Chapter 10

Restrictive and metabolic weight loss strategies in obese females alter functional connectivity network activity in the fasting and postprandial state

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ABSTRACT

Objective

Obesity is associated with enhanced functional connectivity of brain areas implicated in cognitive control, motivation and reward in the fasting state, whereas the eventual response to food intake in these areas is blunted. It is unknown, however, whether these disturbances in functional connectivity are influenced by calorie restriction or roux-en-y gastric bypass surgery (RYGB). The aim of this study was to compare the direct effects of calorie restriction and RYGB in obese females on the response to food intake in functional connectivity brain networks implicated in default mode activity, homeostatic regulation of food intake and reward.

Methods

We performed resting state functional magnetic resonance imaging (MRI) scans on a 3 Tesla MR scanner after 10 hours of fasting and upon food intake in 35 obese subjects before and after 3 weeks of weight loss achieved by calorie restriction or Roux-en-Y gastric bypass surgery.

Results

Calorie restriction enhanced functional hypothalamic connectivity with the putamen in the fasted state, whereas these changes were not seen after RYGB. There was increased positive hypothalamic insula connectivity after restriction, which decreased upon food intake. Furthermore, restrictive weight loss increased connectivity between amygdala and frontal pole after food intake. In RYGB treated subjects, however, connectivity between amygdala and operculum increased after food intake.

Conclusion

Our data suggest that restrictive weight loss is associated with an increased motivation for food after an overnight fast, whereas RYGB is associated with an enhanced postprandial response to food intake in areas implicated in satiety and gustation.

INTRODUCTION

The brain is an important regulator of short and long-term energy homeostasis, integrating signals from peripheral energy stores to adapt food intake and energy expenditure. Food intake is under the control of cortical and subcortical areas related to reward and cognition (1-4). Neuroimaging studies show evidence for an an "obesogenic" pattern of brain activity, including increased activity of brain areas implicated in cognitive control, motivation and reward in the fasting state, and a blunted response after food intake in these areas (5-7). Obesity may be associated with an imbalance between brain circuits that promote reward seeking behavior and those that govern cognitive control. Such "obesogenic" neural activity might hamper intentional weight loss in obese subjects (3;8).

The hypothalamus integrates neural and hormonal signals in order to maintain short and long term energy homeostasis (2;3). Hypothalamic connectivity to reward processing areas, such as the insula and orbitofrontal cortex was altered in obesity and is correlates to circulating insulin levels (9). The amygdala, an important constituent of the brain's emotion and reward network, regulates the hedonic effects of food intake by connecting emotional, rewarding and gustatory cues to aspects of cognition (1). Altered responses of the reward network may trigger excessive motivation for non-homeostatic eating (6;10;11). The PCC network activity reflects a baseline state of brain function (12). Activity in this network is enhanced in obese and formerly obese subjects and is associated with increased appetite (13), and reacts to food related stimuli (14;15).

Subjects who have lost weight show enhanced activity of reward areas in response to visual food cues, suggesting that these subjects are more sensitive to such stimuli, perhaps in response to their low energy stores (16). *In contrast*, other studies reported that patients after weight loss showed decreased activity in reward regions (17) and enhanced activity in impulse control regions in response to visual food cues, in parallel with decreased motivation for food and enhanced cognitive control (18). In accordance to this, it was suggested that the activity in reward regions has a role in the prediction of successful weight loss (19;20).

Calorie restriction or Roux-en-Y-gastric bypass surgery (RYGB) are currently accepted therapies for weight loss and weight loss maintenance. Whereas calorie restriction (i.e. gastric banding or low calorie diet) is very effective on the short term, physiological mechanisms, such as inhibition of catabolic pathways, counteract sustained weight loss (8). The RYGB procedure effectively changes eating behavior and perception of

satiety and taste within weeks, coinciding with altered anorectic gut hormone secretion in the postprandial state (21;22).

The aim of this study was to compare the effects of a meal challenge on functional connectivity of brain networks involved in homeostatic regulation of food intake, reward, and self-referential processing between obese females after calorie restriction (very low calorie diet and gastric banding) and after RYGB.

