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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences

Master of Biomedical Sciences

Master's thesis

Tissue-specific insulin sensitivity and changes in brain reward reactivity in overweight or obese pre-diabetic adults

Benthe Cloosen

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Environmental Health Sciences

SUPERVISOR :
dr. Tanja ADAM

MENTOR :
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*Running title: *Brain reward activity and insulin sensitivity.*

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ABSTRACT

The obesity epidemic increases the global burden of disease. Feeding for pleasure, controlled by the brain's reward system, maybe an important driver of obesity. Insulin is crucial in suppressing food intake, as it regulates appetite by signalling rewarding circuits. Overweight individuals tend to suffer from brain insulin resistance, which diminishes insulins' ability to suppress feeding. Given that the link between peripheral insulin resistance and increased brain reward reactivity to food cues has already been demonstrated, the current research aims to investigate the link with tissue-specific insulin resistance. Therefore, we primarily hypothesize that increased hepatic insulin sensitivity is associated with reduced brain reward reactivity to food cues. fMRI scans were performed to assess changes in brain reward reactivity to (non)food cues in 27 overweight or obese pre-diabetic subjects, before and after a 2-month low-energy diet followed by a 22-month weight maintenance period with dietary guidelines for a high or medium protein content. Results indicate that a trend was observed for the positive association between changes in HOMA-B and changes in brain reward reactivity to food cues. Changes in body weight and fat mass were positively associated with HOMA-IR measured at the study end. No associations with tissue-specific insulin sensitivity were found. A trend was observed for the gender-specific difference in the reactivity of the left amygdala to food cues. This research provides more insight into the role of insulin and the brain's reward system in obesity. Future studies are needed to further explore the role of tissue-specific insulin sensitivity.

1. INTRODUCTION

1.1. Obesity and its related problems

Over the past 50 years, the prevalence of obesity has globally increased, reaching pandemic levels (1). More specifically, obesity rates have more than doubled in most countries, with more than 1.9 billion overweight adults and 650 million obese adults worldwide, according to the World Health Organization (WHO) (2). This metabolic condition represents a sincere public health threat by increasing the prevalence of non-communicable diseases, including type 2 diabetes mellites (T2DM), cardiovascular disease, and some types of cancer (1). In general, the development of obesity occurs when the caloric intake is higher than the energy expenditure, causing a chronic positive energy balance (3). While genetic predisposition increases the likelihood of developing obesity, several other factors can also cause its origination. Environmental factors such as the abundant availability and the relatively inexpensive cost of energy-dense foods, exposure to psychological stressors of society, and reduced physical activity, are considered key contributors to the obesity epidemic (4-6). Fortunately, obesity and its related metabolic diseases are preventable. Many people manage to lose weight with the help of modern strategies that improve dietary habits and energy balance (e.g. portion-controlled meals, low-fat or

low-carbohydrate diets, intermittent fasting, high-intensity training (7, 8)). However, maintaining body weight after weight loss is the biggest challenge, as many people fail to maintain even 50% of their initial weight loss after 1 year (8). Physiological metabolic responses to weight loss, such as increased ghrelin levels (9) (a gut peptide associated with increased appetite), reductions in resting energy expenditure (10), and leptin (a hormone associated with satiety) (11), are designed to protect the human body from the adverse effects of starvation (12) and may therefore be at the root of this weight regain problem. Furthermore, another important factor within this problem may be the changes in neuronal systems that regulate food intake. Previously, it has been suggested that the "abnormal" neuronal responses to food from the brain areas that regulate feeding behaviour in obese and diminished obese individuals may lead to a predisposition to obesity (13).

1.2. Tissue-specific insulin resistance

Obesity is a condition that is firmly linked to insulin resistance and T2DM (14). Interestingly, research has demonstrated that insulin resistance may develop independently in distinct organs (15). Accordingly, it has been suggested that insulin resistance is usually present in various tissues for some time before obvious clinical conditions such as T2D develop (16). Moreover, the liver and skeletal muscle are considered insulin-sensitive organs (15). While reduced hepatic insulin sensitivity results in increased or less suppressed glucose production within the liver, insulin resistance in skeletal muscle is associated with decreased uptake of glucose into the muscle (17, 18). Besides the liver and skeletal muscle, the human brain is also an insulin-sensitive organ (19). Insulin can suppress feeding behaviour by acting on brain regions that regulate appetite and metabolism (20). However, previous studies have demonstrated that peripheral insulin resistance reduces the insulin permeability of the blood-brain barrier by diminishing the number of endothelial insulin receptors, causing insulin resistance of the brain (21, 22). The latter implies a decrease in the ability of insulin to suppress feeding (20).

