Human protein status modulates brain reward responses to food cues

Sanne Griffioen-Roose, Paul AM Smeets, Emmy van den Heuvel, Sanne Boesveldt, Graham Finlayson, and Cees de Graaf

ABSTRACT

Background: Protein is indispensable in the human diet, and its intake appears tightly regulated. The role of sensory attributes of foods in protein intake regulation is far from clear.

Objective: We investigated the effect of human protein status on neural responses to different food cues with the use of functional magnetic resonance imaging (fMRI). The food cues varied by taste category (sweet compared with savory) and protein content (low compared with high). In addition, food preferences and intakes were measured.

Design: We used a randomized crossover design whereby 23 healthy women [mean ± SD age: 22 ± 2 y; mean ± SD body mass index (in kg/m²): 22.5 ± 1.8] followed two 16-d fully controlled dietary interventions involving consumption of either a low-protein diet (0.6 g protein · kg body weight⁻¹ · d⁻¹, ~7% of energy derived from protein, approximately half the normal protein intake) or a high-protein diet (2.2 g protein · kg body weight⁻¹ · d⁻¹, ~25% of energy, approximately twice the normal intake). On the last day of the interventions, blood oxygen level–dependent (BOLD) responses to odor and visual food cues were measured by using fMRI. The 2 interventions were followed by a 1-d ad libitum phase, during which a large array of food items was available and preference and intake were measured.

Results: When exposed to food cues (relative to the control condition), the BOLD response was higher in reward-related areas (orbitofrontal cortex, striatum) in a low-protein state than in a high-protein state. Specifically, BOLD was higher in the inferior orbitofrontal cortex, striatum) in a low-protein state than in a high-protein state. The BOLD response was higher in reward-related areas (orbitofrontal cortex, striatum) in a low-protein state than in a high-protein state.

Conclusions: Protein status modulates brain responses in reward regions to savory food cues. These novel findings suggest that dietary protein status affects taste category preferences, which could play an important role in the regulation of protein intake in humans. This trial was registered at www.trialregister.nl/trialreg/admin/rctview.asp?TC=3288 as NTR3288.

INTRODUCTION

Protein is an indispensable component of the human diet. It provides nitrogen and amino acids, including the 9 amino acids classified as essential, which are needed to preserve and maintain bodily functions and life (1). In humans, the range of protein intakes, in contrast with fat and carbohydrates, has remained relatively constant over time and across populations, both as a percentage of energy in the diet (~10–25%) and as the absolute amount eaten (~40–100 g) (2–4). Both in animals and in humans, protein intake appears to be tightly regulated (5–8). After a protein deficit, when the protein status is low, food intake and food preferences show adaptive changes that suggest compensatory mechanisms aimed at restoring adequate protein status (9, 10).

In humans, sweet and savory are the main attractive taste categories (11). Within our food range, sweet-tasting foods contain more carbohydrates, whereas savory-tasting foods usually contain higher levels of protein (12–14). It has been proposed that sweet taste acts as a signal for energy-rich nutrients and that savory taste allows the recognition of amino acids (15). It is conceived that, through repeated consumption of food during our lifetime, we learn to associate the sensory attributes of foods with their physiologic effects. It has been shown that this associative learning influences selection and intake of food and has been suggested to play a central role in the development of specific appetites (16, 17). The underlying mechanism on the role of sensory attributes of foods in protein intake regulation is far from clear and requires further clarification.

In recent years, both animal studies and human fMRI studies have located brain areas involved in protein intake regulation (18–21). Journel et al (19) showed that high-protein diets lead to greater activation in the nucleus tractus solitaries and in the arcuate nucleus compared with a normal-protein diet. In addition, reward and motivation aspects of eating behavior, controlled mainly by neurons present in the limbic region, appeared to play an important role in the reduced hedonic response after a high-protein diet. Leidy et al (18) showed that a high-protein breakfast led to reductions in hippocampal and parahippocampal activation compared with a normal protein breakfast when exposed to images of food cues. Our study further investigated the specific relation between protein and the underlying neural effects on food reward.

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Our objective was to investigate the effect of human protein status on neural responses to different food cues associated with protein with the use of fMRI. These food cues varied by taste category (sweet compared with savory) and protein content (low compared with high). In addition, we measured food liking and wanting and spontaneous protein intake. We hypothesized that a low-protein status would increase brain-reward responses to food cues associated with protein, affecting food preferences and intake.

