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## Deactivation of the left dorsolateral prefrontal cortex in Prader-Willi syndrome after meal consumption

Martin Reinhardt<sup>1,2</sup>, Angelo Del Parigi<sup>3</sup>, Kewei Chen<sup>4</sup>, Eric M. Reiman<sup>4,5</sup>, Pradeep Thiyyagura<sup>4</sup>, Jonathan Krakoff<sup>1</sup>, Maximilian G. Hohenadel<sup>1</sup>, Duc Son N.T. Le<sup>1</sup>, and Christopher M. Weise<sup>1,7</sup>

<sup>1</sup>Obesity and Diabetes Clinical Research Section, Phoenix Epidemiology and Clinical Research Branch National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Department of Health and Human Services, Phoenix, AZ

<sup>2</sup>Department of Diagnostic and Interventional Radiology, University of Leipzig, Germany

<sup>3</sup>Drexel University, Philadelphia, PA, and Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT

<sup>4</sup>Banner Alzheimer's Institute, Phoenix, Arizona

<sup>5</sup>Neurogenomics Division, Translational Genomics Research Institute, University of Arizona, and Arizona State University, Phoenix, Arizona

<sup>7</sup>Department of Neurology, University of Leipzig, Germany

### Abstract

**Background/Objectives**—Prader-Willi syndrome (PWS) a type of human genetic obesity may inform us about the physiology of non-syndromic obesity. Objective of this study was to investigate the functional correlates of hunger and satiety in individuals with PWS in comparison to healthy controls with obesity, hypothesizing that we would see significant differences in activation in the left dorsolateral prefrontal cortex (DLPFC) based on prior findings.

**Subjects/Methods**—This study compared the central effects of food consumption in 9 individuals with PWS (7 men, 2 women; body fat 35.3%±10.0) and 7 controls (7 men; body fat 28.8%±7.6), matched for percentage body fat. H<sub>2</sub><sup>15</sup>O PET scans were performed before and after consumption of a standardized liquid meal to obtain quantitative measures of regional cerebral blood flow (rCBF), a marker of neuronal activity.

**Results**—Compared with obese controls, PWS showed altered ( $p < 0.05$  FWE cluster-level corrected; voxelwise  $p < 0.001$ ) rCBF before and after meal consumption in multiple brain regions.

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Corresponding author: Martin Reinhardt, MD; National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Disease 4212 N. 16th Street, Phoenix, Arizona 85016, Phone: 602-200-5329, Fax: 602-200-5335, [martin.reinhardt@nih.gov](mailto:martin.reinhardt@nih.gov).

**Conflict of Interest:** The authors have nothing to disclose.

**Authors Contributions.** M.R. and C.M.W. wrote the manuscript and analyzed the data. K.C. and P.T. helped analyzing the data. M.G.H. analyzed parts of the data. A.D.P. was principally involved in designing the study and collected the data. M.R., A.D.P., K.C., E.M.R., P.T., J.K., M.G.H., D.S.N.T.L., and C.M.W. contributed to the interpretations of findings and commented on and edited the drafts. J.K. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

There was a significant differential rCBF response within the left DLPFC after meal ingestion with decreases in DLPFC rCBF in PWS; in controls DLPFC rCBF tended to remain unchanged. In more liberal analyses (voxelwise  $p < 0.005$ ) rCBF of the right orbitofrontal cortex (OFC) increased in PWS and decreased in controls. In PWS, rCBF of the right OFC was associated with changes in appetite ratings.

**Conclusion**—The pathophysiology of eating behavior in PWS is characterized by a paradoxical meal induced deactivation of the left DLPFC and activation in the right OFC, brain regions implicated in the central regulation of eating behavior.

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## Introduction

With a prevalence of 1 in 20,000 live births, Prader-Willi syndrome (PWS) is the most common syndromic type of human genetic obesity (1). PWS is characterized by a variable combination of symptoms, including infant hypotonia and feeding problems followed by excessive or rapid weight gain, characteristic facial features, hypogonadism, global childhood developmental delay and mild to moderate global delay in older children (2). Hyperphagia due to impaired satiation is a hallmark of PWS and results in obesity for 100% of individuals with PWS, if access to food is unrestricted (3). The disrupted satiation mechanism in PWS is characterized by delayed meal termination, early return of hunger and desire to eat after eating, seeking and hoarding of food, and eating of non-food items (4).

Previous studies have investigated the neurostructural and neurofunctional foundations to the hyperphagia in PWS. Neuroimaging studies of PWS individuals report structural abnormalities such as ventriculomegaly (100%), decreased grey matter volume in the parietal-occipital lobe (50%), sylvian fissure polymicrogyria (60%), incomplete insular closure (65%) (5) compared with normal weight siblings and individuals with early-onset morbid obesity, and reduced pituitary height (49%) (6), compared to reference data of healthy individuals. Delayed hypothalamic response to glucose ingestion indicating altered hypothalamic satiety signaling was noted among subjects with PWS (7). The affected segment on chromosome 15 leads to structural alterations of the gamma-aminobutyric acid type-A (GABAA) receptor. Adult PWS subjects also have evidence of reduced (GABAA) receptor binding potential in the anterior cingulate gyrus and the frontal, temporal and insular cortices (8), brain regions previously implicated in hunger and satiety (9,10). We demonstrated that the left dorsolateral prefrontal cortex (DLPFC), a brain region involved in top-down control of behavioral responses and decision making (11), is less activated following a meal (measured as regional cerebral blood flow) in obese compared to lean individuals (12), and has greater post-meal activation in successful dieters vs. non-dieters (13). In PWS subjects compared to non-obese controls, a lack of post-meal neural activation in brain regions previously associated with a satiety response, including the prefrontal cortex, was reported (4). In response to food images after a meal, PWS compared with obese subjects have hyperactivation in subcortical reward circuitry, including the prescribed orexigenic domain (9), and hypoactivation in the DLPFC and orbitofrontal cortex (OFC) (14).

