

## Associations between ghrelin and leptin and neural food cue reactivity in a fasted and sated state



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### ABSTRACT

Food cue exposure can trigger eating. Food cue reactivity (FCR) is a conditioned response to food cues and includes physiological responses and activation of reward-related brain areas. FCR can be affected by hunger and weight status. The appetite-regulating hormones ghrelin and leptin play a pivotal role in homeostatic as well as hedonic eating. We examined the association between ghrelin and leptin levels and neural FCR in the fasted and sated state and the association between meal-induced changes in ghrelin and neural FCR, and in how far these associations are related to BMI and HOMA-IR. Data from 109 participants from three European centers (age  $50 \pm 18$  y, BMI  $27 \pm 5$  kg/m<sup>2</sup>) who performed a food viewing task during fMRI after an overnight fast and after a standardized meal were analyzed. Blood samples were drawn prior to the viewing task in which high-caloric, low-caloric and non-food images were shown. Fasting ghrelin was positively associated with neural FCR in the inferior and superior occipital gyrus in the fasted state. This was partly attributable to BMI and HOMA-IR. These brain regions are involved in visual attention, suggesting that individuals with higher fasting ghrelin have heightened attention to food cues. Leptin was positively associated with high calorie FCR in the medial prefrontal cortex (PFC) in the fasted state and to neural FCR in the left supramarginal gyrus in the fasted versus sated state, when correcting for BMI and HOMA-IR, respectively. This PFC region is involved in assessing anticipated reward value, suggesting that for individuals with higher leptin levels high-caloric foods are more salient than low-caloric foods, but foods in general are not more salient than non-foods. There were no associations between ghrelin and leptin and neural FCR in the sated state, nor between meal-induced changes in ghrelin and neural FCR. In conclusion, we show modest associations between ghrelin and leptin and neural FCR in a relatively large sample of European adults with a broad age and BMI range. Our findings indicate that people with higher leptin levels for their weight status and people with higher ghrelin levels may be more attracted to high caloric foods when hungry. The results of the present study form a foundation for future studies to test whether food intake and (changes in) weight status can be predicted by the association between (mainly fasting) ghrelin and leptin levels and neural FCR.

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## 1. Introduction

Food cues are omnipresent. The responsiveness of individuals to food cues varies. Such food cue reactivity (FCR) is a conditioned anticipatory response to food cues and includes neural activation in brain areas related to food reward and subsequent physiological responses (Boswell and Kober, 2016). FCR is affected by many factors, including hunger and weight status (Charbonnier et al., 2018) and can elicit both homeostatic and hedonic eating (Cornier et al., 2007; Martin et al., 2010). Hedonic eating, also referred to as eating in the absence of hunger, facilitates overconsumption which can result in weight gain and obesity (Jansen et al., 2011; Tetley et al., 2009).

Appetite-regulating hormones such as ghrelin and leptin play an important role in food intake and their fasting levels differ between individuals with normal weight and individuals with overweight (Caro et al., 1996; Myers et al., 2010; Shiiya et al., 2002; Tschöp et al., 2001). Ghrelin is synthesized in the stomach and leptin by adipocytes. Both hormones act on receptors in the central brainstem and hypothalamus, which are key areas involved in the regulation of food intake (Klok et al., 2007). The melanocortin pathway plays a central role in the hypothalamic control of food intake and mediates the effect of ghrelin and leptin on the regulation of food intake. In addition, leptin and ghrelin modulate feeding behavior in the central brainstem by regulating the responsiveness towards satiety signals (for a review: (Shan and Yeo, 2011)). In individuals with normal weight, fasting ghrelin levels are relatively high, rising before an expected meal time and showing a postprandial drop after eating, suggesting an important role in meal initiation (Cummings et al., 2001; Tschöp et al., 2001). Individuals with overweight have lower fasting ghrelin compared to individuals with normal weight, and smaller postprandial decreases (Meyer-Gerspach et al., 2014; Yang et al., 2009). Based on this, Yang et al. (2009) suggest that individuals with overweight might experience lower levels of satiety after a meal, which might promote overconsumption, e.g. by snacking in between meals. This idea is supported by the finding that individuals with overweight consume more in an ad libitum buffet meal than individuals with normal weight after a low ghrelin dose, while intake is similar after a high ghrelin dose (Druce et al., 2005). This suggests that individuals with overweight are more sensitive to the appetite stimulating effects of ghrelin, which may promote overconsumption. Circulating leptin levels scale with adipose tissue mass; leptin provides the brain with information about the energy stores in the body (Considine et al., 1996). Normally, when leptin levels are high or rise, leptin suppresses appetite and increases energy expenditure in order to maintain body weight (Myers et al., 2010). However, in individuals with obesity, high leptin does not lead to such regulation of energy balance, i.e. they are effectively leptin insensitive. This so-called leptin resistance might be a consequence of overconsumption and perpetuate an elevated defense of adiposity levels (Caro et al., 1996; Kolarzinski et al., 1996; Myers et al., 2010; Sahu, 2002).

Recent evidence indicates that ghrelin and leptin not only play a pivotal role in homeostatic eating, but also in hedonic eating (Egecioglu et al., 2010; Grosshans et al., 2012; Malik et al., 2008; Perello et al., 2010; Rosenbaum et al., 2008; Skibicka et al., 2012), which can be triggered by food cues. Brain areas involved in visual FCR include visual areas involved in attention (i.e., middle occipital gyrus, fusiform gyrus, superior parietal lobule), reward-related areas (i.e., dorsal striatum and hypothalamus/ventral striatum), limbic areas (i.e., amygdala and thalamus), and prefrontal areas related to the incentive value of foods (i.e., lateral OFC and mPFC) (Brooks et al., 2013; Cornier et al., 2013; van der Laan, Smeets, de Ridder, and Viergever, 2011; van Meer, van der Laan, Adan, Viergever, and Smeets, 2015). In individuals with normal weight intravenous ghrelin administration increases neural FCR to food images in brain regions related to encoding the incentive value of food cues, such as the amygdala, orbitofrontal cortex, anterior insula, and striatum (Malik et al., 2008). Moreover, Goldstone et al. (2014), found that in individuals with nor-

mal weight both fasting and subcutaneously injected ghrelin in a fed state increased orbitofrontal cortex activation in response to high-energy food viewing, hippocampus activation in response to both high- and low-energy food viewing, and the appeal of high-energy foods. Kroemer et al. (2013) showed that higher fasting ghrelin is associated with greater neural FCR to palatable food images in a bilateral network of visual processing-, reward- and taste-related regions in normal weight adults. In addition to these human studies, many animal studies support the idea that ghrelin acts on mesolimbic reward and motivational pathways to increase FCR (Abizaid et al., 2006; Carlini et al., 2004; Naleid et al., 2005). According to these studies, current knowledge about the relationship between ghrelin levels and FCR is limited to samples including normal weight individuals. To what extent weight status influences the relationship between ghrelin and leptin and FCR has yet to be investigated. Furthermore, many studies state that people with higher BMI have enhanced FCR, however, a recent meta-analysis of Morys et al. (2020) shows limited evidence for this. One explanation might be that BMI is not an accurate proxy for underlying metabolic alterations in people with a higher weight status. It is possible that impaired insulin sensitivity, as e.g. assessed with HOMA-IR, is more appropriate than BMI in this case. Therefore, we included both BMI and HOMA-IR as covariates in the current study

Animal studies established that leptin binds to receptors on dopaminergic midbrain neurons which project to cortical, limbic, and striatal brain areas that are implicated in motivational and reward responses to palatable food cues (Figlewicz, 2016; Hommel et al., 2006). So far, there is some evidence that supports a link between leptin and FCR in reward related brain regions in humans, however, the direction of this effect remains equivocal because results are mixed (Farooqi et al., 2007; Grosshans et al., 2012; Jastreboff et al., 2014; Rosenbaum et al., 2008; Roth et al., 2018; Simon et al., 2014).

