Chapter 4

Obesity is marked by distinct functional connectivity in neural networks involved in the control of food reward and salience

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Abstract

Objective

The brain plays a crucial role in controlling energy balance. We hypothesize that brain circuits involved in reward and salience respond differently to fasting in obese compared to lean individuals. We compared functional connectivity networks related to food reward and saliency, prior to and after 48 hours of fasting, in lean and obese individuals.

Subjects

We included 13 obese (2 males, 11 females, BMI 35.4 ± 1.2, age 31 ± 3) and 11 control subjects (2 males, 9 females, BMI 23.2 ± 0.5, age 28 ± 3). Resting-state functional magnetic resonance imaging (fMRI) scans were made before and after fasting. Functional connectivity of the amygdala, hypothalamus and posterior cingulate cortex was assessed using seed-based correlations.

Results

Fasting affected hypothalamus functional connectivity differently between groups. Before fasting, a stronger connectivity between hypothalamus and left insula was found in obese subjects which decreased upon fasting. Upon fasting, connectivity of the hypothalamus with the dorsal anterior cingulate cortex (dACC) increased in lean subjects and decreased in obese individuals. Amygdala connectivity with the ventromedial prefrontal cortex was stronger in lean subjects at baseline, which did not change upon fasting. No differences in posterior cingulate cortex connectivity were observed.

Conclusion

To conclude, obesity is marked by alterations in functional connectivity networks involved in food reward and salience. Fasting differentially affected hypothalamic connections with the dACC and the insula between obese and lean subjects. Our data supports the idea that food reward and nutrient deprivation are differently perceived and/or processed in obesity.

Introduction

It is currently well recognized that obesity is a growing public health concern [1;2]. Obesity results from excessive energy intake relative to actual metabolic needs. The monitoring and regulation of energy intake depends both on internal factors such as homeostatic signals and cognitive-emotional state as well as on external factors such as the availability of food and social context. The brain plays a crucial role in the decision to eat by integrating the multiple hormonal and neuronal signals [3]. Not surprisingly, there is increasing evidence that obesity is associated with changes in the central nervous system [4-8].

In normal weight subjects, hunger is associated with increased activity in the prefrontal cortex, hypothalamus, thalamus, several limbic/paralimbic areas, basal ganglia, temporal cortex, cerebellum, insula, orbitofrontal and anterior cingulate cortices, striatum, hippocampus, parahippocampal gyrus, and precuneus [6;7;9-15]. This underscores the concept that food intake is controlled by multiple neural networks and their mutual connections [16]. Therefore, it is likely that alterations in the complex interplay between multiple brain regions are involved in the pathophysiology of obesity (i.e. unbalanced food intake). To better understand this disorder, it is important to evaluate how brain regions responsible for food intake interact in healthy controls, and if changes in this circuitry are related to obesity.

Resting-state functional magnetic resonance imaging (RS-fMRI) has become an important tool to study functional interactions in the brain in absence of overt behavior [17;18]. Remote brain regions are considered to be functionally connected when showing coherent signal fluctuations over time. By now, the presence of spatially distinct resting-state networks (RSNs) has been demonstrated consistently. Interestingly, most of these networks also show a remarkable overlap with patterns of brain activity evoked by tasks probing cognitive and emotional function [19]. Thus far, we are aware of only one study that has looked at differences in RS functional connectivity between obese individuals and healthy controls after an overnight fast [20], finding increased functional connectivity of the default mode network with the cuneus and midcingulate cortex in obese participants, while a temporal network demonstrated that functional connectivity strength was decreased in the left insular cortex. Moreover, the connectivity in these networks seems to vary with increasing body mass index (BMI). While

these results shed light on integration of brain circuits after an overnight fast in obese participants, we set out to study whether RSNs involved in food homeostasis, salience and reward respond differently to a prolonged fast in obese compared to lean individuals.

To this end, RS data were acquired after an overnight fast and after a prolonged fast of 48 hours. We compared the response of three different functional connectivity networks to 48 hours of fasting between obese and normal weight volunteers using a seed-based correlation approach. First, hypothalamic functional connectivity was assessed since the hypothalamus controls energy balance by regulating food intake and energy expenditure [3;21;22]. Then we analyzed functional connectivity of the amygdala, as this is a key constituent of the brain's emotion circuitry [23] and as such involved in learning how to translate emotional and rewarding events to behavioral schemes [24]. Alterations in reward-related brain regions are possibly associated with obesity [8]. Finally, we studied the default mode network (DMN). The DMN includes regions which are active when subjects are in an awake, resting state without any task, but whose activity diminishes during specific goal-directed behaviors [25]. The DMN includes the precuneus, posterior cingulate, medial prefrontal, and inferior parietal cortices. It has been suggested previously that alterations in the DMN in obese subjects may originate from parietal and cingulate regions [26;27].