The current study was designed to investigate the functional correlates of hunger and satiety in individuals with PWS compared with appropriate obese controls by analyzing differences in  $H_2^{15}O$ -PET measured regional cerebral blood flow (rCBF), a marker of neuronal activity, before and after consumption of a standardized liquid meal. Based on prior findings, we hypothesized that ingestion of a satiating meal would result in less activation of the left DLPFC in individuals with PWS.

## Subjects and Methods

### Subjects

We invited study participants through targeted mailing to members of the Prader-Willi Syndrome Association of the United States (N=10) and newspaper advertisement in the Phoenix (AZ, USA) metropolitan area (controls, N=8). Complete neuroimaging data was available from 9 white individuals with PWS (7 men and 2 women; White; age  $25.1 \pm 5.5$  y; body fat  $35.3\% \pm 10.0$ ) and 7 controls (7 men; 4 White, 2 Hispanic, 1 Black; age  $30.5 \pm 6.6$ ; body fat  $28.8\% \pm 7.6$ ). All volunteers were not affected by other pathologies, as determined by medical history, physical examination, and laboratory screening tests, and were not taking any medication at the time of admission, except for two individuals with PWS who took testosterone cypionate 200 mg/bimonthly until 14 days prior to admission, and recombinant growth hormone 2.55 mg/day until 19 months prior to admission, respectively. The diagnosis of PWS was confirmed by genetic testing prior to admission. Diabetes was excluded by oral glucose tolerance test (15). Women were studied in the follicular phase of their menstrual cycle. The protocol was approved by the Institutional Review Boards of the NIDDK; all subjects gave written consent before participation.

Psychiatrists and psychologists not associated with the study evaluated each individual with PWS to assess the level of competence to understand all experimental procedures and to provide informed consent. Prior to consenting, individuals with PWS viewed a videotape detailing the main procedures of the study. Upon admission to our clinical research unit (CRU) volunteers were provided a standard weight maintaining diet (WMD) with 50%, 30%, 20% carbohydrate, fat, and protein content, respectively. Individual weight maintaining energy needs were determined based on weight and sex (16). The WMD was provided for at least 3 days prior to neuroimaging procedures and all volunteers were asked not to exercise for the duration of their stay.

### Body weight and body composition measures

Body weight was measured upon admission to the CRU. Body composition (fat mass-FM; fat free mass-FFM) was estimated by total body dual-energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI). Percentage body fat, FM and FFM were estimated as previously described (17).

### Imaging procedures and ratings of hunger and appetite

Neuroimaging procedures were conducted at Banner-University Medical Center (Phoenix, AZ). Structural magnetic resonance imaging (MRI; 1.5T Signa, General Electric, Milwaukee, WI) was performed to rule out gross anatomical abnormalities and images were

used for coregistration in preprocessing of positron emission tomography (PET) images. Data acquisition used three-dimensional pulse sequence [radio-frequency-spoiled gradient recall acquisition in the steady state (SPGR), repetition time (TR) = 33msec, echo time (TE) = 5msec,  $\alpha = 30^\circ$ , number of excitations = 1, field of view = 24cm, imaging matrix = 256×192, slice thickness = 1.5mm, scan time = 13:36min] and consisted of 124 contiguous horizontal slices with in-plane voxel dimension of 0.94×1.25mm.

PET maps of rCBF were obtained for each subject using an ECAT-951/31 scanner (Siemens, Knoxville, TN) and  $^{15}\text{O}$ -labeled water as radiotracer. During each 1-minute scan, subjects rested in the supine position without movement and were asked to keep their eyes closed and positioned as if looking straight ahead. For each scan, a 50-mCi intravenous bolus of  $^{15}\text{O}$ -water was injected. Two scans (F1 and F2, F=fasting) were obtained in resting conditions after a 12 hour fast and two (S1 and S2, S=satiety) after the administration (over 25 minutes) of a satiating amount of a liquid meal (Ensure Plus, 1.5 kcal/mL, 56% carbohydrate, 29% fat, 15% protein; Ross-Abbott Laboratories, Columbus, OH), which provided 50% of calories of the individual resting energy expenditure. There was an interval of 10 minutes between F1 and F2, and S1 and S2. To control for swallowing, 30 seconds before each scan, subjects were asked to retain and swallow 2mL of water at room temperature administered from a syringe through a plastic tube into the mouth. Immediately after each scan in fasting and satiety conditions, subjects were asked to rate feelings of desire to eat, hunger, fullness, thirst and prospective food consumption (i.e., amount of food wanted) on a 100-mm visual analogue scale (VAS), ranging from 0 "not at all hungry", (or full, etc.) to 100 "extremely hungry" (or full, etc.). Six individuals with PWS and seven control subjects filled out all pre and post meal questionnaires. The subjects anticipated being fed until sated, as they were fully familiarized with the experimental protocol in order to minimize the risk of learning-related artifacts. To accustom the participants with the study procedures the feeding protocol was performed at least twice on the CRU prior to the actual imaging session.

