Correlations of macronutrient-induced functional magnetic resonance imaging signal changes in human brain and gut hormone responses¹⁻³

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ABSTRACT

Background: Body energy homeostasis is largely regulated by the interactions between appetite-related brain regions and gut hormones. We hypothesized that the sensitivity of appetite-related brain regions [e.g., hypothalamus, insula, thalamus, parahippocampal/hippocampal cortex, caudate, putamen, amygdala, and orbitofrontal cortex (OFC)] varies for each macronutrient, and the differential sensitivity is associated with gut hormone concentrations in humans.

Design: Brain activation responses to ingested fat, glucose, protein, and water in the above-mentioned 8 brain regions of 14 healthy men were investigated by using functional magnetic resonance imaging. Fasting and postprandial plasma glucose, insulin, ghrelin, cholecystokinin (CCK), and glucagon-like peptide 1 (GLP-1) concentrations were measured. The relation of the blood oxygen level–dependent (BOLD) signal with plasma glucose and hormone concentrations was assessed by using Pearson’s correlation analysis.

Results: Ingested macronutrients similarly reduced the BOLD signal in the middle insula, thalamus, parahippocampal cortex, caudate, and lateral OFC. Protein ingestion reduced the BOLD signal in the amygdala more effectively than did fat and glucose ingestion. BOLD signal changes were positively correlated with circulating ghrelin concentrations and were negatively correlated with circulating insulin, CCK, and GLP-1 concentrations. The findings indicate variations in the correlation between brain activation and plasma hormone concentrations after ingestion of different macronutrients.

Conclusions: The middle insula, thalamus, parahippocampal cortex, caudate, and lateral OFC, but not the amygdala, have similar sensitivities to isocaloric and isovolumetric macronutrient solutions. Differential correlations exist between BOLD signal changes in activated brain regions and postprandial changes in plasma concentrations of different gut hormones in response to the ingestion of different macronutrients. This trial was registered at chictr.org as ChiCTR-TRC-12001945. Am J Clin Nutr 2012;96:275–82.

INTRODUCTION

Body energy homeostasis is largely regulated by hunger and satiety signals resulting from gut-brain interactions, which are triggered by ingested nutrients and appetitive stimuli (1). The gastrointestinal tract releases many hormones that act as a signal to the central nervous system to regulate appetite. Ghrelin is the only factor known to increase the appetite through circulation (2) and has been shown to play a role in the long-term regulation of body weight (3). In contrast, cholecystokinin (CCK)⁴ and glucagon-like peptide 1 (GLP-1) potently reduce food intake in rodents and humans (4, 5). Insulin is one of the key adiposity signals in the brain that regulates energy homeostasis (6). All of these hormones are sensitive to the nutrient content in the gut (7). Carbohydrates may be the most effective suppressor of postprandial circulating ghrelin concentration (8, 9) and stimulator of GLP-1 and insulin release (10, 11). Fat and protein evoke a greater postprandial release of CCK than does carbohydrate (12, 13).

Recent studies using neuroimaging have provided insights into the in vivo function of gut-brain pathways in humans. Ingestion of glucose and satiating meals reduces neuronal activity in homeostatic (e.g., hypothalamus) and nonhomeostatic [e.g., insula, thalamus, hippocampus, caudate, putamen, amygdala, and orbitofrontal cortex (OFC)] brain regions (14–18). The homeostatic system appears to drive food intake based on caloric need or energy balance (19, 20). Neural responses within the nonhomeostatic brain network in response to environmental food cues may override the homeostatic signal, thus contributing to the development and maintenance of obesity (20). Several reports have shown that the infusion of exogenous ghrelin (21), GLP-1 (22), CCK-1 receptor antagonist (23), or intranasal insulin (24) modulates the activity of appetite-related brain regions.

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⁴ Abbreviations used: BOLD, blood oxygen level–dependent; CCK, cholecystokinin; fMRI, functional magnetic resonance imaging; GLP-1, glucagon-like peptide 1; OFC, orbitofrontal cortex; ROI, region of interest; VAS, visual analog scale.

