Fat label compared with fat content: gastrointestinal symptoms and brain activity in functional dyspepsia patients and healthy controls

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ABSTRACT

Background: High-fat meals are associated with dyspeptic symptoms in functional dyspepsia (FD) patients. It is still unclear how fat is processed, or how FD symptoms and neuronal activities are modulated by psychological factors.

Objective: We investigated brain activity by functional magnetic resonance imaging (fMRI) after the ingestion of high- and low-fat foods with correct/incorrect fat information.

Design: We compared 12 FD patients and 14 healthy controls (HCs). We recorded resting-state fMRI on four different days before and after ingestion of four yogurts (200 mL, 10% or 0.1% fat, "low fat" or "high fat" label).

Results: FD patients showed more pronounced dyspeptic symptoms than did HCs, and symptoms were relieved less after consuming high fat–labeled yogurt than low fat–labeled yogurt, irrespective of the actual fat content. This is indicative of either a placebo effect of low-fat information or a nocebo effect of high-fat information on symptom expression. FD patients showed greater activity than did HCs in occipital areas before and after ingestion regardless of fat content and label, as well as greater activity in the middle frontal gyrus before ingestion. In addition, functional connectivity (FC) from the insula to the occipital cortex (I-O) increased after high fat ingestion and decreased after low fat ingestion in FD patients. FC from the insula to the precuneus (I-P) was higher in FD patients than in HCs after ingestion of low fat–labeled yogurt. In FD patients, I-O FC negatively correlated with nausea and I-P FC with FD symptom intensity, food craving, and depression.

Conclusions: Our results endorse the importance of psychological perception of food on the incidence of dyspeptic symptoms and on the altered brain activities. These findings show the importance of cognitive components in perceptions of fat, food craving, depression, and brain functions in pathophysiologic mechanisms of FD. This trial was registered at clinicaltrials.gov as NCT02618070. *Am J Clin Nutr* 2018;108:127–135.

Keywords: functional dyspepsia, fat, nutrient label, functional magnetic resonance imaging, cognitive neuroscience

INTRODUCTION

Functional dyspepsia (FD) is characterized by postprandial fullness, early satiation, epigastric pain, bloating, and nausea symptoms after meals, particularly those containing high-fat food (1, 2), in the absence of any structural abnormalities in the gastrointestinal tract (3, 4). The role of fat in altering the gastrointestinal sensitivity and producing symptoms is a well-known pathophysiologic feature in FD patients. Intraduodenal infusion of lipids, but not glucose or saline, has been shown to induce nausea, bloating, and vomiting symptoms in FD patients (5). After ingestion of a high-fat meal, nausea and pain symptoms were greater than after a high-carbohydrate meal (1) and food diaries revealed that bloating symptoms were related to the amount of fat ingested (2).

Feinle-Bisset et al. (6) showed that a low-fat meal, served to FD patients under the pretense that it was a high-fat meal, caused more severe fullness and bloating symptoms than did a low-fat meal served with the correct fat information. In addition, it has been shown in healthy volunteers that concentrations of ghrelin, as a physiologic marker of satiation, varied after

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Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: ALFF, amplitude of low-frequency fluctuations; FC, functional connectivity; FD, functional dyspepsia; FPQ, Fat Preference Questionnaire; FWE, family-wise error; HC, healthy control; HH, high-fat yogurt with "high fat" label; HL, high-fat yogurt with "low fat" label; LH, low-fat yogurt with "high fat" label; LL, low-fat yogurt with "low fat" label; PFC, prefrontal cortex; VAS, visual analog scale.

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ingestion of identical milkshakes when subjects were informed before ingestion that it was either a high-fat, high-calorie or a low-fat, low-calorie milkshake (7). These findings suggest that the cognitive perception of fat at the central nervous system level plays a prominent role in the secretion of hormones and symptom reporting. The non-specific improvement (or worsening) of symptoms by an inactive treatment or treatment-unrelated cue—the placebo (or nocebo) effect—is because of the repeated pairing of cue and response or expectation of symptom relief (or exacerbation). We propose that if FD patients were aware of a close association between their symptoms and high-fat diet, the information on the amount of fat (more or less) could have an impact on their dyspeptic symptoms.

One hypothesis from the early 1990s proposed that abnormalities of the brain-gut axis are one of the key mechanisms governing FD (8). The presence of the food or nutrient in the gastrointestinal tract is signaled to the central nervous system, which then modulates eating behavior and controls the gastrointestinal symptoms (9). Furthermore, some of the brain's many pathways for controlling the perception of internal and external stimuli might be impaired in FD patients and cause somatic symptoms. A large number of functional neuroimaging studies suggest that there is an alteration in the activation of the cognitive and pain-processing brain regions in FD patients (10). However, little is known about how fat or fat information is processed in the brain, or how it is mediated by pathologic factors in FD patients.