### Functional imaging data processing and statistical analysis

$\text{H}_2^{15}\text{O}$  PET scans were first inspected visually (reader blinded to group membership) to rule out methodological abnormalities in data acquisition. Statistical Parametric Mapping (SPM5; <http://www.fil.ion.ucl.ac.uk>) and implemented automated algorithms were used to align each subject's sequential PET images (18), rigidly co-register functional PET to anatomical MRI scans (18,19), spatially normalize the co-registered images to the stereotactic space as defined by the template provided by the Montreal Neurological Institute (MNI), and to smooth these normalized PET images with a 8mm full-width-at-half-maximum Gaussian filter. Voxelwise statistical analyses were performed using a two-level, random-effects approach (20). An individual contrast image of differences in rCBF in response to the consumption of a satiating liquid meal (i.e., average of the two satiety scans minus average of the two hunger scans) was created for each subject, accounting for whole brain blood flow by proportional scaling, which basically scales each image to a reference count (i.e., the global brain activity) set at a physiologically realistic value of 50 ml/dl/min (20). Then, to test for differences in rCBF between the two groups, averaged whole brain scans of the hunger and satiety condition, and the contrast images were separately analyzed with a two-sample t-test (between groups comparison). Results of whole brain analyses were

considered significant at  $p < 0.05$  FWE corrected on the cluster-level, with a voxel-wise threshold of  $p < 0.001$  and  $k=50$  continuous voxels. For more exploratory analyses the voxel-wise threshold was relaxed to  $p < 0.005$ . To investigate associations of meal induced changes in rCBF with corresponding changes in hunger and appetite ratings, rCBF values of peak voxels of the respective clusters were extracted and analyzed outside the SPM framework.

### Additional statistical analyses

Group differences ( $p < 0.05$ ) in age were assessed by Student's t-test; group differences in percentage body fat by general linear models (GLM) adjusted for age. Within group changes in VAS ratings and extracted rCBF were assessed by Wilcoxon signed ranks tests. Between group differences in VAS ratings were assessed by Wilcoxon rank-sum tests. Associations between extracted rCBF data and changes in hunger and appetite ratings were analyzed using Spearman rank correlations. In addition GLM analysis was performed to test for group interactions using SAS statistical software package (SAS E-guide 5.1 and SAS version 9.3; SAS Institute, Inc, Cary, NC)

## Results

Characteristics of the study population are listed in table 1 and results of the SPM analyses are summarized in table 2.

### Premeal Condition

Compared to controls, PWS showed higher rCBF within the left superior temporal gyrus (STG) and the left insular cortex (Fig. 1A) but widespread lower rCBF within subcortical brain regions, including the bilateral striatum (i.e., caudate nucleus) and the bilateral anterior cingulate gyrus (ACC) extending towards the left precuneus (Fig. 1B).

### Postmeal Condition

PWS had higher rCBF within the right orbitofrontal cortex (OFC) and the left STG, compared to controls (Fig. 2A). Resembling the results of the premeal condition, lower rCBF within the bilateral striatum and bilateral cingulate gyrus was observed in PWS (Fig. 2B).

### Postmeal – Premeal

At the original voxel-wise threshold of  $p < 0.001$  no significant differences ( $p < 0.05$  FWE cluster-level corrected) were observed for the contrasts PWS > Controls. At a more lenient voxel-wise threshold of  $p < 0.005$ , meal consumption resulted in greater rCBF increase ( $p < 0.05$  FWE cluster-level corrected) in the orbitofrontal cortex and within the bilateral posterior cingulate gyrus (PC) in PWS compared to controls (Fig. 3A).

In our specified ROI, we observed a differential activation of the left DLPFC following the liquid meal in PWS compared with controls ( $p < 0.05$  FWE cluster-level corrected; voxelwise threshold  $p < 0.001$ ). On inspection of the directionality of the rCBF by group, this was driven by an actual decrease in rCBF in PWS (Fig. 3B). Within group analyses (i.e. Wilcoxon signed-ranks tests) of extracted postmeal – premeal rCBF (i.e. peak voxels)

showed a significant rCBF decrease of the left DLPFC ( $p = 0.004$ ) and a significant increase within the right OFC ( $p = 0.004$ ) and the bilateral PC ( $p = 0.02$ ) in PWS. In the control group, no significant changes were observed within the left DLPFC ( $p = 0.81$ ), yet significant decreases were observed within the right OFC ( $p = 0.02$ ), and the bilateral PC ( $p = 0.03$ ).

### Hunger and Appetite Ratings

In the pre- versus postmeal state, control subjects had significantly lower ratings of hunger, desire to eat and prospective food consumption and higher ratings of fullness (table 3). Individuals with PWS did not have significantly lower ratings for hunger, thirst and prospective food consumption ( $p > 0.15$ ) but desire to eat was lower and ratings of fullness were higher (both  $p < 0.05$ ). In PWS versus controls, desire to eat was higher in PWS during fasting ( $p < 0.05$ ) and controls had lower ratings of desire to eat post-meal ( $p < 0.15$ ). As expected the delta differences from the pre- to the postmeal state demonstrated the expected patterns of greater reduction in hunger, desire to eat, thirst and prospective food consumption as well as greater increase in fullness compared to PWS, however none reached statistical significance.

Spearman correlation analysis showed a significant positive correlation between rCBF in the OFC and changes in desire to eat ( $r_s=0.89$ ,  $p<0.02$ ) in PWS but not in controls ( $r_s=-0.29$ ,  $p=0.53$ ) with a significant interaction term (GLM;  $p<0.02$ ; Figure 3C). No other significant associations between hunger ratings and extracted rCBF of DLPFC, PC and OFC were observed (all  $p>0.05$ ).

### Discussion

Results of this study indicate a differential activation of the left dorsolateral prefrontal cortex, the orbitofrontal and posterior cingulate cortex in PWS compared to healthy individuals. Of note is our finding of a paradoxical meal-induced deactivation in the left DLPFC, a brain region previously found to have greater meal-induced activation in lean adults compared to individuals with obesity.