In addition to ghrelin and leptin, evidence from rodent studies has shown that additional underlying pathways contribute to differences in FCR (Livneh et al., 2017, 2020). For example, hypothalamic melanocortin pathways gate insular cortex responses to food cues and increasing evidence shows that food cues might even drive neural activity to simulate a future satiety state (Livneh et al., 2017, 2020). However, there are remarkable discrepancies between human and animal research regarding the role of ghrelin and leptin on FCR as well as on the impact of weight status on these relationships. This may be due to several factors including the different levels at which these mechanisms are studied. While human studies are largely limited to correlational analyses, animal studies more often focus on underlying mechanisms. In addition, findings in humans may be more prone to variability present in the different samples studied, which is no issue in animal research.

A key factor that might affect the relationship between appetite-regulating hormones and FCR is satiety. Most neuroimaging research on FCR is performed in a more or less fasted state; only few studies include both a fasted and sated condition. Such studies found that individuals with normal weight have increased neural FCR in response to food cues in a fasted compared to a sated state (Cornier et al., 2007; Frank et al., 2013; Führer et al., 2008; Holsen et al., 2005; Huerta et al., 2014; LaBar et al., 2001; Mohanty et al., 2008). Very few studies have examined the effect of satiety on the relationship between appetite-regulating hormones and neural FCR. Roth et al. (2018) showed that a greater reduction in ghrelin levels after a meal was associated with a greater reduction of neural FCR to high-caloric food cues in normal weight, but not in obese children. In line with this, Sun et al. (2014) demonstrated that individuals with a larger drop in ghrelin after a meal show a greater decline in their neural response to tasting milkshake, i.e. food receipt, in the midbrain, striatum, amygdala and medial OFC. Jastreboff et al. (2014) report positive associations between serum leptin and neural FCR to images of high-caloric foods in the hypothalamus ( $r = 0.45$ ), striatum ( $r = 0.37$ ), amygdala ( $r = 0.46$ ), hippocampus/parahippocampus ( $r = 0.40$ ), angular gyrus ( $r = 0.62$ ), and anterior

and posterior insula ( $r = 0.59$ ) in a group of lean and obese adolescents (total  $n = 40$ ) scanned 2 h after a meal.

In the present study we examined the associations between ghrelin and leptin levels and neural FCR in the fasted and sated state as well as the association between meal-induced changes in ghrelin and neural FCR, and whether these associations are associated with BMI and HOMA-IR. Based on prior studies, fasting ghrelin was hypothesized to be positively associated with FCR in the fasted state (Abizaïd et al., 2006; Kroemer et al., 2013; Malik et al., 2008). Furthermore, it was hypothesized that a greater decline in ghrelin after eating would be related to a greater decline in neural FCR (Roth et al., 2018; Sun et al., 2015, 2014). Leptin levels were hypothesized to be positively related to neural FCR (Farooqi et al., 2007; Grosshans et al., 2012; Rosenbaum et al., 2008; Simon et al., 2014). BMI was not expected to have a significant role in the relation between ghrelin and FCR in the fasted state, but in the sated state a higher BMI was hypothesized to affect the association between ghrelin levels and FCR. This was based on the idea that individuals with a higher BMI might have a smaller decrease of ghrelin after satiation (Yang et al., 2009). Lastly, we hypothesized that BMI and HOMA-IR would affect the relationship between leptin levels and FCR, because individuals with a higher BMI and HOMA-IR might be less leptin sensitive, resulting in increased neural FCR.

## 2. Material and methods

### 2.1. Participants

We included healthy normal weight (BMI 20–25 kg/m<sup>2</sup>) or overweight/obese (BMI  $\geq 27.5$  kg/m<sup>2</sup>) adults. Participants were recruited in The Netherlands, Scotland and Greece. Additional criteria were: right-handed, non-smoking, a stable weight (did not gain or lose  $> 5$  kg in the past 6 months), no use of medication (except aspirin/paracetamol, oral contraceptives, anticoagulants and cholesterol medication) and no current alcohol consumption  $> 28$  units per week. Furthermore, MRI exclusion criteria (e.g. claustrophobia, pregnancy and metal implants in the body) and criteria that might influence the response to food cues (food allergies, special diets, eating disorders, gastrointestinal disorders or metabolic or endocrine disease, highly restraint eating scores on the Dutch Eating Behavior Questionnaire (Van Strien, Frijters, Bergers, and Defares, 1986)) were used.  $n = 142$  eligible participants enrolled in the study. Two participants were excluded because of poor MRI data quality due to excessive head movement. Seven participants were excluded because they did not have a successful food viewing task run for both conditions, 21 participants were excluded because of poor fMRI data quality (see below), and another three were excluded due to missing hormone data. The resulting sample included in the analyses consisted of  $n = 109$  participants, from which a subset of  $n = 88$  was included in the analyses including ghrelin (Table 1).

### 2.2. Study procedures

The study consisted of two morning MRI scan sessions. On both days, participants came in after an overnight fast of at least 10 h. Anthropomorphic measures (age, length, weight, and % body fat and bioimpedance (measured with a TANITA BC-418-MA BIA machine)) were collected at the start of the first session. Mood and menstrual cycle phase, if applicable, were reported at the start of both sessions. During the sated session participants were scanned after the consumption of an individually determined amount of liquid breakfast consisting of a commercially available vanilla-flavored whey protein shake (XXL Nutrition, The Netherlands, nutritional value per 100 ml protein shake prepared with full-fat milk: 110/461 kcal/kJ energy, 3.9 g fats (amount saturated: 2.4 g), 14.1 g protein, 4.9 g carbohydrates). The amount provided contained 25% of the estimated total daily energy requirement, which was obtained by multiplying the basic metabolic rate (BMR), as

**Table 1**  
Participant characteristics ( $n = 109$ ).

Characteristic	Mean $\pm$ SD
Country ratio (NL:SCT:GR), % <sup>2</sup>	65:22:22
Gender (male:female)	53:56
Age, y <sup>1</sup>	49.56 $\pm$ 18.05
BMI, kg/m <sup>2</sup> <sup>1</sup>	26.86 $\pm$ 4.70
Interscan interval (days) <sup>1</sup>	8.96 $\pm$ 3.64
First visit (fasted:sated)	62:47
Amount liquid breakfast consumed in sated condition, ml <sup>1</sup>	519 $\pm$ 94
Ghrelin prior to scan ( $t = \pm 55$ ) in fasted condition, pg/ml <sup>1,3,4</sup>	1228 $\pm$ 688
Ghrelin prior to scan ( $t = \pm 55$ ) in sated condition, pg/ml <sup>1,3,4</sup>	923 $\pm$ 349
Meal induced $\Delta$ Ghrelin ( $t = \pm 55$ minus $t = 0$ ), pg/ml <sup>1,3</sup>	-242 $\pm$ 204
Leptin, pg/ml <sup>1</sup>	17.71 $\pm$ 17.13
Fasting insulin, mg/dl <sup>5</sup>	9.87 $\pm$ 6.07
Fasting glucose, $\mu$ IU/ml <sup>5</sup>	100.71 $\pm$ 9.90
HOMA-IR <sup>5,6</sup>	2.54 $\pm$ 1.69

<sup>1</sup> Mean  $\pm$  SD.

<sup>2</sup> NL: The Netherlands, SCT: Scotland, GR: Greece.

<sup>3</sup>  $n = 88$ .

<sup>4</sup> Significantly different between fasted and sated condition (paired  $t$ -test,  $t(87) = 5.2$ ;  $p < 0.001$ ).

