Functional brain responses in the maintenance of energy balance

Opstal, A.M. van

CHAPTER 9

Changes in brain activity after weight loss in obesity

Submitted

A.M. van Opstal[1]
M.A. Wijngaarden[2]
J. van der Grond[1]
H. Pijl[2]

[1] Leiden University Medical Center, Department of Radiology, Leiden, The Netherlands
[2] Leiden University Medical Center, Department of Internal Medicine, Section Endocrinology, Leiden, the Netherlands

ABSTRACT

Objectives

The regulatory role of the brain in directing glucose homeostasis, energy homeostasis, eating behavior, weight control and obesity is increasingly recognized. Brain activity in (sub)cortical neuronal networks involved in homeostatic cognitive control and hedonic responses is generally increased in obese persons. Currently it is not known whether and which of these functional changes are reversible after dieting. The aim of the current study was to investigate whether prolonged fasting and/or weight loss influences neuronal brain activity in obese persons.

Methods

We included 14 obese participants (2 males, 12 females, BMI 35.2 ± 1.2 kg/m^2) at baseline. Whole brain resting state functional magnetic resonance imaging (fMRI) was performed after an overnight fast (baseline) and after a prolonged 48-h fast in all obese participants. Measurements were repeated after an 8 week weight loss intervention in 10 of the obese participants.

Results

An 8 week weight loss intervention decreased BOLD signal in areas of the brain involved in salience, sensory-motor and executive control. BOLD signal in these areas correlated with leptin levels and BMI. No whole brain voxel-wise changes were observed after the 48-h fast.

Conclusions

An 8 week weight loss intervention decreased activity in brain areas involved in feeding behavior and reward processing, but less in homeostatic regulation of energy balance. Furthermore, our data indicate that these obesity associated alterations in neuronal activity are directly related to excessive bodyweight and might return to more normal level as found in lean subjects after weight loss.

INTRODUCTION

Obesity is an important public health concern [1]. Obesity is marked by a disruption of energy balance leading to increased storage of energy, which can lead to the disruption of a glucose homeostatic and often coincides with disrupted eating behavior [2]. The regulatory role of the brain in directing energy homeostasis, eating behavior, weight control and thus obesity is increasingly recognized [3-5]. Energy homeostasis is centrally regulated by the brain through several interacting neuronal systems. The so-called homeostatic system and the reward and executive control systems are both involved in energy balance [3, 6]. The homeostatic system, consisting, of the hypothalamus and several nuclei in the brainstem, regulates energy intake by combining satiety signals, metabolic and hormonal cues [7]. This is then integrated with signals from the reward system and the executive control system [8]. Hedonic processes however can (completely without awareness) override the homeostatic system and lead to obesity via an excessive energy intake relative to metabolic needs [9, 10].

A large body of work has shown that in obese persons brain function is altered in areas and neuronal networks involved in energy regulation, both the homeostatic and hedonic areas have been shown to be affected [11-15]. Generally, in obese persons brain connectivity and activity in specific (sub)cortical brain areas and neuronal networks is increased compared to lean subjects [11, 14-16]. Furthermore, responses to fasting and food intake have also been shown to be different between obese and lean subjects, predominantly demonstrating an increased activity in brain areas involved in reward systems [11-13, 15]. Therefore, brain alterations in obesity might be involved in an increased energy intake versus the amount of energy needed for a stable body weight [3].

Although these various changes in brain function have been found in obesity in cross sectional studies, it is not known whether and which of these altered brain functions are reversible after significant weight loss. Therefore it remains unknown whether these alterations in brain function are cause or consequence of obesity. The aim of the current study was to investigate whether prolonged fasting and/or weight loss influences ('normalizes') neuronal brain activity in obese participants and if this correlates with alterations in metabolic markers. To distinguish between differences induced by feelings of hunger or weight loss, we examined the effect after prolonged fasting (48h) and after an 8 week diet intervention.