The human left DLPFC has been implicated in top-down processing of behavioral responses and decision making (11). We previously reported a lesser post-meal DLPFC activation in individuals with obesity versus lean controls (21), and in non-dieters compared to successful dieters (13). These and other reports indicate that the DLPFC plays an important role in the central regulation of eating behavior (9,24). A recent meta-analysis of neuroimaging data demonstrated reduced activation of the left DLPFC in individuals with obesity in the context of food related visual stimuli (25). Also, MRI blood oxygenation level dependent (BOLD) measured response of the left DLPFC has been shown to be associated with successful regulation of food-related cravings via DLPFC-striatal pathways (26).

Zhang et al recently demonstrated decreased functional connectivity strength in prefrontal cortical networks including the DLPFC in PWS (27). Lesser left DLPFC activation in response to food images has been reported in individuals with PWS versus controls (14). Our results add to the current literature by indicating actual deactivation of the left DLPFC



in PWS in response to a real physiologic stimulus in form of a full meal, sought to induce satiation. If activation within this region plays a role in the control of food related cravings, our results provide a potential central mechanism of PWS associated hyperphagia. Although PWS and obesity are not characterized by the same behavioral phenotype we believe that the study of PWS patients, who exhibit disinhibited eating behavior and reduced satiation, allows us to draw inferences on which brain regions might be involved in the regulation of eating behavior, particularly on the subject of meal termination and inhibitory control. However, we do acknowledge that, compared to our previous findings, the observed deactivation of the DLPFC differs slightly with respect to the exact location within this region.

Findings from our previous work show that obese women had significantly less activation in the DLPFC than formerly obese women (21), indicating that these changes might be reversible. Whether these changes are a result of weight gain or precede weight gain is not clear. However, a study investigating DLPFC activation, in the same person, before and after weight gain might help answer the question whether interindividual variability in DLPFC activation is a primary- or acquired condition.

Interestingly, Hinton et al proposed PWS-associated hyperphagia to be based upon a disturbed central representation of satiety but not of hunger (4). In our study no significant differences of subjective hunger ratings were observed between PWS and controls, but ratings of desire to eat before the ingestion of a satiating meal were significantly higher in PWS together with a trend for a greater desire to eat after the meal, support the interpretation of an aberrant eating behavior in PWS. We cannot be certain in this setting about the strict discrimination between the meaning of 'desire to eat' vs 'hunger'.

We also observed a relative rCBF increase within the right orbitofrontal cortex (OFC) and left posterior cingulate (PCC) in PWS. Even though these results were significant only at more lenient thresholds (i.e., voxelwise  $p < 0.005$ ), they do confirm a previous report showing increased activation of the medial OFC after consumption of a large meal (i.e., 1200 kcal) in PWS (4). The OFC is a comparably large region containing the secondary taste cortex and, among other functions, has been shown to encode the reward value of food related stimuli (28). In this context our observation of increases in OFC rCBF being associated with less decline in desire to eat in PWS only, seems particularly interesting as this may highlight the OFC's encoding of motivational aspects of behavior, eating in particular. Both medial OFC and DLPFC form part of different networks, with the medial OFC being involved in the salience network and the DLPFC being part of the executive control network. In general, the primary function of the salience network is thought to involve the integration of homeostatically relevant data, including visceral, autonomic, and hedonic information, the executive control network is required to guide flexible and contextually adequate behavior in response to such salient stimuli (29). Considering the functional relevance of these networks allows an intriguing interpretation of our findings with higher activity of the medial OFC and less activity of the DLPFC indicating increased salience processing but reduced cognitive control over such salient stimuli in PWS. This may in part explain the phenotypical hyperphagia of individuals with PWS.

The role of the PCC in the context of eating behavior and PWS respectively is not established. The PCC has been implicated in processing of motivational emotions (30) and several functional imaging studies have demonstrated PCC activity to be modulated by food- and hunger-related tasks (31).

Apart from meal induced changes of rCBF (i.e., contrast of premeal and postmeal condition) we also analyzed premeal and postmeal conditions individually, showing significantly higher rCBF of the left insular cortex in the premeal state in PWS. The insular cortex contains the primary gustatory, olfactory and interoceptive cortex and is considered a key node in the central processing of food related and interoceptive cues. Insular function has been shown to be associated with rewarding and hedonic aspects of food intake (32). Since interoceptive stimuli, such as the feeling of hunger are processed and emotionally validated by the insular cortex (33), the relatively long fast of 12 hours and resulting high hunger levels might have contributed to this finding.

In both, the pre- and postmeal state we observed higher rCBF of the left STG in PWS. Activation of the temporal cortical regions, including the STG have been repeatedly associated with homeostatic challenges including thirst and hunger (34).

Compared to the control group, in the fasting state and after meal consumption, PWS showed reduced rCBF in the bilateral cingulate gyrus (i.e. dorsal ACC), a region involved in the processing of several higher cognitive functions, including reward-based decision making (35), and the caudate nucleus, a region implicated in cognitive processes, such as decision-making, reward and reward based learning (36). Dysfunction of these regions might contribute to the multitude of psychiatric and behavioral issues seen in PWS, including the aberrant eating behavior.

Besides providing additional insight in the complex central pathophysiology of behavioral disturbances in PWS, these results also emphasize specific areas of the brain as a potential targets for the treatment of PWS. Boggio et al. proposed transcranial direct current stimulation (tDCS) to the DLPFC as a potential option to control hyperphagia in PWS, through reduction of cue-induced craving related to food exposition (37), and other authors recently found reduced food-craving and measures of hyperphagia behavior in PWS after tDCS of the right DLPFC (38). A review of three small clinical trials indicate that modulation of the activity of brain circuits involved in eating behavior via tDCS could provide therapeutic benefits in common obesity (39). Indeed, a recently finished study of our group shows that repetitive tDCS stimulation of the left DLPFC in subjects with obesity results in significant weight reduction (40) underscoring the potential of non-invasive brain stimulation techniques in the treatment of behavioral disorders.