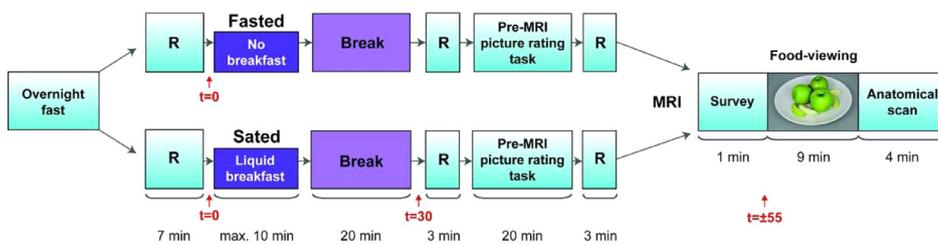
<sup>5</sup> Fasting insulin, fasting glucose and HOMA-IR values were averaged over the fasted and sated session ( $t = 0$ ).

<sup>6</sup> HOMA-IR was calculated using the following equation: (fasting insulin \* fasting glucose)/405.

calculated with the Schofield equation, with 1.4. The Schofield equation estimates BMR based on age, gender and weight (Schofield, 1984). The time between the two scan sessions was 1–2 weeks (Table 1). The conditions (i.e. fasted or sated state) were counterbalanced at each site. On both study days, participants filled out several questionnaires and executed a computerized food image rating task before the scan session (Fig. 1). During this task, participants rated 133 standardized food images (Charbonnier et al., 2016) on liking, perceived caloric content and perceived healthiness on 9-point Likert scales. On the sated day, the food image rating task was executed 20 min after liquid breakfast consumption. The liquid breakfast was consumed between 7:30 and 11:00 a.m. Participants entered the scanner approximately 1 hour after this. Hunger and fullness were measured at baseline (after an overnight fast and before feeding), prior to the pre-fMRI food image rating task, and prior to the scan (after the liquid breakfast in the sated condition) in each session on 9-point Likert scales. For hormone analyses blood was collected through a cannula at the start of both morning sessions ( $t = 0$ ), after ingestion to show the (first) effects of food entering the duodenum and intestines ( $t = 30$ ) and prior to the start of the scan session ( $t = \pm 55$ ; approximately 55 min after  $t = 0$ ). Ghrelin, insulin and glucose were measured at all timepoints ( $t = 0$ ,  $t = 30$  and  $t = \pm 55$ ), but insulin and glucose levels at  $t = 0$  and ghrelin levels at  $t = 0$  and  $t = \pm 55$  were included in the current study. Leptin was only measured at  $t = 0$ . Subsequently, participants underwent a 38-min MRI scan session consisting of four functional MRI runs during which they performed a food image viewing task, a food choice task (two runs) and a monetary reward task. The study was approved by the UMC Utrecht Medical Ethical Committee and registered in the Dutch trial registry ([www.trialregister.nl](http://www.trialregister.nl); NL3512/NTR3644). Ethical approval in Aberdeen was granted by the National Health Service North of Scotland Research Ethics Service and ethical approval in Athens was granted by the Bioethics Committee of Harokopio University and the Greek Ministry of Education ([www.clinicaltrials.gov](http://www.clinicaltrials.gov); NCT01597024). All participants provided written informed consent. The results of the food image viewing task in combination with the hormones ghrelin and leptin are the focus of this paper.

### 2.3. Hormone analyses

All blood samples were placed on ice immediately after venipuncture and centrifuged within 15 min after the blood draw at 3500 g at 4 °C



**Fig. 1.** Schematic overview of the study procedures. Blood was collected at baseline ( $t = 0$ ),  $t = 30$  (only sated condition), and  $t = \pm 55$  min (prior to food viewing task). The 20-min break was optional in the fasted condition. R, Ratings: hunger, thirst, and fullness ratings at the start of the test day, prior to the pre-fMRI image rating task, and prior to the scan session. After the anatomical scan a food choice task and a monetary reward task were performed (not shown), in addition to the food-viewing task, which was the focus of this manuscript.

for ten minutes. After centrifugation, samples were aliquoted and frozen using dry ice within 5 min. Samples were stored at  $-80^{\circ}\text{C}$  prior to duplicate analysis in batches. Leptin samples were analysed in the Department of Clinical Biochemistry in Addenbrooke's Hospital in Cambridge, United Kingdom, in a laboratory accredited for diagnostic clinical use under Clinical Pathology Accreditation (CPA) and ISO-15,189. The leptin blood samples of 2.7 mL per time point were taken into S-Monovette plasma Lithium Heparin tubes (LiHep; 2.7 mL,  $75 \times 13$  mm). Leptin analyses were performed using an in-house two-site DELFIA assay that uses a monoclonal capture antibody and a polyclonal detection antibody with a fluorescent detection using europium-labelled streptavidin. The antibodies and standards were sourced from R&D Systems (R&D Systems Europe, Abingdon, UK). This assay has a lower limit of detection of 0.1 ng/mL and intra-assay CVs of 3.9–7.1%.

Ghrelin samples were analysed at the laboratory of Professor J. Holst at the University of Copenhagen, Panum Institute, Denmark. The ghrelin blood samples of 4.9 mL per time point were taken into S-Monovette hematology tubes (Potassium K-EDTA; 4.9 mL,  $90 \times 13$  mm). Total ghrelin was measured using a commercially available immunological assay (ghrelin Total RIA Kit, catalog no. GHRT-89HK; Linco Research, St Charles, MO, USA) used previously by [Dynesen et al. \(2008\)](#). The lowest level of ghrelin detectable using this assay was 93 pg/mL. The limit of linearity for this assay was 6000 pg/mL. All samples were read using a gamma counter. Typical inter-assay coefficients of variation (CVs) for this method range from 14.7 to 17.8%, and intra-assay CVs between 3.3–10%. All quality controls were within acceptable limits. The samples included substantial variation in ghrelin and leptin levels (see Supplement 3). This is commonly observed, also in other studies that used similar assays ([Dirksen et al., 2013](#); [Martins et al., 2010](#); [Veedefald et al., 2016](#)).

Analyses of plasma glucose and insulin plasma were conducted at the University of Aberdeen, Rowett Institute, Technical Services department. Glucose concentrations were measured using a hexokinase method on a Dimension® clinical chemistry analyzer (Siemens Healthcare GmbH, Erlangen, Germany) with CVs of <2% within the reference range. Insulin was measured using a Liaison® XL automated immunoassay analyzer (DiaSorin, Italy) with a chemiluminescence immunoassay, which had a range of 20–3470 pmol/L and intra-assay CVs of 5.0–6.0% across the analytical range (see also [Crabtree et al. \(2020\)](#)). The average levels of fasting glucose and insulin on the fasted and sated visits ( $t = 0$ ) were used to estimate hepatic insulin sensitivity based on the homeostatic model assessment ( $\text{HOMA-IR} = (\text{fasting insulin} * \text{fasting glucose})/405$ ; [Matthews et al. \(1985\)](#)).

#### 2.4. Food image viewing fMRI task

In the food image viewing task, participants watched 18 blocks of 7 images each: 12 blocks with foods, of which 6 blocks with high- and 6 with low-caloric food images, and 6 blocks with non-food images. The images came from a standardized image set ([Charbonnier et al., 2016](#)) and were pretested on recognizability and liking in the three countries. Each block was followed by an inter-block interval (i.e. black screen with crosshair) with a duration of 3.5–4 s. In total, participants viewed

126 images over 454 s ( $\sim 8$  min). They were given the following task instruction: “In the next task you will see food and non-food products. Please look at the images and pay close attention, since at the end of the MRI session you will be asked a couple of questions regarding the images shown during this task.” After the MRI session, participants were shown 10 images for which they had to indicate whether they had seen them during the task. See [Charbonnier et al. \(2018\)](#) for more details.

