose, *mmol/L*	5.3 ± 0.1	6.2 ± 0.1*
Insulin, *pmol/L*	54.5 ± 10.4	97.3 ± 13.8*
HOMA-IR	2.04 ± 0.28	4.49 ± 0.44*
Ghrelin, *pmol/L*	93.3 ± 3.2	81.5 ± 2.3*
PYY, *pmol/L*	13.7 ± 0.7	13.0 ± 0.9

[1] Values are means ± SEM. *Different from lean, $P < 0.05$.
[2] Blood samples were drawn from fasting participants.

blunted in obese participants compared with lean ones after both meals. The percent suppression in ghrelin at 30 min after each meal was less in obese than in lean participants and less after the HF meal than the HC meals in both lean and obese participants (Fig. 1*B*). Ghrelin concentrations over time differed between obese and lean participants ($P < 0.01$) and between HC and HF meals ($P < 0.01$), but no time × group × meal interaction was found by repeated measures of ANOVA. These outcomes indicate that changes in ghrelin concentrations over time were dependent on both obesity status and the quality of macronutrients consumed during the meal. The decremental AUC for plasma ghrelin was larger in the lean (7506 ± 855 pmol·min/L) than in the obese (3939 ± 505 pmol·min/L) participants after the HC meal ($P < 0.01$) whereas in contrast, they did not differ after the HF meal and were 6132 ± 833 pmol·min/L and 4200 ± 2045 pmol·min/L, respectively.

Although postprandial PYY concentrations increased after both meals, the PYY response differed between HF and HC meals in lean but not in obese participants. Notably, among lean participants, peak PYY concentrations occurred at 60 min after the HF meal but 30 min after the HC meal (**Fig. 2***A*). Obese participants exhibited a similar PYY response pattern with a lower peak at 60 min after both meals. Correspondingly, postprandial PYY concentrations differed over time between the HC and HF diets ($P < 0.01$), but not between obese and lean participants, by repeated measures of ANOVA. However, there was an interaction between group and meal on postprandial PYY concentrations over time. Neither lean nor obese participants significantly differed between meals in the percent increase in PYY concentrations at 30 min after ingestion (Fig. 2*B*). The incremental AUC of PYY was higher in the lean (887 ± 140 pmol·min/L) than obese (585 ± 113 pmol·min/L) participants after the HF meal ($P < 0.05$), whereas they did not differ after the HC meal and were 663 ± 110 pmol·min/L and 619 ± 69 pmol·min/L, respectively.

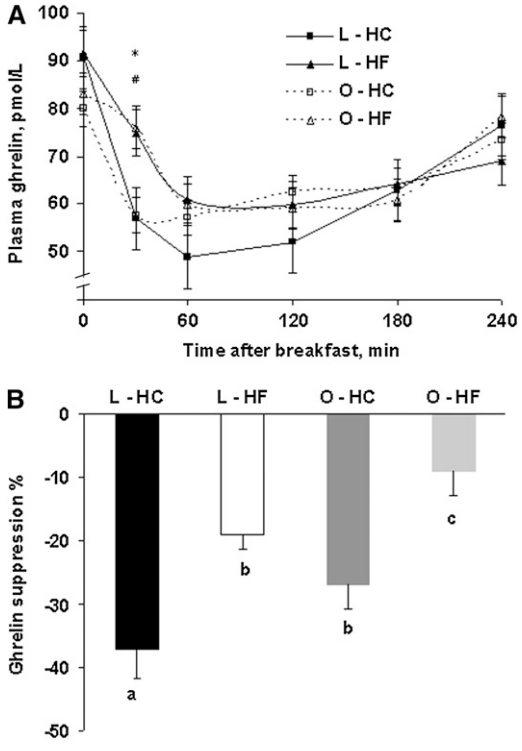

FIGURE 1 Postprandial changes in plasma ghrelin concentrations (*A*) and percent ghrelin suppression at 30 min after ingestion (*B*) in obese and lean participants after they consumed HC and HF meals. Values are means ± SEM, $n = 15$ (obese) or 12 (lean). *Different from corresponding HC in lean participants at that time, $P < 0.05$; #different from corresponding HC in obese participants at that time, $P < 0.05$ (*A*). Means without a common letter differ, $P < 0.05$ (*B*).