Several important limitations in our study need to be acknowledged, including the low statistical power, due to small sample size, a slightly smaller control group, and a suboptimal gender match limiting our ability to detect modest differences in neural activity in other brain regions. Despite these limitations we found significant and relevant differences in rCBF in the left DLPFC due to a meal induced deactivation in those with PWS. We acknowledge the order of pre- and postmeal PET scans was not randomized and therefore



we cannot exclude non-specific time dependent effects on brain activation between the two scans. We would add that given the complexity of PWS phenotype randomization of the order would have been difficult to achieve. Furthermore, PET imaging results have inherent limitations including reduced spatial and contrast resolution and low accuracy of the image deformation algorithm used to compute statistical maps. For the latter case, we attempted to minimize this issue with the use of MRI data. Because specific information about genetic PWS subtypes (chromosome 15q deletion, maternal uniparental disomy 15, other less common mechanisms) was not available for our study group we were not able to investigate potential differences in neural mechanisms between possible subgroups. We also acknowledge that PWS is a complex disorder that, apart from hyperphagia and obesity involves several additional abnormalities (41). In addition study groups were not perfectly balanced with respect to gender, but additional sensitivity analyses did not indicate a significant influence of gender differences on our results.

In conclusion, in individuals with PWS we have demonstrated pre- and postmeal hyperactivation in the primary taste cortex and post-meal hyperactivation in brain areas involved with food reward and decision making. Importantly we demonstrated differences in activation of the left DLPFC in response to ingestion of a satiating meal specifically driven by a deactivation in those with PWS. Our findings, in combination with promising emerging therapeutic options such as tDCS, might support the identification of treatment options for PWS.