METHODS

Study population and design

For detailed description; methods have also been described in an earlier study by Wijngaarden et al. [15]. We included 14 obese but otherwise healthy (e.g., non-diabetic) participants at baseline. Inclusion criteria were: Caucasian, healthy, weight stable, with a normal fasting plasma glucose (≤5.6 mmol/L). Exclusion criteria were: a positive family history of diabetes type 2, smoking, medication use affecting glucose homeostasis and/or brain function,. The study was conducted in accordance with the Declaration of Helsinki, informed consent was obtained from all participants and the study was approved by the local institutional review board of the Leiden University Medical Center and registered in the Netherlands Trial Register under number NTR2401. Functional magnetic resonance imaging (fMRI) was performed after an overnight fast (baseline) and after a prolonged 48-h fast in all participants. Measurements were repeated after an 8 week weight loss intervention in 10 of participants.

MRI data acquisition

MRI scanning was performed on a Philips Achieva 3.0 T scanner using an 8-channel head coil (Philips Healthcare, Best, The Netherlands). For registration purposes anatomical high-resolution 3D T1- weighted images of the whole brain were acquired (ultra-fast gradient echo acquisition, repetition time (TR) 9.78 ms, echo time (TE) 4.59 ms, flip angle 8, 140 axial slices, FOV 224 mm x 244 mm, reconstructed in-plane resolution 0.875 mm x 0.875 mm, slice thickness 1.2 mm) along with a high-resolution T2*-weighted EPI scan (EPI factor 29, 84 axial slices scanned in ascending order, TR 2200 ms, TE 30 ms, flip angle 80, FOV 220 mm x 220 mm, voxel size 1.96 x 1.96 x 2.0 mm). Resting-state scans were acquired with T2*-weighted gradient echo-planar imaging (EPI factor 29, 160 volumes, 38 axial slices scanned in ascending order, TR 2200 ms, TE 30 ms, flip angle 80, FOV 220 mm x 220mm, isotropic voxel size 2.75 mm with a 0.25 mm slice gap).

MRI data preprocessing

MRI data were preprocessed and analyzed using Functional Magnetic Resonance Imaging of the Brain Software Library (FSL) version 5.0.8. [17]. Of all data sets, structural and functional, non-brain structures were removed using Brain Extraction Tool (BET) tool as implemented in FSL. The T1-weighted images were registered to the 2 mm isotropic MNI-152 standard space image (Montreal Neurological Institute, Montreal, QC, Canada) using non-linear registration with a warp resolution of 10 mm. The FMRI Expert Analysis Tool (FEAT) was used for motion correction with MCFLIRT, spatial

smoothing with a full width at half maximum of 3 mm, and high pass temporal filtering with a cut-off frequency of 0.01 Hz. The functional resting state images were registered to the corresponding T1-weighted images using Boundary-Based Registration (BBR) affine registration, using the high-resolution echo planar images as an additional registration step.

MRI data analysis BOLD changes

Whole brain BOLD signal intensities were compared between study visits according to Rombouts et al. [18]. In short, a single volume BOLD signal map was calculated by averaging the time series data. Average cerebral spinal fluid (CSF) signal of the BOLD image was determined by averaging all voxels within the masked CSF. This CSF mask was determined by selecting voxels located in the lateral ventricles on the segmented structural images. This approach decreases the possibility of including unwanted signal of other compartments than CSF. Next, in each subject, a normalized BOLD signal map was calculated by dividing each voxel's signal by the average CSF signal. Voxel-wise comparisons of normalized BOLD signal maps between study visits were done using the Randomize tool of FSL with a paired samples approach and using Threshold-Free Cluster Enhancement (TFCE) [19]. After voxel-wise comparisons mean BOLD signal was calculated for the clusters showing a significant change in BOLD signal compared to baseline. The statistical clusters were binarized and used as masks/region of interest to calculate mean BOLD signal in these clusters per subject for every study time point.

Statistical analysis of quantitative data

Changes in subject characteristics between study time points were analyzed by repeated measures ANOVA. Associations between average BOLD signal in the extracted clusters and metabolic markers were determined using Pearson's correlation coefficient. Associations were determined for glucose, insulin, leptin, weight and BMI. A $p<0.05$ was considered significant.

