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BRIEF REPORT

Effect of Macronutrient Composition on Postprandial Peptide YY Levels

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Background: Peptide YY (PYY) is released from the distal small intestine and colon after meals and reduces appetite by increasing satiety. The amount of PYY released is proportional to calories ingested. Fat ingestion has also been reported to stimulate PYY release.

Objective: The objective of the study was to determine whether macronutrient composition influences postprandial serum PYY levels by comparing 1 wk of a weight-maintenance low-carbohydrate, high-fat (LCHF) diet with a low-fat, high-carbohydrate (LFHC) diet.

Methods: In this randomized crossover study, 18 obese subjects (14 females, 4 males, mean body mass index 35.6 ± 2.9 kg/m^2) were randomly assigned initially to 1 wk of a weight-maintenance LCHF or LFHC diet, after which a test meal of identical composition was given and serum PYY levels were assessed for 2.5 h postprandially. After a 1-wk washout period, subjects were crossed over and retested.

Results: After 1 wk, mean postprandial area under the curve PYY after the LCHF test meal was 1.5-fold greater than after the LFHC test meal ($P < 0.001$). The LCHF diet led to 55% higher levels of postprandial serum PYY levels, compared with the LFHC diet ($P = 0.005$).

Conclusions: These data show that a LCHF diet stimulates PYY secretion more than a LFHC diet in obese individuals. ***(J Clin Endocrinol Metab* 92: 4052–4055, 2007)**

PEPTIDE YY (PYY) is a peptide produced primarily by endocrine L cells in the ileum and colon that inhibits food intake after meals by interacting with receptors in the hypothalamus to signal satiety and suppress appetite (1) and delaying gastrointestinal transit (2). After meals, PYY is released into circulation partly in proportion to calories ingested such that plasma levels rise within 15 min, plateau at approximately 90 min, and peak, depending on calories ingested (3).

Animal studies suggest that fats have a more stimulatory effect on PYY, compared with carbohydrates and proteins (4). We hypothesized that a low-carbohydrate, high-fat diet (LCHF) would result in a greater increase in postprandial serum PYY levels, compared with a low-fat, high-carbohydrate diet (LFHC). To test this hypothesis, we compared fasting and postprandial PYY levels in obese individuals randomized in a crossover design to 1 wk of a LFHC or LCHF diet.

Subjects and Methods

Subjects

Obese adult men and women were recruited by advertisement in the local community. Subjects were eligible if they had a body mass index (BMI) 30–40 kg/m^2, 18–60 yr of age, generally healthy, and weight stable for 3 months or longer before enrollment. Exclusion criteria included uncontrolled diabetes mellitus with hemoglobin A1C greater than 7.9; systolic blood pressure greater than 170 mm Hg or diastolic blood pressure greater than 100 mm Hg; and pregnancy. Only one African-American female had type 2 diabetes mellitus and was on a stable dose of a sulfonylurea. Subjects provided informed, signed consent. All procedures took place at the General Clinical Research Center, and the protocol was approved by the Institutional Review Board of Virginia Commonwealth University.

Study protocol

In a randomized, crossover design, subjects were assigned initially to 1 wk of either a weight-maintenance LCHF diet (≤15% of total calories/day from carbohydrate) or LFHC diet (≤20% of total calories per day from fat). After the week, subjects presented to the General Clinical Research Center and were provided a test meal of identical macronutrient composition as to their previous week's consumption (mean 540 kcal). Serum PYY levels were measured postprandially every 30 min for 150 min. After a 1-wk washout period consisting of their usual diets, subjects were crossed over to the other diet for 1 wk and retested in a similar manner.

Although postprandial PYY secretion is believed to be dependent on meal previously ingested, we tested for postprandial PYY levels after 1-wk periods of specific macronutrient ingestion to potentially prime basal PYY secretion and stabilize insulin and leptin levels. In addition, this manner of testing more closely simulated physiological consumption, compared with a single test meal or direct perfusion of nutrients into the duodenum before testing.

Meals consisted of foods typically found in an American diet and included, among other items, Healthy Choice (low fat) and Life Choice (low carbohydrate; both ConAgra Foods, Omaha, NE) frozen dinners. Food items were supplied to the subjects in 3- to 4-d worth of meals at each pick-up. Body weights were determined at each pick-up and study visit date. Compliance was assessed using detailed interview by the same investigator at each study visit.

