The Effects of a Hypocaloric Diet on Diet-Induced Thermogenesis and Blood Hormone Response in Healthy Male Adults: A Pilot Study

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(Received April 30, 2015)

Summary  Calorie restriction is a common strategy for weight loss and management. Consumption of food and nutrients stimulates diet-induced thermogenesis (DIT), as well as pancreatic and gastrointestinal hormone secretion that may regulate energy metabolism. Yet, little is known about the impact of hypocaloric diets on energy metabolism-related parameters. In this study, we assessed the effects of hypocaloric diets on hormonal variance in relation to DIT in healthy adults. Ten healthy male adults were enrolled in a randomized crossover study comprising three meal trials. Each subject was given a meal of 200 (extremely hypocaloric), 400 (moderately hypocaloric), or 800 kcal (normocaloric). Postprandial blood variables and energy expenditure were measured for 4 h (after the 200- and 400-kcal meals) or 6 h (after the 800-kcal meal). DIT and postprandial changes in blood pancreatic peptide and ghrelin were significantly smaller after the extremely or moderately hypocaloric diet than after the normocaloric diet but were similar between the hypocaloric diets. Postprandial blood insulin, amylin, glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide type-1 (GLP-1) increased in a calorie-dependent manner. Thermogenic efficiency (DIT per energy intake) was negatively correlated with the maximum blood level (C max) (p=0.01) and incremental area under the curve (p=0.01) of the blood GIP response. Calorie restriction thus leads to hormonal responses and lower DIT in healthy adults. Extreme calorie restriction, however, led to greater thermogenic efficiency compared with moderate calorie restriction. The postprandial GIP response may be a good predictor of postprandial thermogenic efficiency.

Key Words  calorie restriction, diet-induced thermogenesis (DIT), energy metabolism, glucose-dependent insulinotropic polypeptide (GIP), gut hormone

The increasing prevalence of obesity and overweight, which are caused by an imbalance between energy intake and consumption, is a major health problem worldwide. Calorie restriction, as well as exercise, is a common strategy for weight loss and management.

Diet-induced thermogenesis (DIT), which is the increase in energy expenditure after meal ingestion, accounts for approximately 10% of the total daily energy expenditure in humans (1). In humans, 20–30% of proteins, 5–10% of carbohydrates, and up to 3% of fat ingested is utilized in DIT (2); dietary protein, carbohydrate, and fat increase DIT by 25–40%, 6–8%, and 3%, respectively (3–5). DIT is also dependent on the total energy content (6) and size (7) of meals.

The scientific evidence accumulated to date indicates that energy metabolism may be controlled by pancreatic and gastrointestinal hormones. Resting energy expenditure (REE) is higher in patients with type 1 diabetes than in healthy subjects but can be rescued by insulin treatment (8). Amylin (9, 10) or pancreatic polypeptide (11) administration increases energy expenditure in rodents. Gastrointestinal hormones, such as ghrelin (12), cholecystokinin (CCK) (13), glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide type 1 (GLP-1) (14, 15), and peptide YY (PYY) (16) have also been shown to have roles in energy metabolism in animals and humans.

Because the endocrine response of these hormones is regulated by nutrients and meal ingestion, the restriction of calorie intake may change the secretion of the energy metabolism-regulating hormones and thereby affect postprandial energy metabolism.

Several studies have shown that an increase in calorie intake or meal size increases DIT (6, 7) and the postprandial change in blood hormone levels (17, 18). Intake of a low-calorie diet might reduce DIT and the postpran-
dial hormone response. However, little is known about the linearity of the decrease in DIT and the hormone response associated with calorie restriction. We hypothesized that DIT or postprandial thermogenesis might decrease nonlinearly with reduced calorie intake, especially after ingestion of an extremely hypocaloric meal, which may be associated with a decrease in energy metabolism-related hormones. A better understanding of postprandial energy metabolism after the ingestion of an extremely low-calorie meal may be beneficial to the design of dietary therapies for weight maintenance or loss.

Accordingly, the aim of this study was to clarify the postprandial energy metabolism and hormone response after consumption of a hypocaloric diet by healthy adults. We also investigated the correlation between DIT or postprandial thermogenesis and hormone release.