RESULTS

Subject characteristics at baseline and after diet intervention

We included 14 obese (2 males, 12 females, BMI 35.2 ± 1.2 kg/m^2) at baseline. Measurements were repeated after a 48-h fast in all participants and after an 8 week diet intervention in 10 of the participants (4 exclusions due to drop-outs and motion artifacts during scanning). Subject characteristics for the study group at the different study time points are shown in table 1.

Table 1. Subject characteristics

	baseline (n=14)	after 48-h fast (n=14)	after diet (n=10)
Age (years)	30 ± 3		
Height (m)	1.74 ± 0.08		
Weight (kg)	107 ± 16	104 ± 4*	95 ± 4*
BMI (kg/m^2)	35.2 ± 4.3	34.4 ± 1.2*	31.5 ± 1.3*
Fasted insulin (mU/L)	19.6 ± 7.9	3.2 ± 3.0*	19.9 ± 8.6
Fasted glucose (mmol/L)	5.0 ± 0.7	4.0 ± 0.6*	4.6 ± 0.7*
Fasted leptin (µg/L)	36.2 ± 13.3	20.6 ± 11.8*	13.7 ± 8*

Values in mean ± standard deviation, *significantly different from baseline at p<0.05 (repeated measure ANOVA)

Weight and BMI decreased significantly after the weight loss intervention with a mean weight loss of 12.7%. Leptin and glucose levels decreased significantly compared to baseline after both the 48-h fast and the weight loss intervention. Insulin levels were significantly decreased after the 48-h fast.

Qualitative and quantitative BOLD signal changes

The 48-h fast intervention did not lead to significant voxel-wise changes. The dietary weight loss intervention resulted in a significant voxel-wise decrease in BOLD signal in parts of the insula, orbito-frontal cortex and in areas of several frontal gyri (figure 1). The quantitative BOLD signal were determined in the clusters showing significant changes after weight loss. The mean quantitative BOLD signals for all three measured time points are shown in figure 2 for the three main clusters containing the OFC, the insula and middle and inferior frontal gyri. The mean BOLD signals in the OFC cluster and the insula clusters were significantly decreased after the diet intervention (p<0.0001) but not show changes in signal after the 48-h fast (p=0.252 and p=0.687 respectively). The mean BOLD signal in the middle and frontal gyri cluster was significantly decreased after weight loss (p<0.0001) but also showed a significant decrease in BOLD signal after the 48-h fast (p=0.043).

Correlation between BOLD activity and metabolic markers

Leptin - At all the three different time points (overnight fast, 48 hour fast and after weight-loss intervention), leptin showed a significant positive correlation with the activity in the orbitofrontal cortex (OFC), insula and frontal gyri. At baseline (overnight fast) the correlation coefficient for the BOLD signal in the OFC was 0.625 (p=0.017), for the insula it was 0.590 (p=0.026) and for the frontal gyri it was 0.745 (p=0.002). After the diet intervention the correlations were similar or stronger; the correlation coefficient