SUBJECTS AND METHODS

Subjects

Twenty-three healthy women completed the study, which ran from March to June 2012 (Table 1). Of the 24 subjects enrolled in the study, 1 subject dropped out after the first intervention period. A supplemental flow diagram of progress through the phases of the study is available online (see Supplemental Figure 1 under “Supplemental data” in the online issue). We recruited healthy, normal-weight women aged 18–35 y. Exclusion criteria were as follows: restrained eating [Dutch Eating Behavior Questionnaire, score >2.80 (23)], lack of appetite, an energy-restricted diet during the past 2 mo, change in body weight >5 kg during the past 2 mo, stomach or bowel diseases, diabetes, thyroid disease or any other endocrine disorder, use of daily medication other than oral contraceptives, difficulties with swallowing and/or eating, taste or smell disorders, being a vegetarian, being allergic or intolerant to products under study, smoking, being pregnant or lactating, being left-handed, and contraindications for MRI scanning.

Potential subjects filled out an inclusion questionnaire, including a medical-history questionnaire. They attended a screening and practice session that included measurement of weight and height and explanation/practice of the different procedures, including fMRI. The subjects were unaware of the exact aim of the study and were informed that we were investigating the effect of specific diets, which varied in macronutrient content, on brain activity. We kept the subjects naive to the fact that we specifically varied the protein levels of the diets and were interested in reward. The study was approved by the Medical Ethical Committee of Wageningen University. Before the study began, a sample size calculation was performed. Because we have executed a similar behavioral study (9), we were able to verify that 24 subject would provide sufficient power for the outcomes of the behavioral measurements. Written informed consent was obtained from all subjects.

Study design

Subjects followed two 16-d fully controlled dietary interventions involving consumption of individualized isocaloric menus providing either a low-protein diet (0.6 g protein · kg body weight$^{-1}$ · d$^{-1}$, ~7% of energy derived from protein, which is about half the amount of normal protein intake) or a high-protein diet (2.2 g protein · kg body weight$^{-1}$ · d$^{-1}$, ~25% of energy; about twice the normal protein intake) in a randomized, counterbalanced, crossover design (Figure 1). Subjects were randomly assigned to 1 of the 2 treatment-order groups by the principal investigator. The 2 interventions were followed by a 1-d ad libitum phase during which a large array of food items was available and protein and energy intakes were measured (our secondary outcome measure).

The 2 interventions were separated by a minimum of a 4-wk washout period during which subjects were instructed to consume their habitual diet. On the last day of the interventions, before the start of the ad libitum phase, and in the fourth week of the washout period (washout measurement), brain responses to odor and visual food cues were measured by using fMRI. After the fMRI session the LFPQ was completed. BW, body weight; LFPQ, Leeds Food Preference Questionnaire; lib, libitum.

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Diets

Protein in the diets was exchanged for carbohydrates, and the amount of fat was kept similar (Table 2). Protein manipulation was achieved by varying commercially available foods in the diets and by changing the protein contents of the foods (e.g., low-protein bread). In addition, whey protein isolate powder (Nectar, pink grapefruit; Syntrax) was added to drinks, desserts, or both, which were consumed during meals to enable the variations in required individual protein amounts. We calculated a taste ratio of the low-protein and high-protein diets by classifying the offered foods as sweet tasting, savory tasting, or neutral tasting: and divided the total amount of food (g) per taste by the total amount (g) of food provided by the diet. The ratio of sweet: savory:neutral for the low-protein diet was 50:16:34 and for the high-protein diet was 54:16:30.

Procedure

During the dietary intervention, we provided the subjects with foods and beverages, except for water, coffee, and tea (ad libitum intake without milk and sugar), which covered ~90% of their estimated daily energy requirement. Subjects chose the remaining 10% of energy from a list of choice items that included virtually protein-free and fat-free foods [a common procedure within our division (25)]. Their choice was recorded in a diary.

Each subject’s total energy requirement was estimated by using the Schofield equation, with age, weight, height, sex, and a physical activity level of 1.6 accounted for (22), which was then verified by comparing the result against the results of a validated food-frequency questionnaire (25) that determines habitual energy intake and that was filled out during screening. These 2 indicators were used to get an accurate estimate of every subject’s energy requirement.

During weekdays at lunch, the subjects visited the research center at the Wageningen University (Wageningen, Netherlands) and consumed their hot meal. All other foods were supplied daily as a meal package and were consumed at home. The home meal package contained 2 bread meals with toppings for dinner and breakfast and beverages, fruit, and snacks. On Fridays, subjects received a home meal package with foods and beverages for the entire weekend plus instructions for the preparation of these foods. Subjects were instructed to eat all foods that were provided. They were allowed to use seasoning and table salt.