MATERIALS AND METHODS

Subjects. Fifteen people were recruited for eligibility screening (inclusion criteria: male, body mass index (BMI) [kg/m²] 22±5, age [y] 25–49, fasting blood glucose [mg/dL] 70–109, fasting blood triglyceride (TG) [mg/dL] 50–199); of these 10 subjects were enrolled in the study (age [y], 36.0±3.0; weight [kg], 68.3±3.5; BMI [kg/m²], 22.9±1.0; body fat [% weight], 22.6±2.2; REE [kcal/d], 1,757.0±72.0). Three subjects were smokers. None of the subjects took medication; had lifestyle interventions (e.g., dietary/nutritional restriction, dietary/nutritional modification, restriction or modification of exercise or physical activity, or exercise therapy), allergies, hypersensitivity to wheat flour or spice; or were heavy users of alcohol.

The study protocol was approved by the Ethics Committee of Kao Corporation (Tochigi, Japan), and the study was conducted according to the guidelines laid down in the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Procedures. This was a non-blinded, randomized crossover study comprising three trials with an interval of at least 6 d between subsequent trials.

The randomization procedure was conducted by a person who was not directly involved in the study, and the subjects and staff were unaware of the assignments for the duration of the study. The subjects were given lunch [900 kcal (3,767 kJ), carbohydrate : fat : protein=15 : 25 : 60 (cal%)] before 8 PM the night before each study session: thereafter only water was allowed until the following morning.

After overnight fasting for 13 to 15 h, at 8:30 AM, the subjects dressed in laboratory uniforms and went to a test room. Blood samples were collected and fasting-REE were measured for 15 min, and the mean values of the data recorded from minutes 5 to 10 were used. Thermogenic efficiency was calculated by dividing DIT by the energy intake.

Blood analysis. Blood samples (5 mL) were collected via the median cubital vein by using the BD™ P700 Blood Collection System for Plasma GLP-1 Preservation (Becton Dickinson, Franklin Lakes, NJ) and immediately mixed with protease inhibitor cocktail (Roche Diagnostics Co., Tokyo, Japan). After centrifugation at 1,300 ×g for 10 min at room temperature, plasma was removed and stored at −80°C until analysis. Blood glucose was determined by using a blood glucose self-monitoring device (Accu-Chek Aviva; Roche Diagnostics Co.) immediately after blood collection. Plasma triglyceride concentrations were measured by using an E-test Wako kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma triglyceride concentrations were measured by using the appropriate ELISA: apolipoprotein B48 (AKHB48; Shibayagi, Gunma, Japan), insulin (10–1113; Mercodia, Uppsala, Sweden), CCK (EK-069-04; Phoenix Pharmaceuticaals, Burlingame, CA), total GIP (EZHGIP-54K; Merck Millipore,
Tokyo, Japan), and active GLP-1 (EGLP-35K; Merck Millipore). Other hormones (active ghrelin, total amylin, pancreatic polypeptide, total PYY, and leptin) were measured by using a Milliplex Map human metabolic hormone panel (HMH-34K; Merck Millipore).

Statistical analysis. Data are expressed as mean ±SE. Time-course data from 0 to 4 h were compared by using two-factor repeated-measures ANOVA to evaluate the meal-by-time interaction and the time effect, and non-repeated measures ANOVA was used to evaluate the meal effect. When a significant meal-by-time interaction was observed, an unpaired t-test was used for intergroup comparison at each time point during the analytical period. All other statistical tests were performed by using the Tukey-Kramer method. p values of less than 0.05 were considered statistically significant. Thermogenic efficiency was calculated and used in the regression analyses as an index for the efficiency of diet-induced thermogenesis. All statistical analyses were performed by using the GraphPad Prism version 6.0 (GraphPad Software, La Jolla, CA).