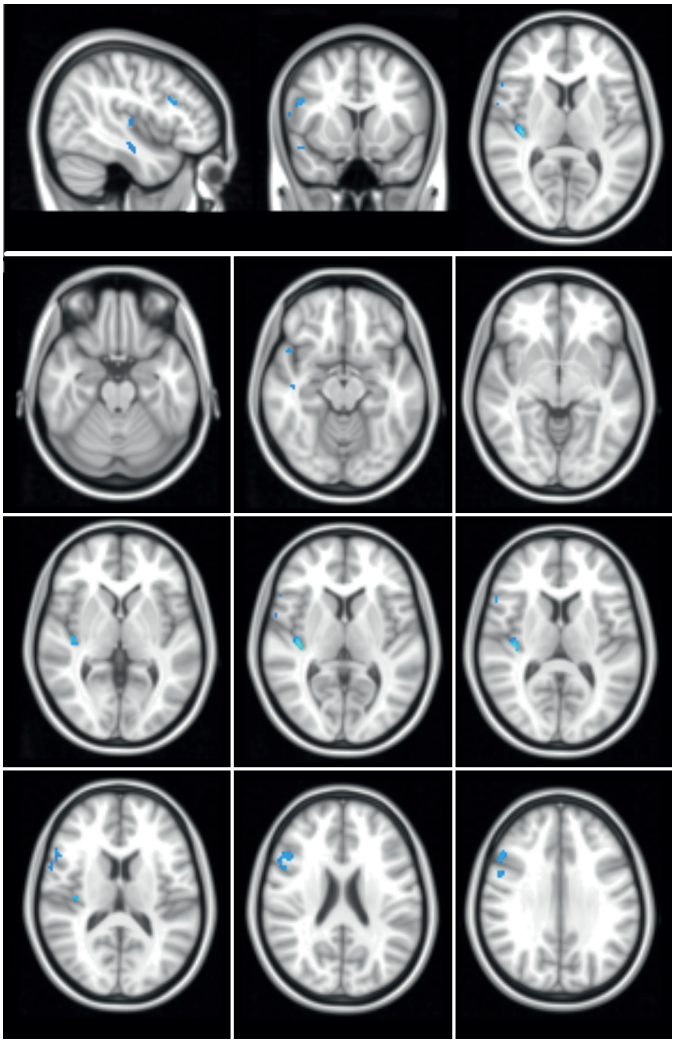

Figure 1. Brain areas with significant changes in BOLD signal/neuronal activity after diet intervention. Decreases in BOLD signal are shown in blue scale (FWE corrected).

for the BOLD signal in the OFC was 0.639 (p=0.047), for the insula it was 0.768 (p=0.009) and for the frontal gyri it was 0.742 (p=0.014).

BMI - BMI showed a significant positive correlation with the activity in the OFC and frontal gyri, but not the insula, at several but not all of the measured study time points. At baseline the correlation coefficient for the BOLD signal in the OFC was 0.433 (p=0.122) and 0.683 (p=0.007) for the frontal gyri. After the diet intervention the

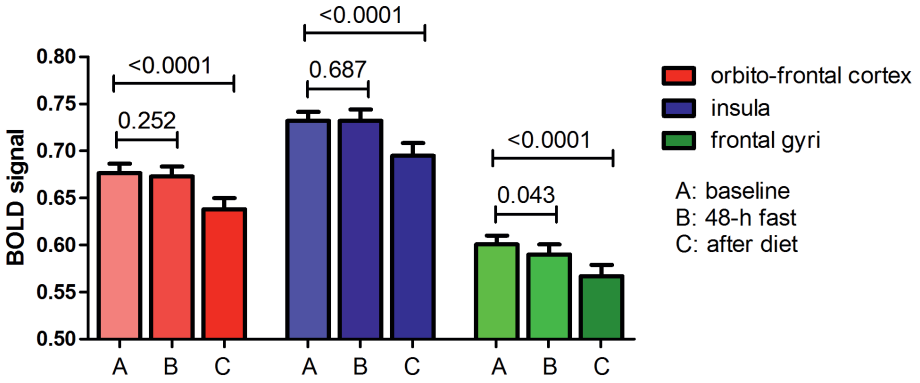

Figure 2. Quantitative BOLD signal in significantly affected clusters per study timepoint. Data depicted as mean with standard error.

correlation coefficient for the BOLD signal in the OFC was 0.684 (p=0.029) and 0.696 (p=0.026) for the frontal gyri. Scatter plots for the correlation at baseline between leptin and BMI with the BOLD signals in the clusters are shown in figure 3. Insulin/glucose - At baseline no association was found between insulin and glucose with the BOLD signal. After the 48-h fast only, insulin levels showed a positive correlation with the activity in all three clusters. The change in leptin, glucose, insulin and BMI after weight loss did not show a correlation with the change BOLD signal in the affected clusters.

DISCUSSION

In this study we compared fMRI data from obese individuals after an overnight fast with a 48-h fast and an 8-week weight loss intervention (again after an overnight fast). Our data show that an 8-week weight loss intervention, but not a 48-h fast, significantly decreased BOLD signal in parts of the insula, orbito-frontal cortex and in areas of several frontal gyri, indicating a decrease in neural activity in these brain areas. Additionally, we found that the neuronal activity in these areas was correlated with leptin and BMI.