#### 2.5. Image acquisition and processing

In all countries imaging was performed on a Philips Achieva 3.0 T MRI scanner (Philips Healthcare, Best, NL). Functional images were obtained with an 8-channel SENSE head-coil using a 2-D echo planar imaging (EPI) sequence with the following parameters: voxel size 4 mm isotropic; repetition time (TR) = 1400 ms; echo time (TE) = 23 ms; flip angle =  $70^{\circ}$ ; 30 axial slices; SENSE-factor = 2.4 (anterior-posterior). 316 functional images were acquired. A high-resolution anatomical image was obtained with the use of a  $T_1$ -weighted 3D-turbo-field-echo sequence with the following parameters: TR/TE = 8.4/3.8 ms; flip angle =  $8^{\circ}$ ; voxel size =  $1 \times 1 \times 1$  mm; FOV =  $175 \times 288 \times 288$  mm; 175 sagittal slices; total scan duration 4.7 min.

#### 2.6. Data analyses

##### 2.6.1. Behavioral and hormone analyses

Behavioral and hormone data were analyzed using SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA). Hunger and fullness ratings collected from the Likert scale were analyzed with paired t-tests between the fasted and sated condition at the start of the day (baseline) and prior to the scan session. Differences in ghrelin levels between  $t = 0$  and  $t = \pm 55$  (i.e. prior to and after the intake of the liquid breakfast) in the sated condition were analyzed with paired t-tests.

##### 2.6.2. Image preprocessing

Image preprocessing and analyses were carried out with SPM12 (Wellcome Department of Imaging Neuroscience, London, United Kingdom, version 7487), run with MATLAB R2018b (The Mathworks Inc, Natick, MA). After slice timing correction and realignment, the structural scan was registered to the mean functional scan. Next, the structural scan was segmented using unified segmentation, and normalization parameters were estimated. A study-specific anatomical template was created using DARTEL ([Ashburner, 2007](#)), and DARTEL was used to normalize this template and the functional scans to MNI space. The data were then smoothed with an isotropic 8-mm full width at half maximum Gaussian kernel. The ArtRepair toolbox (<http://cibsr.stanford.edu/tools/ArtRepair/ArtRepair.html>) was used to detect and repair anomalously noisy volumes. Volumes that moved more than 1 mm/TR were repaired. None of the datasets was excluded from the analyses because of too many volumes that had to be repaired (threshold set at >30% replacements).

##### 2.6.3. Subject and group level analyses

Statistical maps were calculated for each dataset by fitting a boxcar function to the fMRI time series, convolved with the canonical hemody-

dynamic response function. Data were high-pass filtered with a cutoff of 128 s. The following conditions were modelled versus implicit baseline (crosshair viewing): high-caloric food image viewing, low-caloric food image viewing and non-food image viewing. First, the average brain activation across these three conditions was assessed (image viewing versus crosshair viewing). Since visual inspection of these data showed limited or even absent brain activation during image viewing for several datasets we decided to employ an additional data quality criterion based on overall visual cortex activation: the number of voxels active at  $p < 0.001$  in a combined visual processing region of interest (ROI) composed of four anatomical masks covering the fusiform gyrus, lingual gyrus, occipital gyrus and extended visual cortex was calculated for each dataset. Participants with the 10% lowest number of active voxels in this visual processing ROI for one or both study days were excluded from the analysis ( $n = 21$  excluded with on average  $291 \pm 290$  active ROI voxels; the remaining  $n = 109$  had  $765 \pm 378$  active ROI voxels). Subsequently, the average brain activation for food minus non-food image viewing and for high- minus low-caloric food image viewing was calculated across conditions (mean fasted and sated) and between conditions (fasted – sated) for each participant with the use of the SPM image calculator. These beta images were then submitted to one-sample t-tests in combination with the nuisance covariates site (NL/SCT/GR, coded with two dummy variables), and both covariates of interest (ghrelin and leptin). All analyses were also done with BMI as an added covariate. To correct for differences in the amount of breakfast consumed (delta) ghrelin levels were divided by the amount of liquid breakfast in mL for all analyses in the sated state and those using the difference in ghrelin between the fasted ( $t = 0$ ) and the sated ( $t = \pm 55$ ) ghrelin concentrations in the sated state.

The statistical parametric maps generated were masked with a gray matter mask based on the DARTEL group template brain. Cluster extent thresholds were determined for each analysis at  $p = 0.001$  with the SPM cluster size threshold tool available at [https://github.com/CyclotronResearchCentre/SPM\\_ClusterSizeThreshold](https://github.com/CyclotronResearchCentre/SPM_ClusterSizeThreshold) to determine the minimum cluster size needed for an FWE-corrected  $p = 0.05$  across the analysis volume. In addition, we report results at a secondary arbitrary threshold of  $p = 0.001$ ,  $k > 19$  contiguous voxels to allow for meta-analysis. Such a threshold inflates the risk of false positives, but it is more stringent than the arbitrary threshold used by many studies (Eklund et al., 2016) and much more stringent than recommended by Lieberman and Cunningham (2009). For selected significant clusters average parameter estimates were extracted for each participant with the use of the MarsBar toolbox (<http://marsbar.sourceforge.net/>) for visualization of effects.

In order to assess the effect of hunger state on neural FCR as well as possible interactions between ghrelin and leptin and hunger state on neural FCR we used a functional region of interest (fROI) approach, which combines a priori anatomical areas of interest with a functional criterion based on an overall minimum level of responsiveness to the food-viewing task (see e.g. Griffioen-Roose et al. (2014) and Mehta et al. (2012)). Anatomical ROIs were the following brain areas implicated in food cue processing (Dagher, 2012, 2017), areas in which fasting ghrelin correlated with neural FCR (Kroemer et al., 2013) and areas where ghrelin administration increases neural FCR (Malik et al., 2008): hypothalamus, amygdala, hippocampus, insula, striatum, brainstem including the dopaminergic midbrain (ventral tegmental area and substantia nigra), prefrontal cortex (orbitofrontal cortex including the ventromedial prefrontal cortex, anterior cingulate cortex, dorsolateral prefrontal cortex and a number of visual areas (pulvinar, fusiform gyrus, lingual gyrus, middle occipital gyrus, superior occipital gyrus). We combined all available masks from the WFU Pickatlas toolbox (Maldjian et al. (2003), version 3.0.5) with prefrontal cortex masks defined according to Gozzi et al. (2009) into one ROI mask. To obtain functional ROIs we used this mask in one-sample t-tests on the  $F > NF$  and  $HC > LC$  contrast images from the fasted state in combination with a threshold of  $p = 0.01$  (uncorrected),  $k \geq 8$ . The resulting fROIs were

transformed to MarsBaR toolbox (version 0.44) ROIs and average parameter estimates were extracted for each fROI for both the fasted and the sated state. We used the fasted state for fROI definition because in that state the greatest neural FCR might be expected. Next, we ran generalized linear mixed models in R (version 3.6.1; lme4 package) for each fROI in the  $F > NF$  and  $HC > LC$  contrast with ghrelin and leptin and hunger state as covariates in all models.

### 3. Results

#### 3.1. Appetite ratings

Baseline hunger and fullness ratings did not differ significantly between the conditions (Table 2). As expected, hunger ratings prior to the scan were significantly lower on the sated compared to the fasted day, while fullness ratings were significantly higher on the sated compared to the fasted day. Liking ratings were significantly lower in the sated versus fasted condition for both the high and low calorie food images.