After enrollment, subjects were instructed by one of two dietitians to complete a food preferences questionnaire and 3-d food diary. Their average daily total energy requirements were estimated by total energy

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Abbreviations: AUC, Area under the curve; BMI, body mass index; HOMA, homeostasis model assessment; LCHF, low carbohydrate, high fat; LFHC, low fat, high carbohydrate; PYY, peptide YY.

expenditure = basal metabolic rate (calculated with the Harris-Benedict equation) × activity factor (sedentary = 1.3, some regular exercise = 1.5, and regular exercise or demanding job = 1.7). From these estimates, weight-maintenance meal plans were designed. Daily caloric intake was adjusted as needed to maintain body weight stable within 2.0 kg of baseline weight.

The mean macronutrient ratio of the actual diets consumed for the LFHC diet was 25% fat to 65% carbohydrate to 10% protein, and for the LCHF diet was 74% fat to 6% carbohydrate to 20% protein. Macronutrient compositions were calculated using the Nutrition Data System for Research (version 4.04; Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN).

Assays

Serum glucose concentrations were measured on an YSI glucose analyzer (YSI Inc., Yellow Springs, OH) using oxidative methodology and serum insulin using a double-antibody RIA. For PYY determinations, aprotinin (Sigma-Aldrich, Inc., St. Louis, MO) at a concentration of 1 μg/ml and dipeptidyl peptidase IV inhibitor (Linco Research Inc., St. Louis, MO) at a final concentration of 100 μM were added to the serum and samples stored at −70 C until assays were performed. All PYY samples were assayed in duplicate for measurement of total PYY ($PYY_{3–36}$ and $PYY_{1–36}$) using a specific and sensitive RIA (Linco Research). This assay used ^{125}I-labeled PYY and PYY antiserum to determine the level of active PYY in serum by the double-antibody technique. The lower limit of detection was 10 pg/ml, and the coefficients of variation were 9.4% within and 8.5% between assays. Serum leptin and adiponectin were measured with commercially available ELISA kits (Diagnostic System Laboratories, Inc., Webster, TX).

Statistical analysis

The study was designed to detect at least a 35% difference in area under the curve (AUC) of PYY between the two groups at 80% power with $\alpha = 0.05$. All data are presented as means ± SD. Baseline measurements were assessed with unpaired *t* tests. Comparisons between diets in parameters over time were analyzed using random-effects, repeated-measures ANOVA with time and diet as main effects. Correlations were tested by Pearson's correlation coefficient. AUC PYY was calculated with the trapezoidal method. The homeostasis model assessment (HOMA) was determined by fasting insulin (milliunits per liter) × fasting glucose (millimoles per liter)/22.5. All statistical analyses were made using JMP (version 6.0; SAS Institute Inc., Cary, NC), with $P < 0.05$ regarded as statistically significant.

Results

Baseline characteristics and weight changes

There were no dropouts throughout the study. The mean age of study subjects was 41.3 ± 11.7 yr and the mean BMI 35.6 ± 2.9 kg/m^2. Other than a higher systolic blood pressure in those assigned initially to the LCHF group (LCHF group: 141.9 ± 4.9 *vs.* 124.5 ± 5.5 mm Hg, $P < 0.05$), no other significant differences were observed. There were no differences in BMI based on sex (females: 35.5 ± 3.2 *vs.* males: 36.3 ± 1.5, P = NS) or ethnicity [African-Americans (n = 10): 36.1 ± 2.9 *vs.* Caucasians (n = 8): 35.1 ± 3.0, P = NS]. Mean baseline values (n = 18) were as follows: glucose 95.6 ± 12.4 mg/dl, insulin 10.6 ± 4.8 μU/liter, PYY 102.5 ± 50.4 pg/ml, leptin 39.1 ± 19.3 ng/ml, and adiponectin 6.8 ± 2.6 μg/ml.

Both diets were well tolerated. Two subjects admitted to noncompliance by consuming additional food items not on the menu a mean of three times per individual. There were no differences in mean weight loss by diet (LFHC diet: −1.4 ± 1.3 kg *vs.* LCHF diet: −1.3 ± 1.3 kg).