Material & Methods

Subjects

Participants were recruited by advertisements in the public space. The participants had to be Caucasian, healthy, weight-stable, with a fasting plasma glucose ≤ 5.6 mmol/l. Exclusion criteria were: a positive family history of type 2 diabetes mellitus, smoking, recent blood donation and general MRI contraindications. Participants who used medication that could affect glucose homeostasis and/or brain function were excluded. Lean and obese individuals were matched with respect to age and sex. 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 study was approved by the Medical Ethical Committee of the Leiden University Medical Centre and registered in The Netherlands Trial Register (NTR2401). All volunteers gave written informed consent before participation.

Study Design

During the 48-hour fast, all participants were admitted to our research center after an overnight fast. On the first study day, the overnight fasted subjects underwent baseline MRI scanning. After the MRI, participants consumed a standardized breakfast – after which several metabolic parameters were assessed which will be published elsewhere - and then fasted for the next 48 hours. Drinking water and non-caffeinated tea was allowed ad libitum. The second MRI session was conducted after 48 hours of fasting. Participants were asked to lie still and relaxed with their eyes open during acquisition of each resting-state scan.

MRI 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). Whole-brain resting-state scans were acquired using T2*-weighted gradient-echo echo-planar imaging (EPI, EPI factor 29,160 volumes, 38 axial slices scanned in ascending order, repetition time (TR) 2200 ms, echo time (TE) 30 ms, flip angle 80^0, field of view 220x220 mm, 2.75 mm isotropic voxels with a 0.25 mm slice gap).

A high-resolution T_1-weighted anatomical image (ultra-fast gradient-echo acquisition, TR 9.78 ms, TE 4.59 ms, flip angle 8^0, 140 axial slices, FOV 224x224 mm, in-plane resolution 0.875x0.875 mm, slice thickness 1.2 mm) and a high resolution T_2^*-weighted EPI scan (TR 2200 ms, TE 30 ms, flip angle 80^0, 84 axial slices, FOV 220x220 mm, in-plane resolution 1.96x1.96 mm, slice thickness 2.0 mm) were acquired for registration purposes.

fMRI data preprocessing

All data was analyzed using FSL Version 4.1.3. (FMRIB's Software Library, www.fMRIb.ox.ac.uk/fsl) [28;29]. 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 registered to the high resolution EPI scan, the high resolution EPI scan to the T_1-weighted anatomical image, and the T_1 image to the 2 mm isotropic MNI-152 standard space (T_1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal,

QC, Canada). 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 (MNI to resting-state data).

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. Using the MNI standard space image, binary spherical regions of interest (ROIs) with a 2.5 mm radius were created around the center voxels of the bilateral hypothalamus (left seed: x=-4, y=-1, z=-13, right seed: x=5, y=-1, z=-13), and with a 3.5 mm radius of the amygdala (left seed: x=-23, y=-4, z=-19, right seed: x=23, y=-4, z=-19) [30], and PCC (seed: x=-5, y=-49, z=40 [31]). Using the inverse transformation matrix, 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 associated with the different seeds: 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 analyzed using FEAT (FMRI Expert Analysis Tool) version 5.98, part of FSL (FMRIB's Software Library [28]). 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 and corresponding images of variance were resliced into 2 mm MNI space and fed into a higher level mixed effects analysis to assess within-group (one sample *t*-test) and between-groups (independent samples *t*-test) effects at baseline, and the difference between baseline and the post-fast time point (repeated measures analysis of variance). 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 $p<0.05$ [32]. Regions of interests (ROIs) were drawn on significant and contiguous voxels found with the functional connectivity analysis. Average *z*-scores were calculated within these ROIs for each individual and then averaged per group to create bar graphs to illustrate the strength and directionality of the functional connectivity effects.

All data shown is depicted as mean ± standard error of the mean (SEM).

Results

Fourteen obese (BMI>30 kg/m^2) and 12 lean (BMI 19-25 kg/m^2) participants were included. Two scans were excluded because of excessive motion (>3 mm in any direction) resulting in large imaging artifacts (one of an obese and one of a lean participant). Therefore, we compared fMRI results of 13 obese (2 males, 11 females, BMI 35.4 ± 1.2, age 31 ± 3) and 11 lean individuals (2 males, 8 females, BMI 23.2 ± 0.5, age 28 ± 3).