In the current study, we aimed to determine the effects of fat and information of fat content (high fat and low fat) in FD patients and healthy controls (HCs). Furthermore, we tested how the psychological factors influence symptoms and brain activity in FD patients.

METHODS

Participants

Twelve FD patients (5 men, mean ± SE age: 46.46 ± 5.64 y) and 14 age- and BMI-controlled healthy subjects (HCs, 5 men, mean ± SE age: 45.79 ± 4.71 y) participated in the study. Right-handed volunteers within the age range of 18–75 y and with a BMI (kg/m^2) of 19–29 were included. FD patients were diagnosed on the basis of the ROME III criteria (11), as well as an unsuspicious endoscopy documented in their medical records. Volunteers with non-removable metal implants, claustrophobia, severe psychiatric illness, substance dependence and abuse, and any food allergy or intolerance were excluded from the study. The ethics committee of the Medical Faculty of the University of Tübingen approved the study (633/2,015,802) and the study was registered at clincialtrials.gov as NCT02618070 before it began. All participants provided informed consent and all experiments were conducted ethically according to the principles of the Declaration of Helsinki.

Test food

Two plain yogurts, low fat (0.1% fat, 200 mL, 106 kcal, 13.8 g of carbohydrate, 11 g of protein) and high fat (10% fat, 200 mL, 266 kcal, 14 g of carbohydrate, 6 g of protein, Weihenstephan) were used. Congruent or incongruent labels were attached to each yogurt [high-fat yogurt with "high fat" label (HH), high-fat yogurt with "low fat" label (HL), low-fat yogurt with "high fat" label (LH), low-fat yogurt with "low fat" label (LL)].

Study design

This crossover study was designed for subjects to eat four different yogurts during four separate visits. Each participant was examined in the morning (0700–1100) on four separate occasions following an overnight fast from 10PM the night before. Smoking and consumption of alcohol, coffee, or tea were prohibited during the fasting period. Participants completed a visual analog scale (VAS; 0 = no symptoms at all, 10 = very severe symptoms) to assess hunger, appetite, abdominal fullness, satiation, nausea, vomiting, abdominal pain, and uncomfortable, burning, and bloating (baseline FD symptoms). The same VAS ratings were assessed again immediately (Post1), 10 min (Post2), and 20 min (Post3) after the yogurt consumption. Between the pre- and postyogurt fMRI sessions, participants were permitted to exit the scanner and were served one of the 4 yogurts (HH, HL, LH, LL) in randomized order (www.randomizer.org). Participants were told they had a 50% chance of receiving correctly labeled yogurt and were asked to eat a whole portion of yogurt within 5 min. At the end of the study, patients indicated their dyspepsia symptom intensity, and their disease-related quality of life was measured with the use of the Nepean Dyspepsia Index (12). Depression and anxiety levels were evaluated with the use of the Beck Depression Inventory (13) and the State-Trait Anxiety Inventory (14). Furthermore, the Eating Disorders Examination Questionnaire (15), Food Cravings Questionnaire (16), and Fat Preference Questionnaire (FPQ) (17) were used to evaluate their eating behavior (**Figure 1**).

Imaging protocol

All images were obtained with a 3 T scanner (Siemens MAGNETOM Prisma). On the first day, a high-resolution T1-weighted anatomical image (magnetization-prepared rapid gradient echo) was recorded (repetition time = 2300 ms, echo time = 4.18 ms, 176 slices, matrix = 256 × 256, voxel size = 1 × 1 × 1 cm^3). Whole brain blood oxygenation level–dependent data were obtained with the use of the standard T2*-weighted echo planar sequence (160 vol, repetition time = 2000 ms, echo time = 30 ms, 30 slices, matrix = 64 × 64, flip angle = 80°, voxel size = 3 × 3 × 3.4 cm^3) before and after ingestion.

Imaging processing

Preprocessing of the blood oxygenation level–dependent signal was performed with the use of the Data Processing Assistant for Resting-State fMRI, version 2.2 (http://restfmri.net) (18) and SPM8 (Wellcome Trust Centre for Neuroimaging). Images for each subject were assessed to identify any excessive movement (>2 mm or 2°) and the first 4 volumes were discarded for signal equilibrium and adaptation. Slice time correction and head motion correction were applied to raw images, and functional images were realigned and coregistered with the structural image.