#### 3.2. Hormone data

See Table 1. Ghrelin levels prior to the scan in both the fasted ( $1228 \pm 688$ ) and sated condition ( $923 \pm 349$ ), and the difference between ghrelin levels before and after intake of the liquid breakfast in the sated state ( $-242 \pm 204$ ) were available for 88 participants. Ghrelin levels in the fasted state were significantly higher than those in the sated state ( $p < 0.001$ ). Leptin levels were available for all 109 participants ( $17.7 \pm 17.1$ ). Fasted and sated ghrelin levels prior to the scan session ( $t = \pm 55$ ) were negatively correlated with BMI ( $r = -0.22$ ,  $p = 0.044$  and  $r = -0.37$ ,  $p < 0.001$ , respectively). Leptin ( $t = 0$ ) was positively correlated with BMI ( $r = 0.62$ ,  $p < 0.001$ ).

#### 3.3. Food versus non-food and high- versus low-caloric food image viewing

First, brain responses were assessed for food versus non-food image viewing. In the fasted state there was significant activation in the right calcarine sulcus, the middle part of the right cingulate cortex (mCC), and the bilateral angular gyri. In the sated state there was significant neural activation in the right calcarine sulcus, the left posterior cingulate cortex (pCC), the right middle temporal gyrus, the medial part of the left superior frontal gyrus, the left thalamus and the left angular gyrus. No significant differences in brain activation were found between the fasted and sated state (Supplement 1).

For high- versus low-caloric food image viewing there was no significant brain activation in both the fasted and the sated state. Except for greater activation in the right pallidum (MNI (20, 4, 0),  $Z = 4.27$ , Supplement 2) in the fasted versus the sated state there were no significant differences in brain activation between high- versus low-caloric food image viewing when comparing the hunger states.

#### 3.4. Whole brain results

##### 3.4.1. Correlations between ghrelin and fcr

In the fasted state there were positive correlations between fasting ghrelin and neural FCR in the left inferior occipital gyrus and right superior occipital gyrus (Table 3, Fig. 2). However, although physiologically plausible, these correlations were outlier driven and were no longer significant after removal of the two outlier values (both  $> 2$  SD above the mean). Additionally, adding BMI or HOMA-IR to the model diminished the cluster sizes below the extent threshold of  $k = 67$ . Thus, this association is partly explained by BMI and HOMA-IR, although there is residual variation explained by ghrelin. BMI and HOMA-IR were not significantly associated with FCR. No correlations were found between ghrelin and FCR in the sated state, nor between pre- and post-prandial difference in ghrelin levels ( $t = \pm 55$  minus  $t = 0$ ) in the sated state and FCR in the sated state or the fasted versus sated state.

**Table 2**  
Hunger, fullness and liking ratings in the fasted and sated condition (mean  $\pm$  SD)<sup>1</sup>.

	Fasted condition	Sated condition	Fasted vs. sated condition <sup>2</sup>	
			t	p
<b>Hunger ratings</b>				
Baseline	5.31 $\pm$ 2.20 <sup>a</sup>	4.99 $\pm$ 2.07 <sup>a</sup>	1.79	0.077
20 min after consumption	N.A.	2.65 $\pm$ 1.75 <sup>a</sup>		
Prior to scan	5.89 $\pm$ 2.08 <sup>b</sup>	3.05 $\pm$ 1.80 <sup>c</sup>	12.23	<0.001
<b>Fullness ratings</b>				
Baseline	3.17 $\pm$ 1.81 <sup>a</sup>	3.22 $\pm$ 1.76 <sup>a</sup>	-0.20	0.846
20 min after consumption	N.A.	6.52 $\pm$ 1.82 <sup>a</sup>		
Prior to scan	2.76 $\pm$ 1.57 <sup>b</sup>	5.71 $\pm$ 2.23 <sup>c</sup>	-11.05	<0.001
<b>Liking ratings</b>				
High calorie food images	4.63 $\pm$ 1.90 <sup>d</sup>	3.68 $\pm$ 1.76	5.79	<0.001
Low calorie food images	5.92 $\pm$ 1.63 <sup>d</sup>	5.19 $\pm$ 1.90	4.84	<0.001

<sup>1</sup> Ratings were scored on 9-point Likert scales.<sup>2</sup> Differences between the fasted and sated state were tested with paired *t* tests.<sup>a</sup> *n* = 107.<sup>b</sup> *n* = 99.<sup>c</sup> *n* = 94.<sup>d</sup> *n* = 108.**Table 3**  
Positive correlation between ghrelin and brain activation during food versus non-food viewing in the fasted state<sup>1,2,3</sup>.

Brain region	Peak voxel coordinate (MNI)			Peak <i>p</i> <sub>fwe</sub>	Z-score	Cluster size ( <i>k</i> )
	x	y	z			
L inferior occipital gyrus	-44	-72	-8	0.010 <sup>4</sup>	4.64	74
R superior occipital gyrus	24	-72	32	0.024 <sup>4,6,7</sup>	4.43	72
R middle occipital gyrus	28	-84	16	0.334	3.65	
R supramarginal gyrus	36	-40	36	0.033 <sup>5,6</sup>	4.35	52
R inferior parietal gyrus	40	-40	44	0.052	4.23	
R inferior parietal gyrus	28	-44	40	0.079	4.11	
R inferior occipital gyrus	44	-68	-12	0.036 <sup>5,6</sup>	4.33	43
R inferior temporal gyrus	52	-48	-16	0.296	3.69	

<sup>1</sup> *n* = 88.<sup>2</sup> Site and leptin were included as covariates in the model.<sup>3</sup> Cluster extent threshold *k* = 68.<sup>4</sup> No longer above the cluster extent threshold of *k* = 67 for the model with BMI or HOMA-IR added: L inferior occipital gyrus: BMI *k* = 58, *p*<sub>fwe</sub> = 0.013, HOMA-IR *k* = 54, *p*<sub>fwe</sub> = 0.019; R superior occipital gyrus: BMI *k* = 32, *p*<sub>fwe</sub> = 0.036, HOMA-IR *k* = 27, *p*<sub>fwe</sub> = 0.069.<sup>5</sup> No longer significant with BMI added to the model: R supramarginal gyrus, *p*<sub>fwe</sub> = 0.066; Right inferior occipital gyrus, *p*<sub>fwe</sub> = 0.105.<sup>6</sup> No longer significant with HOMA-IR added to the model: R superior occipital gyrus, *p*<sub>fwe</sub> = 0.069; R supramarginal gyrus, *p*<sub>fwe</sub> = 0.089; R inferior occipital gyrus, *p*<sub>fwe</sub> = 0.087.<sup>7</sup> Cluster-level *p*<sub>fwe</sub> = 0.042.

In both the fasted and the sated state there were no correlations between fasting ghrelin and high calorie FCR. In addition, there were no correlations between the difference between the pre- and post-prandial ghrelin levels ( $t = \pm 55$  minus  $t = 0$ ) and high calorie FCR in the sated state or the fasted versus sated state. Adding BMI or HOMA-IR to the model did not alter this.