MATERIAL & METHODS

Subjects

We included obese females who agreed to participate in a weight loss trial comparing the effects of different weight loss strategies. A concise description of the study population has been published. In short, subjects were all Caucasian, with a mean BMI 42.8 ± 4.1 kg/m^2 (range 35-51kg/m^2) and age 49.0 ± 6.1 yr (range 35-54y). Exclusion criteria were smoking, any chronic disease other than diabetes, including psychiatric illness, the use of medication that could affect brain function and general MRI contraindications. Eighty percent of subjects were postmenopausal. The subjects had either normal fasting glucose (NGT) or type 2 diabetes. All diabetic subjects were treated with oral medication only (metformin, sulfonylurea derivatives). Subjects who reported the use of weight loss medications within 90 days prior to enrollment of the study were excluded. Body weight of all subjects had been stable for at least 3 months prior to inclusion. Participants were allowed to use cholesterol lowering statins and antihypertensive medication.

Ethics

The study was performed in accordance with the principles of the revised Declaration of Helsinki (as amended in Seoul (2008) and including the clarifications added in Washington (2002) and Tokyo (2004)). The protocol was approved by the medical ethics committee of the Leiden University Medical Center, and all subjects provided written informed consent before participation.

Study design

All oral glucose-lowering agents were discontinued 48 hours prior to scanning. Scans were made within a month before surgery/start of the diet, and again between 2 and 3 weeks after surgery/start of the diet. Subjects were given a liquid meal (266 milliliters Nutridrink®, 400 kcal; 49 energy % carbohydrate (48,9 g, dextrine maltose / sacharose),

35% lipids (15,4 g), 16% protein (15,9 g)) while remaining in supine position within the MRI scanner.

Surgery

During RYGB, a 25 milliliters gastric pouch was created and connected to a 100cm Roux-en-Y limb. Gastric banding entailed placement of a standard silicone LapBand® (Inamed, Allergan, Santa Barbara, CA) around the stomach to create a 15 milliliters pouch. On the first day after surgery, patients were prescribed a clear liquid diet for 4-5 days. For the first 3 weeks after surgery, progressively less liquid food was consumed, containing ground or mashed protein sources and vegetables. Our prescribed dietary suggestions ascertained an equivalent calorie intake between the two intervention groups.

Very low calorie diet

Commercially available Prodimed® (Prodimed Benelux BV, Valkenswaard, The Netherlands) is a high-protein-low-calorie meal replacement plan (VLCD), with an average calorie intake of 600 kcal/day. The meal replacement plan consists of sachets (~90 kcal each of which ~18 g protein, ~2.5-5 g carbohydrates, 0,5-2 g fat) soluble powder for preparation of meals. Subjects were allowed 4-5 sachets a day, and an additional choice of selected vegetables.

FMRI data acquisition

MRI scans were acquired on a Philips Achieva 3.0 Tesla scanner using an eight-channel SENSE head coil for radiofrequency reception (Philips Healthcare, Best, The Netherlands). A high-resolution T_1-weighted anatomical image (ultra-fast gradient-echo acquisition, TR 9.78ms, TE 4.59ms, flip angle 8^0, 140 axial slices, FOV 224x224mm, in-plane resolution 0.875x0.875mm, slice thickness 1.2mm) and a high resolution T_2^*-weighted EPI scan (TR 4400ms, TE 30ms, flip angle 80^0, 84 axial slices, FOV 220x220mm, in-plane resolution 1.96x1.96 mm, slice thickness 2mm) were acquired for registration purposes. Whole-brain resting-state scans were acquired using T_2^*-weighted gradient-echo echo-planar imaging (EPI, 160 volumes, 38 axial slices scanned in ascending order, repetition time (TR) 4400ms, echo time (TE) 30 ms, flip angle 80^0, field of view 220x220mm, 2.75mm isotropic voxels with a 0.25mm slice gap). A whole-brain-resting state scan was performed in the fasting state, and when subjects had finished intake of the meal (meal intake took an average of 7 minutes). Although we used a longer TR time than is often used, our sampling frequency is above the Nyquist rate for sampling the low frequency range of interest in resting-state connectivity studies (i.e., 0.1-0.01Hz).