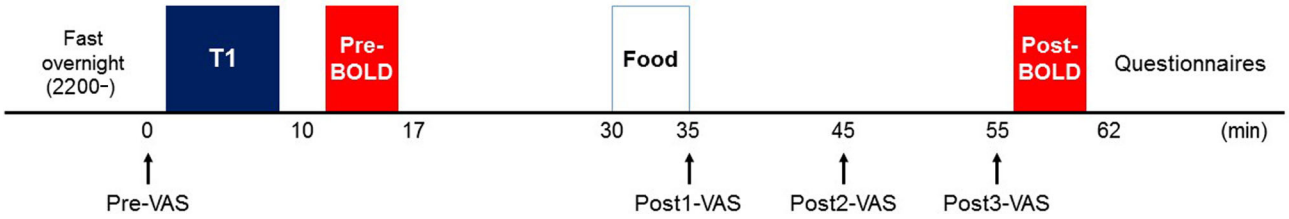

FIGURE 1 Study procedure. Schematic illustration of the study procedure with timeline. Following an overnight fast the study commenced in the morning (0700–1100). Baseline (pre-VAS) and three subsequent dyspeptic symptoms after ingestion (Post1, 2, 3-VAS) were assessed every 10 min with the use of VAS. BOLD, blood oxygen level–dependent contrast imaging; T1, T1-weighted image for structure imaging; VAS, visual analog scale.

Images were normalized into the Montreal Neurological Institute space and smoothed with a Gaussian kernel full width at half maximum of 6 mm. Following preprocessing, amplitude of low-frequency fluctuations (ALFF) analysis within the low frequency band (0.01–0.1 Hz) was performed with the use of the Data Processing Assistant for Resting-State fMRI. The time series data of each voxel was transformed into the frequency domain, and the power spectrum amplitude was calculated. The square root was calculated at each frequency of the power spectrum, and the average square root was then obtained across 0.01–0.1 Hz at each voxel. This average square root was taken as the ALFF, and the mean ALFF was calculated as the original ALFF value/averaged ALFF across all voxels.

For seed-based functional connectivity (FC) analysis, 8-mm sphere regions of interest of the left and right middle-posterior insula were defined by peak coordinates ($x = -42$, $y = -33$, $z = 17$; $x = 36$, $y = -15$, $z = 13$, respectively) of clusters from a resting-state ALFF map [family-wise error (FWE) corrected $P < 0.05$, cluster dimension $k > 10$ voxels]. The averaged time course was then obtained from the regions of interest and the correlation analysis was performed in a voxel-wise fashion. Finally, the correlation coefficient map was converted into z maps by Fisher's r-to-z transform to improve the normality (zFC). For correlation and mediation analysis, the first eigenvariate of each cluster that survived the threshold from the mean ALFF and zFC maps was extracted.

Statistical analysis

All statistical analyses were performed with IBM SPSS statistics 24.0 (IBM Corp.). An independent two-sample t test was used to compare sample characteristics between the groups. Baseline differences of FD symptoms were determined with a mixed ANOVA with visit (baseline of visits 1, 2, 3 and 4) and group (HC, FD) factors. To examine the effect of yogurt consumption on the changes of VAS ratings of FD symptoms, a mixed ANCOVA was conducted with time (Post1, Post2, Post3), group (HC, FD), fat (high, low), and label (high, low) factors, and with the baseline VAS scores as covariate. For ALFF and FC maps, SPM8 second-level t tests between the groups (HC, FD), fat content (high, low), and labels (high, low) were performed with the use of baseline-corrected images with the ImCalc function implemented in SPM8. Two-tailed Pearson's partial correlation analysis was also performed between questionnaire variables and the intensity of ALFF and FC from the baseline fMRI measurement of the first visit, controlling for age and BMI. To examine whether the association between variables were mediated by other variables, bootstrapped mediation analysis of a linear regression model was performed as described by Hayes (19) with the use of the PROCESS macro. Only variables showing a significant correlation with others during correlation analysis were included in the mediation analysis as independent, dependent, or mediator variables. Age and BMI were included as covariates. The significance analysis is based on 1000 bootstrap realizations. The statistical significance level was set at $\alpha = 0.05$, and FWE correction for fMRI analysis and Bonferroni correction for the analysis of behavior data were applied to account for multiple comparison.

RESULTS

Sample characteristics

Sample characteristics and questionnaire scores are presented in **Table 1**. We ascertained no significant differences in age and BMI between the groups ($P > 0.5$). FD patients showed a significantly higher dyspeptic symptom score and lower quality of life score ($P < 0.001$) than did HCs. FD patients also had significantly higher depression, anxiety state, and trait levels ($P < 0.01$, 0.001, 0.05, respectively) and higher food craving state scores ($P < 0.05$) than did HCs. Among the FPQ subscales (FPQ_TASTE: percentage of high-fat food which tastes better than low-fat food, FPQ_FREQ: percentage of high-fat food which is eaten more frequently than low-fat food, FPQ_DIFF: FPQ_TASTE – FPQ_FREQ), only the FPQ_TASTE score was significantly higher in FD patients than in HCs. No significant differences were observed between the groups in Eating Disorders Examination Questionnaire scores.

