Comparison of Postprandial Profiles of Ghrelin, Active GLP-1, and Total PYY to Meals Varying in Fat and Carbohydrate and Their Association With Hunger and the Phases of Satiety

Catherine Gibbons, Phillipa Caudwell, Graham Finlayson, Dominic-Luc Webb, Per M. Hellström, Erik Näslund, and John E. Blundell

Biopsychology Group (C.G., P.C., G.F., J.E.B.), Institute of Psychological Sciences, University of Leeds, Leeds, United Kingdom LS2 9JZ; Department of Medical Sciences, Gastroenterology and Hepatology (D.-L.W., P.M.H.), Uppsala University, 753 12 Uppsala, Sweden; and Department of Clinical Sciences (E.N.), Danderyd Hospital, Karolinska Institutet, SE-171 77 Stockholm, Sweden

Context: The relationship between postprandial peptides at circulating physiological levels and short-term appetite control is not well understood.

Objective: The purpose of this study was first to compare the postprandial profiles of ghrelin, glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) after isoenergetic meals differing in fat and carbohydrate content and second to examine the relationships between ghrelin, GLP-1, and PYY with hunger, fullness, and energy intake.

Design: Plasma was collected before and periodically after the meals for 180 minutes, after which time ad libitum food was provided. Simultaneous ratings of hunger and fullness were tracked for 180 minutes through phases identified as early (0–60 minutes) and late (60–180 minutes) satiety.

Setting: This study was conducted at the Psychobiology and Energy Balance Research Unit, University of Leeds.

Participants: The participants were 16 healthy overweight/obese adults.

Main Outcome Measures: Changes in hunger and fullness and metabolic markers were indicators of the impact of the meals on satiety.

Results: Ghrelin was influenced similarly by the 2 meals [$F_{(1, 12)} = 0.658$, $P = .433$] and was significantly associated with changes in hunger ($P < .05$), which in turn correlated with food intake ($P < .05$). GLP-1 and PYY increased more by the high-fat meal [$F_{(1, 15)} = 5.099$ and $F_{(1, 14)} = 5.226$, $P < .05$]. GLP-1 was negatively associated with hunger in the late satiety phase and with energy intake ($P < .05$), but the PYY profile was not associated with hunger or fullness, nor was PYY associated with food intake.

Conclusions: The results demonstrate that under these conditions, these peptides respond differently to ingested nutrients. Ghrelin and GLP-1, but not PYY, were associated with short-term control of appetite over the measurement period. **(*J Clin Endocrinol Metab* 98: E847–E855, 2013)**

In the context of obesity, there is considerable interest in the role of gut peptides on appetite control (1). The most interest has focused on the episodic peptides and their relationship with hunger and the short-term (meal-to-meal) control of appetite (2). Research strategies involve either the exogenous administration of appetite-re-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received November 6, 2012. Accepted March 4, 2013.
First Published Online March 18, 2013

Abbreviations: GLP-1, glucagon-like peptide-1; PYY, peptide YY; VAS, visual analog scale.

lated peptides with the subsequent measurement of postadministration action (3, 4) or the monitoring of physiological concentrations of peptides in the blood after specific nutrient loads or controlled meals. Most studies using the former strategy have demonstrated an action of the peptides on appetite and food intake, but the effect may depend on the attainment of supraphysiological peptide levels. The second strategy has been less commonly used.

Much research has been conducted on 3 peptides, ghrelin, glucagon-like peptide-1 (GLP-1), and peptide YY (PYY), that fluctuate episodically and are believed to have contrasting actions on appetite control (5, 6). Episodic hormones are those that change throughout the day, particularly before and after meals. Ghrelin is the only known circulating orexigenic hormone and should be considered separately from the anorexigenic, satiety-related hormones. Ghrelin has been described as the "hunger hormone" (7) with episodic changes in profiles of hunger sensations and levels being similar throughout the day: increasing during fasting and decreasing after food intake. Exogenous administration of ghrelin has been shown to stimulate appetite and food intake in rats and humans (4, 8–11). Ghrelin has also been described as a meal initiator on the basis of a study in which ghrelin and hunger sensations were measured in relation to food intake and revealed an overlap between the 2 variables in the interval between meals (2). However, the actual correlation between the variables was not reported. Furthermore, there was no association between area under the curve ghrelin levels and energy intake at either the lunch or dinner meals or with the subjects' chosen intermeal interval. In other research, ghrelin has been shown to be suppressed more by carbohydrates than by fat (12, 13); however, this is not always the case. Other authors have argued that ghrelin suppression is dependent on the energy content of the meal consumed (14) and not on the proportion of macronutrients.

In contrast to ghrelin, GLP-1 and PYY are released into the circulation after a meal and are reduced during periods of fasting. GLP-1 is secreted from the same gut endocrine cells that synthesize PYY in the distal small and large intestine and therefore is released into the circulation after a meal (15). Infusion of GLP-1 has been shown to reduce hunger levels and energy intake (16). GLP-1 is a potent incretin, that is, a stimulator of insulin release, and peripheral administration of GLP-1 inhibits appetite in animals and humans (17). Studies have shown reduced postprandial GLP-1 release in severely obese subjects, which normalizes with weight loss (18), but others have failed to replicate these findings (19). The role of GLP-1 as an incretin indicates a greater response to ingested carbohydrates (20).

PYY infusion has previously been shown to reduce food intake in normal-weight (6) and obese humans (21), and repeated administration to rodents has been shown to attenuate weight gain (6). Ingestion of fat has been shown to produce the greatest release of PYY, followed by protein and then carbohydrate (22, 23). In contrast, only one study has shown the greatest increase in PYY after carbohydrate consumption (24). GLP-1 and PYY are 2 of many satiety hormones released throughout the gastrointestinal tract; eg, cholecystokinin and others are also thought to play a role in satiety (25).