### 3.4.2. Correlation between leptin and FCR

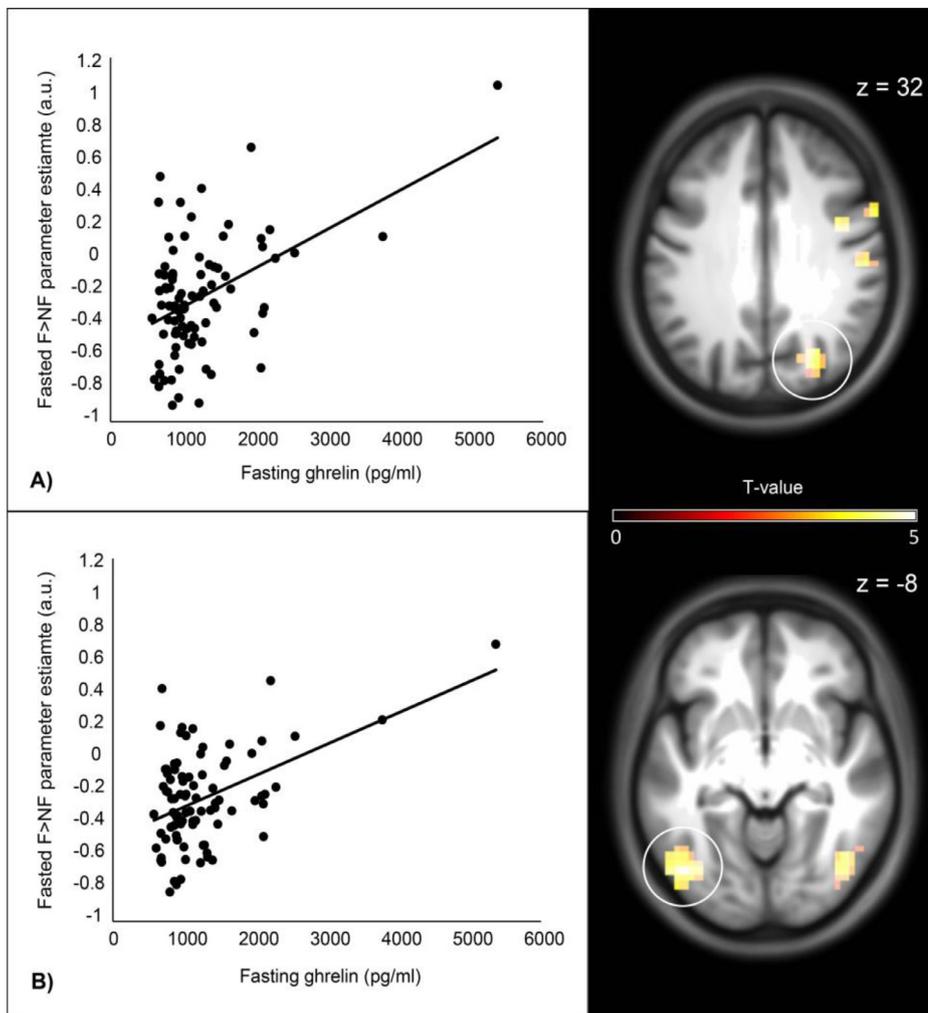
There was no correlation between leptin and neural FCR in the fasted or sated state. Adding BMI or HOMA-IR as a covariate did not alter this. Leptin was positively correlated with neural FCR in the left supramarginal gyrus in the fasted versus sated state, but only when taking HOMA-IR into account (Table 4, Fig. 3, Supplement 5). In addition, there was no association between BMI or HOMA-IR and neural FCR in either the fasted, sated or fasted versus sated state.

Leptin correlated significantly with activation in the right medial PFC (superior frontal gyrus) during high calorie FCR in the fasted state, but only when taking BMI into account (Table 5, Fig. 4, Supplement 5). For the sated and the fasted versus sated state there were no significant correlations between leptin and high calorie FCR. Adding BMI or HOMA-

IR to the models did not alter the results. We did not find an association between BMI and high calorie FCR in the fasted, sated or the fasted versus sated state. HOMA-IR was positively associated with high calorie FCR in the left mid cingulate gyrus in the sated state and with high calorie FCR in the right rolandic operculum in the fasted versus sated state.

### 3.4.3. *f*ROI results

General linear mixed models showed an effect of hunger state (i.e., fasted versus sated) on high calorie FCR in the left putamen ( $B = -0.05$ ,  $SE = 0.016$ ,  $t = -3.17$ ,  $p = 0.002$ ), right caudate nucleus ( $B = -0.05$ ,  $SE = 0.019$ ,  $t = -2.98$ ,  $p = 0.004$ ), right pallidum ( $B = -0.07$ ,  $SE = 0.016$ ,  $t = -4.27$ ,  $p < 0.001$ ), and right vmPFC ( $B = -0.17$ ,  $SE = 0.054$ ,  $t = -3.08$ ,  $p = 0.003$ ), with higher levels of neural FCR to high versus low calorie food pictures when fasted versus sated. There was no effect of hunger state on neural FCR for food versus non-food images. In addition, there were no interactions between either ghrelin or leptin and hunger state on neural FCR. Adding BMI and HOMA-IR to the models did not alter the results.



**Fig. 2.** Left: Scatterplot of the positive correlation between ghrelin levels and (A) right superior occipital gyrus and (B) left inferior occipital gyrus activation during food versus non-food image viewing in the fasted state. Right: Color-coded T-map thresholded at  $T = 3.19$  ( $p = 0.001$ ) and superimposed on the average normalized anatomical image. It is of note that, although physiologically plausible, the correlations were outlier driven and were no longer significant after removal of the two highest ghrelin values (both  $>2$  SD above the mean). Scatterplots are intended for illustration purposes only.

**Table 4**

Positive correlation between leptin and neural FCR in the fasted versus sated state (controlling for HOMA-IR) <sup>1,2,3</sup>.

Brain region	Peak voxel coordinate (MNI)			Peak $p_{fwe}$	Z	k
	X	Y	Z			
L supramarginal gyrus	-60	-28	36	0.229 <sup>4</sup>	3.83	133
L supramarginal gyrus	-48	-36	28	0.318	3.71	
L Postcentral gyrus	-44	-32	48	0.380	3.64	

<sup>1</sup>  $n = 109$ .

<sup>2</sup> Site and differential ghrelin levels ( $t = \pm 55$  minus  $t = 0$ ) were also included as covariates in the model. Without HOMA-IR in the model there was no significant effect.

<sup>3</sup> Cluster extent threshold  $k = 58$ .

<sup>4</sup> Cluster-level  $p_{fwe} = 0.003$ .

**Table 5**

Positive correlation between leptin and high- versus low-caloric food viewing activation in the fasted state (controlling for BMI) <sup>1,2,3</sup>.

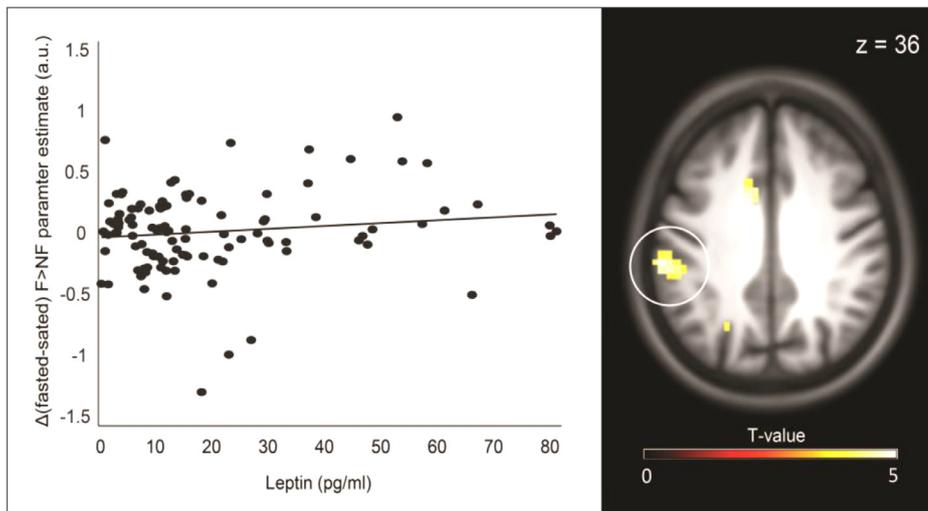
Brain region	Peak voxel coordinate (MNI)			Peak $p_{fwe}$	Z	k
	X	Y	Z			
R superior frontal gyrus, medial part/medial PFC	4	32	40	0.078 <sup>4</sup>	4.11	95
L superior frontal gyrus, medial part/medial PFC	-8	36	32	0.213	3.80	
L superior frontal gyrus, medial part/medial PFC	-4	44	24	0.303	3.68	

<sup>1</sup>  $n = 109$ .