Food-induced FD symptoms

FD symptom ratings are described in **Supplemental Table 1**. There were no significant differences in baseline FD symptoms between each visit ($P > 0.05$), but FD patients reported significantly increased burning, discomfort, pain, bloating, nausea, and fullness symptoms than did HCs at baseline ($P < 0.05$). ANCOVA showed the significant main effect of time was in satiation, pain, and vomiting ($P < 0.01$, 0.05, 0.001, respectively). The significant main effect of group was found in nausea, vomiting, and bloating (FD > HC, $P < 0.05$), and that of label was found in satiation, discomfort, and burning symptoms ("high fat" label > "low fat" label, $P < 0.01$, 0.05, 0.05, respectively). Significant main effects of both group (FD > HC,

TABLE 1
Overview of study sample characteristics[1]

	HC	FD patients	*P* value
Gender, M/F	5/9	5/7	
Subgroup	—	PDS: 4/6, EPS: 5/4	
FD duration, mo	—	156 ± 57.24	
Age, y	45.79 ± 4.71	46.46 ± 5.64	
BMI, kg/m^2	23.79 ± 0.91	22.93 ± 0.63	
NDI_Symptom	4.5 ± 1.35	64.5 ± 9.10	<0.001
NDI_QOL	49.44 ± 0.25	31.56 ± 3.74	<0.001
EDE-Q Total	1.30 ± 0.26	0.80 ± 0.34	NS
Restraint	1.13 ± 0.18	0.38 ± 0.17	NS
Eating concern	0.44 ± 0.22	0.20 ± 0.13	NS
Weight concern	1.71 ± 0.39	0.86 ± 0.36	NS
Shape concern	1.87 ± 0.49	1.33 ± 0.44	NS
BDI-II	3.07 ± 1.70	13.17 ± 2.57	<0.01
STAI_state	29.64 ± 1.97	43.17 ± 2.78	<0.001
STAI_trait	30.63 ± 2.17	41.92 ± 3.76	<0.05
FCQ_state	31.82 ± 1.72	38.07 ± 1.94	<0.05
FCQ_trait	77.71 ± 8.47	87.17 ± 9.82	NS
FPQ_TASTE	46.48 ± 4.03	67.56 ± 12.24	<0.01
FPQ_FREQ	40.63 ± 4.54	55.67 ± 5.60	NS
FPQ_DIFF	5.85 ± 3.57	11.88 ± 6.52	NS

[1]Values are means ± SEs. *P* value determined by independent two-sample *t* test, FD vs. HC. BDI-II, Beck Depression Inventory; EDE-Q, Eating Disorder Examination Questionnaire; EPS, epigastric pain syndrome; FCQ, Food Cravings Questionnaire; FD, functional dyspepsia; FPQ, Fat Preference Questionnaire; FPQ_DIFF, high-fat restriction (FPQ_TASTE – FPQ_FREQ); FPQ_FREQ, percentage of high-fat food which is eaten more frequently than low-fat food; FPQ_TASTE, percentage of high-fat food which tastes better than low-fat food; HC, healthy control; NDI, Nepean Dyspepsia Index; PDS, postprandial distress syndrome; QOL, quality of life; STAI, State Trait Anxiety Inventory.

$P < 0.01$) and label ("high fat" label > "low fat" label, $P < 0.01$) were found for abdominal pain. Interaction between time and group was foundfor the symptoms pain, burning, bloating, and nausea ($P < 0.05$, 0.05, 0.001, 0.05, respectively). Interaction between time and label was found for bloating, between group and label for satiation, and between group and fat for fullness ($P < 0.05$). No adverse events were recorded.

Resting-state brain activity

Baseline ALFF (preyogurt session)

FD patients showed a significantly greater ALFF in the bilateral middle frontal gyrus, left middle, and right inferior occipital gyrus and a significantly lower ALFF in the left superior frontal gyrus and left middle cingulate gyrus than did HCs (all $P < 0.001$, FWE corrected, **Table 2**).

Changes of ALFF (postyogurt compared with preyogurt session)

Following yogurt ingestion, significant group differences of changes of ALFF were observed in the left middle occipital gyrus and right cerebellum (ALFF increased in FD patients regardless of the type of yogurt consumed but decreased in HCs compared with baseline). The ALFF of the left middle occipital gyrus was significantly higher in FD patients than in HCs, particularly in the HH state (all $P < 0.05$, FWE corrected, Table 2).

Functional connectivity

In FD patients, FC of the left insula to the right insula and the left inferior occipital gyrus increased significantly after eating high-fat yogurt (HH, HL) and decreased after eating low-fat yogurt (LH, LL), regardless of the label ($P < 0.05$). There were no significant differences in changes in the FC of HCs (**Table 3**, **Figure 2**).

In comparison to HCs, FD patients showed significantly increased FC between the right insula and the bilateral precuneus after they had eaten "low fat"–labeled yogurt ($P < 0.05$, Table 3, Figure 2).