The areas of the brain we found showing decreased neuronal activity after weight loss are brain areas that have previously been shown to be affected in obesity [11-15]. Most of these studies show that the activity and/or connectivity in these areas is increased in obesity compared to lean subjects. Therefore it might be that the decreases in activity

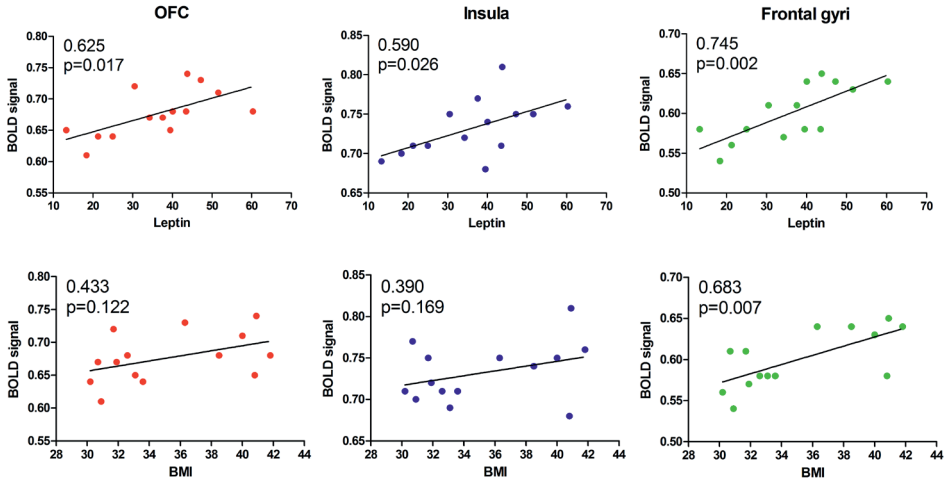

Figure 3. Scatter plots indicating the correlation between leptin and BMI and BOLD signal in the significantly affected clusters. Leptin in µg/L, BMI in kg/m

we found after weight loss in these areas is an indication of normalization of brain activity in these areas. However, one could also speculate that these alterations are the result of weight-loss that might even drive food intake and cause the so-called yo-yo effect. Interestingly, our findings are in line with a recent study showing alterations in the same brain areas (insula and frontal cortex) upon a weight loss intervention [20]. This study did look at changes in functional connectivity instead of BOLD activity, which is a different measure of brain function, but the areas affected by the weight loss intervention are nonetheless very similar. We found no voxel wise changes in BOLD signal after the 48-h fast in the current study. This suggests that the decreases in neuronal activity might not be caused by short term metabolic changes or changes in feelings of hunger and satiety caused by fasting, but are more likely related to actual decreases in body weight. However, in an earlier we study we did find several changes in functional connectivity between the hypothalamus and the insula and anterior cingulate cortex after an 48-h fast in obese participants using a seed-based approach [15]. In the current study, although we did not find whole brain effects of the 48-h fast, when looking at the mean BOLD signal in the affected clusters we did find a significant decrease in the frontal gyri cluster after the 48-h fast. A possible explanation for the results in our current and previous study is that the effects of fasting lead to more subtle changes in brain function, which can be detected with more specific region of interest analysis but do not lead to whole brain effects.

The brain areas showing decreases in activity after weight loss are known to be involved in regulation of energy balance and feeding behavior [6]. As mentioned, both the homeostatic control and hedonic and executive control systems work in concert to regulate energy balance [3, 6]. The areas we find showing a decrease in activity after weight loss are mainly involved in the hedonic and executive control systems and less in the homeostatic system. The insula is involved in determining salience of incoming stimuli [21] and in taste perception [22]. Due to the combination of these functions the insula is therefore involved in palatable feeding. The insula has been shown to have a stronger BOLD response to ingestion of sucrose in obese adolescents compared to lean subjects [23]. Similar to the insula, the orbito-frontal cortex is also involved in determining salience due to its role in the limbic system and executive functioning [24, 25]. The orbito-frontal cortex is important in determining reward and affective value of food via stimuli including taste [26]. Furthermore, the orbito-frontal cortex has been indicated as an important structure in termination of food intake and this function has been shown to be disrupted in obesity [27]. The decreases in neuronal activity in both the insula and orbito-frontal cortex found in our study could indicate a change in reward and salience response after weight loss. Furthermore, the areas we found responding to weight loss showed overlap with brain areas that responded to glucose ingestion in an earlier study by our group [28]. In this study we found the areas that decrease in activity in the insula, thalamus, anterior cingulate gyrus, orbito-frontal cortex, amygdala, hippocampus and the occipital cortex in response to glucose, these areas are involved in hunger and satiety, indicating a satiety response after energy ingestion. This indicates that the decrease neuronal activity in these brain areas might lead to a different satiety response after weight loss.