The ad libitum phase that followed the dietary interventions started with a hot lunch; the subjects were allowed to select the foods themselves and eat until comfortably satiated. A large meal package was provided for consumption at home and contained >200% of the estimated energy requirements. The home meal package consisted of foods that were not available during the intervention and included buns with toppings for dinner and breakfast and beverages, fruit, and snacks. The foods were provided in unusual portion sizes to prevent habitual intake (26). In addition, many foods were offered in both a low-protein and high-protein version to enable selective protein intake. It was not specifically indicated to the subjects that there were low-protein and high-protein foods. Individual food intakes were measured by weighing the remainders of food on the plate (during lunch) and the meal package the next day. Ad libitum energy intake and macronutrient selection were calculated by using food-composition tables (27).

**Table 2**
The mean nutritional composition (energy content and macronutrient composition) of the low-protein and high-protein diets

<table>
<thead>
<tr>
<th></th>
<th>Low-protein diet</th>
<th>High-protein diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ/d)</td>
<td>9.5</td>
<td>9.6</td>
</tr>
<tr>
<td>Protein (g/kg body weight)</td>
<td>0.6</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>143</td>
</tr>
<tr>
<td>(% of energy/d)</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>Carbohydrates (g/d)</td>
<td>323</td>
<td>215</td>
</tr>
<tr>
<td>(% of energy/d)</td>
<td>58</td>
<td>38</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>83</td>
<td>87</td>
</tr>
<tr>
<td>(% of energy/d)</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>Alcohol (g/d)</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>(% of energy/d)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>28</td>
<td>26</td>
</tr>
</tbody>
</table>

1 Duplicate portions of the provided diets were collected every day, stored at ~20°C, and analyzed for energy and macronutrient compositions after the experiment. Nitrogen was determined by using the Kjeldahl method (43; method 920.87), and the amount of protein was calculated by using a conversion factor of 6.25, of fat by using the acid hydrolysis method (43, method 14.019), and of available carbohydrate by subtracting moisture, ash, protein, and dietary fiber and fat from the total weight. The energy content was calculated from the macronutrient composition by using the following energy conversion factors: protein, 17 kJ/g; fat, 37 kJ/g; carbohydrate, 17 kJ/g; and alcohol, 29 kJ/g. The average of the calculated composition of the free-choice items (10%), which were recorded in a diary by all subjects (n = 23), was added.

fMRI

On day 17 of the interventions, before the start of the ad libitum phase and in the fourth week of the washout period (washout measurement), subjects were scanned between 1000 and 1400 at the Hospital de Gelderse Vallei (Ede, Netherlands). All experimental measurements of one individual took place at the same time. Subjects were instructed to have refrained from eating ≥2 h before the test. The scan session consisted of 2 functional runs during which 350 functional volumes were acquired by using a T2*-weighted gradient echo image, acquired with BOLD contrast on a 3-Tesla Siemens Magnetom Verio fMRI scanner equipped with a 32-channel head coil. Whole-brain fMRI data were obtained with a T2*-weighted 2D echo-planner imaging sequence (repetition time = 2140 ms, echo time = 25 ms, 90° flip angle, field of view = 192 × 192 mm, 43 axial slices, ascending order, voxel size 3 × 3 × 3 mm). The imaging volume was tilted at an oblique angle of 30° to the anterior-posterior commissure line to reduce signal dropout in the orbitofrontal and ventral temporal lobes (28). In addition, a high-resolution T1-weighted anatomical MRI scan (MPRAGE: repetition time = 1900 ms, echo time = 2.26 ms, 9° flip angle, field of view = 256 × 256 mm, 192 sagittal slices, voxel size = 0.5 × 0.5 × 1 mm) was acquired between the 2 functional runs.