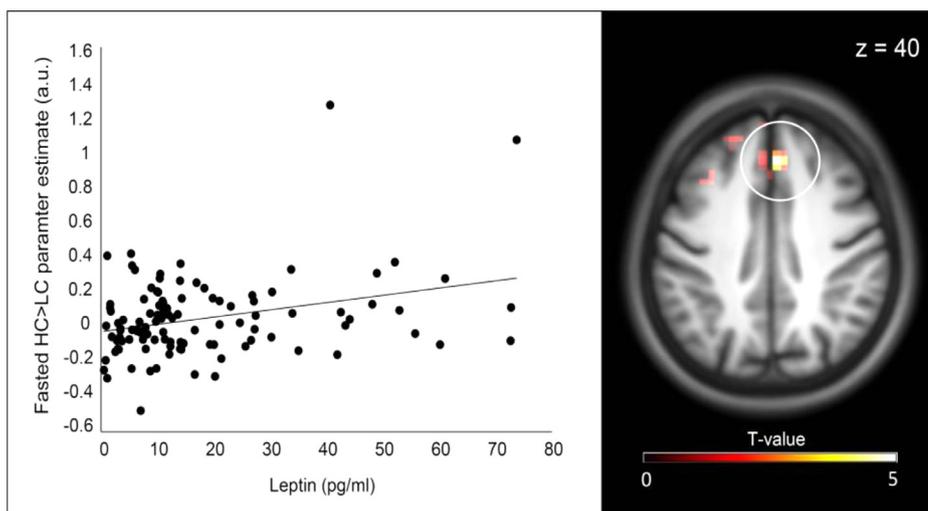
<sup>2</sup> Site and ghrelin were also included as covariates in the model. Without BMI in the model there was no significant effect.

<sup>3</sup> Cluster extent threshold  $k = 68$ .

<sup>4</sup> Cluster-level  $p_{fwe} = 0.019$ .



**Fig. 3.** Left: Scatterplot of the positive correlation between leptin and left supramarginal gyrus activation during food versus non-food image viewing in the fasted versus sated state, controlled for HOMA-IR. Right: color-coded T-map thresholded at  $T = 3.17$  ( $p < 0.001$ ) superimposed on the average normalized anatomical image. Scatterplots are intended for illustration purposes only.



**Fig. 4.** Left: Scatterplot of the positive correlation between leptin and right medial PFC (superior frontal gyrus) activation during high- versus low-caloric food image viewing in the fasted state, controlled for BMI. Right: color-coded T-map thresholded at  $T = 3.17$  ( $p < 0.001$ ) superimposed on the average normalized anatomical image. Scatterplots are intended for illustration purposes only.

## 4. Discussion

We examined the association between ghrelin and leptin levels and neural FCR in the fasted and sated state as well as the association between meal-induced changes in ghrelin and neural FCR and in how far these associations are related to BMI. Ghrelin was positively associated with neural FCR in the inferior and superior occipital gyrus in the fasted state. This effect was partly attributable to BMI. Leptin was positively associated with neural FCR to high-caloric foods in the medial PFC in the fasted state, but only when controlling for BMI. There were no associations between ghrelin and leptin and neural FCR in the sated state, nor between meal-induced changes in ghrelin and neural FCR.

### 4.1. Ghrelin and FCR

The positive association between ghrelin levels and neural FCR in the inferior and superior occipital gyrus in the fasted state is consistent with our hypothesis and with previous studies (Abizaid et al., 2006; Kroemer et al., 2013). The inferior occipital gyrus is involved in visual processing and attention processes and has been implicated specifically in FCR (Meng et al., 2020; van der Laan et al., 2011; van Meer et al., 2015). A possible explanation for the increased activation in these brain areas during food versus non-food image viewing in a fasted state is that food images are more salient and elicit heightened attention in comparison to non-foods (Killgore and Yurgelun-Todd, 2007). Inspec-

tion of the whole-brain statistical maps further indicates a spatially diffuse enhancement of food-cue reactivity by ghrelin that is more pronounced in brain regions that are involved in food-cue reactivity in general (see <https://neurovault.org/collections/VAYWVURJ/>). It is of note, however, that there is an ongoing debate on whether activity in visual cortices during task-related fMRI might also represent a certain level of arousal or engagement of participants in the task, rather than being interpreted in the context of the task (Roth et al., 2020). This indicates that the activity we found in the inferior and superior occipital gyrus might additionally be explained by heightened levels of arousal or engagement in the food viewing paradigm due to the higher saliency of food versus non-food images as suggested previously for the fusiform and lingual gyri (van der Laan et al., 2011; van Meer et al., 2015).

There was no association between sated ghrelin levels or meal-induced changes in ghrelin levels and FCR in the sated state, which is in line with the literature (Kroemer et al., 2013; Sun et al., 2014), who also did not find these associations after either a sugar load or a protein rich meal, respectively. This could be the result of the lower saliency of food cues due to satiety and is in line with the idea that ghrelin is involved in meal initiation. We also did not find an association between meal induced changes in ghrelin and the corresponding differences in neural FCR between the fasted and the sated state. This contrasts with several studies (Roth et al., 2018; Sun et al., 2015, 2014), and might be attributed to methodological differences. Studies that found such an association used highly palatable food stimuli versus non-palatable stim-

uli (non-food objects and tasteless solutions), while the present study focused on differences between food versus non-food and high- versus low-caloric food images. This could have led to smaller changes in neural FCR in the present study. Moreover, Roth et al. (2018) only found a significant association between pre- to post-meal reduction in acetylated ghrelin and FCR in their healthy weight but not in their obese group. While in children with a healthy weight greater reductions in acetylated ghrelin after a meal were associated with a greater reduction in neural activation to high-caloric food cues, this effect was absent in overweight children, despite substantial reductions in ghrelin (Roth et al., 2018). Across all participants there was no significant association between post-meal reductions in ghrelin and neural FCR, similar to what we found. Based on this finding, we additionally analyzed participants with a normal weight (BMI 20–25 kg/m<sup>2</sup>, *n* = 47) and with overweight (BMI ≥ 27.5 kg/m<sup>2</sup>, *n* = 41) separately. However, there were no significant associations between meal induced changes in ghrelin and the corresponding difference in neural FCR between the fasted and the sated state in either group, nor were there higher levels of FCR in people with higher levels of BMI or HOMA-IR (see Supplement 4). Thus, we find an overall lack of association between ghrelin responses to food and neural FCR and no evidence for differential sensitivity to ghrelin in different weight status groups. There is also a difference in the way ghrelin is measured between the studies. Roth et al. (2018) measured acetylated ghrelin, while the current study measured total ghrelin levels (sum of acetylated and deacetylated ghrelin). However, the studies of Sun and colleagues used the same assay as the current study to measure ghrelin levels (Sun et al., 2015, 2014).

There was no association between ghrelin and FCR to high- versus low-caloric foods in the fasted state. This is in contrast with our expectations. We did find an association between ghrelin and neural FCR to foods versus non-foods in the visual cortex (inferior and superior occipital gyri). This suggests that after an overnight fast there is heightened attention for food in general, irrespective of its caloric content.