A main limitation of our study is the study population. The sample size of our study is limited and consist mostly of female participants due to a low response to our advertisement, especially by male participants. Furthermore, it would be an interesting – but probably unethical – experiment to compare the effects of weight loss we found in our obese participants to the effects of weight loss in lean subjects to determine if the effects are caused by loss of excess weight or weight loss in general. Additionally it would be interesting to follow up our participants after the weight loss to determine if the effects on the brain activity are maintained when participants return to a less restrictive diet.

In addition to our finding of significant changes in neuronal activity after weight loss we found that the neuronal activity in the responsive areas was correlated with hormonal and metabolic markers of obesity. Central regulation of energy balance is known to be influenced by peripheral hormonal signal, both in response to energy intake but also in response to long term signals of energy balance such as leptin.[7,

29] Earlier research has shown that various functional brain responses are affected by hormonal signals such as insulin and leptin.[30, 31] Our data show a positive correlation between leptin and BMI and the BOLD signal in the affected brain areas, indicating that high BMI and high leptin levels are associated with a high activity in these brain areas. The change in leptin, glucose, insulin and BMI after weight loss did not show a correlation with the change BOLD signal in the affected clusters, perhaps due to our small sample size and therefore limited statistical power. However, weight loss did lead to decreases in BMI and leptin levels and to decreases in neuronal activity in our study. This further substantiates that the activity in these brain areas might be related to body weight instead of as leptin, insulin and BMI are all correlated with fat mass and body weight.

Taken together, we studied brain activity before and after a weight-loss intervention. We found a decreased activity in brain areas important for feeding behavior and reward processing. Furthermore, the neuronal activity in these brain areas was correlated with metabolic markers of obesity. These results might indicate that alterations in neural activity are reversible, although we did not compare our data with lean individuals in this study. Besides, since it is well known that a lot of obese patients cannot maintain weight-loss for a longer period of time, the exact connotations of these findings still need to be established.

Acknowledgments and conflicts of interest

We would like to thank Bep Ladan-Eygenraam and Mirjam Lips for their practical assistance. This work was supported by the Netherlands Consortium for Systems Biology (NCSB) and Center for Medical Systems Biology (CMSB) within the framework of the Netherlands Genomics Initiative (NGI/NWO). Authors have no personal conflict of interest to disclose.