During each functional run, odors of 6 different food products were delivered. These food cues varied in taste category (sweet compared with savory) and protein content (low compared with high) (see Supplemental Table 1 under “Supplemental data” in...
the online issue): chocolate mousse (sweet low-protein), apple turnover (sweet low-protein), curd with fruit flavor (sweet high-protein), potato röstis rounds (savory low-protein), beef with gravy (savory high-protein), and pizza (savory high-protein). One nonfood odor (grass) was included to account for general odor effects (International Flavors & Fragrances Inc). Before the study began, these odors were matched on perceived intensity and diluted to medium to high intensity [55–75 on a 100-mm visual analog scale (VAS)]. The sweet odors and nonfood odor were diluted with 1,2-propanediol (curd 1:1000, chocolate mousse 1:100, apple turnover 1:1000, grass 1:500), and the savory odors were diluted with deionized water (beef 1:1000, pizza 1:1000, potato röstis rounds 1:1000). Odors were presented with a computer-controlled 8-channel olfactometer (Burghart) while a picture of the product was simultaneously shown. The stimulus presentation program E-Prime 2.0 Professional (Psychology Software Tools Inc) was used to trigger the olfactometer and present the visual cues.

In each functional run, all 7 odors were presented 7 times, which led to a total of 14 events per odor. Following a fixation cross (1 s), odorants were delivered for 3 s embedded in a constant nonodorous airflow (total flow 8 L/min, relative humidity 80%, 36°C) while a picture of the food was shown simultaneously, followed by an “evaluation screen” (4 s) during which subjects were instructed to mentally evaluate the product. Afterward, there was either a rest period (5–9 s) or the food product was rated on liking (“How pleasant would you find the taste of this product right now?”) or wanting (“How much do you want to consume this product right now?”) on a 100-mm VAS with the use of a button box (7 s). In each functional run, every product was assessed once on liking and once on wanting.

Before starting the scan session, subjects filled out a questionnaire, including a question about the date of their last menstrual cycle. Analyses on these data showed that the different phases of the menstrual cycle were roughly equally distributed over the treatment groups. In addition, entering the menstrual cycle phase as a covariate in our analyses did not affect our results.

LFPQ

After the fMRI, the subjects completed the LFPQ (24) to measure food liking and food wanting. It included photographs of 16 foods that varied in taste category (sweet compared with savory) and protein content (low compared with high). These 4 categories (sweet low-protein, sweet high-protein, savory low-protein, and savory high-protein) were matched on energy density, fat content, and type of food (each category contained one sandwich, one snack, one cookie, and one meal item). A picture of each food was shown, and subjects had to rate their liking (“How pleasant would you find the taste of this product right now?”) on a 100-mm VAS. In addition, foods were presented in randomized pairs, and subjects had to select their most wanted food (“select the food which you most want to eat right now”) as quickly and accurately as possible. During the latter procedure reaction time was measured. Reaction times were transformed to a standardized "d-score" with the use of a validated algorithm (29): the smaller the d-score, the greater the wanting for that food category relative to other categories in the task.

Body weight, urinary nitrogen excretion, and analytic methods

Body weight was measured twice a week before subjects ate their hot meal, while they were wearing no shoes or heavy clothing. If a subject’s weight fluctuated by >0.2 kg from baseline, the research dietitian decided whether energy intake needed to be adjusted for weight maintenance.

As an independent, objective marker of dietary compliance, total urinary nitrogen excretion was measured from two 24-h urine collections made during day 16 of each intervention. Total urinary nitrogen excretion in the low-protein state was 77 ± 12 mg · kg body weight−1 · d−1 and in the high-protein state was 303 ± 26 mg · kg body weight−1 · d−1). These data confirm that the low-protein diet was inadequate and contained protein amounts below the average daily recommendation and that the high-protein diet contained more protein than needed (the average daily recommendation is equivalent to 105 mg N · kg body weight−1 · d−1) (30). Completeness of the two 24-h urine samples that were collected on day 16 of the interventions was verified by recovery of three 80-mg doses of para-aminobenzoic acid given with the meals (31). Analyses showed an average recovery rate of 94.4%. Nitrogen in urine was determined colorimetrically according to the Kjeldahl method (96; method 920.87) on a Vitros 250 Chemistry System (Ortho-Vlinical Diagnostics).

Although we relied primarily on the total nitrogen excretion data as an independent, objective marker of dietary compliance, we also used other means to promote compliance. These included instructing subjects to keep a diary to record any deviations from the diet, illness, and use of drugs. Subjects were urged not to change their physical activities, which were also monitored by assessing the number of steps taken each weekday with pedometers (Yamax Digi-Walker).