Functional connectivity of hypothalamus

Positive connectivity analysis at baseline

Areas that were connected with the hypothalamus in both groups were: the brainstem, medial prefrontal cortex, amygdala, insula, posterior part of the middle temporal gyrus, subcallosal cortex, orbitofrontal cortex, hippocampus and the nucleus accumbens. Data for lean individuals at baseline is shown in supplementary Figure 1. At baseline there were no differences in functional connectivity between lean and obese individuals.

Negative connectivity analysis at baseline

The regions that were connected with the hypothalamus in both groups were: the cuneal cortex, the frontal poles, the lateral occipital cortices and the inferior frontal gyri (supplementary Figure 1). Again, there were no differences between obese and lean individuals at baseline.

Effects of 48-hours of fasting

After fasting, connectivity of the hypothalamus with the left insula and the superior temporal gyrus decreased in obese subjects (Figure 1A) compared with the baseline scan. Fasting did not significantly affect hypothalamic connectivity in lean individuals. An interaction between Group and Time was found for hypothalamic connectivity with the left insula and the dorsal anterior cingulate cortex (dACC), demonstrating differentially affected connectivity by the prolonged fast between lean and obese participants (Figure 2, Table 1). The (positive) connectivity between hypothalamus and left insula was reduced in the obese group to levels comparable to the lean individuals. In addition, the connectivity between the hypothalamus and the dACC increased in lean subjects, whereas it decreased in obese individuals (Figure 2).

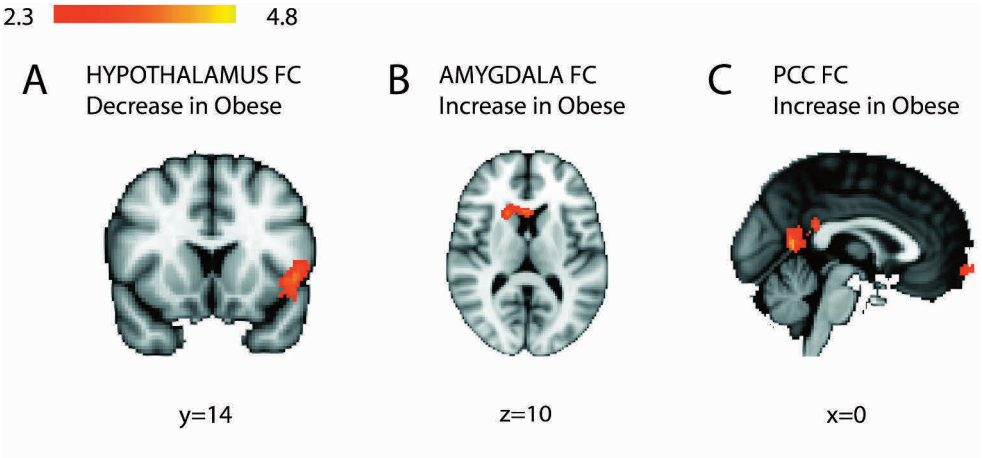


Figure 1: Fasting compared to baseline: Fasting induced changes in hypothalamus, amygdala and posterior cingulate cortex functional connectivity networks in obese subjects only.
Fasting had a significant effect on all three (positive analysis) functional connectivity networks studied, but only in the obese subjects. In this figure we show that fasting A) reduced functional connectivity between hypothalamus and left insula and hypothalamus and superior temporal gyrus in obese individuals, B) increased amygdala functional connectivity with the left caudate nucleus in obese subjects and C) increased PCC functional connectivity with the frontal pole, the precuneus cortex and the PCC itself in obese individuals. All results are projected on 2mm MNI standard space. Effects are thresholded at $z>2.30$, $p<0.05$. The left side of the brain in the figure corresponds with the right side in reality and vice versa. Abbreviations: PCC, posterior cingulated cortex; MNI standard space, T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada.

Functional connectivity of the amygdala

Positive connectivity analysis at baseline

Areas that were connected with the amygdala in both groups included: the brainstem, hippocampus, hypothalamus, globus pallidus, fusiform gyrus, orbitofrontal cortex and both temporal lobes. Data for lean individuals at baseline is shown in supplementary Figure 2. At baseline, lean individuals demonstrated increased amygdala functional connectivity with the ventromedial prefrontal cortex (vmPFC) (Figure 3, Table 2). Moreover, increased connectivity was observed between the amygdala and superior temporal gyrus in lean individuals compared with obese individuals (Figure 3, Table 2).