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Fat, carbohydrate, and protein are the 3 primary calorogenic nutrients and play essential roles in energy homeostasis regulation. Gut hormones have shown various sensitivities to different macronutrients (25), although whether different macronutrients have different effects on the activation of appetite-related brain regions remains unknown.

In the current study, we hypothesized that the sensitivity of the appetite-related brain regions (eg, hypothalamus, insula, thalamus, parahippocampal/hippocampal cortex, caudate, putamen, amygdala, and OFC) varies for each macronutrient, and the differential sensitivity is associated with gut hormone concentrations in humans.

SUBJECTS AND METHODS

Subjects

Fourteen healthy young men [mean age: 23 y; range: 21–25 y; mean BMI (in kg/m²): 21.2 (range: 19.7–23.5)] were recruited from the East Normal University of China in Shanghai via posters on campus. All subjects were right-handed and were nonsmokers. They had low dietary restraint (Three-Factor Eating Questionnaire restraint score ≤13) (26), had no allergies to any food, and had no endocrine or gastrointestinal disorders. Their weights were stable (<3-kg change over the previous 3 mo) according to their self-report, and they were not taking any medications that were likely to confound the study outcomes. The subjects were not informed about the true purpose of the current study and were blinded to the nature of the infusions. All subjects gave written informed consent before their inclusion in the study. All procedures were compliant with the Declaration of Helsinki, and the protocol was approved by the Ethical Committee of Harbin Medical University.

Test beverages

The 4 test beverages consisted of 300 mL of each of the following: 250 g glucose solution/L (BeiXing; Heping Pharmaceutical Corp), 1255 kJ; 257 g whey protein solution/L (Aishente), 1255 kJ; 111 g soybean oil emulsion/L (Intralipid; Sino-Swed Pharmaceutical Corp Ltd), 1255 kJ; and 0 kcal distilled water/L. To eliminate the confounding effect of taste, all 4 solutions were identical in taste. One of the test beverages consisted of commercial Intralipid (containing soybean oil, 22% fat; Intralipid, Sino-Swed Pharmaceutical Corp Ltd). The minimum detectable concentration of soybean oil was 0.05% (w/w), the Intralipid concentration was 10% (w/w), and the Intralipid solution was diluted to 5% (w/w) before use.

Preparation of plasma samples

Blood samples were collected into chilled tubes containing sodium EDTA2Na and aprotinin (60 µL, 0.6 trypsin-inhibiting units/mL blood), which were then gently shaken several times. All blood samples were chilled in an ice bath until centrifugation at 1600 x g for 15 min at 4°C. Plasma was collected and stored at −80°C until assayed.

Glucose and hormone assays

Plasma glucose concentrations were detected with the oxidase/peroxidase method (Nanjing Jiancheng Bioengineering Institute). Plasma insulin, total ghrelin, CCK(26–33), and GLP-1(7–36) concentrations were measured by using ELISA kits (Phoenix Pharmaceuticals). The minimum detectable concentrations of these assays were 0.78 µIU insulin/mL, 0.02 ng ghrelin/mL, 0.05 ng CCK(26–33)/mL, and 0.14 ng GLP-1(7–36)/mL. Intrassay variation was 5–10%, and interassay variation was 15% for all hormone assays.

Image and statistical analysis

Imaging data were preprocessed and analyzed by using Statistical Parametric Mapping 8 (http://www.fil.ion.ucl.ac.uk/spm) and Data Processing Assistant for Resting-State fMRI V2.0 Basic Edition (29) implemented in MATLAB 7.8 (The Mathworks Inc). The first 12 time points were removed because of signal equilibrium and to allow the participants to adapt to the scanning...
noise. The remaining 420 volumes for every subject were pre-processed with slice timing correction, realignment of all images to the first volume, spatial normalization to Montreal Neurological Institute 152 template with \( 3 \times 3 \times 3 \) mm\(^3\) resampling, spatial smoothing with a 4-mm full-width half maximum isotropic Gaussian kernel, and elimination of linear drift. Data with motion >1 mm or rotation of >1° during scanning were excluded.