Analyses

Data are presented as means ± SEs unless otherwise specified. fMRI data were preprocessed and analyzed with the SPM8 software package (Wellcome Department of Imaging Neuroscience, London, United Kingdom) in conjunction with the MarsBar toolbox (http://marsbar.sourceforge.net/) run with MATLAB 7.12 (The Mathworks Inc). The functional volumes of every subject were realigned, globally normalized to Montreal Neurological Institute space, and spatially smoothed with an isotropic Gaussian kernel of 8 mm full width at half maximum. Eight conditions were modeled: delivery of the 7 odors and rating. The responses to rating were ignored in further analyses. Before the analysis, it was established that subjects were to be excluded when head motion exceeded 3 mm displacement (one voxel). None of the measurements exceeded this limit; thus, no one was excluded. To regress out motion-related variance, the motion-correction parameters from the realignment procedure were added to the model as regressors.

For analyses, food products were either divided on taste category (sweet compared with savory) or protein content (low compared with high). For every subject, 12 contrast images (parameter estimations) were calculated: perception and evaluating sweet food cues (7 s) compared with control (grass), perception and evaluating savory food cues (7 s) compared with control, perception and evaluating low-protein food cues (7 s) compared with control (grass), and perception and evaluating high-protein food cues (7 s) compared with control (grass). Analyses showed an average recovery rate of 94.4%. Nitrogen in urine was determined colorimetrically according to the Kjeldahl method (96; method 920.87) on a Vitros 250 Chemistry System (Ortho-Vlinical Diagnostics).
compared with control, and perception and evaluating high-protein cues (7 s) compared with control, in a low-protein state, in a high-protein state, or at the end of the washout.

To test our hypothesis, 2 whole-brain statistical F-maps were created by performing an ANOVA (see Supplemental Table 2 under “Supplemental data” in the online issue). The first F-map contained the independent variables intervention (low protein status, high-protein status, or end of the washout period) and taste category of food cue (sweet food cues and savory cues). The second F-map contained the independent variables intervention and protein content of food cue (low-protein food cues and high-protein food cues). We used a functional region of interest (fROI) approach that combined a priori anatomical areas of interest with a functional criterion based on a minimum level of responsiveness to food cues and learning (32). Anatomical areas of interest included olfactory areas identified in a meta-analysis, which included piriform and orbitofrontal cortex (OFC), amygdala, anterior insula, and ventral putamen (we used the complete activation map available at http://flavor.monell.org/~jlundstrom/index_ALE.html) (33). In addition, we added the striatum (caudate, putamen, and pallidum). These areas play a prominent role in primary reward processing and found to be involved in protein regulation (eg, 18, 34). Mask images were obtained from the WFU Pickatlas 9 (35) and dilated one voxel to account for anatomical variation. To identify fROIs for both created F-maps, a threshold with a significance level of P < 0.05 and a cluster size of k > 8 contiguous voxels were used. On the subject level, the measured changes in BOLD signal were modeled, and the general linear model (GLM) was used to identify voxels in which the BOLD signal time courses could be explained by a linear combination of regressors. For a given condition (eg, the response to a food cue), the expected change in BOLD signal was modeled by using a canonical hemodynamic response function. This yielded an estimate of the β value for each condition (parameter estimate). This estimated β value is proportional to the magnitude of the BOLD response and is referred to here as “β value.”

The mean β value in each fROI was calculated with the use of MarsBar and submitted to an ANOVA (GLM procedure) in SAS (SAS Institute). This allowed us to test the effects of the intervention (low-protein status, high-protein status, or end of washout) and taste category (sweet or savory) or the intervention and protein content (low or high) as independent variables within each fROI. This technique represents an unbiased approach to test a priori hypotheses and avoids problems of circularity (32). Results of the LFPQ and intake during the ad libitum phase were analyzed by ANOVA (GLM procedure).

Subjects were included in all models as a random factor. The analyses showed that the addition of treatment order as a covariate did not affect the results. Tukey’s test was used for post hoc comparisons. The analyses were conducted with the use of SAS, 9.1 (SAS Institute Inc).

RESULTS

Brain responses

The fROI analyses of the brain responses to food cues (relative to the control condition) showed a main effect of intervention in parts of the OFC, the striatum, and the hippocampus/parahippocampal gyrus (Figure 2, Table 3): the BOLD response was increased in the left and right orbital part of the inferior frontal gyrus, left and right caudate, and right putamen in a low-protein state compared with a high-protein state. Specifically, the BOLD response to savory food cues was higher in both the left and right orbital part of the inferior frontal gyrus in a low-protein state than in a high-protein state (Figure 3). Compared with baseline, the BOLD response was lower in the left hippocampus and right parahippocampal gyrus in a high-protein state.