Negative connectivity analysis at baseline

The following areas were connected with the amygdala in both groups: the frontal poles, the precuneal cortices and the occipital cortices (supplementary Figure 3).

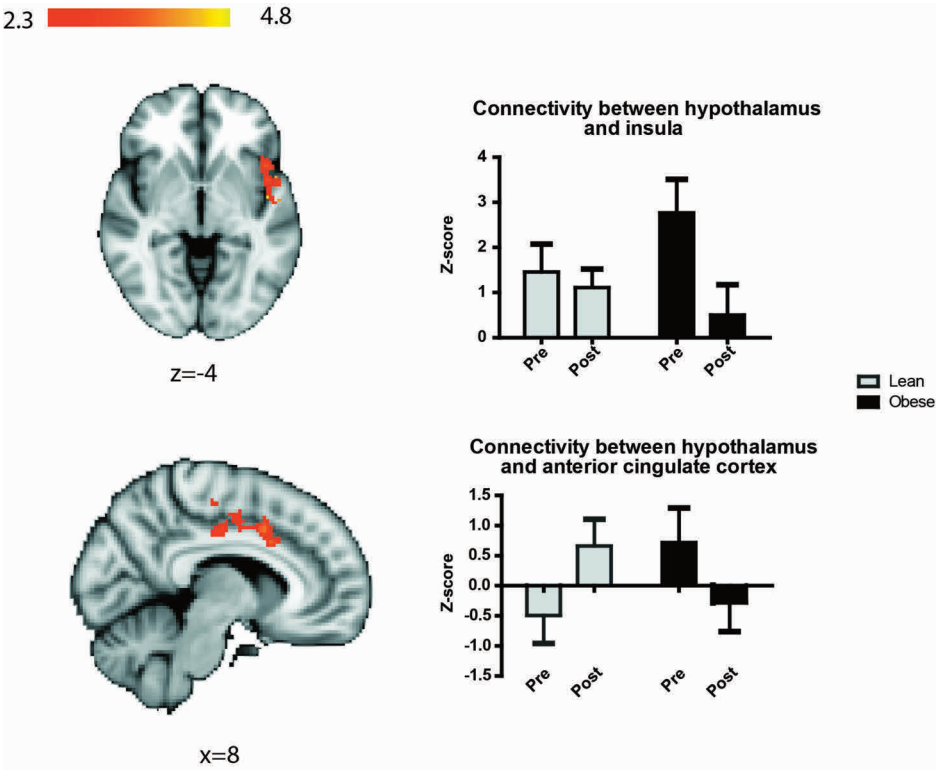


Figure 2: The effect of fasting (compared to baseline) between lean and obese subjects: Fasting affected hypothalamic connectivity with the left insula and dACC differently in lean compared to obese participants.

Fasting had a different effect on hypothalamic functional connectivity with the left insula and the dorsal anterior cingulate cortex in lean compared to obese individuals. In the top figure, the difference in hypothalamic connectivity with the insula is depicted. In the bottom figure, we show the difference in hypothalamic connectivity with the dorsal anterior cingulated cortex.

Between group effects of hypothalamus functional connectivity are projected here on 2mm MNI standard space. Between group effects are thresholded at $z>2.30$, $p<0.05$. The left side of the brain in the figure corresponds with the right side in reality and vice versa. Error bars depict standard errors of the mean. Abbreviations; MNI ,T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada.

Effects of 48-hours of fasting

Fasting increased connectivity between the amygdala and left caudate nucleus in obese individuals (Figure 1B). Fasting did not affect the connectivity of the amygdala in lean subjects. However, these differences did not reach statistical significance.

Table 1 The effect of fasting compared to baseline between lean and obese subjects

Region	Hemisphere	Peak voxel coordinates (MNI) x	y	z	Z-value
Differentially affected regions by the fast between lean and obese (interaction group x time)					
Anterior cingulate gyrus	R	4	-8	42	3.97
Posterior cingulate gyrus	R	6	-18	36	3.24
Inferior frontal gyrus	L	-50	12	0	4.57
Insula	L	-42	-10	4	3.44

Only the significant results are shown. All Z-values are corrected for multiple comparisons ($p<0.05$). Abbreviations: R, right; L, left; MNI standard space, T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada.