Research on the nutritional aspects of appetite control has focused considerable attention on the effects of fat and carbohydrate (26). Fat is often regarded as having a much weaker action on satiety than carbohydrate (27). However, the effect of these 2 nutrients is more apparent when foods are consumed under ad libitum conditions, when high-fat foods tend to lead to high-fat hyperphagia (28) or "passive overconsumption" (29). However, when foods varying in fat and carbohydrate are delivered under controlled, isoenergetic conditions in fixed amounts, the effects on appetite are very similar, although not always identical (30). This methodological issue is important in the design of studies to examine postprandial effects of macronutrients, for which it is essential to measure effects after controlled iso-energetic meals.

The aim of the present study was to clarify the action of ghrelin, GLP-1, and PYY in the immediate postmeal period, by comparing their natural physiological profiles after the delivery of isoenergetic nutrient meals containing comparable amounts of protein but varying in fat and carbohydrate content.

Patients and Methods

Subjects

Sixteen participants, consisting of 5 men and 11 women (aged 45.6 ± 6.2 years with body mass index [BMI] of 29.8 ± 2.9 kg/m^2), took part in the study. Body weight and composition were measured using air displacement plethysmography (Bodpod, Concord, California). Participants were recruited via e-mail and poster advertisements. They were initially screened to ensure they met the inclusion criteria: BMI of 27 to 38 kg/m^2, nonsmoking, nonactive (<1 session of moderate intensity exercise per week), and not taking any medication. The BMI criterion was used because of the relevance of gut peptides to appetite in the context of obesity and weight gain.

Study design

A within-subject crossover design was used for this study. Participants visited the laboratory on 2 mornings (separated by at least 3 days) in a fasted state, having eaten nothing from 10:00

PM the previous night. Standardized pasta meals were provided to the participants to eat the night before the study days. The order of the 2 conditions was randomized to eliminate a condition effect. On 1 of the 2 mornings, body composition measurements were taken while the participants were in a fasted state.

Upon arrival at the laboratory, an iv cannula was inserted into the antecubital vein for serial measurements of metabolic and appetite peptides. A fasting blood sample and fasting appetite ratings were completed before the fixed breakfast was provided. Participants were provided with either a high-fat/low-carbohydrate (>50% energy from fat) or high-carbohydrate/low-fat (<4% energy from fat) breakfast meal. Because the meals were deliberately designed to be isocaloric, the high-fat breakfast could also be seen as low-carbohydrate and the high-carbohydrate breakfast as low fat. The adjustments in carbohydrate were necessary for the meals to remain isocaloric (we chose not to vary protein for theoretical reasons). A time period of 10 minutes was allowed for consumption of the breakfast meal, therefore matching the rate of consumption among individuals. During the following 3 hours, participants stayed in the laboratory in separate cubicles to ensure that no social influences took place. Blood sampling and visual analog scale (VAS) appetite sensation measures were taken at specific time points until an ad libitum lunch meal was provided (Figure 1). Early satiety phase was defined as the period between 0–60 minutes whereas the late satiety phase was defined as the period between 60–180 minutes.

Test meals

During pilot testing, the breakfast meals were compared on pleasantness and found to be equally palatable. The fixed breakfast meal consisted of yogurt, honey, and fruit accompanied by a choice of tea or coffee. The 2 conditions were matched for energy content (590 kcal) and weight (685 g) but differed in energy density (high-fat/low-carbohydrate, 50.3% fat, 38.0% carbohydrate, and 11.7% protein; high-carbohydrate/low-fat, 3.2% fat, 83.6% carbohydrate, and 13.2% protein). Participants were instructed to consume all the food and drink provided within 10 minutes.

An identical ad libitum lunch meal was provided after 3 hours on both study days to directly measure eating behavior after the macronutrient challenge. The meal consisted of a tomato and herb risotto and strawberry yogurt. Participants were instructed to eat until they were comfortably full.

Subjective appetite measures

VASs have been used in clinical and research settings to continuously monitor a range of subjective appetite sensations (31). We recently developed a personal digital assistant–based system to measure various appetite ratings. This method has been validated against the standard pen and paper technique and an alternative handheld computer system (32). Questions regarding subjective states of hunger and fullness were assessed. Appetite ratings were completed immediately before and after food consumption and also immediately before each blood sample as shown in Figure 1.

Blood parameters

Venous blood samples were collected into EDTA-containing Monovette tubes. These tubes contained a mixture of inhibitors (dipeptidyl peptidase IV inhibitor [10 μL/mL blood], aprotinin [50 μL/mL blood], and Pefabloc SC [50 μL/mL blood]) to prevent degradation of the peptides to be measured.

Samples were drawn eight times during the morning at 0 minutes and after breakfast at +10, +20, +30, +60, +90, +120, and +180 minutes for the measurement of metabolic and appetite peptide levels (Figure 1). After collection, blood samples were centrifuged for 10 minutes at 4°C and 3500 rpm. Samples were immediately pipetted into Eppendorf tubes and stored at −80°C until analysis.