Glucose, insulin, and HOMA of insulin resistance (HOMA-IR)

Mean fasting glucose level was slightly higher after 1 wk of the LFHC diet, compared with the LCHF diet (92.6 ± 12.6 *vs.* 91.3 ± 12.9 mg/dl, $P = 0.01$, respectively). No differences were observed in mean fasting insulin levels (LFHC diet: 7.6 ± 5.1 μU/liter, LCHF diet: 7.7 ± 4.9 μU/liter, P = NS). Mean postprandial glucose levels were approximately 2-fold higher and mean postprandial insulin levels 3-fold higher after the LFHC test meal (Figs. 1 and 2). HOMA-IR did not differ between the two groups.

PYY

Men had higher mean baseline fasting PYY levels than women [men (n = 4): 168.4 ± 52.7 pg/ml, women (n = 14): 83.7 ± 31.1 pg/ml, $P = 0.0008$]. The observed sex differences persisted postprandially, with AUC PYY lower in women than men after receiving either the LFHC ($P = 0.012$) or LCHF ($P = 0.026$) test meals.

There were no differences in fasting PYY levels at baseline by diet. Likewise, mean fasting PYY levels did not differ after the LCHF diet, compared with the LFHC diet (95.3 ± 47.6 *vs.* 98.4 ± 57.6 pg/ml, P = NS). However, mean postprandial AUC PYY after the LCHF test meal was 1.5-fold greater than after the LFHC test meal ($P < 0.001$, Fig. 3). Regardless of diet, mean postprandial PYY concentrations increased shortly after ingesting a test meal. However, PYY concentrations over time were significantly higher after the LCHF test meal, compared with the LFHC test meal ($P = 0.005$). Significant differences occurred between 60 and 150 min, with up to 55% higher postprandial PYY levels on the LCHF diet *vs.* the LFHC diet.

Correlations between PYY and insulin, leptin, and adiponectin

In exploratory analysis, a negative correlation between baseline fasting PYY and leptin levels was detected, with higher leptin levels correlating with lower PYY levels (r =

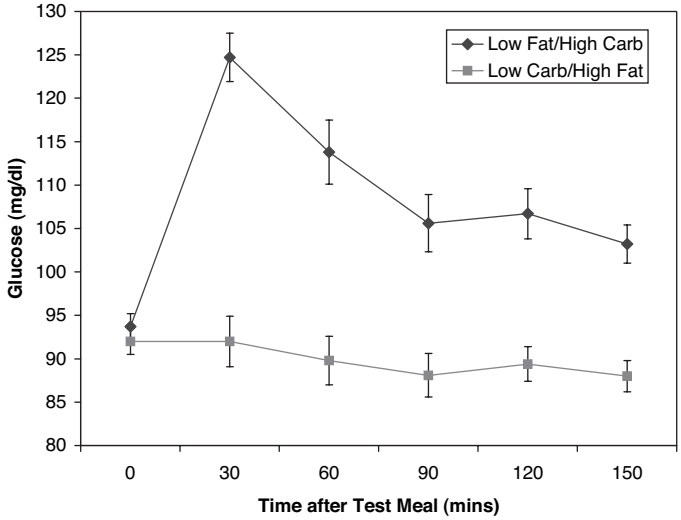

FIG. 1. Change in postprandial glucose levels in 18 subjects after a LCHF diet and a LFHC diet ($P < 0.001$ between 30 and 150 min).

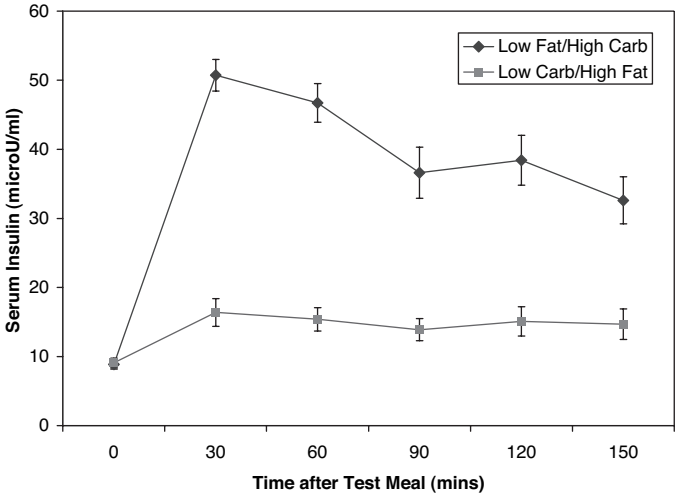

FIG. 2. Change in postprandial insulin levels in 18 subjects after a LCHF diet and a LFHC diet ($P < 0.001$ between 30 and 150 min).