FMRI data preprocessing

All data was analyzed using FSL Version 4.1.3. (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) (23;24). The resting-state scans were preprocessed by applying motion correction, brain extraction (to remove non-brain data), spatial smoothing (Gaussian kernel of 6mm full width at half maximum), a grand-mean intensity normalization of the entire data set by a single scaling factor, and a high pass temporal filter with a cutoff of 0.01Hz. Each resting-state data set was first registered to the high resolution T2*-weighted EPI scan, which was subsequently registered to the high resolution T_1-weighted anatomical image, which was finally registered to the 2mm isotropic MNI-152 standard space (T_1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada). Next, the resulting transformation matrices were concatenated to describe the registration of the resting-state data to MNI standard space, and the inverse matrix was calculated. Scans were excluded because of excessive motion (>3 mm translation or >3° rotation in any direction), large image artifacts, or incomplete scans (e.g., due to claustrophobia during the scan).

fMRI time course extraction and statistics

A seed-based correlation approach was used to study resting-state functional connectivity with the hypothalamus, amygdala and PCC. Binary spherical regions of interest (ROIs), with a 4mm radius, were created of the hypothalamus (left seed: x=-4, y=-1, z=-13, right seed: x=5, y=-1, z=-13), amygdala (left seed: x=-23, y=-4, z=-19, right seed: x=23, y=-4, z=-19) and PCC (seed: x=-5, y=-49, z=40 (25)). Our hypothalamus seed was based upon the T1-weighted 1mm isotropic standard space image of the Montreal neurological Institute (MNI). We identified the middle voxel of the hypothalamus in both hemispheres and created a sphere around these. The 0-coordinate on the x-axis is not the exact midline between the two hemispheres, but is slightly closer to the left hemisphere. Therefore, we had to move 1 mm to the right to arrive at a comparable midpoint of the right hypothalamus as for the left. Using the inverse transformation matrix, the ROIs were registered to each participant's preprocessed resting-state data set.

Then, the mean time course within each ROI was calculated and used as regressor in a general linear model (GLM). Separate GLMs were set up to probe each of the three networks: the first contained regressors for the left and right hypothalamus seeds, the second contained regressors for the left and right amygdala seeds, the third contained the regressor for the PCC seed. In addition, white matter signal, cerebrospinal fluid (CSF) signal, six motion parameters (3 translations and 3 rotations), and the global

signal were used as nuisance regressors. For each individual the three GLMs were calculated using FEAT (FMRI Expert Analysis Tool) version 5.98, part of FSL (FMRIB's Software Library (23)). Except for the PCC, contrasts were made for the left and right seed separately, as well as for both seeds together. The resulting parameter estimate maps were resliced into 2mm MNI space and fed into a higher level mixed effects analysis to assess within-group and between-groups effects in fasted and postprandial condition and at baseline and after the interventions. Whole-brain *z*-score statistical images were thresholded with an initial cluster-forming threshold of $z>2.3$ and a corrected cluster significance threshold of $p<0.017$, bonferroni corrected for multiple testing (three regions of interest) (26). Masks were created from the significant and adjacent voxels where we found differences in the functional connectivity analyses. Next, the average *Z*-scores were extracted from these masks for each individual, and used to plot the significant whole-brain corrected connectivity differences.

RESULTS

Patient characteristics and weight loss

Forty-one obese females were included: 18 subjects (11 DM / 7 NGT) in the calorie restriction group (BMI 41.4 ± 3.8 kg/m^2, age 49.3 ± 5.8yrs, comprising subjects receiving VLCD or GB) and 23 subjects (13 DM / 10 NGT) in the RYGB group (BMI 43.6 ± 3.8 kg/m^2, age 49.3 ± 36.8yrs). Due to excessive motion (>3mm in any direction) and large image artifacts we excluded 3 scans (1 restriction, 2 RYGB). Three subjects experienced symptoms of dumping syndrome in response to the test meal, with nausea, sweating and palpitations occurring within 20 minutes after consumption. We excluded these incomplete scans from the analysis (2 NGT, 1 DM). Three weeks after intervention, weight loss was comparable between groups (-7,6 ± 1,2 kg in restriction patients, -8,9 ± 1,8 kg in RYGB patients, $p=0.10$). Conceivably, three weeks after treatment, all subjects were still obese (BMI after restriction 39,0 ± 5,4 kg/m^2, BMI after RYGB 40,6 ± 3,7 kg/m^2).