Functional connectivity of the posterior cingulate cortex

Positive connectivity analysis at baseline

We observed PCC functional connectivity with the following regions in both groups: the posterior division of the right middle temporal gyrus, an extensive frontal area

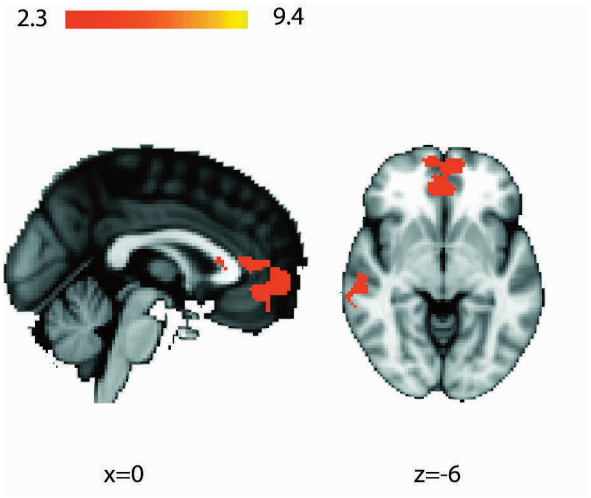


Figure 3: Differences at baseline in amygdala functional connectivity between groups: Significant functional connectivity between amygdala and mPFC was found in lean but not in obese individuals.
Here we show the difference in amygdala functional connectivity with the medial prefrontal cortex in lean compared to obese subjects at baseline. Increased connectivity was observed in the lean subjects. Results are projected on 2mm MNI standard space. Between group effects are thresholded at $z>2.3$, $p<0.05$ (cluster corrected). The left side of the brain in the figure corresponds with the right side in reality and vice versa. Abbreviations: mPFC, medial prefrontal cortex. MNI, T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada.

Table 2 Baseline differences in amygdala functional connectivity between groups

Region	Hemisphere	Peak voxel coordinates (MNI)			Z-value
		x	y	z	
Lean > Obese Baseline					
Medial prefrontal cortex	R	10	62	-8	4.27
Superior temporal Gyrus	R	56	-32	2	3.75

Only the significant results are shown. All Z-values are corrected for multiple comparisons ($p<0.05$). Abbreviations: R, right; L, left; MNI standard space, T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada.

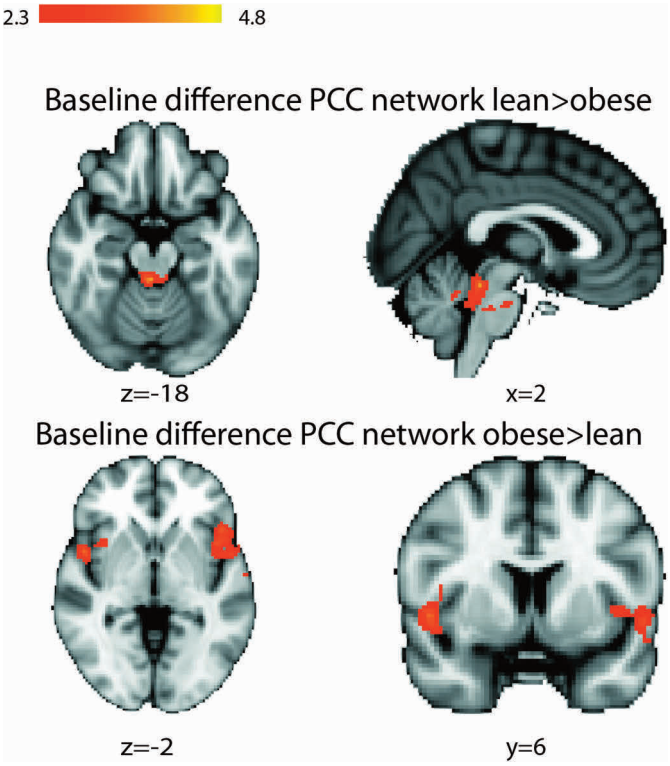


Figure 4: Differences at baseline in posterior cingulate cortex functional connectivity between groups: Stronger connectivity between the PCC and brainstem in lean subjects and stronger connectivity with the bilateral frontal opercular cortices, extending into the insula, in obese subjects. Baseline differences in posterior cingulate cortex functional connectivity between lean and obese individuals, projected on 2mm MNI standard space. Here we show that connectivity between the PCC and brainstem and between the PCC and pons were stronger in lean subjects, whereas PCC connections with left and right frontal opercular cortices were stronger in obese individuals at baseline. Between group effects are thresholded at $z>2.3$, $p<0.05$ (cluster corrected). The left side of the brain in the figure corresponds with the right side in reality and vice versa. Abbreviations: MNI standard space, T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada.