-0.55, $P = 0.017$). There was also a negative relationship between AUC PYY and AUC leptin for the whole group after the LFHC diet ($r = -0.48$, $P = 0.049$) but not after the LCHF diet. No other significant correlations were detected.

Discussion

Our study is the first to demonstrate enhanced postprandial PYY secretion after consumption of a LCHF diet, compared with a LFHC diet. Although some animal studies have shown that bile acids play a major role in regulation of PYY (5–7), other studies have reported a more crucial role of nutrients in PYY regulation (8).

Fat appears to be the most potent stimulus of PYY and carbohydrate the least potent (8), supporting the results of the present study. Ileal infusion of the fatty acid oleic acid in animals (8) and humans (9) significantly increases plasma PYY levels. One investigation demonstrated that fat digestion is necessary for stimulation of PYY in humans (10). Batterham *et al.* (11) recently reported that high-protein intake stimulated PYY intake in humans and mice. However, we found no difference in results after statistically adjusting for protein.

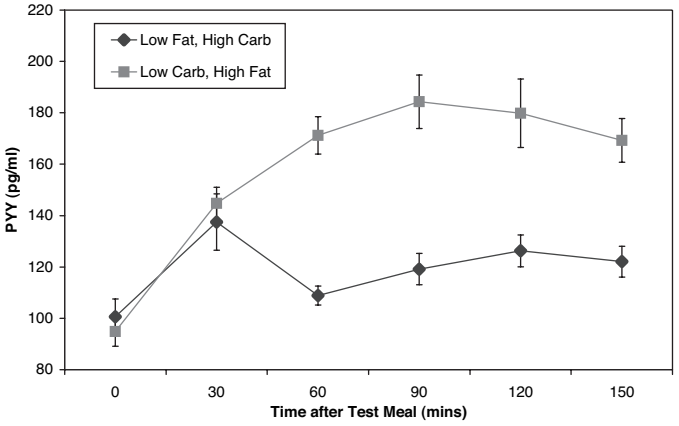

FIG. 3. Change in postprandial PYY levels in 18 subjects after a LCHF diet and a LFHC diet ($P < 0.001$ between 60 and 150 min).

PYY regulates satiety via interaction with receptors in the arcuate nucleus (2) and by slowing ileal transit of digested food (3). Although we did not measure satiety, our findings of higher postprandial PYY levels after the LCHF diet, compared with the LFHC diet, possibly suggest that those in the LCHF group may have experienced greater postprandial satiety, which in turn can facilitate weight loss. Of interest, 6-month studies have reported greater weight loss with low-carbohydrate diets, compared with low-fat diets (12).

Fasting PYY levels correlated negatively with fasting leptin levels and a negative correlation postprandially was noted with the LFHC diet, implying that PYY secretion may be mediated by leptin or vice versa. Although leptin regulates the small intestinal response to cholecystokinin in leptin-deficient obese mice (13), leptin did not appear to regulate PYY in humans (14). Our results also revealed sex differences in PYY levels, with women having lower fasting and postprandial PYY levels. To our knowledge, only one other study has reported gender differences in PYY secretion (15), although the gender differences contrasted with our results. Further investigations are needed to evaluate the relationship between PYY and leptin and evaluate possible sex differences in PYY secretion.

A limitation to our study was that satiety was not measured using visual analog scales. Furthermore, the possibility exists that postprandial responses to PYY may have been influenced by the test meals only rather than by the previous weeks' meals. However, the finding that fasting PYY levels were higher, although not significantly, after 1 wk of the LCHF meals, compared with the LFHC meals makes this less likely. Another limitation is that due to the differing proportions of protein and fat in the two diets, it is difficult without complex statistical modeling to definitely differentiate between the effects of increases in dietary fat and protein on PYY levels.

In summary, the results of this study indicate that PYY is regulated by the macronutrient composition of foods to which endocrine cells lining the ileum and colon are exposed. LCHF foods stimulate PYY secretion to a much greater degree than do LFHC foods.

Acknowledgments

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