1.3. The brain's reward system

Food intake and body weight regulation are mainly determined by the hypothalamus, representing the centre of homeostatic food intake regulation. It coordinates a diversity of signals to regulate neuronal, hormonal, and nutritional processes(23). The reward system regulates the hedonic (non-homeostatic) control of feeding behaviour and can possibly override the homeostatic control of food intake (24). The brain's reward system is made up of multiple structures that play a critical role in establishing proper goal-directed behaviour by coordinating aspects of reward, cognition, procedural learning, and motor control (25). The mesocorticolimbic pathway plays a central role within this neural system. It originates from dopaminergic neurons in the ventral tegmental area (VTA) of the midbrain, inducing descending projections to the regions in the limbic forebrain, most notably the nucleus accumbens, as well as the pre-frontal cortex (26). The pre-frontal cortex in turn provides descending projections to the nucleus accumbens and the VTA (27). When the body runs low on nutrients, appetite signalling will initiate food intake. However, food also elicits pleasurable and rewarding signals, mediated in part via dopamine release in the mesolimbic circuitry, which can override satiety and further stimulate appetite (28). For instance, the consumption of foods rich in sugar or fat stimulates the brain's reward system promoting eating far beyond the individual's nutritional needs (29). This hedonic signalling in response to palatable calorically dense foods is increasingly discussed as a major underlying cause of the worldwide increase in obesity (30). Therefore, the brain's reward system received a lot of attention as it may be an important driver in the development of obesity (31).

1.4. Brain insulin signalling

Insulin, a hormone produced by pancreatic β-cells, has the ability to alter feeding behaviour by relaying information about peripheral fat stores to the hypothalamus (32, 33). When the energy targets are reached and adipose tissue is restored, the insulin hormone acts as a satiety signal to reduce food intake (20). Separate from the hypothalamic brain circuits, insulin receptors have also been found in

the striatum, a part of the dopamine system, substantiating a role for insulin in the rewarding or hedonic aspects of feeding (34). More specifically, it has been suggested that insulin has the ability to regulate dopamine release and reuptake within the brain's reward system (35). In addition, a study in healthy, sated humans demonstrated that intranasal insulin administration reduces the responses of dopamine-affected brain regions to food images (36). Likewise, the food palatability ratings in mesolimbic regions decreased after administration in normal insulin-sensitive individuals (37). Furthermore, higher body weight has already been shown to be associated with insulin resistance in the human brain (19, 38). Consistently, a study has shown that insulin can regulate the striatal function in lean men, whereas overweight men do not respond to insulin, indicating insulin resistance of the brain (39). Additionally, another study has also demonstrated that having T2DM increases the brain responses to food pictures in comparison with healthy individuals (40). Lately, many advances have been made in understanding the role of brain insulin signalling in food behaviour. In addition to animal studies, many human studies have been conducted on this topic. For instance, one study revealed that reward-related brain regions show stronger functional connectivity in subjects with lower insulin sensitivity indices, which can be postulated as "over-activation" of these brain areas within the reward system (14). Furthermore, other studies have also shown that decreased insulin sensitivity or insulin resistance is positively associated with higher brain reward reactivity to food cues in overweight or obese subjects (40, 41). Although previous research has already demonstrated the important link between peripheral insulin sensitivity and brain reward responsivity, no detailed investigation has yet been conducted to examine the effect of hepatic and muscle insulin sensitivity on the brain's reward system. Nevertheless, investigating this possible link between tissue-specific insulin resistance and brain reward reactivity could be interesting, as insulin resistance may often occur in insulin-sensitive organs before systemic insulin resistance develops.

1.5. Weight loss

Eating food is necessary for survival. The brain's reward system is believed to have evolved to drive these behaviours essential to survival (42). However, several studies have already proposed that disrupted brain reward pathways may not only be involved in drug addiction but also be associated with overconsumption of palatable foods, which can eventually lead to obesity (43-45). Previously, it has been shown that overweight individuals experience stronger appetitive sensations in response to visual or olfactory palatable food stimuli compared to lean individuals (46). This increased sensitivity to food rewards in overweight or obese individuals, caused by either a hyperactivation of the hedonic system (47, 48) or a dopaminergic hypofunction (49, 50), is blamed for excessive food consumption and weight gain. In addition, neuroimaging studies have identified obesity-related changes in brain activity and connectivity, supporting the importance of brain alterations in the pathophysiology of obesity (51, 52). More precisely, associations have been shown between increased body weight and structural and functional changes in areas of the core reward network (such as the orbital frontal cortex and nucleus accumbens) but also the expanded reward network (53). Consistently, a study within obese individuals has revealed that losing weight appears to reduce neuronal responses of brain reward areas to high-calorie food picture stimuli (54). These findings were further confirmed by a study in which weight loss induced by bariatric surgery also reduced the responsiveness of brain reward regions to food images, which reflects the positive influence of weight loss on the brain response (55). Furthermore, weight loss is recognized as one of the most powerful strategies to reduce insulin resistance (56-59). However, further studies should also confirm these results.