Independent of type of intervention, a main effect of taste category of the food cues (relative to the control condition) was found in the amygdala and the insula (Table 3), whereby insula activation was higher for sweet food cues. In the amygdala, the BOLD response was higher with savory food cues. The protein content of the food cues did not modulate the BOLD response.

Food liking and wanting

The diet interventions significantly altered both liking [F (2, 242) = 8.6; P < 0.001] and wanting [F (2, 242) = 5.0; P < 0.01] for foods according to their taste category (Figure 4); the liking for savory food cues was higher in a low-protein state than in a high-protein state (P < 0.01). In addition, in a high-protein state, sweet food cues were more liked (P < 0.001) and tended to be more wanted (P = 0.06) than savory food cues. The protein content of the food cues had no effect on liking or wanting.

Protein and energy intakes

Total protein intake (g) in the ad libitum phase was 8% higher in a low-protein state (103 ± 31 g) than in a high-protein state (95 ± 33 g): F 1,22 = 4.8, P < 0.05. Total energy intake (MJ) during the ad libitum phase in a low-protein state (14.1 ± 4.3 MJ) did not differ from intake in a high-protein state (14.5 ± 4.5 MJ): F 1,22 = 0.5, P > 0.05.

DISCUSSION

The objective of this study was to investigate the effect of human protein status on neural responses to different food cues associated with protein with the use of fMRI. In addition, we measured food preferences and intake.

In summary, we found that protein status modulated the BOLD response in parts of the reward system in response to food cues (relative to the control condition), whereby the BOLD response was higher in reward-related areas (OFC, striatum) in a low-protein state than in a high-protein state. Specifically, when exposed to savory food cues, the BOLD response was higher in the inferior OFC. In contrast, the protein content of the food cues did not modulate the BOLD response. In addition, protein status affected food liking and wanting and modulated spontaneous protein intake; all increased in a low-protein state.

We hypothesized that a low-protein status would increase brain-reward responses to food cues associated with protein. The results show that low-protein status (when the subjects where protein deprived) and high-protein status (when the protein was overconsumed) oppositely affected the BOLD response. The finding that both protein deprivation and protein overconsumption had a modulating effect is not surprising because both can damage an organism in the long term.
In a high-protein state, the BOLD response decreased when the subjects were exposed to food cues (relative to the control condition), which agrees with recent published studies showing that protein intake reduces the BOLD response in reward-related areas (18–20). In our study, however, this reduction in BOLD response was not accompanied by a subsequent decrease in total energy intake in the ad libitum phase, but led to a change in specific food selection (as shown by the similar energy intakes but different protein intakes in the low-protein and high-protein states). Given the current findings, we postulate that organisms regulate their protein intake by selecting foods differing in nutrient content, instead of decreasing or increasing their total

**FIGURE 2.** Mean (±SEM) changes in the BOLD response for the fROIs of the left and right inferior frontal gyrus, orbital part (A); caudate (B); putamen (C); and left hippocampus and right parahippocampal gyrus (D) when exposed to odor and visual food cues (relative to the control condition) in a low-protein state, in a high-protein state, or at the end of washout. The interventions significantly altered the BOLD response in the left ($F_{2,110} = 9.8$, $P < 0.001$) and right ($F_{2,110} = 9.8$, $P < 0.001$) inferior frontal gyrus, left ($F_{2,110} = 4.4$, $P < 0.05$) and right ($F_{2,110} = 6.1$, $P < 0.01$) caudate, right ($F_{2,110} = 4.3$, $P < 0.05$) putamen, and left hippocampus ($F_{2,110} = 6.1$, $P < 0.01$) and right parahippocampal gyrus ($F_{2,110} = 4.3$, $P < 0.05$). $n = 23$. The changes in BOLD response for the fROIs were compared by means of ANOVA (general linear model procedure). Next to each graph the corresponding fROI is shown in black on a representative slice of the mean anatomical magnetic resonance imaging of all subjects. *Significantly different ($P < 0.05$) from the other bars. An accompanying horizontal line indicates that the 2 bars at the extreme ends are significantly different. fROI, functional region of interest.
energy intake, when given the opportunity via access to a variety of food options. This specific food-selection effect was supported by the finding that protein status specifically modulated the BOLD response to food cues differing in taste category: the BOLD response to savory food cues was higher in the inferior OFC in a low-protein state than in a high-protein state. This was also reflected in the results of the LFPQ. Protein status altered both liking and wanting for foods according to their taste category; liking for savory food cues was higher in a low-protein state than in a high-protein state. In addition, sweet food cues were more liked and tended to be more wanted than savory food cues in the high-protein state. Again, the protein content of the food cues had no effect on liking or wanting.