Before analysis, plasma was thawed, and an additional protease inhibitor cocktail was added (final concentration: 1× Sigma-SIGMAFAST[1×] and dipeptidyl peptidase IV inhibitor KR-62436 (0.5 μM), catalog nos. S8820 and K4264, respectively; Sigma-Aldrich, St Louis, Missouri). Glucose and triglycerides were analyzed by the Department of Clinical Chemistry at Uppsala University Hospital (Uppsala, Sweden). Reference intervals for plasma were as follows: triglycerides (adult), <1.8 mmol/L; and glucose (adult), 4.0 to 6.0 mmol/L. Total ghrelin was assayed with a commercial ELISA kit (catalog no. EZGRT-89K; Millipore, Billerica, Massachusetts) and a Tecan Infinite M200Pro plate reader (Tecan, Männedorf, Switzerland). The inter- and intra-assay coefficients of variation were 5.9% and 3.4%. Insulin, GLP-1 (active), and PYY (total) were analyzed using a magnetic bead–based multiples kit (catalog. no. HMHMAG-34K-06; Millipore). The plate reader was a Luminex MagPix (Millipore), and the plate washer was a Tecan Hydroflex (Tecan) fitted with a magnetic holder. The inter- and intra-assay coefficients of variation were 12.5% and 8.3%.

Total and acylated ghrelin were both measured during this study. Both forms of ghrelin gave the same results; therefore, only total ghrelin is reported. PYY (total) was measured due to feasibility. Because the overwhelming composition of circulating total PYY is known to be PYY_{3-36}, the present PYY (total) assay effectively measured PYY_{3-36}. A separate study showed an es-

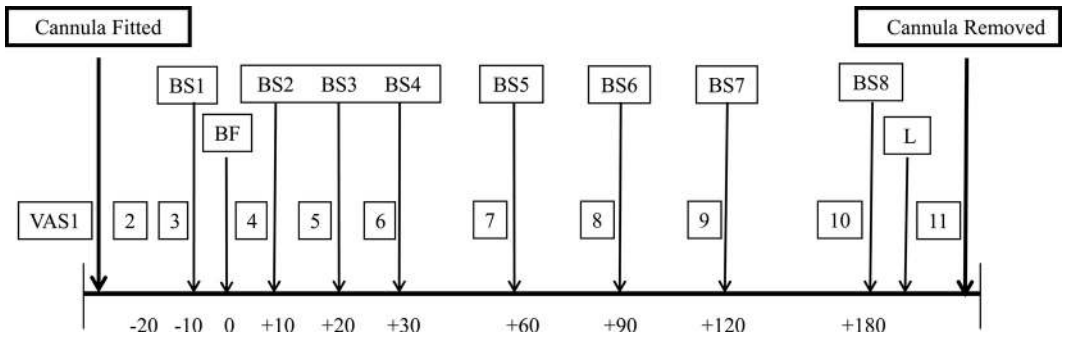

Figure 1. Schematic diagram of postprandial study protocol. BF, breakfast; L, lunch; BS, blood sample.

sentially perfect correlation between this PYY (total) assay and a $PYY_{3–36}$ selective RIA. The relevant antibodies for PYY (total) used in the present study (originally from Linco, St. Charles, Missouri), have been used by others to demonstrate the effects of $PYY_{3–36}$ (33). Glucose and insulin were analyzed not as markers of metabolic health of the participants but rather as indicators of appetite control.

Statistics

Because of the large individual variations in fasting levels of metabolic and appetite hormones, we computed the change from baseline at each time point for each individual for all of the variables. The postprandial period was separated into early and late satiety. The early phase was 0 to 60 min, and the late phase was 60 to 180 min. Repeated-measures (2 × 7) ANOVAs were performed. Statistical paired *t* tests were used to analyze the effects of macronutrient condition on ad libitum energy intake on the 2 separate study days. There was no significant effect of sex on fasting metabolic or appetite hormone levels; therefore, men and women were analyzed together. Although this study was not powered to detect differences between men and women, we have closely inspected the data and can confirm that for GLP-1 and PYY there was no suggestion of a sex difference for either fasting or postprandial levels. For total ghrelin, fasting values were slightly higher in women than in men, but the range was larger. There were 3 outliers who had no detectable ghrelin (that is, far below the 100 pg/mL lowest standard and at least 1 order of magnitude below the average for the group that otherwise deviated less than 2-fold) at numerous time points and 1 outlier for PYY who showed ~10 fold greater levels of PYY compared with the other participants. These participants were therefore excluded from the analysis of these particular hormones but were included in other analyses in which they were not outliers. All statistics represent means ± SEM.

Ethics

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures were approved by the NHS Leeds (West) Research Ethics Committee, United Kingdom (no. 09/H1307/7). Peptide analysis procedures were approved by the regional ethics committee in Stockholm, Sweden (no. 2011/1956-31/2). Written informed consent was obtained from all subjects. This study obtained International Standard Randomized Controlled Trial Registry authorization (ISRCTN47291569) in compliance with guidelines from the World Health Organization (WHO) and CONSORT.

Results

Participant characteristics

A total of 16 overweight or obese participants took part in both conditions for this acute study; 11 were women and 5 were men. Their mean age was 45.6 ± 6.2 years, BMI was 29.8 ± 2.9 kg/m^2, body mass was 86.9 ± 8.7 kg, percent body fat was 39.5 ± 8.2%, fat mass was 34.29 ± 8.0 kg, fat-free mass was 52.6 ± 9.2 kg, and waist circumference was 101.8 ± 7.8 cm. Because the clinical cutoff points for fat mass and waist circumference are different for men and women, the respective sex values were fat mass of 28.1 ± 3.48 kg for men and 37.1 ± 8.0 kg for women. Waist circumferences were 102.8 ± 3.7 cm for men and 101.4 ± 9.2 cm for women.