Pearson's correlation analysis

Significant negative correlations were established between the intensity of FD symptoms and disease-related quality of life ($r = -0.85$, $P < 0.01$), and positive correlations were established between the intensity of FD symptoms and state depression level ($r = 0.52$, $P < 0.05$) in FD patients. Baseline resting-state brain activity (ALFF) in the left middle frontal gyrus was significantly negatively correlated with the intensity of FD symptoms ($r = -0.77$), the food craving state score ($r = -0.78$, $P < 0.01$), and depression ($r = -0.73$, $P < 0.001$) and significantly positively correlated with quality of life ($r = 0.73$, $P < 0.05$) in FD patients. FC intensity before ingestion (pre-yogurt session) between the right insula and right precuneus was significantly negatively correlated with the FD symptom intensity, food craving ($r = -0.70$ and -0.69, respectively, $P < 0.01$), and depression level ($r = -0.64$, $P < 0.05$), and significantly positively correlated with quality of life ($r = 0.68$, $P < 0.05$) in FD patients. The FC intensity of the postyogurt session between the left insula and the left inferior occipital gyrus was significantly negatively correlated with the nausea symptom rating in FD patients ($r = -0.64$, $P < 0.05$, **Table 4**).

Mediation analysis

To assess the relation of FD-related psychological symptoms, mediation analysis was performed. The models and the investigated variables are described in **Figure 3**. The total effect of the quality of life on depression was significant (path c, $P < 0.05$), and was fully mediated by FD symptoms (path a, $P < 0.001$; path b, $P < 0.05$; path c', not significant; standardized indirect effect = −0.51, 95% CI: −1.07, −0.25) in FD patients (Figure 3B, Model 1). The total effect of depression on the quality of life was also significant (path c $P < 0.01$) and fully mediated by FD symptoms (path a, $P < 0.01$; path b, $P < 0.001$; path c', not significant; standardized indirect effect = −0.43, 95% CI: −0.59, −0.26) in FD patients (Figure 3B, Model 2). We also found that the significant total effect of food craving state score on the baseline resting-state brain activity in the left middle frontal gyrus (path c, $P < 0.001$) is fully mediated by depression (path a, $P < 0.01$; path b, $P < 0.01$; path c', not significant; standardized indirect effect = −0.17, 95% CI: −0.29, −0.07) in FD patients (Figure 3B, Model 3).

TABLE 2
Brain regions showing significant differences in ALFF at baseline (preyogurt) and changes in ALFF (postyogurt – preyogurt) between groups[1]

Regions	*z* Score of peak voxel	Coordinates of peak voxel in MNI space	*P* value
Preyogurt FD > HC			<0.001
Left mid. frontal gyrus	6.12	−48, 30, 38	
Right mid. frontal gyrus	5.68	36, 15, 60	
Right inf. occipital gyrus	5.48	39, −93, −13	
Left mid. occipital gyrus	5.23	−36, −75, 8	
Preyogurt HC > FD			<0.001
Left sup. frontal gyrus	6.14	−12, 15, 72	
Left mid. cingulate cortex	5.45	−9, 18, 34	
Postyogurt vs. preyogurt FD > HC			<0.05
Left mid. occipital gyrus	4.36	−33, −81, 34	
Right cerebellum	4.06	48, −60, −30	
Postyogurt vs. preyogurt FD > HC, HH			<0.05
Left mid. occipital gyrus	4.43	−30, −81, 38	

[1]Two-sample *t* test with the use of SPM8, FWE-corrected *P* value, cluster dimension $k > 10$ voxels. ALFF, amplitude of low-frequency fluctuations; FD, functional dyspepsia; FWE, family-wise error; HC, healthy control; HH, high-fat yogurt with "high fat" label; inf., inferior; mid., middle; MNI, Montreal Neurological Institute; sup., superior.

DISCUSSION

Our data demonstrate: *1*) an expectancy effect of the information about the fat content on symptom severity, *2*) altered resting-state brain activities in the prefrontal, occipital, cingulate, and cerebellum cortices, *3*) high fat induced changes in the FC of the insula-inferior occipital gyrus in comparison with low fat, and there was a group difference of the changes in FC between the insula-precuneus in response to "low fat" label, *4*) negative correlations between symptoms, food craving, depression, middle frontal gyrus activity, nausea, and the FC amplitude of the insula-inferior occipital gyrus, and *5*) a mediation effect of depression on the influence that food craving has on the middle frontal gyrus activity in FD patients.

Psychological factors in FD patients

In this study, anxiety, depression, and food craving state were more intense in FD patients than in HCs. In a bid to understand the psychological processes in FD patients, mediation analysis was performed. We found that the bidirectional effect between depression and quality of life scores is mediated by FD symptom severity. This indicates that increased depression reduced the quality of life of FD patients and the effect was mediated by worsened dyspeptic symptoms, and vice versa. Moreover, the effect of increased craving for food (state) on the inhibited prefrontal brain activity is also mediated by depression, leading to the plausible hypothesis that food craving enhances depression and suppresses the brain activity involved in executive control in FD patients.