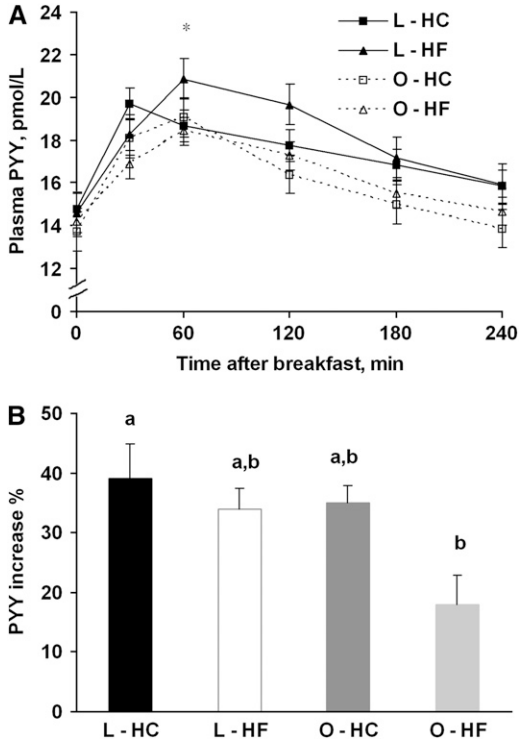

FIGURE 2 Postprandial changes in plasma PYY concentrations (*A*) and percent PYY increase at 30 min after ingestion (*B*) in obese and lean participants after they consumed HC and HF meals. Values are means ± SEM, $n = 15$ (obese) or 12 (lean). *Different from corresponding HC in lean participants at that time, $P < 0.05$ (*A*). Means without a common letter differ, $P < 0.05$ (*B*).

Effects on appetite. The VAS satiety scores revealed lower satiety at 30 min after the HF meal than after the HC meal in both obese and lean participants (**Fig. 3***A*). However, by the end of the test (240 min), only obese patients reported higher hunger scores after the HF meal than the HC meal; lean participants reported no difference in subjective hunger rating between meals (Fig. 3*B*).

Correlations. Fasting PYY concentrations correlated negatively with fasting ghrelin concentrations in both lean ($r = -0.69$; $P < 0.01$) and obese ($r = -0.41$; $P < 0.05$) participants. In lean, but not obese, participants, the increase in insulin levels correlated with the decrease in ghrelin ($r = -0.37$; $P < 0.05$) and the increase in PYY ($r = 0.38$; $P < 0.05$) over the first 30 min. The decremental AUC of ghrelin correlated with the incremental AUC of insulin ($r = -0.66$; $P < 0.01$) and incremental AUC of glucose ($r = -0.44$; $P < 0.05$) in lean participants, but these correlations were not significant in obese participants.

Discussion

Obesity is the result of an energy intake that exceeds energy expenditure. Dietary composition effects that may modulate GI responses are likely important in appetite regulation and, thereby, body weight. Although a variety of studies have investigated the effects of diet composition on ghrelin and PYY secretion, with rather diverse findings (4,11,20,26–34,39–43), few have assessed effects on subjective appetite and examined effect differences between lean and obese individuals. To our knowledge, this is the first study to compare the relative effects of isocaloric meals that differed in the amount of carbohydrate and fat on the postprandial ghrelin and PYY response as well as their effects on subjective appetite between obese and lean Chinese participants.

Notably, we found that dietary macronutrient composition influenced postprandial circulating ghrelin and PYY concentrations in both lean and obese participants. There was less ghrelin suppression in the first 30 min after a HF meal than a HC meal in both groups and less ghrelin suppression in obese individuals than lean ones after each meal. Consistent with those findings, both lean and obese participants reported less satiety via VAS after the isocaloric HF meal than the HC meal at 30 min after ingestion. These outcomes support the hypothesis that a HF meal is less filling than an isocaloric HC meal in both lean and obese individuals and that HF diets may decrease satiety and contribute to overeating in obese individuals who showed a greater preference for HF foods (44,45).

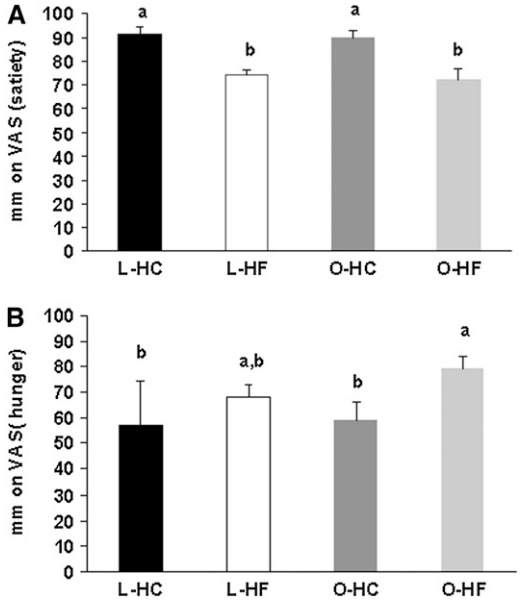

FIGURE 3 VAS (mm) for perceived satiety at 30 min after initiation of each meal (*A*) and for hunger at 240 min after each test meal (*B*). Values are means ± SEM, $n = 15$ (obese) or 12 (lean). Means without a common letter differ, $P < 0.05$.