Fasting peptide and subjective appetite levels

There was no difference in the fasting peptide levels between the high-fat/low-carbohydrate and high-carbohydrate/low-fat breakfast conditions (all $P > .05$) as shown in Table 1.

Plasma glucose and insulin

For both glucose and insulin levels, there was a main effect of condition [$F_{(1, 15)} = 6.200$, $P < .05$ and $F_{(1, 15)} = 32.688$, $P < .001$, respectively] with the high-carbohydrate/low-fat breakfast causing a greater increase than the high-fat/low-carbohydrate breakfast. There was also an effect of time throughout the morning [$F_{(6, 90)} = 10.720$, $P < .001$ and $F_{(6, 90)} = 11.137$, $P < .001$] and a condition × time interaction [$F_{(6, 90)} = 7.340$, $P < .001$ and $F_{(6, 90)} = 4.171$, $P < .05$].

Episodic appetite hormones

For ghrelin, the effect of condition was not significant [$F_{(1, 12)} = 0.658$, $P = .433$]. There was a main effect of time with the meals having a suppressive effect on ghrelin levels throughout the morning [$F_{(6, 72)} = 17.637$, $P < .001$], but there was no condition × time interaction [$F_{(6, 72)} = 0.382$, $P = .797$]. Similar results were seen with acylated

Table 1. Absolute Fasting Levels of Glucose, Insulin, Total and Acylated Ghrelin, GLP-1 (active), and PYY (total) and Ratings of Hunger and Fullness Before Consumption of the Breakfast

Fasting Levels	High-Fat/Low-Carbohydrate	High-Carbohydrate/Low-Fat	*P* Value
Glucose, mmol/L	5.21 ± 0.3	4.81 ± 0.3	.260
Insulin, ng/L	906.98 ± 143.9	879.81 ± 128.8	.653
Total ghrelin, pg/mL	545.07 ± 57.4	552.71 ± 53.3	.612
Acylated ghrelin, pg/mL	70.90 ± 35.8	66.43 ± 35.5	.820
GLP-1, ng/L	20.04 ± 3.16	22.00 ± 3.91	.588
PYY, ng/L	72.62 ± 20.3	67.15 ± 19.8	.475
Hunger, mm VAS	63.56 ± 6.3	61.63 ± 6.3	.539
Fullness, mm VAS	21.06 ± 4.1	20.69 ± 3.7	.921

Data are means ± SEM.

ghrelin (data not shown). However, for GLP-1 there was a main effect of condition [$F_{(1, 15)} = 5.099$, $P < .05$] and time [$F_{(6, 90)} = 6.839$, $P < .01$], but there was no condition × time interaction [$F_{(6, 90)} = 0.488$, $P = .710$]. This finding demonstrated that the high-fat/low-carbohydrate foods created a greater rise in GLP-1 than the high-carbohydrate/low-fat foods. Likewise, for PYY levels, the effect of condition was significant with the high-fat/low-carbohydrate breakfast causing a greater rise in PYY than the high-carbohydrate/low-fat breakfast [$F_{(1, 14)} = 5.226$, $P < .05$]. The effect of time was not quite significant [$F_{(6, 84)} = 1.978$, $P = .078$], and there was no condition × time interaction [$F_{(6, 84)} = 0.800$, $P = .572$] (Figure 2).

Subjective appetite sensations

There was no effect of macronutrient condition on changes in hunger levels throughout the morning [$F_{(1, 15)} = 0.505$, $P = .488$] or a condition × time interaction [$F_{(6, 90)} = 0.540$, $P = .645$]. There was an effect of time [$F_{(6, 90)} = 33.387$, $P < .001$], with hunger being suppressed after food consumption before a gradual rise until the lunch meal. Likewise, there was no difference between the 2 conditions when fullness levels [$F_{(1, 15)} = 2.277$, $P = .152$] were examined or a condition × time interaction [$F_{(6, 90)} = 1.240$, $P = .306$]. However, there was a significant effect of time [$F_{(6, 90)} = 30.615$, $P < .001$] with both breakfasts stimulating an immediate rise in fullness levels before a steady decline until the lunch meal (Figure 3).

Relationship between episodic peptides and subjective appetite

There was a strong relationship between ghrelin and hunger during the early and late satiety phases after both the high-fat/low-carbohydrate (early phase: $r = 0.556$, $P < .05$; late phase: $r = 0.620$, $P < .05$) and high-carbohydrate/low-fat (early phase: $r = 0.671$, $P < .05$; late phase: $r = 0.506$, $P = .078$) breakfasts. Again, acylated ghrelin showed similar relationships (data not shown).

There was no relationship between GLP-1 and hunger during the early phase of satiety; however, there was a relationship during the late phase on the high-fat/low-carbohydrate day ($r = -0.523$, $P < .05$), but this relationship was not quite significant on the high-carbohydrate/low-fat day ($r = -0.428$, $P = .09$). There was no relationship between GLP-1 and fullness over the whole morning or early and late satiety ($r = 0.063–0.372$, all $P > .05$).