Expectancy effect of fat label on FD symptom

The effect of high-fat food on symptom aggravation was not established in this study. For the symptoms reduced after consuming yogurt (pain, discomfort, burning), "low fat"–labeled food induced more symptom relief than "high fat"–labeled food, whereas satiation was increased significantly more by "high fat"–labeled food than by "low fat"–labeled food. This result demonstrates an expectancy effect of the information about fat content; these may be called placebo or nocebo effects (20). While other dyspeptic symptoms, including nausea, vomiting, and bloating, were higher in FD patients than in HCs, they remained unchanged for the different yogurts. This may indicate that some, but not all, of the visceral symptoms can be modulated by cognitive factors.

TABLE 3
Significant changes in FC (postyogurt – preyogurt) within and between groups[1]

Condition	Seed region	Regions of significant FC changes	*z* Scores of peak voxel	Coordinates of peak voxel in MNI space	*P* value
High fat > low fat in FD	Left insula	Right insula	5.09	39, 18, −4	<0.05
		Left inf. occipital gyrus	4.30	−33, −87, −4	
FD > HC "low fat"-labeled yogurt	Right insula	Left precuneus	3.71	−6, −57, 13	<0.05
		Right precuneus	3.71	21, −51, 21	

[1] Two-sample *t* test with the use of SPM8, FWE-corrected *P* value, cluster dimension $k > 10$ voxels. FC, functional connectivity; FD, functional dyspepsia; FWE, family-wise error; HC, healthy control; inf., inferior; MNI, Montreal Neurological Institute.