First-level analysis was carried out on each subject for each session. The preingestion and postingestion scans were divided into 7 consecutive 5-minute time bins (eg, T0 to T6). The baseline time bin (T0) consisted of 60 preingestion scans (5 min). For each subject, T0 was deducted from 6 postingestion time bins (T1 to T6) for each session (ie, fat, glucose, protein, or water). First-level analysis yielded 6 first-level images (Tn – T0, Tn minus T0) corresponding to each time bin for each subject.

On the basis of previous studies, we a priori selected 8 regions of interest (ROIs): hypothalamus, insula, thalamus, parahippocampal/hippocampal cortex, caudate, putamen, amygdala, and OFC (14–18). To determine whether there were significant differences in the changes in the ROIs between subjects after ingestion of fat, glucose, protein, or water, a second-level analysis (between subjects) was carried out by using a \( 4 \times 6 \) full-factorial ANOVA with test beverage as an independent factor and time bin as a repeated-measures nonindependent factor. The correction thresholds correspond to a corrected \( P < 0.05 \) determined by Monte Carlo simulations with the use of the program AlphaSim in Analysis of Functional NeuroImages. Only activations exceeding an uncorrected threshold of \( P < 0.010 \) on individual voxel and a minimal cluster size of 10 voxels were considered significant. The activated regions were identified by using REST 1.4 software (30), and the time course of each activated region was extracted. At every time point (1 min), the mean gray value of each activated region was calculated. These mean gray values were normalized to the mean of the 5-min baseline to determine the percentage blood oxygen level–dependent (BOLD) signal change from the mean baseline. Because drinking can cause artifactual signal changes, the BOLD signal during ingestion was not included in the statistical analyses. The AUC\(_{0-30\,\text{min}}\) was calculated by using the trapezoidal rule to quantify the overall BOLD response to the test beverages, which reflected both the amount and the duration of the response.

Statistical analyses of BOLD signal changes, appetite sensations, and hormone and glucose concentrations were analyzed by using SPSS 16.0 (SPSS Inc) with \( \alpha = 0.05 \). Differences in VAS, glucose, and hormone responses and the AUC of BOLD signal changes between all test beverages were analyzed by 1-factor ANOVA, followed by a Tukey post hoc test.

The time course of BOLD signal changes among all test beverages was analyzed by 2-factor repeated-measures ANOVA, with test beverage and time as main effects, followed by a Tukey post hoc test. The relations between changes in BOLD signal of activated regions and changes in VAS, glucose, and hormones were assessed by using Pearson’s correlation analysis.

RESULTS

Hunger and satiety ratings

Preingestion sensation ratings were equivalent across all 4 study sessions. Ingestion of the 4 test beverages did not induce nausea at any time during the procedure. The beverages produced a small reduction in the sensation of hunger and a small increase in satiety. No significant differences in the sensation of hunger among the 4 ingested beverages were found. Ingested fat, glucose, and protein beverages induced significantly higher satiety than did ingested water (Table 1).

Plasma glucose, insulin, ghrelin, GLP-1, and CCK concentrations

As shown in Table 1, fasting glucose and hormone concentrations were equivalent across the 4 study sessions. Plasma glucose concentrations increased significantly after glucose ingestion, whereas no plasma glucose elevation occurred after ingestion of fat and protein beverages compared with ingestion of water. Plasma insulin and GLP-1 concentrations after glucose and protein ingestion were significantly higher than those after water ingestion. Moreover, glucose ingestion produced higher plasma insulin concentration than did protein ingestion. Fat ingestion did not elevate plasma insulin and GLP-1 concentrations. Plasma ghrelin concentration after glucose ingestion was significantly lower than that after water ingestion. In contrast, plasma ghrelin concentrations were not significantly decreased 30 min after fat and protein ingestion compared with those after water ingestion. Plasma CCK concentrations decreased significantly after ingestion of each macronutrient compared with those after water ingestion. Moreover, the plasma CCK concentration after fat ingestion was significantly higher than that after glucose and protein ingestion.