(medial and superior frontal gyruses, frontal pole), an extensive posterior cortical area (posterior cingulate cortex, precuneus cortex) and the lateral occipital cortices. Data for lean individuals at baseline is shown in supplementary Figure 3. Connectivity between the PCC and brainstem was stronger in lean subjects, whereas PCC connections with the bilateral frontal opercular cortices, extending into the insula, were stronger in obese individuals at baseline (Figure 4, Table 3).

Negative connectivity analysis at baseline

The following areas showed connectivity with the PCC in both groups: bilateral precentral gyrus, bilateral insula, bilateral occipital fusiform gyrus, and the cerebellum (Supplementary Figure 3).

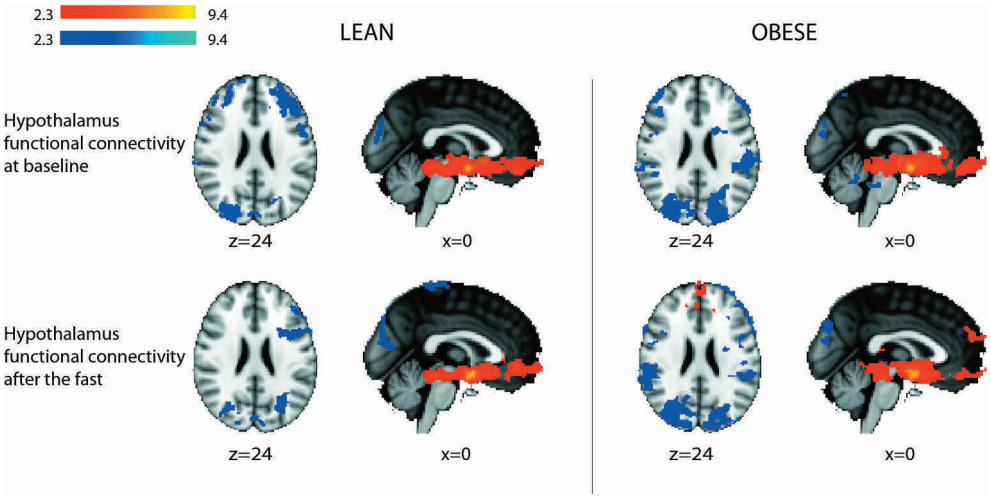
Effects of 48-hours of fasting

After fasting, PCC connectivity with the frontal pole, the posterior cingulate gyrus and the precuneus cortex was increased in the obese group (Figure 1C). Although fasting did not impact functional connections of the PCC in lean subjects, the different reactions in lean and obese individuals did not reach statistical significance.

Table 3 Baseline differences in PCC functional connectivity between groups

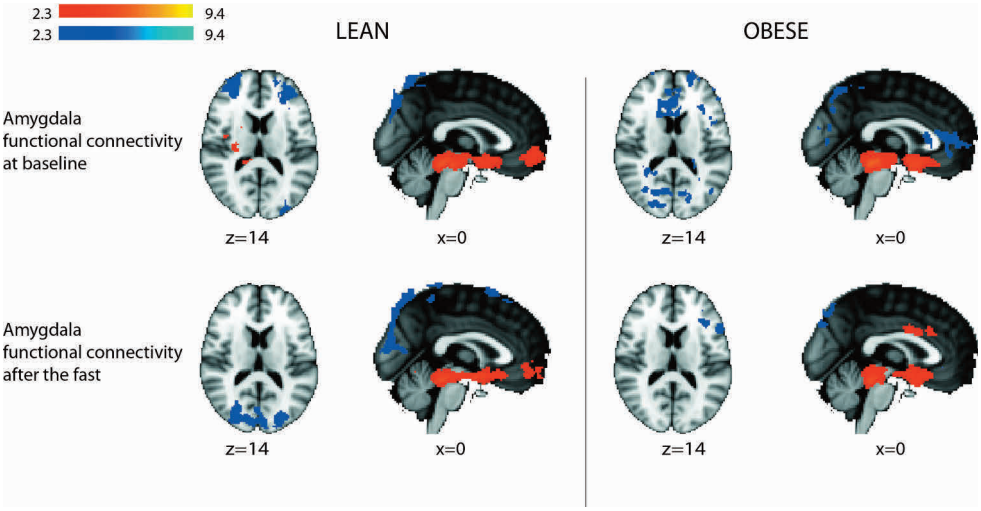
Region	Hemisphere	Peak voxel coordinates (MNI)			Z-value
		x	y	z	
Lean > Obese					
Baseline					
Brain stem	R	2	-36	-18	4.03
Pons	L	-10	-18	-28	2.74
Obese>Lean					
Baseline					
Central opercular cortex	L	-52	6	-2	2.62
Central opercular cortex	R	50	6	-2	3.53

Only the significant results are shown. All Z-values are corrected for multiple comparisons ($p<0.05$). Abbreviations: PCC, posterior cingulate cortex; R, right; L, left; MNI standard space, T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada.