Although the percent change of PYY concentrations did not differ in either group at 30 min after ingestion of either meal, we observed a somewhat different time course of PYY response between meals and a blunt overall PYY secretion in obese participants compared with lean ones after a HF meal. A HF meal induced greater PYY secretion in lean individuals than in obese ones, whereas HC meals produced no such difference. Other data show similar elevation in PYY concentrations after both carbohydrate- and fat-rich meals in nonobese men (46) and impaired postprandial PYY release in obese participants (17,20). Because PYY responses differ between macronutrients, whether the alteration reduces satiety after meals and leads to increased hunger in obese people warrants further investigation.

The physiological mechanisms that modulate plasma ghrelin and PYY releases are not well understood. Most studies indicate that postprandial insulin suppresses ghrelin secretion (27,47–50), but 2 studies question the inhibitory role of insulin on ghrelin (51,52). It has also been suggested that acute increases in plasma glucose regulate plasma ghrelin independent of the insulin (53). In our study, we confirmed a significant inverse association between postprandial ghrelin and insulin concentrations in lean but not obese participants. This result is consistent with another study (11), which found that nutrient-induced insulin secretion did not suppress ghrelin in obese participants.

Although $PYY_{3–36}$ infusion is known to suppress energy intake and significantly decrease circulating ghrelin in humans (17), the relationship between PYY and ghrelin concentrations has not been consistent (21,54). Our study showed a significant inverse correlation between fasting PYY and fasting ghrelin concentrations but no such correlation between the changes in PYY and ghrelin levels after test meals. The interplay between ghrelin and PYY in relation to appetite is poorly understood. Further studies are warranted to explore whether changes in ghrelin suppression and PYY secretion after different macronutrients might contribute to increased energy intake in obese participants. Studies in animals and humans indicate that GI motor response is reduced after exposure to a HF diet, although how the slower gastric emptying affects ghrelin and PYY secretion also warrants further investigation (55–57).

One limitation of the present study is that total ghrelin concentrations were assessed rather than the active acylated form of ghrelin, and total PYY was measured instead of $PYY_{3–36}$. This raises the possibility that changes in total ghrelin and PYY concentrations may not exactly reflect changes in active ghrelin and $PYY_{3–36}$. However, a strong positive association exists between total and acyl-ghrelin (58). Furthermore, although only the acylated form of ghrelin was thought to be biologically active, the current perspective is that unacylated ghrelin also exerts some biological activities (59–61), thereby supporting total ghrelin as more relevant overall. Meanwhile, studies have shown that the ratio of circulating $PYY_{1–36}$:$PYY_{3–36}$ is similar in lean and obese participants (20).

Another limitation is that we provided both lean and obese participants with the same quantity of test meals based on gender rather than ad libitum intake. Although the VAS ratings of satiety did not differ between obese and lean participants after either a HC or HF meal, we cannot exclude the possibility that either overfeeding in the lean or underfeeding in the obese may have affected the study outcomes to some extent. Because appetite-induced changes in total energy intake mediate in part gut-brain satiety hormones and weight gain, differences can be expected. The test meals were not calibrated in form and size, which may have resulted in different consumption times, chewing intensity, saliva excretion, and other such factors. Finally, because all our participants were Chinese young adults, it is unclear whether the results can be generalized to other populations.

In summary, both lean and obese individuals groups reported lower satiety after an isocaloric HF meal than a HC meal. The decreased ghrelin levels and increased PYY levels had different temporal patterns postprandially after fat intake compared with carbohydrate intake, suggesting that different, yet unknown, mechanisms may contribute to the regulation of postprandial ghrelin and PYY. Impaired ghrelin response in obese participants may contribute to impaired satiety and may be a factor contributing to overeating in obese participants. Further studies are warranted to elucidate the mechanisms that underlie ghrelin and PYY responses and their association with hunger and satiety in both lean and obese subjects.

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