RESULTS

Postprandial thermogenesis after consumption of a hypocaloric diet
Consumption of each test meal increased the REE postprandially until 120 to 150 min after the meal consumption (Fig. 1A). Then the postprandial-REE gradually declined and reached the baseline level at 210 min after the 200- or 400-kcal meal or 330 min after the 800-kcal meal (Fig. 1A). A significant meal-by-time interaction (M×T) was found for the increase in postprandial-REE (Fig. 1A). The increase in postprandial-REE (DIT) after the 800-kcal meal was significantly greater than that after ingestion of the 200- or 400-kcal meal (p<0.05). However, DIT after the 200- or 400-kcal meal was similar. The energy balance (energy intake minus postprandial-REE) for 4 h after meal ingestion was negative (−129 kcal) only after the extremely hypocaloric diet (200 kcal) (Fig. 1A). The energy balance was positive (+75 or 437 kcal) after the moderately hypocaloric (400 kcal) or the normocaloric (800 kcal) diet, respectively (Fig. 1B). The thermogenic efficiency after
Fig. 2. (A–K) Time course of blood variable levels after ingestion of each test meal containing 200 (open circles), 400 (closed triangles), or 800 kcal (open squares) in overnight-fasted, healthy, male subjects. (A) Glucose, (B) triglycerides (TG), (C) ApoB48, (D) insulin, (E) amylin, (F) pancreatic polypeptide (PP), (G) ghrelin, (H) total glucose-dependent insulino- tropic polypeptide (GIP), (I) cholecystokinin (CCK), (J) active glucagon-like peptide type-1 (GLP-1), (K) peptide YY (PYY). (right-upper inset) Relative incremental area under the curve (iAUC) or decremental AUC (dAUC) of each blood variable after meal ingestion. Two-factor repeated measures ANOVA was used to evaluate the meal (M)-by-time (T) interaction and the time effect, and non-repeated-measures ANOVA was used to evaluate the meal effect. Data are presented as means ± SE. a, b, c: Means that do not share a given letter differ significantly (\( p < 0.05 \)).
the extremely hypocaloric meal was significantly greater ($p=0.04$) than that after the moderately hypocaloric diet (Fig. 1C).

**Postprandial responses of blood variables**

Postprandial blood variable levels changed depending on the meal size (Figs. 2A–K), with the exception of the leptin levels, which did not change postprandially (data not shown).

Blood levels of insulin, amylin, total GIP, and active GLP-1 increased in proportion to the energy content of the test meal (Figs. 2D, E, H, and J, respectively). In contrast, postprandial changes in blood TG, ApoB48, pancreatic polypeptide (PP), ghrelin, CCK, and PYY (Figs. 2B, C, F, G, I, and K) were similar for the 200- and 400-kcal meals, whereas these changes were significantly greater after the normocaloric (800-kcal) meal compared with the hypocaloric meals. Postprandial blood glucose responses after the 400-kcal or 800-kcal meal were comparable to each other and higher than that after the 200-kcal meal.

**Correlation of postprandial thermogenesis with blood variables**

Thermogenic efficiency (DIT per energy intake) was negatively correlated with only blood GIP response (both maximum blood level ($C_{\text{max}}$) and iAUC) (Figs. 3A and B, respectively) and was not associated with the caloric content of the meal (data not shown). A correlation analysis after ingestion of each test meal did not find any significant correlation between thermogenesis and the GIP response. We did observe a trend toward a negative correlation between thermogenic efficiency and blood GIP response ($C_{\text{max}}$ and iAUC) after the 400-kcal meal ($p=0.16$ and 0.11, respectively). DIT tended to be correlated with the blood PYY response ($C_{\text{max}}$) after the 800-kcal meal ($r$ [Pearson’s correlation coefficient]$=0.59$, $p=0.08$).

**DISCUSSION**

The present study had three major findings. First, this study showed for the first time that caloric reduction decreases postprandial thermogenesis (DIT) nonlinearly.

The extremely hypocaloric diet (200 kcal) induced similar DIT with the moderately hypocaloric (400 kcal) diet, which led to a negative energy balance and greater thermogenic efficiency. DIT has been shown to be dependent on meal size (6, 7). However, this was shown in studies with test meals that contained over 400 kcal (6, 7). To our knowledge, the current study is the first to show that DIT after an extremely hypocaloric meal was equivalent to that after a moderately hypocaloric meal.

Second, this study revealed that consumption of an extremely hypocaloric diet leads to reduced blood responses for insulin and incretin (GIP and GLP-1) compared with a moderately hypocaloric diet; however, with respect to other blood hormones, the two diets yielded similar responses.