In contrast, there was no relationship between PYY and hunger (range $r = -0.057–0.294$, all $P > .05$) or fullness in either the early or late satiety phases after the high-fat/low-carbohydrate (early phase: $r = -0.187$, $P = .505$; late phase: $r = -0.307$, $P = .265$) or high-carbohydrate/low-

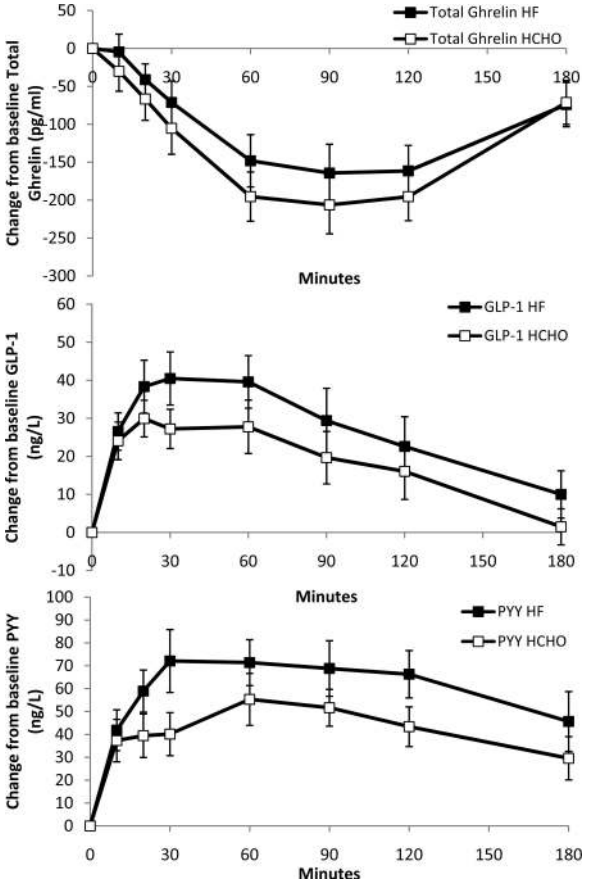

Figure 2. Postprandial profiles of total ghrelin (top), active GLP-1 (middle), and total PYY (bottom) after consumption of a high-fat/low-carbohydrate (HF) and high-carbohydrate/low-fat (HCHO) condition breakfast.

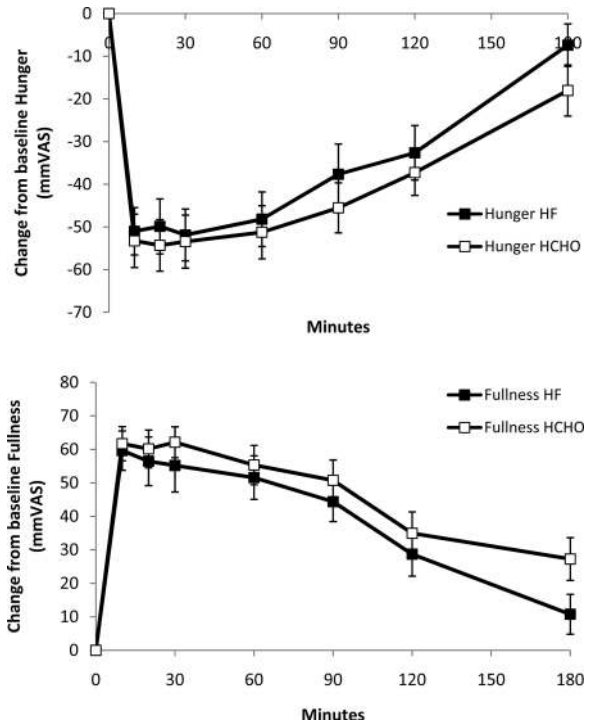

Figure 3. Postprandial profiles of hunger (top) and fullness (bottom) after consumption of a high-fat/low-carbohydrate (HF) and high-carbohydrate/low-fat (HCHO) condition breakfast.

fat (early phase: $r = 0.174$, $P = .534$; late phase: $r = 0.225$, $P = .421$) breakfast condition.

Ad libitum energy intake

There was no difference in ad libitum energy intake at the standard lunch meal after either the high-fat/low-carbohydrate (947 kcal) or high-carbohydrate/low-fat (939 kcal) breakfasts [$t_{(14)} = 0.201$, $P = .844$]. However, there were significant correlations between meal size and changes in both ghrelin and hunger across the 180-minute time period on both the high-fat/low-carbohydrate (ghrelin: $r = 0.659$, $P < .05$; hunger: $r = 0.577$, $P < .05$) and high-carbohydrate/low-fat (ghrelin: $r = 0.731$, $P < .01$; hunger: $r = 0.522$, $P = .067$) condition days. Changes in ghrelin and hunger immediately before the lunch meal (at 180 minutes) were also associated with energy intake (ghrelin, high-fat/low-carbohydrate: $r = 0.564$ and high-carbohydrate/low-fat: $r = 0.597$, both $P < .05$; hunger, high-fat/low-carbohydrate: $r = 0.461$, $P = .073$ and high-carbohydrate/low-fat: $r = 0.550$, $P < .05$). A greater suppression of ghrelin and hunger was associated with a smaller meal size. In addition, there was a relationship between GLP-1 and fullness and energy intake on both the high-fat/low-carbohydrate (GLP-1: $r = -0.547$; fullness: $r = -0.514$, $P < .05$) and high-carbohydrate/low-fat days (GLP-1: $r = -0.526$; fullness: $r = -0.605$, $P < .05$), showing that a greater increase in both GLP-1 and fullness was associated with a smaller meal size (all scatterplots are shown in Figure 4). However, the rise in PYY was not associated with energy intake on the high-fat/low-carbohydrate (PYY: $r = -0.178$, $P = .525$) or high-carbohydrate/low-fat (PYY: $r = 0.284$, $P = .304$) conditions days.