REFERENCES

1. Withrow, D. and D.A. Alter, *The economic burden of obesity worldwide: a systematic review of the direct costs of obesity.* Obes. Rev, 2011. 12(2): p. 131-141.
2. Spiegelman, B.M. and J.S. Flier, *Obesity and the regulation of energy balance.* Cell, 2001. 104(4): p. 531-543.
3. Morton, G.J., T.H. Meek, and M.W. Schwartz, *Neurobiology of food intake in health and disease.* Nat. Rev. Neurosci, 2014. 15(6): p. 367-378.
4. Schwartz, M.W. and D. Porte, Jr., *Diabetes, obesity, and the brain.* Science, 2005. 307(5708): p. 375-379.
5. Schwartz, M.W., et al., *Central nervous system control of food intake.* Nature, 2000. 404(6778): p. 661-671.
6. Morton, G.J., et al., *Central nervous system control of food intake and body weight.* Nature, 2006. 443(7109): p. 289-295.
7. Kim, K.S., R.J. Seeley, and D.A. Sandoval, *Signalling from the periphery to the brain that regulates energy homeostasis.* Nat. Rev. Neurosci, 2018. 19(4): p. 185-196.
8. Blouet, C. and G.J. Schwartz, *Hypothalamic nutrient sensing in the control of energy homeostasis.* Behav. Brain Res, 2010. 209(1): p. 1-12.
9. Volkow, N.D., R.A. Wise, and R. Baler, *The dopamine motive system: implications for drug and food addiction.* Nat. Rev. Neurosci, 2017. 18(12): p. 741-752.
10. Berthoud, H.R., H. Munzberg, and C.D. Morrison, *Blaming the Brain for Obesity: Integration of Hedonic and Homeostatic Mechanisms.* Gastroenterology, 2017. 152(7): p. 1728-1738.
11. Garcia-Garcia, I., et al., *Reward processing in obesity, substance addiction and non-substance addiction.* Obes. Rev, 2014. 15(11): p. 853-869.
12. Garcia-Garcia, I., et al., *Alterations of the salience network in obesity: a resting-state fMRI study.* Hum. Brain Mapp, 2013. 34(11): p. 2786-2797.
13. Kullmann, S., et al., *Functional network connectivity underlying food processing: disturbed salience and visual processing in overweight and obese adults.* Cereb. Cortex, 2013. 23(5): p. 1247-1256.
14. Lips, M.A., et al., *Resting-state functional connectivity of brain regions involved in cognitive control, motivation, and reward is enhanced in obese females.* Am. J. Clin. Nutr, 2014. 100(2): p. 524-531.
15. Wijngaarden, M.A., et al., *Obesity is marked by distinct functional connectivity in brain networks involved in food reward and salience.* Behav. Brain Res, 2015. 287: p. 127-134.
16. Zhang, B., et al., *Altered baseline brain activity differentiates regional mechanisms subserving biological and psychological alterations in obese men.* Sci. Rep, 2015. 5: p. 11563.
17. Jenkinson, M., et al., *FSL.* Neuroimage, 2012. 62(2): p. 782-790.
18. Rombouts, S.A., et al., *Whole brain analysis of T2* weighted baseline FMRI signal in dementia.* Hum. Brain Mapp, 2007. 28(12): p. 1313-1317.
19. Smith, S.M. and T.E. Nichols, *Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference.* Neuroimage, 2009. 44(1): p. 83-98.
20. Garcia-Casares, N., et al., *Brain Functional Connectivity Is Modified by a Hypocaloric Mediterranean Diet and Physical Activity in Obese Women.* Nutrients, 2017. 9(7).
21. Uddin, L.Q., *Salience processing and insular cortical function and dysfunction.* Nat. Rev. Neurosci, 2015. 16(1): p. 55-61.
22. Small, D.M., *Taste representation in the human insula.* Brain Struct Funct, 2010. 214(5-6): p. 551-61.
23. Boutelle, K.N., et al., *Increased brain response to appetitive tastes in the insula and amygdala in obese compared with healthy weight children when sated.* International Journal Of Obesity, 2014. 39: p. 620.
24. Kringelbach, M.L. and E.T. Rolls, *The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology.* Prog Neurobiol, 2004. 72(5): p. 341-72.
25. Rolls, E.T. and F. Grabenhorst, *The orbitofrontal cortex and beyond: from affect to decision-making.* Prog Neurobiol, 2008. 86(3): p. 216-44.
26. Small, D.M., et al., *The role of the human orbitofrontal cortex in taste and flavor processing.* Ann N Y Acad Sci, 2007. 1121: p. 136-51.
27. Shott, M.E., et al., *Orbitofrontal cortex volume and brain reward response in obesity.* Int J Obes (Lond), 2015. 39(2): p. 214-21.
28. van Opstal, A.M., et al., *Brain activity and connectivity changes in response to glucose ingestion.* Nutr. Neurosci, 2018: p. 1-8.
29. Liu, S. and S.L. Borgland, *Regulation of the mesolimbic dopamine circuit by feeding peptides.* Neuroscience, 2015. 289: p. 19-42.
30. Labouebe, G., et al., *Insulin induces long-term depression of ventral tegmental area dopamine neurons via endocannabinoids.* Nat. Neurosci, 2013. 16(3): p. 300-308.
31. Pan, W.W. and M.G. Myers, Jr., *Leptin and the maintenance of elevated body weight.* Nat. Rev. Neurosci, 2018. 19(2): p. 95-105.