Brain activation

A conjunction analysis was used to identify where the significant differential activations responding to different macronutrients were located in the ROIs. Significant main effects of test beverages on BOLD signal changes were found in the middle insula, thalamus, parahippocampal cortex, caudate, amygdala, and lateral OFC (Figure 1, Table 2). The mean BOLD signal changes in the middle insula, thalamus, parahippocampal cortex, caudate, amygdala, and lateral OFC as a function of time are shown in Figure 2. At the beginning of ingestion (0 min), significant signal increases occurred in all treatments. These represent artifacts caused by swallowing and slight movements of the head during drinking. Afterward, fat, glucose, and protein treatments induced prolonged signal decreases in all of the activated regions, whereas water treatment returned to baseline. Protein ingestion induced a greater decrease in the amygdala signal than did glucose and fat ingestion. No significant differences were observed in the BOLD signal changes in the thalamus, parahippocampal cortex, middle insula, lateral OFC, and caudate in response to fat, glucose, and protein ingestion (Figure 2).

Relations between metabolites/hormones, subjective ratings of satiation, and postprandial changes in BOLD signal

Correlation analyses were performed for all activated regions that showed different BOLD signal changes in response to the 4 test beverages. All the significant correlation coefficients (\( P < 0.05 \)) for all of the treatments are shown in Table 3. After
ingestion of fat, changes in postprandial plasma CCK concentrations were negatively correlated with AUC5–30 min of BOLD changes in the thalamus (r = −0.563, P = 0.029) and caudate (r = −0.471, P = 0.035), whereas, changes in postprandial plasma ghrelin concentrations were positively correlated with AUC5–30 min of BOLD changes in the amygdala (r = 0.550, P = 0.025), middle insula (r = 0.661, P = 0.010), and lateral OFC (r = 0.548, P = 0.021). After glucose ingestion, changes in postprandial plasma glucose, insulin, CCK, and GLP-1 concentrations were negatively correlated with AUC5–30 min of BOLD changes in the middle insula (r = 0.548, P = 0.021). After protein ingestion, changes in postprandial plasma ghrelin concentration changes were positively correlated with AUC5–30 min of BOLD changes in the amygdala (r = 0.563, P = 0.029) and caudate (CCK: r = −0.544, P = 0.044), middle insula (insulin: r = −0.754, P = 0.001; GLP-1: r = −0.543, P = 0.036), and lateral OFC (insulin: r = −0.650, P = 0.001; GLP-1: r = −0.579, P = 0.015), whereas postprandial plasma ghrelin concentration changes were negatively associated with AUC5–30 min of BOLD changes in the thalamus (CCK; r = −0.601, P = 0.014), caudate (insulin: r = −0.519, P = 0.037), and lateral OFC (GLP-1: r = −0.487, P = 0.033), whereas changes in postprandial ghrelin concentrations were positively correlated with AUC5–30 min of BOLD changes in the

**FIGURE 1.** Brain areas showing significant changes in BOLD signal responses after ingestion of the test beverages. am, amygdala; BOLD, blood oxygen level–dependent; ca, caudate; in, middle insula; OFC, orbitofrontal cortex; pa, parahippocampal cortex; th, thalamus.
amygdala \( (r = 0.698, \ P = 0.005) \). Plasma glucose and hormone concentrations did not exhibit a correlation with BOLD signal changes in activated regions after ingestion of water (Table 3).

**DISCUSSION**

To our knowledge, this was the first study to investigate the effects of pure macronutrients on the activation of appetite-related brain regions and to further assess the relations between brain activation and plasma glucose, insulin, ghrelin, CCK, and GLP-1 concentrations in humans.

The pattern of brain activation we observed is consistent with that in previous studies, showing that fasting increases the activation of limbic areas (eg,insula, parahippocampal cortex, OFC, and amygdala), thalamus, and caudate (15, 31–33). The limbic system controls various aspects of hedonic and reward-driven eating. The insula is associated with gustatory processing, reward, desire, and cravings (34), whereas the parahippocampal cortex and the amygdala are involved with the development of short- and long-term memories, including memories of food (34). Activity of the OFC and amygdala, which signals the current appetite value of a food or food cue (35, 36), increases in hunger (21) and decreases in satiety (37). The thalamus acts as a relay zone between the subcortical and cortical areas (38) and is activated under hypoglycemic conditions (39–41). The nutrient-induced BOLD signal response in the caudate is associated with the motivational properties of food (42). In the current study, the deactivation of the ROIs after ingestion of macronutrients reflects the physiologic functions of these brain regions in appetite regulation. However, several studies report opposite results, showing that the BOLD signal increases after ingestion of glucose or l-glutamate in rats (1, 43, 44) and after infusion of dodecanoic acid in humans (23). These inconsistencies may be attributed to species differences, the different test substances, experimental protocols, and statistical methods.