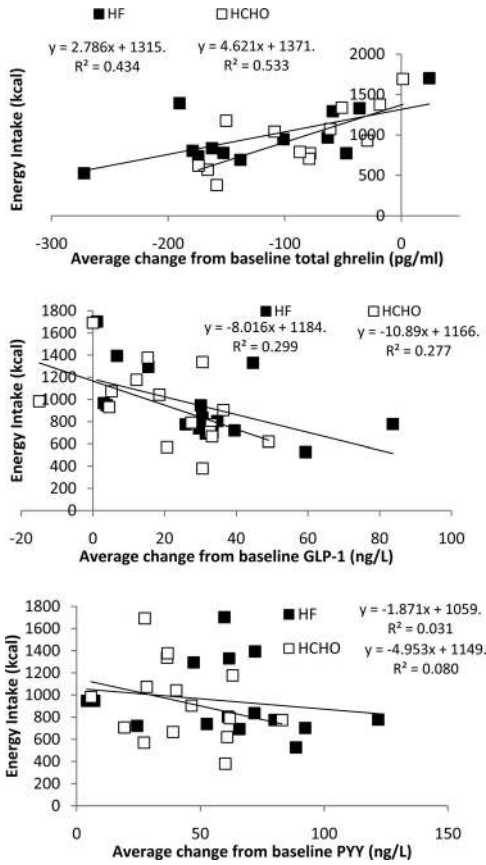

Figure 4. Relationships between postprandial changes in total ghrelin (top), active GLP-1 (middle), and total PYY (bottom) over 180 minutes with energy intake at the ad libitum test meal 3 hours after consumption of a high-fat (HF) or high-carbohydrate (HCHO) breakfast.

Discussion

These data demonstrate certain clear relationships among the fat/carbohydrate content of meals, postprandial profiles of hunger and fullness, concentrations of peptides in blood, and subsequent food intake. The 2 semisolid meals differed markedly in fat and carbohydrate content but were of equal energy value, weight, and protein content and of similar perceived palatability. Glucose and insulin profiles clearly demonstrated different metabolic responses to the meals and reflected the different macronutrient composition. The high-carbohydrate (and therefore low-fat) breakfast resulted in a greater response in both glucose and insulin. The meals did not differ in their effects on the postprandial profiles of hunger or fullness or in the amount of food consumed at the test meal. These meals therefore had similar actions on the phases of satiety and on satiation (meal size).

Ghrelin was suppressed equally by the 2 semisolid meals, and this outcome was consistent with a number of previous reports that have found similar effects on ghrelin after fat- and carbohydrate-rich meals (14). Over the 180-minute period the suppression of ghrelin was significantly correlated with the suppression of hunger after both breakfast meals. This finding extends those of previous studies that have shown similar temporal profiles of ghrelin and hunger without confirming an association (2). In addition, ghrelin was significantly correlated with hunger during both the early and late phases of satiety. After each of the semisolid meals, the change in ghrelin was significantly correlated with the amount eaten at the test meal. Therefore, the profile of ghrelin was closely associated with hunger throughout the postprandial period and both ghrelin and hunger predicted the amount eaten. There was also a relationship between ghrelin levels immediately before the ad libitum meal and the amount eaten at that meal. Taken together, these findings are consistent with an action of ghrelin on meal-to-meal appetite control. This finding is interesting because the relationships were disclosed under natural feeding circumstances with levels of ghrelin

within the physiological range. These data therefore confirm the findings that an effect on appetite has been shown after infusions of ghrelin.

GLP-1 levels increased more after the high-fat meal than after the high-carbohydrate meal, which is in contrast to previous studies. However, the high-fat meal did contain some carbohydrate; therefore, the rise may be an effect of fat plus carbohydrate. GLP-1 was associated with hunger in the late satiety phase only and the rise in GLP-1 over the morning was associated with lower energy intakes at the lunch meal, which is consistent with an influence of GLP-1 on short-term appetite control (16). PYY levels also increased more after the high-fat meal than after the carbohydrate meal, which has been shown previously (22). Again, the presence of some carbohydrate in the high-fat meal may have modulated the response of PYY. In contrast to the effect of ghrelin and GLP-1, PYY was not correlated with hunger or fullness over the full 180-minute period or during the early or late phases of satiety. This was true for PYY release measures and for the changes in PYY after the semisolid meals. Various analyses of the data failed to disclose any significant associations. In addition, measures of postprandial PYY were not significantly associated with the amount of food eaten at the next meal. Despite application of data treatments to PYY similar to those applied to ghrelin and GLP-1, there was no evidence that postprandial PYY was associated with hunger or fullness, the phases of satiety, or with satiation. Greater increases in fullness after consumption of the semisolid meals were negatively associated with energy intake at the lunch meal. Consequently, the natural profile of PYY after eating does not appear to be involved in the short-term control of appetite during the 3-hour period after eating. This outcome is in contrast to some studies that have shown an effect of infusions of PYY on appetite and energy intake (6, 21). However, these infusions reached supraphysiological levels (33), and this may account for the different outcomes.

The difference between the findings for GLP-1 and PYY might be explained by gastric emptying; GLP-1 has been shown to have a greater effect in slowing gastric emptying than PYY (34, 35); therefore, gastric emptying might be a mediating factor between the relationship of GLP-1 and short-term appetite control. We measured total PYY, which has been shown to have a temporal pattern similar to that of $PYY_{3–36}$ after meals (36). The total PYY assay used in this study was chosen for its ability to integrate into a multiplexing assay system (ie, Luminex MagPix). It has 100% immunoreactivity with $PYY_{3–36}$ and was already known to us to parallel a $PYY_{3–36}$-selective RIA (catalog no. PYY-67HK; Millipore). Hence, a singleplex RIA would not have added any new or different information. This is likely because most circulating PYY is in the $PYY_{3–36}$ form. It should also be noted that historically, methods to quantify individual components of PYY were not entirely specific to one component over the other (33).