1.6. Gender differences

Currently, our understanding of the biological mechanisms underlying obesity in humans is confined. An obstacle to progress is the poor understanding of gender differences in these underlying mechanisms of obesity (60). Worldwide, women are 2 percent more likely to become overweight or obese compared to men (2).

In addition, they are also more likely to suffer from obesity-related diseases (61). An explanatory mechanism could be gender differences in craving. Food craving is strongly related to eating and weight gain (62), potentially leading to an increased risk of obesity (63). Additionally, meta-analyses of brain imaging studies state that key brain regions involved in cue-reactivity to alcohol, drugs of abuse, smoking, and eating largely overlap and are part of the reward processing brain network (64, 65), suggesting that food cravings mediate addictive eating behaviour and thus increase body weight (66). Sex differences have been observed in the types of foods craved, while women report more cravings for sweets, men crave more salty foods (67, 68). Furthermore, gender differences in the intensity and frequency of cravings have also been reported, with women reporting more cravings (69, 70). Lastly, more women than men indicate that the ability to regulate or limit cravings cognitively is difficult (69). Additionally, women appear to be more sensitive to neuronal adaptations associated with obesity, which may also contribute to the gender-related difference in the prevalence of obesity and other eating disorders (71). However, at this point, there is still much room for further research on these gender-related issues.

1.7. The PREVIEW study and hypothesis

In summary, the effects of weight loss and insulin sensitivity on the brain's reward system need further clarification and substantiation. Besides, more research on gender differences in brain reward reactivity could clarify the differences in obesity prevalence rates between males and females For the current thesis, data of the Prevention of Diabetes through Lifestyle Intervention and Population Studies in Europe and around the World (PREVIEW) intervention study will be used to assess the aforementioned research gaps. Within the PREVIEW study, overweight or obese individuals with impaired fasting glucose, impaired glucose tolerance, or both, were enrolled. During the study, the participants were asked to follow a low-energy diet that lasted for 8 weeks, followed by a 22-month weight maintenance period, with dietary guidelines for either medium or high protein intake (72). Furthermore, the homeostatic model assessment for insulin resistance (HOMA-IR) was used to determine the peripheral insulin sensitivity of the individuals. Consequently, Drummen *et al.* have demonstrated a positive association between increased peripheral insulin resistance and higher brain reward reactivity to food cues within the PREVIEW participants (73). To expand this further, the aim of the present thesis was to investigate whether there is an association between tissue-specific insulin resistance and brain reward reactivity in response to food cues and whether brain reward reactivity to food cues differs between genders. Consequently, we hypothesize that increased hepatic insulin sensitivity is associated with reduced brain reward reactivity to food cues. Secondly, we hypothesize that brain reward responses to food cues are higher in females in comparison to males. Eventually, this study provides a novel contribution to science by providing new insights into the potential gender-specific role of the brain's reward system within obesity and how tissue-specific insulin sensitivity can be linked with this.

2. EXPERIMENTAL PROCEDURES

2.1. Study population

The PREVIEW study included 27 overweight (n=6) and obese (n=21) participants (13 women, 14 men; mean age±SD: 53.6 ± 10.27). To classify participants as overweight (BMI ≥25 and <30) or obese (BMI ≥30), body mass index (kg/m^2) was used. In order to be eligible to participate in the current study, a subject had to meet all of the following criteria: participants needed to have an age of 25 up to and including 70 years old with a BMI equal to or more than 25 kg/m^2 (no upper limit), and must be pre-diabetic (a fasting plasma glucose of 5.6-6.9 mmol/L and/or plasma glucose concentration of 7.8-11.0 mmol/L at 2 hours after an oral glucose tolerance test (OGTT)). Ultimately, participants needed to show a willingness to undergo fMRI procedures. Ultimately, specialized fMRI-related exclusion criteria were constructed for the fMRI scans: subjects that suffer from claustrophobia; left-handedness; and neurological disorders. Furthermore, a detailed description of the recruitment and all (fMRI-related) exclusion criteria can be found in the supplementals.