In the current study, isocaloric fat, glucose, and protein beverages showed similar inhibitory effects on BOLD signal in the middle insula, thalamus, parahippocampal cortex, caudate, and lateral OFC. These results indicate that these 5 regions are more sensitive to the calorie than to the macronutrient content in a meal. However, protein decreased the BOLD signal in the amygdala more effectively than did isocaloric glucose and fat. Min et al (31) also confirm that the amygdala is more sensitive to protein content than to the total caloric content of a meal in rats. Therefore, the amygdala may play a more important role than other regions in mediating dietary protein-induced satiety.

To determine how macronutrients affect the activity of appetite-related brain regions, we further analyzed the associations between the BOLD signal and glucose and gut hormones.

Insulin plays a role in food-related information processing and might terminate food intake in the postprandial state (24). In this study, changes in plasma insulin concentrations are negatively correlated with changes in the BOLD signal in the middle insula, thalamus, amygdala, and lateral OFC. In contrast, changes in plasma glucose concentrations are negatively correlated with changes in the BOLD signal in the thalamus after ingestion of glucose only. In general, these findings are consistent with previous reports that changes in plasma insulin concentrations are negatively correlated with changes in regional cerebral blood flow in several regions inhibited by satiation (16, 45). Some studies suggest a correlation between increased plasma insulin concentrations, but not blood glucose concentrations, and the BOLD signal in the forebrain (1, 14, 46). These results explain the presence of correlation between insulin and the BOLD signal in more brain regions than the correlations between glucose and the BOLD signal, even though the plasma glucose concentration is correlated with plasma insulin concentration.

Changes in plasma ghrelin concentrations were positively correlated with BOLD signal changes in the amygdala, middle insula, and lateral OFC after ingestion of fat; with BOLD signal changes in the middle insula and lateral OFC after ingestion of glucose; and with BOLD signal changes in the amygdala after ingestion of protein. Our results are consistent with those of previous studies, indicating that intravenous ghrelin during fMRI increases the neural response to food pictures in healthy volunteers (21).

A previous study showed that the administration of CCK-1 receptor antagonist abolishes lipid-induced brain activation (23). In the current study, changes in plasma CCK concentrations were negatively correlated with BOLD signal decreases in the thalamus induced by ingestion of fat and protein and in the caudate after ingestion of fat and glucose. Our findings may contribute to a better understanding of the roles of CCK in nutrient-induced brain activation.

Administration of GLP-1 (7–36) amide to fasted human subjects leads to a reduction of brain activity in appetite centers, as observed physiologically after feeding (22). A previous study showed that the postprandial GLP-1 response is positively associated with changes in the left dorsolateral prefrontal cortex, which is implicated in satiety and food intake regulation in humans (47).
FIGURE 2. Mean (±SD) time course (percentage of BOLD signal changes from baseline per minute) and AUC from 5 to 30 min in activated brain regions for each test beverage. \( n = 14 \). Repeated-measures ANOVA showed that time (\( P < 0.001 \)) and test beverage (\( P < 0.001 \)) had significant effects on BOLD signal changes in the middle insula, thalamus, parahippocampal cortex, caudate, amygdala, and lateral OFC. The differences in AUC_{5–30\text{ min}} between the 4 test beverages were analyzed by 1-factor ANOVA, followed by a Tukey post hoc test. *Significantly different from water AUC, \( P < 0.001 \). #Significantly different from fat and glucose AUC, \( P < 0.01 \). Time (0 min) corresponds to the onset of ingestion, and the black bar indicates the duration of ingestion. BOLD, blood oxygen level–dependent; OFC, orbitofrontal cortex.
relations were found between BOLD signal changes in activated brain regions and postprandial changes in plasma concentrations of different gut hormones in response to the ingestion of different macronutrients. Our findings pave the way for further research on the relations between macronutrients, gut hormones, and brain activation.

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REFERENCES