Therefore, the difference between the outcomes of the present study and some previous reports does not appear to be due to the measurement of total PYY or $PYY_{3–36}$. The blood concentration of PYY may be important. There are at least 2 situations in which larger changes in PYY are associated with changes in appetite. After Roux-en-Y surgery, very high levels of PYY are seen, and this state is associated with a reduction in hunger and a decrease in appetite (37–39). In addition, after a substantial body weight loss PYY levels are significantly lowered (40), and this is associated with an increase in hunger. Consequently, under certain physiological conditions, larger changes in PYY may be a signal for changes in appetite. However, these circumstances are very different from the more natural physiological condition and normal feeding circumstances in the current study. It is possible, however, that even under the normal conditions of the present study, PYY changes could be associated with appetite beyond the 3-hour postmeal measurement period. However, this would extend the inference beyond a common intermeal interval.

In the present study, GLP-1 and PYY levels were significantly influenced by the semi-solid meals, and both were significantly increased after the high-fat/low-carbohydrate condition compared with the increase after high-carbohydrate/low-fat condition. However, because the 2 meals showed similar effects on satiety and satiation, the increased levels of GLP-1 and PYY after the high-fat breakfast did not exert any additional suppressive action on hunger or enhanced effect on fullness. GLP-1 was associated with reduced hunger, most markedly in the late satiety phase, whereas PYY was not correlated with hunger or fullness after the high-fat or high-carbohydrate breakfast even though PYY levels were significantly higher after the high-fat load. PYY was therefore sensitive to the effects of food ingestion, but this higher concentration of PYY after high-fat food did not lead to greater suppression of food intake at the test meal. However, we wish to point out that this study was conducted on overweight and obese participants. Because lean participants were not included, we cannot be sure that the effects demonstrated here would be true for all individuals irrespective of body fat content.

Ghrelin was not altered selectively by the nutritional composition of the semisolid meals, suggesting that postprandial ghrelin levels are influenced by the overall energy value of the meal (the 2 semisolid meals were isoenergetic) or by weight or volume of the food (both of which in this

study were similar) or by some combination of these. In each case, the effect of the semisolid meals on hunger was mirrored by the effect on ghrelin. Hunger and ghrelin were closely associated, and both were significantly associated with the amount of food subsequently eaten.

In conclusion, our data suggest that ghrelin and GLP-1 are significant biomarkers of the phases of satiety and may have a determining influence on hunger and food consumption. In contrast, PYY seems to be a significant biomarker of the nutrient composition of food (indicated here by the fat content). This result suggests that PYY may play a role in the intestinal response to fat consumption. However, this natural physiological response to fat does not appear to influence any aspect of satiety or satiation, suggesting that PYY is not involved in the meal-to-meal control of appetite.

Acknowledgments

Address all correspondence and requests for reprints to: Catherine Gibbons, Biopsychology Group, Institute of Psychological Sciences, University of Leeds, Leeds, United Kingdom LS2 9JZ. E-mail: c.gibbons@leeds.ac.uk.