FC in the fasted state or in response to food intake in groups before treatment:

Hypothalamus/Amygdala/PCC: no differences in connectivity in the overnight fasted state or in response to meal intake were observed between the groups before RYGB or restrictive intervention. For this study, we were primarily interested in the effect of the different interventions. However, as we had included obese subjects with normal glucose tolerance and with type 2 diabetes mellitus we first ruled out differences between obese NGT subjects and obese T2DM subjects (Lips *et al.*, American Journal of Clinical Nutrition 2014).

FC in fasted state, before versus after treatment

In patients treated by calorie restriction there was increased hypothalamic FC with the right putamen in the fasting state after the intervention (table 1 / figure 1). The RYGB group, in contrast, did not show any FC changes between hypothalamus and putamen, however no statistically different effect between the RYGB and calorie restriction group was observed.

Table 1 - Within group effects of calorie restriction on hypothalamic connectivity.

	Region	Peak Voxel Coordinates (x/y/z)	Peak Voxel Intensity		
In (overnight) fasting state					
Restriction Pre > Post	Right Putamen	22/16/-06	3.72		Fig 1
In response to food intake					
Restriction Pre > Post	L Occipital pole	4/-88/26	3.48	0.0005	Fig 2A
Restriction Post > Pre	Left Insula	-36/-06/-12	3.01	0.0026	Fig 2B
RYGB Pre > Post	R Operculum	56/-22/14	3.44	0.0006	Fig 2C

All Z-values are corrected for multiple comparisons (p<0.017). Z-values in italics were calculated using an uncorrected threshold of p<0.05. R, right; L, left; MNI standard space, T_1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada.

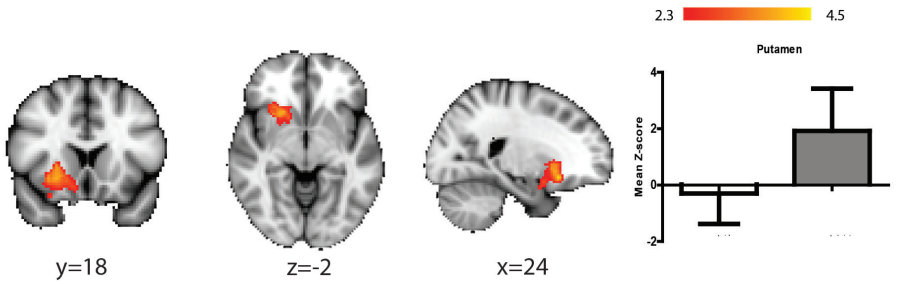

Figure 1. Whithin group effects of calorie restriction on fasting hypothalamic connectivity with the putamen.

Results are projected on 2 mm MNI standard space. Between group effects are thresholded at z>2.3, p<0.017 (cluster corrected). White bar depicts pre intervention and grey bar depicts post intervention. Values are depicted as mean plus/min standard deviation. The left side of the brain in the figure corresponds with the right side in reality and vice versa. MNI, T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, Canada.