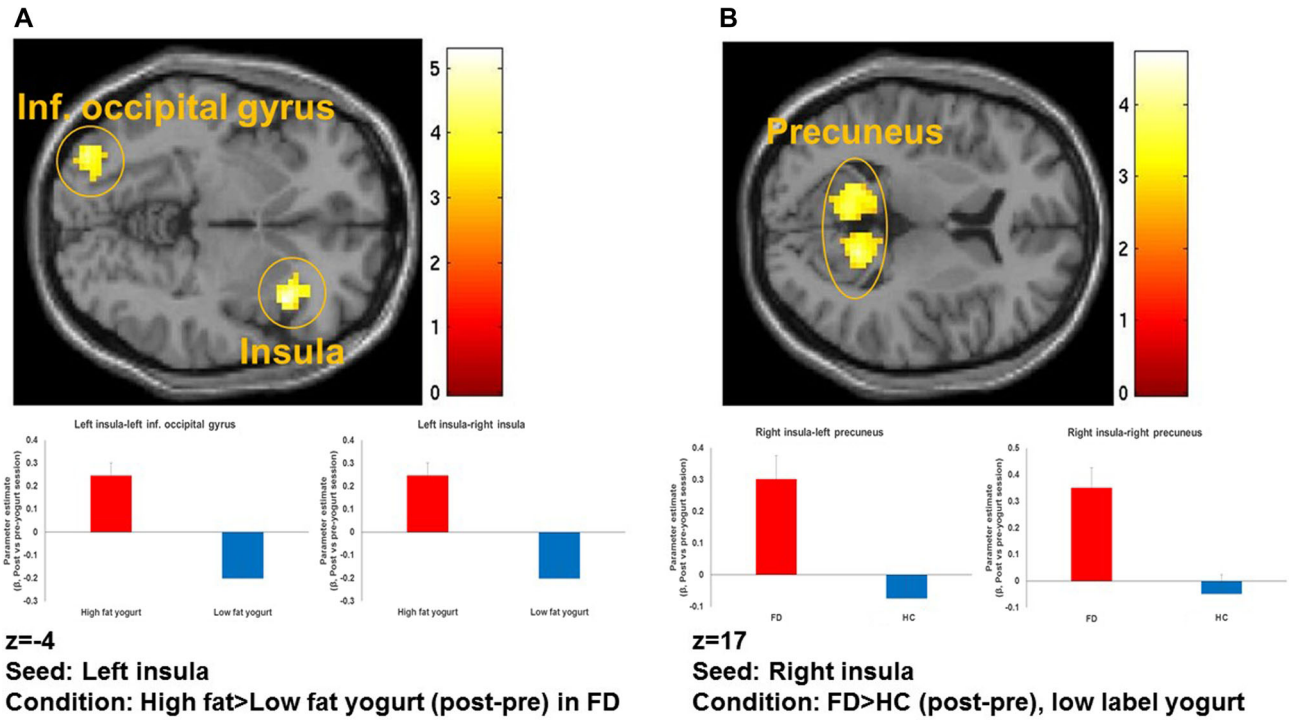

FIGURE 2 Seed-based FC analysis. Effects of fat and fat information on FC between the left insula and the right insula and left inferior occipital gyrus, and between the right insula and the bilateral precuneus. (A) High-fat yogurts (HH, HL) increased the FC of the left insula to the left inferior occipital gyrus and the right insula, whereas low-fat yogurts (LH, LL) reduced the strength of identical connections after ingestion ($P < 0.05$) in FD patients. (B) "Low fat"-labeled yogurts (HL, LL) increased the FC of the right insula to the bilateral precuneus in FD patients, whereas the identical connections decreased in HCs ($P < 0.05$). Two-sample t test, FWE-corrected, cluster dimension $k > 10$ voxels. FC, functional connectivity; FD, functional dyspepsia; FWE, family-wise error; HC, healthy control; HH, high-fat yogurt with "high fat" label; HL, high-fat yogurt with "low fat" label; inf., inferior; LH, low-fat yogurt with "high fat" label; LL, low-fat yogurt with "low fat" label.

The behavior results are inconsistent with a previous study in which both a high fat content and information of high fat (HH, LH) caused higher fullness and bloating ratings than did "low fat"–labeled low-fat yogurt (LL) in FD patients (6). Furthermore, the effect of label was for both high- and low-fat yogurt in our study, whereas previous findings did not demonstrate the effect of "low fat" label for high-fat yogurt (no differences between HH and HL). This might be because of the total amount of fat in the high-fat yogurt used in our study (18 g compared with 23.6 g) (6) and different sample characteristics. The threshold of fat amount and the varieties of symptoms that are affected by psychological factors, together with the role of expectation and previous experience of food in the placebo/nocebo effect on visceral symptoms in FD patients, will require further investigation.

Functional connectivity between the insula and the precuneus

Functional connectivity of the insula-precuneus was negatively correlated with FD symptoms, food craving, and depression in a hunger state. This is enhanced in response to "low fat"–labeled

TABLE 4
Significant results from the Pearson's partial correlation analysis[1]

	Quality of life	Anxiety (state)	Preyogurt ALFF left mid. frontal gyrus	Preyogurt FC right insula-right precuneus	Postyogurt FC left insula-left inf. occipital gyrus
Dyspeptic symptoms	−0.85**	NS	−0.77**	−0.70**	NS
Food craving (state)	NS	0.72*	−0.78**	−0.69**	NS
Depression	NS	NS	−0.73***	−0.64*	NS
Nausea (Post3)	NS	NS	NS	NS	−0.64*
Quality of life		NS	0.73*	0.68*	NS

[1]Two-tailed Pearson's partial correlation analysis, correlation coefficients with P values (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, respectively). Age-, sex-, and BMI-controlled and multiple comparison–corrected. ALFF, amplitude of low-frequency fluctuations; FC, functional connectivity; inf., inferior; mid., middle.