This work was supported by funding from the Biotechnology and Biological Sciences Research Council (Diet and Health Research Industry Club [DRINC]) (Grant BB/G005524/1) and the European Union Seventh Framework Programme (FP7/2007-2013) under Grant Agreement 266408. E.N. was supported by the Novo Nordisk Foundation and Karolinska Institutet Diabetes Theme Center.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Wren AM, Bloom SR.** Gut hormones and appetite control. *Gastroenterology*. 2007;132:2116–2130.
2. **Cummings D, Frayo RS, Marmonier C, Aubert R, Chapelot D.** Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time-and food-related cues. *Am J Physiol Endocrinol Metab*. 2004;287:E297–E304.
3. **de Graaf C, Blom WAM, Smeets PA, Stafleu A, Hendriks HF.** Biomarkers of satiation and satiety. *Am J Clin Nutr*. 2004;79:946–961.
4. **Druce MR, Wren AM, Park AJ, et al.** Ghrelin increases food intake in obese as well as lean subjects. *Int J Obes (Lond)*. 2005;14:1130–1136.
5. **Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS.** A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*. 2001;50:1714–1719.
6. **Batterham RL, Cowley MA, Small CJ, et al.** Gut hormone PYY_{3-36} physiologically inhibits food intake. *Nature*. 2002;418:650–654.
7. **Kojima M, Kangawa K.** Ghrelin: structure and function. *Physiol Rev*. 2005;85:495–522.
8. **Kojima M, Hosada H, Date Y, Nakarzato M, Matsuo H, Kangawa K.** Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 1999;402:656–660.
9. **Nakarzato M, Murakami N, Date Y, et al.** A role for ghrelin in the central regulation of feeding. *Nature*. 2001;409:194–198.
10. **Wren AM, Seal LJ, Cohen MA, et al.** Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab*. 2001;86:5992–5995.
11. **Wren AM, Small CJ, Abbott CR, et al.** Ghrelin causes hyperphagia and obesity in rats. *Diabetes*. 2001;50:2540–2547.
12. **Monteleone P, Bencivenga R, Longobardi N, Serritella C, Maj M.** Differential responses of circulating ghrelin to high-fat or high-carbohydrate meal in healthy women. *J Clin Endocrinol Metab*. 2003;88:5510–5514.
13. **Tannous dit El Khoury D, Obeid O, Azar S, Hwalla N.** Variations in postprandial ghrelin status following ingestion of high-carbohydrate, high-fat, and high-protein meals in males. *Ann Nutr Metab*. 2006;50:260–269.
14. **Callahan H, Cummings D, Pepe M, Breen P, Matthys C, Weigle D.** Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. *J Clin Endocrinol Metab*. 2004;89:1319–1324.
15. **Murphy KG, Bloom SR.** Gut hormones and the regulation of energy homeostasis. *Nature*. 2006;444:854–859.
16. **Näslund E, Barkeling B, King N, et al.** Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. *Int J Obes Relat Metab Disord*. 1999;23:304–311.
17. **Drucker DJ, Nauck MA.** The incretin system: glucagon-like peptide-1 agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet*. 2006;368:1696–1705.
18. **Verdich C, Toubro S, Buemann B, Lysgard MJ, Juul HJ, Astrup A.** The role of postprandial releases of insulin and incretin in meal-induced satiety—effect of obesity and weight reduction. *Int J Obes Relat Metab Disord*. 2001;25:1206–1214.
19. **Feinle C, Chapman IM, Wishart J, Horowitz M.** Plasma glucagon-like peptide-1 (GLP-1) responses to duodenal fat and glucose infusions in lean and obese men. *Peptides*. 2002;23:1491–1495.
20. **Herrmann C, Göke R, Richter G, Fehmann HC, Arnold R, Göke B.** Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion*. 1995;56:117–126.
21. **Batterham R, Cohen M, Ellis S, et al.** Inhibition of food intake in obese subjects by peptide YY3–36. *N Engl J Med*. 2003;349:941–948.
22. **Essah PA, Levy JR, Sistrun SN, Kelly SM, Nestler JE.** Effect of macronutrient composition on postprandial peptide YY levels. *J Clin Endocrinol Metab*. 2007;92:4052–4055.
23. **MacIntosh CG, Andrews JM, Jones KL, et al.** Effects of age on concentrations of plasma cholecystokinin, glucagon-like peptide 1, and peptide YY and their relation to appetite and pyloric motility. *Am J Clin Nutr*. 1999;69:999–1006.
24. **Pedersen-Bjergaard U, Høt U, Kelbaek H, et al.** Influence of meal composition on postprandial peripheral plasma concentrations of vasoactive peptides in man. *Scand J Clin Lab Invest*. 1996;56:497–503.
25. **Kissileff HR, Carretta JC, Geliebter A, Pi-Sunyer FX.** Cholecystokinin and stomach distension combine to reduce food intake in humans. *Am J Physiol Regul Integr Comp Physiol*. 2003;285:R992–R998.
26. **Blundell J, Stubbs R.** High and low carbohydrate and fat intakes: limits imposed by appetite and palatability and their implications for energy balance. *Eur J Clin Nutr*. 1999;53:S148–S165.
27. **Lawton C, Burley V, Wales J, Blundell J.** Dietary fat and appetite control in obese subjects: weak effects on satiation and satiety. *Int J Obes (Lond)*. 1993;17:409–416.
28. **Stubbs RJ, Harbron CG, Murgatroyd PR, Prentice A.** Covert manipulation of dietary fat and energy density: effect on substrate flux and food intake in men eating ad libitum. *Am J Clin Nutr*. 1995;62:316–329.
29. **Blundell JE, Macdiarmid JI.** Fat as a risk factor for overconsumption: satiation, satiety, and patterns of eating. *J Am Diet Assoc*. 1997;97:S63–S69.
30. **Raben A, Agerholm-Larsen L, Flint A, Holst JJ, Astrup A.** Meals with similar energy densities but rich in protein, fat, carbohydrate,

or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. *Am J Clin Nutr*. 2003;77:91–100.
31. **Stubbs RJ, Hughes DA, Johnstone AM, et al.** The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *Br J Nutr*. 2000;84:405–415.
32. **Gibbons C, Caudwell P, Finlayson G, King N, Blundell J.** Validation of a new hand-held electronic data capture method for continuous monitoring of subjective appetite sensations. *Int J Behav Nutr Phys Act*. 2011;8:57–64.
33. **Degen L, Oesch S, Casanova M, et al.** Effect of peptide $YY_{3–36}$ on food intake in humans. *Gastroenterology*. 2005;129:1430–1436.
34. **Näslund E, Gutniak M, Skogar S, Rossner S, Hellstrom P.** Glucagon-like peptide 1 increases the period of postprandial satiety and slows gastric emptying in obese men. *Am J Clin Nutr*. 1998;68:525–530.
35. **Witte AB, Grybäck P, Holst JJ, et a;.** Differential effect of PYY1–36 and PYY3–36 on gastric emptying in man. *Regul Pept*. 2009;158: 57–62.
36. **Batterham RL, Heffron H, Kapoor S, et al.** Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell Metab*. 2006;4:223–233.
37. **le Roux CW, Welbourn R, Werling M, et al.** Gut hormones as mediators of appetite and weight loss after Roux-en-Y gastric bypass. *Ann Surg*. 2007;246:780–785.
38. **Korner J, Bessler M, Cirilo L, et al.** Effects of Roux-en-Y gastric bypass surgery on fasting and postprandial concentrations of plasma ghrelin, peptide YY, and insulin. *J Clin Endocrinol Metab*. 2005;90:359–365.
39. **Falkén Y, Hellström PM, Holst JJ, Näslund E.** Changes in glucose homeostasis after Roux-en-Y gastric bypass surgery for obesity at day three, two months, and one year after surgery: role of gut peptides. *J Clin Endocrinol Metab*. 2011;96:2227–2235.
40. **Sumithran P, Prendergast LA, Delbridge E, et al.** Long-term persistence of hormonal adaptations to weight loss. *N Engl J Med*. 2011; 365:1597–1604.