2.2. Dietary interventions

First, all the participants were enrolled in a 2-month low-energy diet (LED) based on a range of formula products of the Cambridge Weight Plan (Northants, UK). Eventually, participants were required to lose at least 8% of their initial body weight using the LED, otherwise, they were excluded from the study. After the LED, participants were enrolled in a 22-month weight maintenance period during which they were randomly assigned to one of two dietary intervention groups. Both groups focused on protein intake, with one comprising a moderate protein (MP) and the other a high protein (HP) group. The diets were consumed ad libitum concerning energy, but participants were instructed to maintain the weight loss achieved after the LED phase. Besides, additional reductions in BMI were allowed. A detailed description of the dietary intervention can be found in the supplementals.

2.3. Procedures

2.3.1. Clinical investigation days

After that participants were recruited, screened for eligibility, and had given informed consent, they were scheduled for 2 clinical investigation days (CIDs) at the beginning (CID1) and the end of the study (CID6). During these CIDs, participants were asked to be in a fasted state for a minimum of 10 hours. Furthermore, different anthropometric measurements were completed. In addition, fasting blood samples were collected and a 2-hour Oral Glucose Tolerance Test (OGTT) was conducted. For the OGTT, participants were asked to consume a drink containing 75 g glucose in approximately 5 min. Subsequently, blood samples were collected at time points T=0, T=30, T=60, T=90, and lastly, T=120 min. Furthermore, fMRI assessments were scheduled together with CID 1 (baseline) and 6 (month 24, end of the study).

2.3.2. Tissue-specific insulin sensitivity

To determine tissue-specific insulin sensitivity, the Hepatic Insulin Resistance Index (HIRI) and Muscle Insulin Sensitivity Index (MISI) were calculated using a 5-time point OGTT. During the first 30 minutes after the 75 g glucose ingestion, the HIRI is calculated as the product of the area under the curves (AUCs) for glucose and insulin (74). Since the (in)ability of insulin to suppress hepatic endogenous glucose production is reflected by the magnitude of the rise in blood glucose and insulin concentrations during the first 30 minutes (75). The subsequent decrease in blood glucose concentrations after 60 minutes reflects the uptake of glucose by peripheral tissues, primarily the skeletal muscle (75). Accordingly, the MISI is calculated as the rate of glucose concentration decay during the OGTT divided by the mean insulin concentration during the OGTT (74).

2.3.3. fMRI assessment

To investigate brain reward reactivity, functional magnetic resonance imaging (fMRI) was performed to obtain blood-oxygen-level-dependent (BOLD) data from the participants. For the fMRI assessment, participants were instructed to be in an overnight fasted state for a minimum of 10 hours. During the scanning, 9 blocks of each 10 images were shown to the subjects. Each image was displayed for 2 s, and between the 9 blocks, a white cross was shown on a black screen for 10 s. These blocks consisted of high-calorie food images, low-calorie food images, and non-food images. The International Affective Picture and numerous other websites were used to select the images (76). High-calorie food images included fries, mac-and-cheese, hamburgers, donuts, etc. While low-calorie food images included fruit salad, cucumbers, carrots, broccoli, etc. All the participants viewed the same set of images, but in a randomized order. To avoid preference, and learning effects, 60 different images of food were used and randomly shown. Furthermore, participants were instructed to focus on how much they liked the images. All functional and structural fMRI data were obtained with a 3 Tesla scanner (Magneto, Siemens, Erlangen, Germany) using a 64-channel head coil.

2.4. MRI data analysis

For the analysis of the fMRI data, Brain Voyager 20.6 (Brain Innovation B.V, Maastricht, The Netherlands) was used. To investigate the activation of predefined reward processing brain regions, the Regions Of Interest (ROI) analysis was performed. The insula, anterior cingulum, amygdala, and striatum (caudate, putamen,

pallidum) were taken up within the constructed ROI mask (28, 77, 78).

2.5. Statistical analysis

All the analyses were performed using the IBM SPSS statistics version 23 program. All the variables were checked for normality and log-transformed when applicable. Pearson's correlation analysis was used to determine the relationship between (tissue-specific) insulin sensitivity indices and changes in the reactivity of selected brain reward areas. The same was performed to determine the association between changes in weight or fat mass and changes in the reactivity of predefined brain reward regions. Furthermore, Pearson's correlation analysis was also used to determine the relationship between changes in body weight or fat mass and (tissue-specific) insulin sensitivity indices. Eventually, partial correlation analysis was used to determine the previous relationships when adjusted for BMI at baseline, age, and gender. All analyses were run with and without outliers, from which it could be concluded that this made no difference (72). To investigate differences in weight change, fat mass change, or changes in the reactivity of selected brain reward areas between the sexes, univariate analysis was used. To correct for multiple comparisons between the selected brain reward regions, a $p < 0{,}004$ was considered significant.