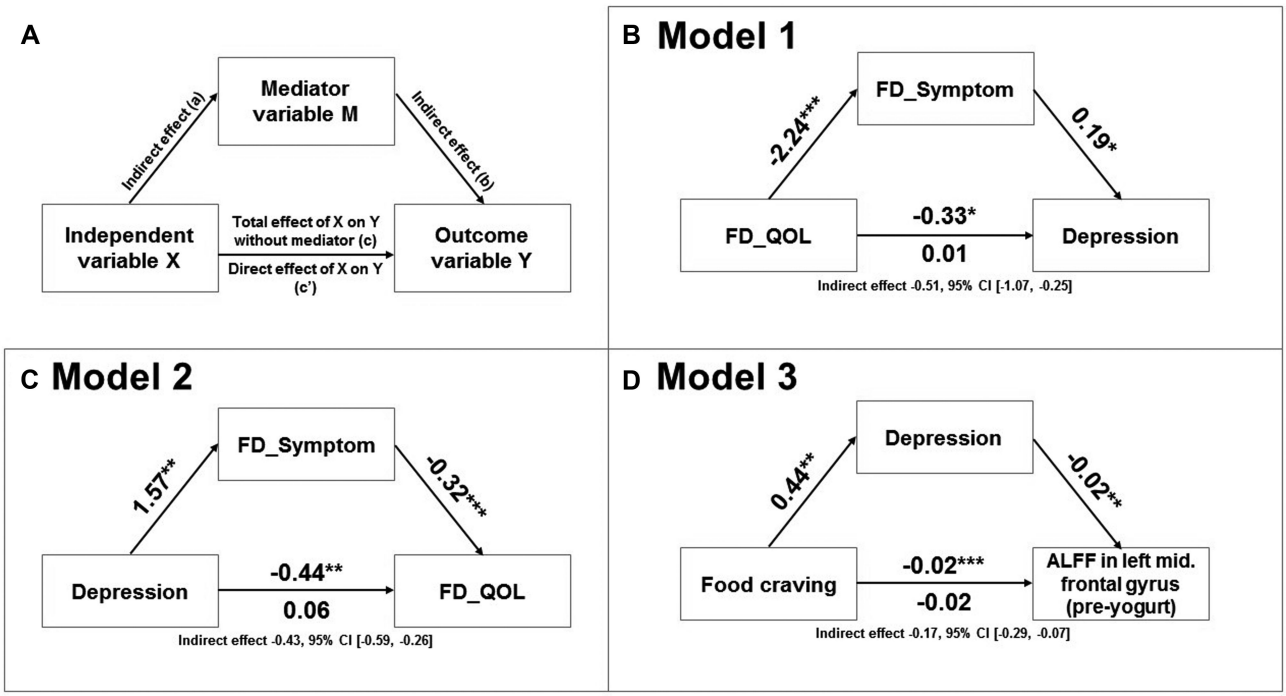

FIGURE 3 Conceptual diagram of mediation analysis. (A) Conceptual diagram of a mediation analysis model with one mediator, as used in this study. Total effect of X on Y (c) = indirect effect of X on Y through M (ab) + direct effect of X on Y (c'). (B) Model 1: FD_QOL (X), FD_Symptom (M), depression (Y). (C) Model 2: depression (X), FD_Symptom (M), FD_QOL (Y). (D) Model 3: food craving (X), resting-state brain activity in left middle frontal gyrus before eating yogurt (Y), depression (M). Path coefficients with P values (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, respectively). ALFF, amplitude of low-frequency fluctuations; FD, functional dyspepsia; mid., middle; QOL, quality of life.

yogurt in FD patients compared with HCs. The precuneus and insula are known to be functionally connected during resting (21) and activated in response to smoking cues in smokers (22). The insula is the core region of the visceral sensory (23, 24) and interoceptive networks (25–27), and is believed to be involved in ingestive behavior (28). The precuneus is related to episodic memory retrieval (29, 30), appetite control (31, 32), appraisal of food (33), and reappraisal of the benefits of eating a food (34), and comprises the default mode network (35). Taken together, the insula-precuneus connection may be affected by visceral symptoms and psychological factors, and strengthened by the food signal processing in the reward context ("low fat" label) by retrieving previous memories of food.

Food craving

We isolated two hypersensitized brain regions—the middle frontal gyrus in the prefrontal cortex (PFC) and the inferior occipital gyrus—which probably subserve different functions. We found a higher craving for food in FD patients than in HCs in a hunger state. Furthermore, food craving influenced the middle frontal gyrus activity indirectly via depression. Food craving and depression affect each other reciprocally, and FD symptoms mediate the influence. Food craving, an intense urge to eat a particular food, is related to the restraint or deprivation of food and calories (36, 37) or a negative emotional state (38). The role of food craving has not yet received sufficient attention in FD patients. The PFC is well known for the inhibitory regulation of cravings for drugs (39), smoking (40), and food (28). It has been used for transcranial direct current stimulation to reduce food craving and calorie intake (41, 42). With mediation analysis results, it is plausible that the long-term experience of FD symptoms and consequent dietary restriction lead to higher food craving, and that craving disrupts the functional demands of the PFC.

Nausea and the occipital cortex

The amplitude of FC between the insula and the inferior occipital gyrus was negatively correlated with the nausea ratings after food ingestion. Although the occipital cortex is one of the most frequently reported brain areas in functional neuroimaging studies in FD patients (10), the underlying cause remains unclear. Previous studies showed that visually induced nausea was correlated with occipital gyrus activity (43) and that a gastric electrical stimulation with an antiemesis effect increased the brain activity in the occipital cortex (44). A study on food-induced nausea and occipital cortex activity would provide insight into the central mechanisms of nausea in patients.

In summary, our results showed placebo or nocebo effects of fat information, a reward cue–related change in FC of the insula-precuneus, food craving–induced activity in the PFC, and nausea-related FC of the insula-occipital cortex. However, the results should be interpreted with caution owing to several limitations. The sample size was small, and only one kind of food was used. Yogurt was selected, since it had already proved successful in

inducing FD symptoms in patients in an earlier study, and its fat composition is easily modulated. However, patients suffering from lactose intolerance were unable to participate. The brain regions and functional connectivities shown in Tables 2 and 3 were drawn from *t* tests and no clusters or connectivities survived after multiple comparison correction of *F* tests. This might be a result of the small sample size and a modest influence of yogurt consumption on the resting state neuronal activity. Therefore, larger sized studies are required to comprehend the central mechanisms of responses to food in FD patients.

In conclusion, individuals with FD have latent impairments in their cognitive perception of high-fat food, and may have altered resting-state brain activity and FC which are influenced by psychological factors. Cognitive perception of fat, food craving, depression, and altered brain functions, as well as a possible role of the brain-gut axis, should be deemed important pathologic characteristics of FD.

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