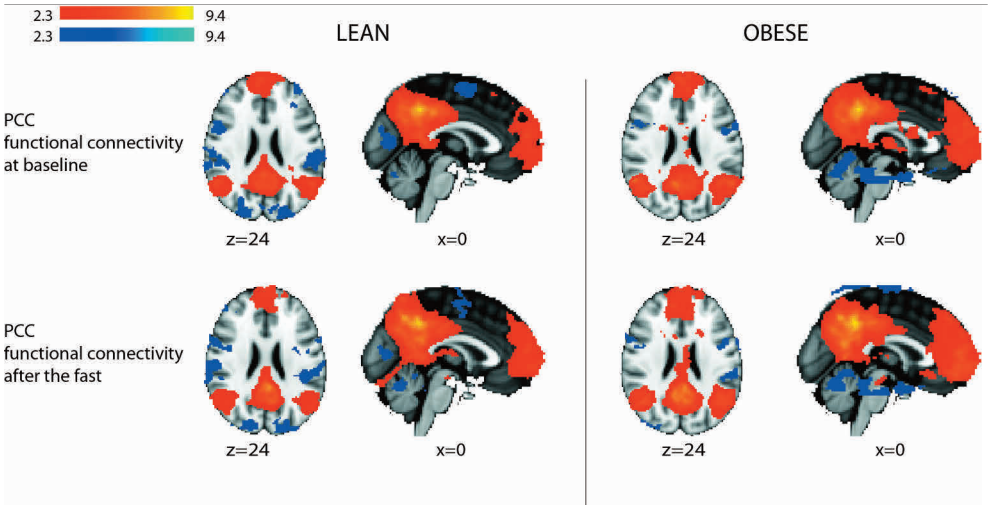
Supplementary Figure 1 Hypothalamus functional connectivity in lean and obese subjects at baseline and after the fast

Within group results of the hypothalamus functional connectivity network at baseline and after the fast. Results are projected on 2mm MNI standard space. Positive connectivity analysis results are depicted in red, negative connectivity analysis results in blue. Effects are thresholded at z>2.30, p<0.05. The left side of the brain in the figure corresponds with the right side in reality and vice versa. Abbreviations: MNI, T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada.



Supplementary Figure 2 Amygdala functional connectivity in lean and obese subjects at baseline and after the fast

Within group results of the amygdala functional connectivity network at baseline and after the fast. Results are projected on 2mm MNI standard space. Positive connectivity analysis results are depicted in red, negative connectivity analysis results in blue. Effects are thresholded at z>2.30, p<0.05. The left side of the brain in the figure corresponds with the right side in reality and vice versa. Abbreviations: MNI standard space, T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada.



Supplementary Figure 3 Posterior cingulated cortex functional connectivity in lean and obese subjects at baseline and after the fast
Within group results of the posterior cingulated cortex functional connectivity network at baseline and after the fast. Results are projected on 2mm MNI standard space. Positive connectivity analysis results are depicted in red, negative connectivity analysis results in blue. Effects are thresholded at $z>2.30$, $p<0.05$. The left side of the brain in the figure corresponds with the right side in reality and vice versa. Abbreviations: MNI standard space, T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada.

Discussion

We compared the effects of 48 hours of fasting on functional connectivity of the hypothalamus and amygdala, brain regions involved in homeostatic control of body weight and/or metabolism and salience, in lean and obese individuals. Additionally, the so called 'default mode network' was examined, as this network's resting state architecture has been studied extensively in both health and disease [33;34] and alterations in this network have been shown in obese subjects previously [20].

At baseline, the patterns of hypothalamus, amygdala and PCC functional connectivity in lean and obese individuals generally corresponded with what is known about these networks in the available literature [30;31;35-37]. There were no apparent differences in the hypothalamic functional connectivity of lean versus obese subjects at baseline. However, we did find differences in baseline connectivity of amygdala and PCC networks between lean and obese subjects. The amygdala was connected to the ventromedial prefrontal cortex (vmPFC) and the superior temporal gyrus in lean, but not in obese individuals. Additionally, in the default mode network, the connections be-

tween PCC and brainstem, and between PCC and pons were stronger in lean subjects, whereas the connections with left and right frontal opercular cortices were stronger in obese individuals.