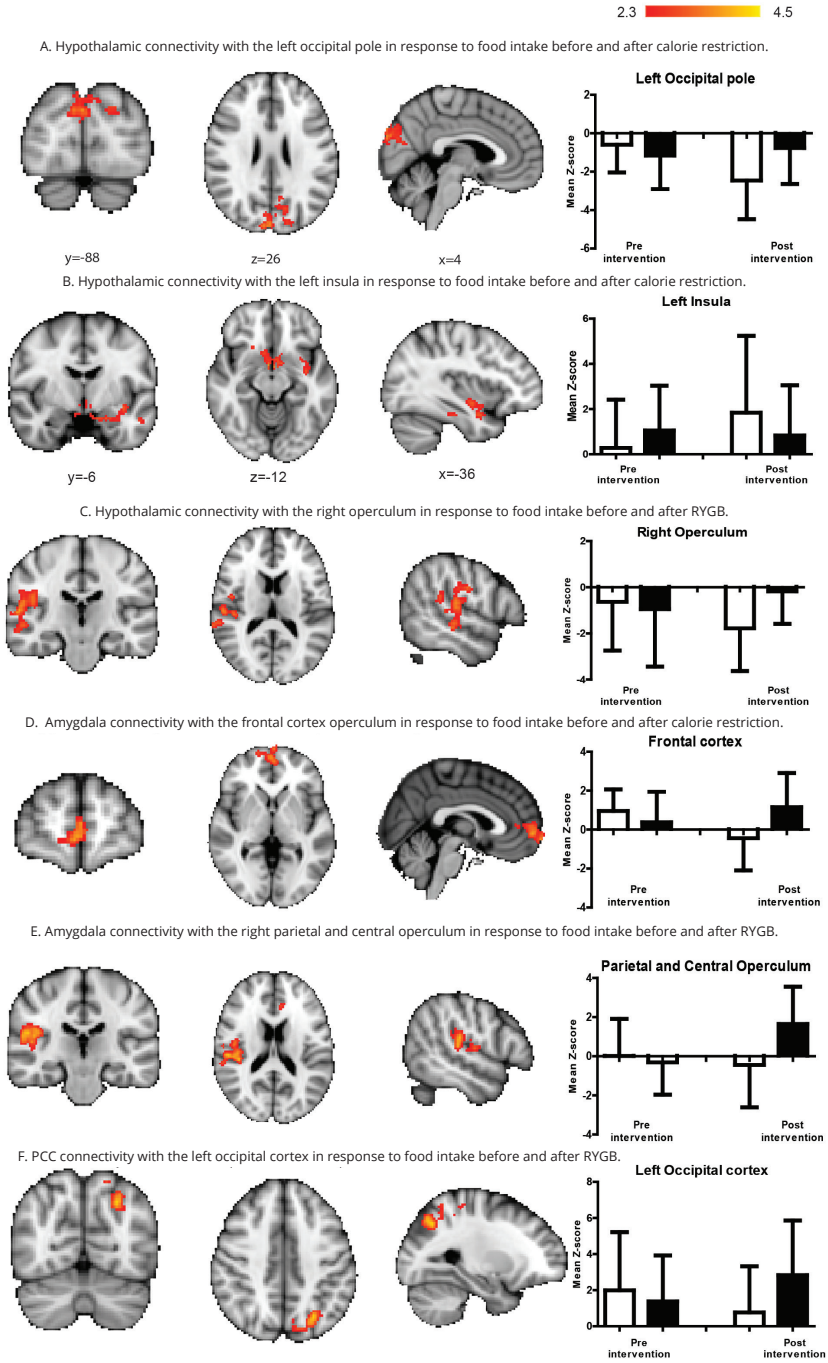

Figure 2. Within group effects of interventions on hypothalamus, amygdala and PCC connectivity in response to food intake. Results are projected on 2 mm MNI standard space. Between group effects are thresholded at z>2.3, p<0.017 (cluster corrected). Values are depicted as mean plus/min standard deviation. White bars depict fasting condition, black bars depict after food intake. The left side of the brain in the figure corresponds with the right side in reality and vice versa. MNI, T1-weighted standar brain averaged over 152 subjects; Montreal Neurological Institute,Montreal,Canada.

FC in fasted state versus after meal intake, before versus after treatment:

Hypothalamus (table 1 / figure 2 A-C): Hypothalamic connectivity with the left occipital pole was not affected by meal intake before intervention; whereas after restriction there was a decrease in connectivity postprandial (figure 2A). Furthermore, connectivity between hypothalamus and left insula decreased postprandial after calorie restriction (figure 2B). There was no effect of RYGB on connectivity in these or other areas, yet no corrected meal x intervention interaction was found. While RYGB did not affect FC between the hypothalamus and operculum in the fasting state, FC was significantly blunted by food intake after RYGB (figure 2C). Again, there was no meal x intervention interaction.

Table 2 - Within group effects of calorie restriction on amygdala connectivity.

	Region	Peak Voxel Coordinates (x/y/z)	Peak Voxel Intensity		
In (overnight) fasting state					
No effects of calorie restriction were observed in the fasting state					
In response to food intake					
Restriction Pre > Post	Prefrontal cortex	02/60/00	3.48	0.0005	Fig 2D
RYGB Pre > Post	R Parietal operculum	54/-24/18	3.89	0.0001	Fig 2E
RYGB Pre > Post	R Central operculum	46/-12/16	3.69	0.0002	

All Z-values are corrected for multiple comparisons ($p<0.017$). Z-values in italics were calculated using an uncorrected threshold of $p<0.05$. R, right; L, left; MNI standard space, T_1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada.