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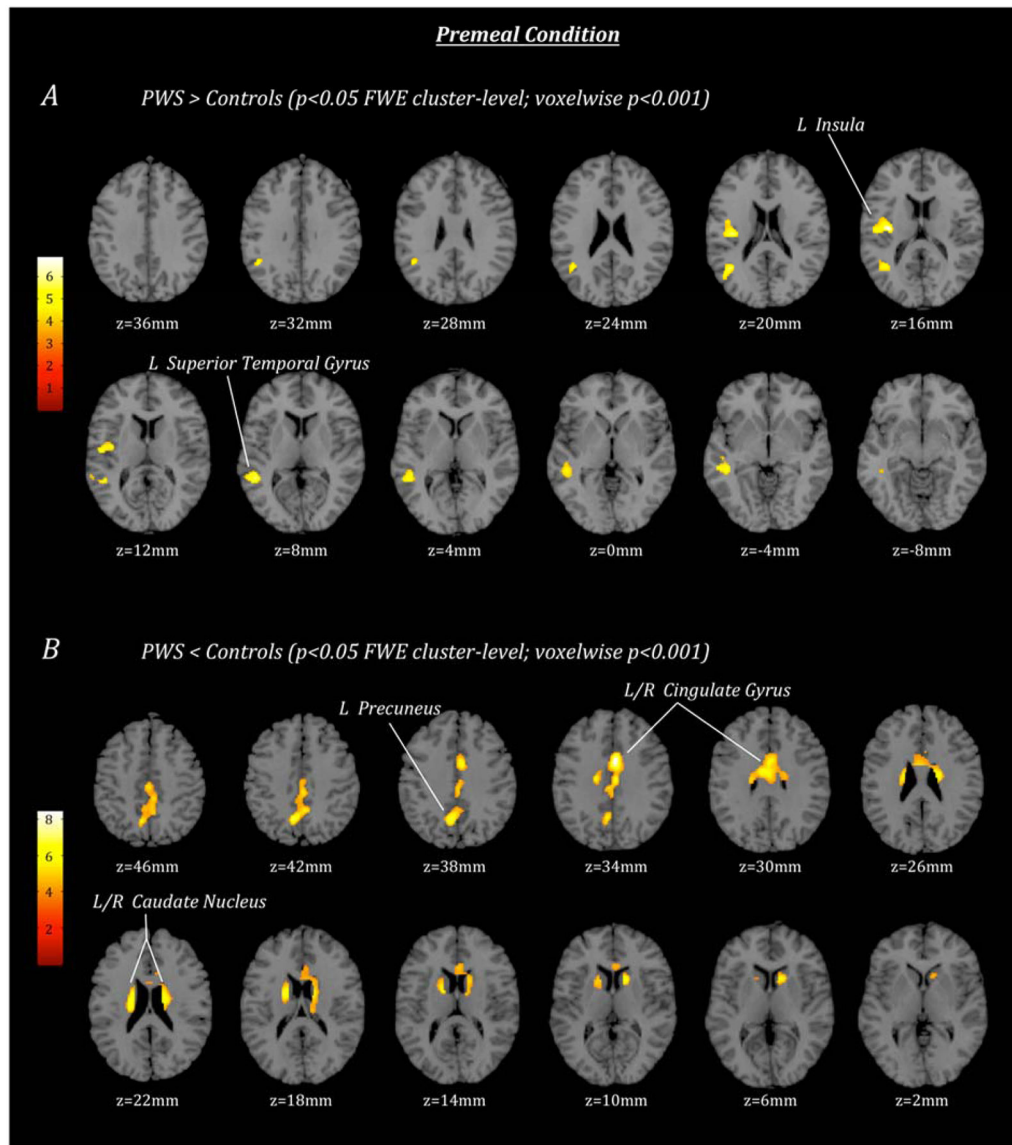
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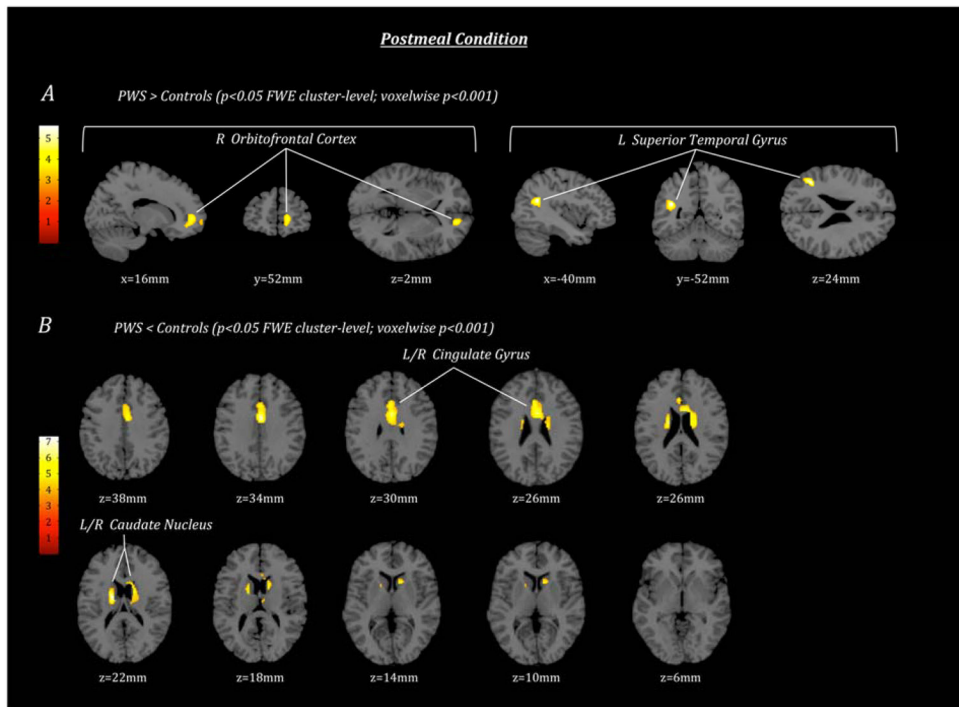
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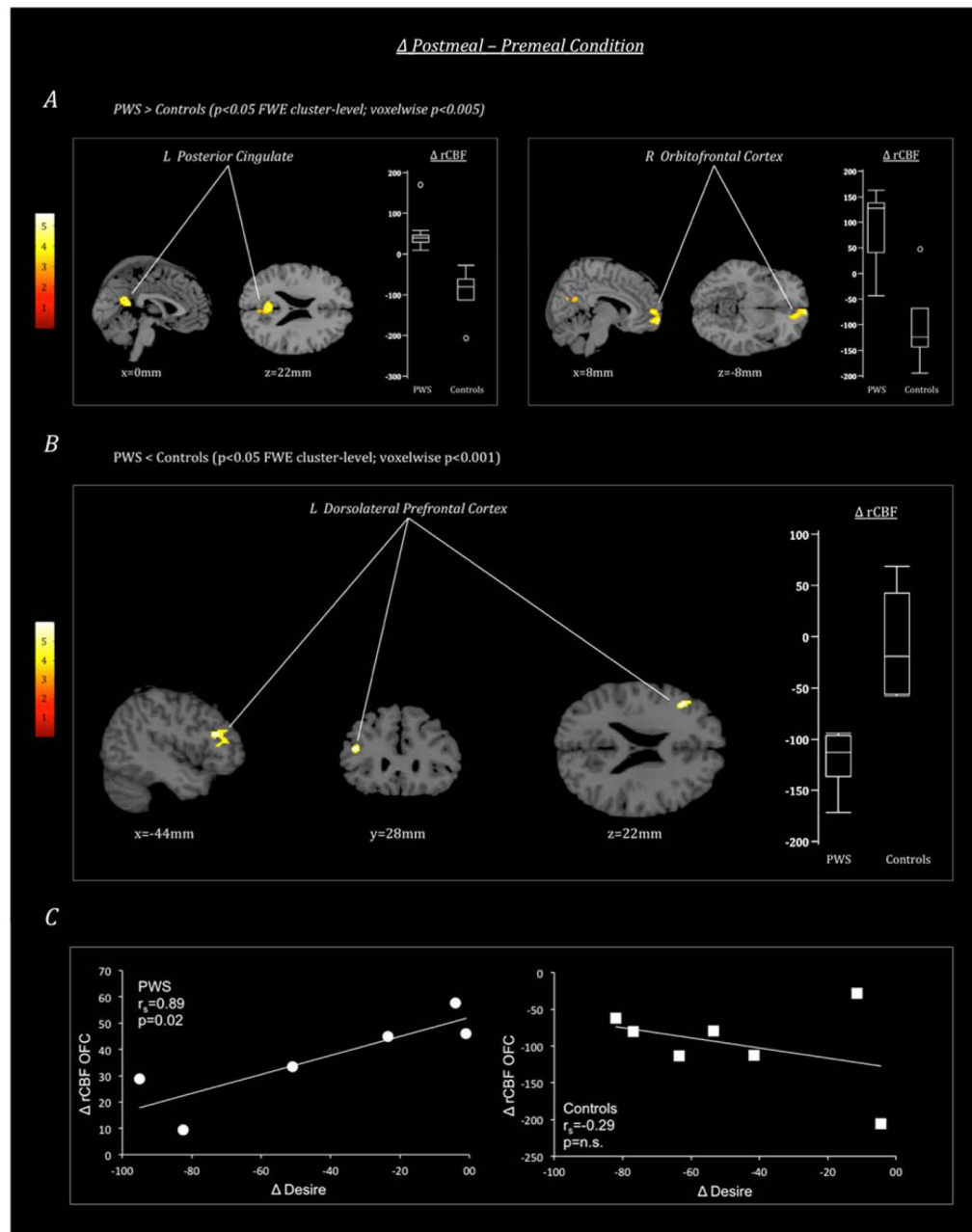
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**Figure 1.** T-score maps of significantly higher (A) and lower (B) rCBF in PWS compared to healthy controls after a 12 hour fast (i.e. premeal condition). Color bars indicate T-score. L left; R right.