Fasting induced changes in all three networks, primarily in the obese group. Obese, but not lean, participants demonstrated decreased hypothalamic connectivity with the left insula, and the superior temporal gyrus. In addition, increased functional connectivity between amygdala and left caudate nucleus was observed in obese, but not in lean individuals. Finally, increased connectivity between the PCC and frontal pole, posterior cingulate gyrus and precuneus cortex was observed in obese, but not in lean subjects.

Most importantly, the 48-hour fast affected hypothalamic connectivity with the left insula and dACC differently in lean compared to obese participants. The strong functional connectivity between hypothalamus and left insula observed at baseline was reduced to a large extent in obese subjects, to a comparable level observed in lean controls. The insula is heavily involved in interoceptive processing, connecting interoception (bodily awareness) to emotion and motivation [38;39]. In addition, functional connectivity between the hypothalamus and the dACC increased in lean subjects after fasting, whereas it decreased in obese individuals. The hypothalamus, dACC and insula are all part of the so called “salience network” [40]. This network is proposed to perceive internal and external cues and define the most relevant among them to adapt behavior and/or physiology accordingly [41]. Therefore, any difference between obese and normal weight subjects in the salience network’s response to fasting may indicate that the neuronal perception or processing of calorie-imbalance is different between these phenotypes. The physiological and behavioral ramifications of the observed differences remain to be established. However, it is tempting to speculate that functional connectivity specifics of the saliency network might drive weight gain in predisposed individuals.

Our baseline analysis showed that functional connectivity between the amygdala and the vmPFC was stronger in lean than in obese individuals. Both the vmPFC and amygdala are involved in the response to motivationally relevant cues such as hunger [24]. Moreover, the amygdala plays an important role in food reward [24] and stimulation of amygdala neurons induces hyperphagia and weight-gain in rodents [42;43]. Notably, the

response of the amygdala and vmPFC to food cues is increased in obese women [44]. It is conceivable that the lack of functional connection between these nuclei in obese individuals hampers mutual control of activity which might affect reward sensations.

Our data also show that PCC functional connectivity with the brainstem is stronger in lean compared to obese participants at baseline. Neurons of the solitary tract, located in the brainstem, are involved in satiety [45] whereas the pons plays a role in tasting [46]. We also show stronger PCC functional connectivity with the left and right frontal opercular cortices in obese compared to lean individuals at baseline. The opercular cortices cover the insula and the frontal operculum is involved in gustation [47]. The physiological function of the default mode network includes cognition and tasks with an internal focus [48]. With respect to our data, it is tempting to speculate that the changes associated with obesity reflect alterations in the control of satiety and possibly taste and/or palatability in these subjects, given the well known functional properties of brainstem and opercula [46].

Our baseline (overnight fasted) results differ from those of previous studies with respect to differences between lean and obese subjects. Importantly, these previous studies mainly focused on the default-mode network. For example, it was demonstrated that the DMN of obese individuals is characterized by increased connectivity with the precuneus and decreased connectivity with the ACC [20] compared to lean subjects. In addition, it has been shown that obese individuals that lost considerable weight have enhanced activity in regions within the DMN: the PCC and the left lateral inferior parietal cortex [7]. The differences with our results might be explained by the analytical methods (independent component analysis versus our seed-based approach) [20], the inclusion of weight-reduced obese subjects [7] and differences in scanning procedure (scans acquired during picture viewing versus our “resting” paradigm) [7].

It is important to discuss some limitations. First, negative correlations might be artificially induced by global signal regression[51]. Therefore, no conclusions can be drawn from the observed sign of the connectivities (positive or negative). As such, differences in connectivity are real but no conclusions about the direction of connectivity can be drawn.

Furthermore, hypothalamic and amygdala functional connectivity may be influenced by noise from arteries surrounding these areas [49]. To reduce this effect, we used the global signal as confound regressor in our analysis, because this signal is largely re-

lated to physiological noise in fMRI data [50]. Due to the relatively low spatial resolution, it is unfortunately impossible to distinguish the different hypothalamic regions that have distinct homeostatic properties. Furthermore, quantification of hunger and other relevant behavioral and cognitive parameters during fasting in future studies would allow correlation of these features with changes in functional connectivity.

In conclusion, we studied resting-state functional connectivity of three brain regions that are related to the behavioral and metabolic control of energy balance in humans. We describe changes in these networks both at baseline and in response to fasting between lean and obese individuals. Although the ramifications of these changes in network connectivity remain to be established, our results are in keeping with the notion that food reward and nutrient deprivation are differentially perceived and /or processed by obese individuals.

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