#### 4.2. Leptin and FCR

Leptin levels were not associated with FCR during food versus non-food viewing in either the fasted or sated state. However, we found a positive association between leptin levels and neural FCR in the fasted minus the sated state in the left supramarginal gyrus, but only when controlling for HOMA-IR. Although the direction of the association was the same, Grosshans et al. (2012) and Simon et al. (2014) found a positive association between leptin and neural FCR in the striatum. This divergence in results might have several reasons, including differences in methodology. Grosshans et al. (2012) reported an association between leptin levels and FCR in the ventral striatum, but only performed a ROI analysis, while the present study used a whole brain approach. In an additional functional ROI analysis on the ventral striatum we could not replicate this result. In addition, in their study activation in the ventral striatum was primarily related to BMI ( $r = 0.47$ ,  $p = 0.001$ ) and the association with leptin was less pronounced ( $r = 0.27$ ,  $p = 0.040$ ). We did not observe an association between BMI or HOMA-IR and neural FCR.

We found a positive association between leptin and neural FCR in the fasted state for high- versus low-caloric food viewing in the medial PFC (peak MNI (4, 32, 40)), but only when controlling for BMI. The medial PFC is involved in the processing of the relative value of an anticipated reward (Amodio and Frith, 2006; Stoeckel et al., 2008). The direction of this effect is in line with prior studies that used similar paradigms (Grosshans et al., 2012; Rosenbaum et al., 2008; Simon et al., 2014) but only Rosenbaum et al. (2008) found such an association in the same PFC region (MNI (6, 26, 40)). However, in a meta-analysis examining differences in brain responses between normal-weight and obese participants in response to food stimuli, Brooks et al. (2013) reported enhanced neural activation in response to food cues in individuals with overweight versus individuals with a normal weight in a similar medial PFC region (TAL (−4, 51, 24)/MNI (−4, 51, 29)). In ad-

dition, Scharmuller et al. (2012) reported enhanced activation during food-viewing in individuals with obesity in the same PFC region that we found (MNI (8, 30, 44)), but only when they actively reappraised the reward value of the images, by imagining that the presented food items were not real but plastic models. In light of these findings, the association between leptin and neural FCR in the present study suggests that in the fasted state higher leptin levels are associated with a higher degree of reward evaluation for high-caloric foods, which are more salient, but not foods in general. This is in line with Raynaud et al. (1999) who reported a positive correlation between fasting serum leptin and palatability, independent of BMI, body fat mass or percentage of body fat. Whether such increased neural responsivity or appraisal of foods also promotes food intake remains to be tested.

#### 4.3. Hunger and weight status

We found greater neural responses to FCR in the fasted versus sated state in reward-related brain regions (i.e., the putamen, caudate nucleus, pallidum) and vmPFC, but only in response to high (versus low) caloric food images. Charbonnier et al. (2018) found effects of hunger state on brain response to high caloric food images in dorsal prefrontal regions (bilateral dorsomedial and right dorsolateral prefrontal cortex) but not in the striatum or vmPFC in normal weight individuals of varying age (range 8 - 75 y). The current findings suggest that hunger specifically increases the reward value of high caloric foods, despite the fact that self-reported liking was higher for both image types in the fasted condition. In addition, we found different significant associations between ghrelin and leptin and neural FCR after controlling for BMI and HOMA-IR, showing that they do have different modulatory effects. While BMI affected the association between ghrelin and neural FCR, HOMA-IR affected more the relation between leptin and neural FCR. Prior evidence emphasizes that BMI might not be a good proxy for underlying metabolic alterations in people with obesity (Morys et al., 2020); HOMA-IR, in contrast, is a measure of insulin sensitivity and our findings underscore the interactions between insulin and leptin signaling in the brain (Belgardt and Brüning, 2010). However, the present study shows that it is relevant to take both BMI and HOMA-IR into account.

Strengths of the present study are the relatively large number of participants and their broad age and BMI ranges. Although we tailored the size of the milkshake load for each participant based on estimated BMR, using age, gender and weight, achieving a similar subjective level of satiety for all participants turned out to be challenging. This may in part be due to the fact that the Schofield equation does not take body composition into account. A larger caloric load could lead to a stronger suppression in ghrelin. To correct for effects of meal size on ghrelin responses we divided ghrelin levels after satiation by the amount of liquid breakfast consumed. However, this does not account for possible mismatches between estimated and actual energy requirement due to body composition differences. It is of note that some findings of the present study are not in line with prior studies about neural FCR to food viewing and associations with appetite-regulating hormones. Most of these studies have relatively small sample sizes, which, especially in combination with liberal statistical thresholds inflates the risk of false positive findings. In addition, part of the studies that focused on the association between leptin and neural FCR included, besides individuals with normal weight, individuals with morbid obesity, which might have resulted in a different relationship between appetite-regulating hormones and FCR than seen in the general population. Also, the simple regression analyses used in the current study might not optimally handle the more extreme hormone levels, which may have added to the divergence in findings. Future studies in this field should consider using more robust inference methods to mitigate reproducibility risks. Furthermore, recent weight history is an often-neglected variable in appetite-regulating hormone research and may have affected the results of prior studies. Changes in weight status, especially when they occur rapidly, often lead to long periods of hormonal restabilization (Strohacker et al., 2014; Sumithran et al., 2011).

Thus, weight history may confound the relationship between appetite-regulating hormones and FCR. This could also have affected our results although we tried to mitigate this by selecting weight-stable (at least 6 months) non-dieting participants.

## 5. Conclusions

We found that ghrelin and leptin levels are associated with neural FCR to foods in general and high-caloric foods, respectively, but only in a fasted state. In addition, associations between FCR and ghrelin were partly attributable to BMI. Individuals with higher fasting ghrelin show enhanced FCR in the inferior and superior occipital gyrus in a fasted state, indicating heightened levels of attention to food cues during fasting. Furthermore, individuals with higher leptin levels show greater medial PFC activation during high- versus low-caloric food viewing when fasted, independent of BMI and HOMA-IR, suggesting that individuals with higher leptin levels have a higher degree of reward evaluation for high-caloric foods, which are more salient, but not for foods in general. In conclusion, we show modest associations between ghrelin and leptin and neural FCR in a relatively large sample of European adults with a broad age and BMI range. The results of the present study form a foundation for future studies to test whether food intake and (changes in) weight status can be predicted by the association between (mainly fasting) ghrelin and leptin levels and neural FCR.

## Data and code availability statement

Group level statistical parametric maps are available at <https://neurovault.org/collections/9487/>

Other anonymized data will be provided upon request for non-commercial research use under a data transfer agreement between UMC Utrecht, who represents the Full4Health consortium partners that collected the data and analyzed the blood samples, and the receiving party.

## Credit author statement

**Mirjam Wever:** Conceptualization; Investigation; Formal analysis; Writing - original draft; Writing - review & editing. **Floor van Meer:** Investigation; Roles/Writing - original draft; Writing - review & editing. **Lisette Charbonnier:** Methodology; Investigation; Formal analysis; Writing - review & editing. **Daniel Crabtree:** Investigation; Writing - review & editing. **William Buosi:** Investigation; Writing - review & editing. **Angeliki Giannopoulou:** Investigation. **Odysseas Androutsos:** Investigation; Supervision; Writing - review & editing. **Alexandra Johnstone:** Funding acquisition; Resources; Investigation; Supervision; Writing - review & editing. **Yannis Manios:** Funding acquisition; Resources; Investigation; Supervision; Writing - review & editing. **Claire L. Meek:** Resources; Investigation; Writing - review & editing. **Jens Holst:** Resources; Investigation; Writing - review & editing. **Paul Smeets:** Funding acquisition; Conceptualization; Resources; Investigation; Methodology; Project administration; Formal analysis; Supervision; Roles/Writing - original draft; Writing - review & editing; Data curation.

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## Supplementary materials

Supplementary data associated with this article can be found, in the online version, at [10.1016/j.neuroimage.2021.118374](https://doi.org/10.1016/j.neuroimage.2021.118374).

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