**Figure 2.** T-score maps of significantly higher (A) and lower (B) rCBF in PWS compared to healthy controls in response to a satiating meal (i.e. postmeal condition). Color bars indicate T-score. L left; R right; C. illustrates correlations between changes in rCBF of the right orbitofrontal cortex with desire to eat in PWS (left) and controls (right).

**Figure 3.**

T-score maps comparing  $rCBF$  in response to a meal (i.e. postmeal  $rCBF$  minus premeal  $rCBF$ ) with relatively higher (A) and lower (B)  $rCBF$  in PWS compared to healthy controls. Boxplots illustrate  $rCBF$  of the corresponding brain region. Color bars indicate T-score. L left; R right.



**Table 1**

	<b>PWS (7M, 2F)</b>	<b>Control (7M)</b>
Race	9C	4C, 2H, 1B
Age (years)	25.1 ± 5.5	30.5 ± 6.6
Body weight (kg)	78.1 ± 16.1	112.9 ± 24.0 <sup>**</sup>
Body fat (%)	35.3 ± 10.0	28.8 ± 7.6
Fat mass (kg)	28.6 ± 11.6	33.6 ± 13.6
Fat free mass (kg)	49.5 ± 7.3	79.3 ± 13.6 <sup>**</sup>
BMI (kg/m <sup>2</sup> )	31.0 ± 7.0	36.2 ± 8.1
Waist circumference (inches)	42.0 ± 7.5	46.1 ± 7.8

General, anthropometric, and body composition characteristics of the study population.

Values are presented as mean ± SD. Differences between PWS patients and the control group were assessed by two sample t-test

\* = p < 0.05;

\*\* = p < 0.01. Abbreviations: C = Caucasian; H = Hispanic; B = Black; BMI = Body mass index

Table 2

Region, Brodman Area	MNI <sup>a</sup> coordinates			P <sub>FWE</sub>	k
	x	y	z		
<i>Premal Condition: PWS &gt; Controls</i>					
<b>L Superior Temporal Gyrus BA 22</b>	<b>-40</b>	<b>-56</b>	<b>20</b>	<b>&lt;0.001</b>	<b>621</b>
L Middle Temporal Gyrus BA 22	-48	-44	8		
L Superior Temporal Gyrus BA 22	-46	-34	-4		
<b>L Insula BA 13</b>	<b>-34</b>	<b>-14</b>	<b>16</b>	<b>0.004</b>	<b>318</b>
L Postcentral Gyrus BA 43	-50	-14	14		
L Insula BA 13	-42	-4	20		
<b>R Medial Frontal Gyrus BA 10</b>	<b>18</b>	<b>52</b>	<b>4</b>	<b>0.36</b>	<b>86</b>
<i>Premal Condition: PWS &lt; Controls</i>					
<b>R Cingulate Gyrus, BA 24</b>	<b>6</b>	<b>6</b>	<b>34</b>	<b>&lt;0.001/&lt;0.05*</b>	<b>2665</b>
L Caudate Nucleus, Body	-18	-4	20		
L Precuneus, BA 7	-8	-60	42		
<b>L Precuneus, BA 19</b>	<b>-34</b>	<b>-72</b>	<b>36</b>	<b>0.17</b>	<b>121</b>
L Inferior Parietal Lobule, BA 40	-40	-54	44		
L Angular Gyrus, BA 39	-34	-58	34		
<b>L Putamen</b>	<b>-34</b>	<b>-18</b>	<b>-6</b>	<b>0.34</b>	<b>88</b>
L Hippocampus	-34	-20	-14		
<b>L Parahippocampal Gyrus, BA 34</b>	<b>-12</b>	<b>4</b>	<b>-22</b>	<b>0.35</b>	<b>87</b>
<b>R Inferior Parietal Lobule, BA 40</b>	<b>54</b>	<b>-50</b>	<b>52</b>	<b>0.68</b>	<b>53</b>
<i>Postmeal Condition: PWS &gt; Controls</i>					
<b>R Anterior Cingulate, BA 10</b>	<b>16</b>	<b>52</b>	<b>2</b>	<b>0.009</b>	<b>294</b>
R Anterior Cingulate, BA 32	14	52	-6		
R Medial Frontal Gyrus, BA 10	12	68	-2		
<b>L Superior Temporal Gyrus, BA 39</b>	<b>-40</b>	<b>-52</b>	<b>24</b>	<b>0.025</b>	<b>233</b>
<b>R Insula, BA 13</b>	<b>52</b>	<b>-40</b>	<b>10</b>	<b>0.45</b>	<b>79</b>
<b>L Insula, BA 13</b>	<b>-40</b>	<b>-4</b>	<b>16</b>	<b>0.47</b>	<b>77</b>