The finding that the altered preferences observed after the interventions were guided by taste category rather than by absolute protein content of the food cues did not agree with our hypothesis. We had expected that the protein content of the food would independently influence food reward responses. However, a recent review concluded that there is no convincing evidence of the existence of a unique “protein taste” system, and hypothesized that learned associations between taste and postoral signals could play a role in protein intake regulation (34). This is supported by studies that have shown that direct intragastric infusion of protein may be sufficient to establish a learned preference for the protein (36). Our study showed that the changes in food preferences caused by manipulation of protein status were underpinned by brain reward responses to relevant food cues. This neural modulation appears to precede the selection of foods with beneficially different macronutrient ratios. We therefore postulate that these changes in food preferences rely on existing learned associations between primary taste categories and macronutrient availability. Our results led us to hypothesize that protein status modulates brain reward responses to savory food cues, which results in changes in taste preferences and leads to adaptations in protein intake. The causality of this process is yet to be established. These novel findings, however, provide the strongest evidence to date that taste is important for the regulation of protein intake in humans.

### Table 3

Identified fROIs and results of analysis on change in BOLD response in each fROI when exposed to odor and visual food cues in a low-protein state, in a high-protein state, or at the end of washout.

<table>
<thead>
<tr>
<th>fROI</th>
<th>Coordinates of fROI</th>
<th>P-main effect</th>
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<tbody>
<tr>
<td></td>
<td>x</td>
<td>y</td>
</tr>
<tr>
<td>F-map (intervention × taste category)²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olfactory map</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L inferior frontal gyrus, orbital part</td>
<td>−21</td>
<td>26</td>
</tr>
<tr>
<td>L amygdala</td>
<td>−18</td>
<td>2</td>
</tr>
<tr>
<td>L insula</td>
<td>−36</td>
<td>2</td>
</tr>
<tr>
<td>R inferior frontal gyrus, orbital part</td>
<td>21</td>
<td>35</td>
</tr>
<tr>
<td>R amygdala/parahippocampal gyrus</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>R insula</td>
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<td>11</td>
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<tr>
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<td>L caudate</td>
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<td>5</td>
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<td>17</td>
</tr>
<tr>
<td>R caudate</td>
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<td>8</td>
</tr>
<tr>
<td>F-map (intervention × protein content)²</td>
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<tr>
<td>Olfactory map</td>
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<td></td>
</tr>
<tr>
<td>L parahippocampal gyrus</td>
<td>−15</td>
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<tr>
<td>R inferior frontal gyrus, orbital part</td>
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<tr>
<td>R hippocampus</td>
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<tr>
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<tr>
<td>L caudate</td>
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<tr>
<td>L putamen</td>
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<td>L pallidum</td>
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<tr>
<td>L insula</td>
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<tr>
<td>R caudate</td>
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<td>−16</td>
</tr>
<tr>
<td>R putamen</td>
<td>27</td>
<td>8</td>
</tr>
</tbody>
</table>

1 BA, Brodmann area; fROI, functional region of interest; L, left hemisphere; R, right hemisphere.

²Two whole-brain statistical F-maps were created. The F-map (intervention × taste category) contained the independent variables intervention (low-protein status, high-protein status, and end of washout) and taste category of food cue (sweet food cues and savory food cues). The F-map (intervention × protein content) contained the independent variables intervention (low-protein status, high-protein status, and end of washout) and protein content of food cue (low-protein food cues and high-protein food cues). The last 3 columns contain the results of the ANOVA (general linear model procedure), in which we analyzed the change in BOLD response in each fROI (relative to the control condition). For the fROIs of F-map (intervention × taste category), the main effects of the intervention and taste category of food cue are shown. For the fROIs of F-map (intervention × protein content), the main effects of the intervention and protein content of food cue are shown. The F-maps had a threshold of F = 2.17 (P < 0.05), uncorrected for multiple comparisons, with a cluster extent threshold k > 8 contiguous voxels.
That the subjects ingested different amounts of protein, but similar amounts of energy in the ad libitum phase, leads us to conclude that protein status specifically modulates food preferences, to restore adequate protein status. Several studies have shown that protein seems to be more satiating than the isoenergetic ingestion of carbohydrate or fat (37), which in our case could bias the energy intake measurement. However, the relation between protein and short-term satiety is still ambiguous, with studies showing different outcomes (38–40). In addition, in both groups, the energy intake during the 1-d ad libitum phase greatly exceeded the habitual intake; therefore, we think it is unlikely that the difference in protein intake in both groups biased the total energy intake.