Amygdala (table 2 / figure 2 D,E): Connectivity between the amygdala and prefrontal cortex was increased postprandial after calorie restriction, whereas no effect of food intake was seen before calorie restriction (figure 2D). In RYGB treated patients, connectivity between amygdala and parietal / central operculum increased after food intake, whereas there was no effect of meal intake at baseline (figure 2E). There was no meal x intervention interaction.

PCC (table 3 / figure 2F): Functional connectivity between PCC and left occipital cortex increased after food intake in RYGB patients. There was no meal x intervention interaction that reached statistical significance.

Table 3 - Within group effects of RYGB on PCC connectivity.

	Region	Peak Voxel Coordinates (x/y/z)	Peak Voxel Intensity	
In (overnight) fasting state				
No effects of calorie restriction were observed in the fasting state				
In response to food intake				
RYGB Pre > Post	Occipital cortex	-26/-68/42	4.08	**<0.0001**

All Z-values are corrected for multiple comparisons (p<0.017). Z-values in italics were calculated using an uncorrected threshold of *p*<0.05. R, right; L, left; MNI standard space, T_1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada.

Interactions between the two interventions: as described above, no significant meal x intervention interactions were observed. As this may have been caused by our small number of subjects we explored possible significant interaction effects in an uncorrected threshold. These results are described in the supplementary text and tables.

DISCUSSION

In this study we compared the short term effects of calorie restriction and RYGB on brain FC networks in response to a liquid meal. We studied the hypothalamus and amygdala FC network and the PCC FC network because of its importance in resting state FC. Given the neuroendocrine changes after RYGB, known to affect satiety and gustation, we were especially interested in the effects of this intervention.

Comparing the effect of the two different treatments, we did not find a meal x intervention interaction that was statistically significant. We did, however, find effects of the interventions within both groups, in different regions after calorie restriction or RYGB. We showed that in the calorie restriction group, FC between the hypothalamus and putamen increased in the fasting state. Moreover, hypothalamus connectivity with the insula decreased upon food intake after calorie restriction, whereas there was no effect after RYGB. Furthermore, calorie restriction only increased postprandial connectivity between amygdala and frontal cortex. In RYGB subjects, amygdala connectivity with the central and parietal operculum increased after food intake, whereas no effect of food intake was seen in this area in patients after restriction. Also in RYGB treated

subjects, functional connectivity between PCC and occipital pole increased after food intake, whereas there had been no effect of food intake before intervention.

Calorie restriction enhanced hypothalamic connectivity with the putamen in the fasting state. The putamen is part of a central orexigenic network, which has an established role in cognitive control. Neural activity in the putamen is suggested to potentiate the termination of feeding by inhibiting the (para)limbic regions of this network (27;28). Increased activity within this network in the fasting state has been associated with an increased motivation to eat after an overnight fast and after skipping breakfast (27;29) and in - weight stable – subjects after weight loss (30). Even so, we described increased FC between the hypothalamus and putamen in the fasting state in obese subjects ad compared to lean (Lips *et al.*, accepted for publication). Our new data shows increased putamen connectivity with the hypothalamus after restriction, which may enhance appetite in response to a negative energy. Evidence shows that obese subjects after weight loss show increased responsiveness to food intake in areas associated with reward and fail to adapt their responses to overeating, which could contribute to excessive intake and bare a risk for weight regain. Since this state is marked by relative leptin deficiency (31), and leptin is known to powerfully dampen appetite (31), leptin may play a pivotal role in the central mediation of hunger associated with maintenance of a reduced weight. Interestingly, the before mentioned hypothalamic connectivity with reward regions increased only after calorie restriction, whereas no effect was seen after RYGB. One previous study assessed the fMRI response to visual food cues after RYGB and showed decreased activation in reward related areas (32). Although activity and connectivity are clearly not the same, our findings imply that the previously described decreased activity in reward related areas after RYGB does not coincide with changes in connectivity between reward and homeostatic control areas.