In this study, DIT was positively associated with blood PYY levels after consumption of the normocaloric meal, which is consistent with the results of Doucet et al. (16), which showed that energy expenditure was associated with the hormone response in healthy women. Sloth et al. (19) further showed that PYY infusion increased energy expenditure both in obese and lean subjects, suggesting that the postprandial blood PYY response may regulate DIT in humans. In the present study, however, consumption of the extremely hypocaloric diet did not stimulate the PYY response, whereas the meal with over 400 kcal elevated blood PYY levels in the healthy male adults. These results suggest that blood PYY was not responsible for DIT after consumption of the extremely hypocaloric diet but instead may regulate postprandial energy expenditure after sufficient energy intake.

Finally, and perhaps most interestingly, this study demonstrated that thermogenic efficiency was inversely correlated with the postprandial blood GIP response in healthy male adults, suggesting that lower blood GIP levels lead to greater postprandial thermogenesis. Accordingly, the postprandial GIP response may be a good predictor of postprandial thermogenic efficiency.

Postprandial GIP release is stimulated by macronutrients, especially dietary carbohydrate (20) and fat (21). GIP facilitates energy storage as fat by stimulating glu-
cose or fatty acid transport into adipose tissues (22). Recently, genome-wide association studies have identified a single-nucleotide polymorphism in the human GIP receptor gene that is related to the risk of obesity (23). Inhibition of GIP signaling by targeted ablation of either GIP-secreting cells or GIP receptors leads to elevated energy expenditure and prevents diet-induced obesity in mice (24, 25). Moreover, an increase in blood GIP levels via intravascular infusion decreased energy expenditure in healthy human volunteers (26). Taken together, these findings suggest that the postprandial blood GIP response may be a negative regulator of postprandial thermogenesis.

Although not significant, a potential weakness of the present study is that our study population was small and included individuals whose BMI and body fat were varied. Therefore, we cannot rule out the possibility of type II errors that may have led to the lack of statistical significance seen for the associations between certain blood variables and energy metabolism. Our correlation analysis after each test meal trial did not reveal a statistically significant correlation between the postprandial thermogenesis and the GIP response. Therefore, it would be worthwhile to study a larger population to clarify the relationship between the postprandial hormone response and energy metabolism. Another limitation is that we cannot exclude the potential influence of spice in the test meal on postprandial thermogenesis. Capsaicin (3.6 mg) has been shown to increase DIT (approximately 10 kcal/270 min) in humans (27), whereas piperine (23 mg) does not change energy expenditure in humans (28). Although the effects of the small amounts of these spices (<0.01 mg capsaicin and 0.8 mg piperine) in the test meal on DIT were slight or negligible, we cannot rule out the possible influence of other spicy ingredients. A further limitation is that the present study revealed interrelationships, not causal relationships, between blood hormones and DIT. We did not address the mechanisms underlying the negative correlation between the postprandial GIP response and thermogenesis. Even though GIP is widely known as an incretin, which potentiates glucose-induced insulin secretion from pancreatic β-cells, the blood insulin response was not associated with DIT. Accordingly, the extrapancreatic action of GIP—rather than its insulinotropic action—may be related to the downregulation of postprandial thermogenesis. However, the GIP receptor is highly expressed in energy-anabolic adipose tissues but not in energy-catabolic tissues such as liver and skeletal muscles. Further studies are required to clarify how blood GIP lowers postprandial thermogenesis.

To conclude, this study provides evidence that consumption of an extremely hypocaloric diet leads to a negative energy balance with greater thermogenic efficiency than a moderately hypocaloric diet in healthy male adults. In addition, the postprandial blood GIP response may be a good predictor of postprandial thermogenic efficiency. Calorie reduction that lowers the postprandial response of the anabolic hormone GIP could be beneficial in the design of therapeutic diets for weight management. However, the negative energy balance after ingestion of the extremely hypocaloric diet in this study was obtained in the acute phase after meal ingestion under strict conditions. Intensive calorie restriction without satiety results in hyperphagia that may increase long-term calorie intake. Further studies under practical and long-term conditions are warranted.

Acknowledgments

We thank Junko Suzuki and Aki Yamasaki for technical assistance and Yoshiaki Ichikawa for advice on statistical analyses.

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