In this study we aimed to further explore the specific relation between protein and the underlying neural effects on food reward. In our previous study, we established that human protein status modulates food preferences (9). It has been shown that the sense of odor is involved in the choice of which food we want to eat. We therefore decided that, for this particular study, it would make sense to measure the effect of food odors (in combination with congruent images) on brain reactivity, rather than the effect of tasting the food. Regarding the differences in neural processing of odors and taste, it has been shown that there are modality-specific activations of the primary taste cortex and the primary olfactory cortex (41). However, regarding the processing of reward, both taste and odor studies have shown that similar brain areas are involved in this process (42), eg, the OFC, where we also found a response in our study. The choice to use odors rather than taste also enabled us to use several solid food cues that varied in taste category and protein content. It would not have been feasible to measure the effect of tasting the solid foods in the scanner because the act of chewing causes head movements.

To obtain more insight into the direction of the effects that we were interested in, an fMRI and LFPQ measurement were included when the subjects were eating their normal diet, at the end of the 4-wk washout period (washout measurement). Because of practical constraints, it was not possible to include a measurement before the start of the treatments, which would have provided a true baseline. Although the addition of treatment order as a covariate in our analyses did not affect the results of the washout period, our design limited us in claiming that subjects returned to their normal protein status after the 4-wk washout.

**FIGURE 3.** Mean (±SEM) changes in the BOLD response for the fROIs of the left (A) and right (B) orbital part of the inferior frontal gyrus when exposed to odor and visual food cues (relative to the control condition) in a low-protein state, in a high-protein state, or at the end of washout. When exposed to savory food cues, the BOLD response was higher in both the left ($P < 0.01$) and right ($P < 0.05$) orbital parts of the inferior frontal gyrus in a low-protein state than in a high-protein state. $n = 23$. The changes in BOLD response for the fROIs were compared by means of ANOVA (general linear model procedure). Next to each graph, the corresponding fROI is shown in black on a representative slice of the mean anatomical MRI scan of all subjects. *Significant difference ($P < 0.05$) between the 2 bars (horizontal line) at the extreme ends. fROI, functional region of interest.

**FIGURE 4.** Food liking (A) and food wanting (B) responses to sweet and savory food cues in a low-protein state, in a high-protein state, or at the end of washout. Food liking responses were assessed by using a 100-mm visual analog scale. Food wanting responses are expressed as a standardized “d-score,” which is a validated algorithm to transform reaction time (29). A smaller d-score means a greater wanting for that food category relative to other categories in the task (reversed y axis). The interventions significantly altered both liking ($F_{2,242} = 8.6, P < 0.001$) and wanting ($F_{2,242} = 5.0, P < 0.01$) for foods according to their taste category: the liking for savory food cues was higher in a low-protein state than in a high-protein state ($P < 0.01$). In addition, sweet food cues were explicitly more liked ($P < 0.001$) in the high-protein state and tended to be more wanted ($P = 0.06$) than savory food cues. Values are means ± SEMs ($n = 23$). Food liking and food wanting were analyzed by using ANOVA (general linear model procedure). *Significant difference ($P < 0.05$) between the 2 bars (horizontal line) at the extreme ends. VAS, visual analog scale.
Note, in the interpretation of the fMRI results, it does not necessarily mean that an increased BOLD response represents higher neural activation and a lower BOLD response represents a lower neural activation. There is still some debate about this topic, eg, one hypothesis is that, given the nature of the BOLD signal, a decrease in BOLD could mean that the local neurons are firing more, and they become efficient at extracting oxygen from the surrounding vasculature. As mentioned previously, our results concur with those of earlier published studies that protein intake, or a high-protein status, reduces the BOLD response in reward-related areas. Whether this reduced response actually represents reduced brain activation needs to be confirmed. In conclusion, protein status modulates brain responses in reward regions to savory food cues. These findings suggest that dietary protein status affects taste category preferences, which could play a role in the regulation of protein intake in humans. To our knowledge, we are the first to provide neural evidence for changes in food preference driven by macronutrient depletion.

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The authors’ responsibilities were as follows—SG-R, PAMS, SB, and CG: designed the study; SG-R and EvdH: collected data; SG-R and PAMS: analyzed the data; SG-R: wrote the manuscript; and all authors: interpreted the results and critically revised the manuscript. None of the authors had a personal or financial conflict of interest.

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