Food intake decreased connectivity between hypothalamus and insula in subjects after calorie restriction, compared to no effect of food intake on connectivity before the intervention. The insula, as mentioned before, is implicated in reward processing and reacts to satiety factors such as taste and gut hormones (33). Thus, reduction of FC between the hypothalamus and insula in response to food intake after calorie restriction might indicate reduced hypothalamic-insula interaction in nutrient sensing. In contrast, it is tempting to speculate that the (uncorrected) increase in postprandial hypothalamus-insula connectivity after RYGB facilitates relay of satiety signals. Although connectivity studies have not reported on this area before, altered regional cerebral blood flow in both the hypothalamus and insula were observed before in response to a

meal (28;34;35). Moreover, it has been shown that hypothalamic activity in the context of manipulation by PYY, one of the satiety factors abundantly secreted in response to food intake after RYGB, is predictive of subsequent food intake (36).

Interestingly, increased functional connectivity between the amygdala and right parietal and central operculum was observed in response to food intake after RYGB. The opercular cortices cover the insula and have been related to gustation and reward (14;37). The amygdala's response to food intake is suggested to depend on the hedonic value that individuals assign to food (7), i.e. it corresponds to the reward predicting value assigned to a stimulus (38). Although the physiological implications of the changes in amygdala functional connectivity remain to be established, it is tempting to speculate that enhanced amygdala-opercular connectivity is involved in the altered perception of taste/gustation and satiety reported after RYGB (39).

Calorie restriction increased postprandial FC between amygdala and prefrontal cortex. Enhanced activity in prefrontal regions is associated with increased dietary restraint in successful dieters, suggesting that neural activity in this area facilitates restrictive behavior (40). Typically, the prefrontal cortex (dorsolateral OFC and dorsal ACC) is associated with cognitive and emotional control, and enhanced activity of these brain regions could promote self-regulation necessary to maintain a more restrictive diet. Enhanced postprandial amygdala frontal FC after calorie restriction may therefore indicate an enhanced amygdala influence on activity in the frontal areas, which could stimulate postprandial sensations of hunger. Of interest, RYGB treated patients showed no effect of intake in amygdala-prefrontal connectivity, possibly reflecting less hunger sensation in the postprandial state.

Several limitations of the current study need to be addressed. First, because of the low response rate of male subjects and to avoid a gender bias (41;41), we decided to include female subjects only. As a consequence, our results can only be generalized to females. The twenty percent premenopausal subjects of our population were not scanned in a particular phase of their menstrual cycle, which may have slightly affected our results. Second, hypothalamic and amygdala functional connectivity may, in addition to changes in homeostasis, be influenced by noise from surrounding arteries (42). To reduce the influence of physiological noise as much as possible, we used the global signal in our analysis as a nuisance regressor, which has shown strong correlations with physiological noise sources in fMRI data before (43). Because global signal regression could mathematically induce negative correlations or even introduce

between-group differences (44;45), no strict conclusions can be drawn from the observed sign of the connectivity. However, global regression increases the signal to noise and connectivity specificity and as such, differences in connectivity are real. Third, physiological stimulus we evoked by using a mixed meal, could be a restriction in the generalizability of our results, because different brain signaling is evoked depending on the type of macronutrients (46). Fourth, we were unable to find any significant correlations between connectivity and physiological (i.e. HOMA-IR, glucose, insulin, gut peptides) or subjective parameters and, which is possibly due to the small number of subjects studied.

In sum, we studied subjects in the first phase of their weight loss intervention, either shortly after RYGB or after restrictive interventions. We did so to be able to differentiate between the effects of calorie restriction *per se* (independent of weight loss) versus those of calorie restriction plus additional effects of RYGB (i.e. bypass of proximal duodenum and increase of gut hormone release). As minor weight loss had occurred by the time of imaging and the amount of weight lost was similar in both groups, the observed within group effects are probably intrinsic to the intervention type indeed.

In conclusion, our data show effects of RYGB or calorie restriction in the neural response to food intake in obese subjects. Calorie restriction increases neuronal connectivity of brain areas associated with motivation to eat in fasting condition. RYGB reinforces postprandial connectivity between areas implicated in satiety and gustation. These data will have to be replicated in larger cohorts, and during weight stabilization and maintenance of the reduced body weight. However, our results do suggest that calorie restriction and RYGB induce specific alterations in brain functional connectivity that may impact on their therapeutic effects.

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