Region, Brodman Area	MNI <sup>a</sup> coordinates			P <sub>FWE</sub>	k
	x	y	z		
<b>R Middle Temporal Gyrus, BA 21</b>	<b>54</b>	<b>-16</b>	<b>-22</b>	<b>0.50</b>	<b>73</b>
<b>L Middle Temporal Gyrus, BA 22</b>	<b>-48</b>	<b>-44</b>	<b>6</b>	<b>0.54</b>	<b>69</b>
<b>L Medial Frontal Gyrus, BA 10</b>	<b>-8</b>	<b>54</b>	<b>10</b>	<b>0.58</b>	<b>65</b>
L Medial Frontal Gyrus, BA 10	-2	66	0		
<b>R Posterior Cingulate, BA 23</b>	<b>2</b>	<b>-56</b>	<b>14</b>	<b>0.64</b>	<b>59</b>
<i>Postmeal Condition: PWS &lt; Controls</i>					
<b>R Cingulate Gyrus, BA 24</b>	<b>4</b>	<b>-2</b>	<b>34</b>	<b>&lt;0.001</b>	<b>1278</b>
R Caudate Nucleus, Body	16	14	10		
L Cingulate Gyrus, BA 24	0	18	28		
<b>L Caudate Nucleus, Body</b>	<b>-16</b>	<b>-4</b>	<b>18</b>	<b>0.02</b>	<b>248</b>
L Caudate Nucleus, Body	-14	8	10		
<b>L Superior Temporal Gyrus, BA 39</b>	<b>-34</b>	<b>-56</b>	<b>34</b>	<b>0.24</b>	<b>111</b>
<b>L Superior Temporal Gyrus, BA 22</b>	<b>-58</b>	<b>2</b>	<b>-6</b>	<b>0.52</b>	<b>71</b>
<b>L Cerebellum Anterior Lobe, Culmen</b>	<b>-2</b>	<b>-50</b>	<b>-16</b>	<b>0.64</b>	<b>59</b>
<i>Postmeal-Premeal: PWS &gt; Controls</i>					
<b>L Posterior Cingulate, BA 23</b>	<b>0</b>	<b>-54</b>	<b>22</b>	<b>0.05</b>	<b>115</b>
L Posterior Cingulate, BA 29	-6	-44	12		
<b>R Superior Frontal Gyrus, BA 10</b>	<b>8</b>	<b>68</b>	<b>-8</b>	<b>0.18</b>	<b>80</b>
R Medial Frontal Gyrus, BA 10	6	60	4		
<i>Postmeal-Premeal: PWS &lt; Controls</i>					
<b>L Middle Frontal Gyrus, BA 46</b>	<b>-44</b>	<b>28</b>	<b>22</b>	<b>0.04</b>	<b>120</b>
L Middle Frontal Gyrus, BA 46	-42	32	12		
L Middle Frontal Gyrus, BA 46	-44	40	26		

Results are listed that survived a voxel-wise threshold of  $p < 0.001$  and an extent threshold (k) of 50 continuous voxels. Bold data indicate primary peak within a cluster; Non-bold data indicate secondary peaks. P<sub>FWE</sub>: p-values after whole brain correction on the cluster-level, unless otherwise indicated;

\* whole brain corrected for multiple comparisons on the voxel-level; R Right; L Left.

**Table 3**

Visual Analogue Scale ratings (0–100)	PWS (4M, 2F) Fasted/Fed	Control (7M) Fasted/Fed	Fasted PWS/Control	Fed PWS/Control	Delta change Fasted to Fed PWS/Control
<b>Hunger</b>	96 (6, 100) vs 17 (5, 95) <sub>n.s.</sub>	75 (65, 86) vs 22 (3, 31) <sup>*</sup>	96 (6, 100) vs 75 (65, 86) <sub>n.s.</sub>	17 (5, 95) vs 22 (3, 31) <sub>n.s.</sub>	-27 (-83, -1) vs -63 (-84, -17)
<b>Desire to eat</b>	99 (98, 100) vs 33 (4, 94) <sup>*</sup>	75 (64, 85) vs 8 (23, 32) <sup>*</sup>	99 (98, 100) vs 75 (64, 83) <sup>*</sup>	33 (4, 94) <sup>*</sup> vs 8 (2, 29) <sub>n.s.</sub>	-37 (-83, -4) vs -53 (-77, -12) <sub>n.s.</sub>
<b>Fullness</b>	3 (1, 6) vs 68 (44, 95) <sup>*</sup>	9 (7, 24) vs 84 (64, 88) <sup>*</sup>	3 (1, 6) vs 9 (7, 24) <sub>n.s.</sub>	68 (44, 95) vs 84 (80, 88) <sub>n.s.</sub>	44 (13, 91) vs 76 (40, 81)
<b>Thirst</b>	54 (6, 98) vs 28 (6, 50) <sub>n.s.</sub>	74 (54, 83) vs 42 (33, 64) <sub>n.s.</sub>	54 (6, 98) vs 74 (33, 64) <sub>n.s.</sub>	28 (6, 50) vs 42 (33, 64) <sub>n.s.</sub>	-1 (-48, -1) vs -39 (-47, 3)
<b>Prospective food consumption</b>	86 (54, 100) vs 31 (17, 46) <sub>n.s.</sub>	80 (76, 91) vs 21 (6, 27) <sup>*</sup>	86 (54, 100) vs 76 (68, 88) <sub>n.s.</sub>	31 (17, 46) <sub>n.s.</sub> vs 21 (6, 27) <sub>n.s.</sub>	-44 (-84, 14) vs -70 (-83, -24)

Visual analogue Scale ratings (0–100) in 7 PWS and 7 control subjects that completed all VAS ratings. Ratings of Hunger, Desire to eat, Fullness, Thirst and prospective food consumption (i.e. amount of food that subjects could envision to eat at time of rating). Values are expressed in Median (interquartile range). n.s. = non-significant (p < 0.05);

‡ = trend (p < 0.15);

\* = p < 0.05.