Table 1 – *Characteristics of the study population at baseline (N=25)*

	No tissue-specific IR	Liver IR	Muscle IR	M + L IR	p^*
N	6	9	2	8	
Male/Female (%)	33.3/66.7	88.9/11.1	100/0	87.5/12.5	
Age (years)	60.0 ± 1.0	54.1 ± 3.51	44.0 ± 11.0	49.0 ± 4.0	
Weight (kg)	85.4 ± 8.08	106.3 ± 3.53	100.5 ± 7.60	88.0 ± 5.19	0.038
BMI (kg/m^2)	29.8 ± 1.72	32.6 ± 0.91	31.7 ± 0.33	32.1 ± 1.25	0.474
Waist circumference (cm)	97.4 ± 7.74	110.0 ± 2.34	101.5 ± 0.50	98.8 ± 3.42	0.128
Hip circumference (cm)	103.9 ± 2.87	110.8 ± 2.10	106.0 ± 1.5	109.8 ± 3.23	0.329
Fat mass (kg)	34.9 ± 3.89	38.8 ± 3.42	40.0 ± 0.97	39.8 ± 3.42	0.797
Body fat (%)	40.98 ± 2.31	36.1 ± 2.35	40.0 ± 2.05	45.07 ± 1.98	0.053
Fasting glucose (mmol/L)	6.1 ± 0.26	6.3 ± 0.08	6.9 ± 0.40	6.3 ± 0.20	0.339
Fasting insulin (mU/L)	6.9 ± 1.22	14.3 ± 1.57	15.8 ± 3.35	18.9 ± 2.05	0.002
HOMA-IR	1.9 ± 0.35	4.0 ± 0.46	4.8 ± 0.74	5.3 ± 0.69	0.003
HOMA-B	53.6 ± 8.62	102.5 ± 10.63	96.2 ± 30.85	135.3 ± 11.94	0.001
MISI	0.25 ± 0.03	0.17 ± 0.02	0.05 ± 0.002	0.04 ± 0.01	< 0.000
Log-transformed	-0.6 ± 0.07	-0.7 ± 0.04	-1.2 ± 0.02	-1.4 ± 0.12	
HIRI	282.7 ± 48.74	742.8 ± 56.9	457.4 ± 38.5	943.9 ± 100.93	< 0.000

This table shows the characteristics of all the individuals within the PREVIEW study sample. In total, 13 women and 14 men participated in the study. However, this table only shows 25 participants (12 women and 13 men) due to missing values of the OGTT. The subjects were divided into 4 different insulin resistance (IR) groups: no insulin resistance (No IR), liver insulin resistance (Liver IR), muscle insulin resistance (Muscle IR), and insulin resistance of the muscle and liver (M + L IR). For each group, the means and standard deviation values of the various measurements performed at baseline (CID1) are reported, including body weight (kg), Body Mass Index (kg/m^2), waist circumference (cm), hip circumference (cm), body fat mass (kg), total body fat percentage (%), fasting glucose (mmol/L), fasting insulin (mU/L), Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), Homeostatic Model Assessment for β-cell function (HOMA-B), Muscle Insulin Sensitivity Index (MISI), and Hepatic Insulin Resistance Index (HIRI). * Based on analysis of variance for normally distributed data (one-way ANOVA).

3. RESULTS

The present study examines the changes in the reactivity of selected brain reward regions between baseline (CID1) and the end of the intervention (CID6). The activity of the selected brain reward areas to food images was contrasted with non-food images. To evaluate peripheral insulin sensitivity the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and the Homeostatic Model Assessment for ß-cell function (HOMA-B) were used. While MISI and HIRI were used to assess tissue-specific insulin sensitivity. Furthermore, all the baseline (CID1) characteristics of 25 participants are shown in Table 1. Due to missing OGTT values, this table contains 25 instead of 27 participants. Within Table 2, it is shown how the participant characteristics have changed over the 2-year intervention period. For the whole population, there was a significant reduction in all the anthropometric measurements (see Table 2). In addition, the insulin sensitivity measured by the different peripheral and tissue-specific indices significantly increased, except for MISI and HOMA-B (see Table 2). Furthermore, the correlations between the different (tissue-specific) insulin sensitivity indices measured at different time points can be found in Table S1 within the supplementals. These time points include baseline (CID1), the end of the study (CID6), and changes between baseline and the end of the study (see Table S1 in supplementals).

3.1. Association between peripheral insulin sensitivity and brain reward reactivity to food cues

To examine whether peripheral insulin sensitivity affects brain reward reactivity, we assessed whether there was a correlation between HOMA-IR and HOMA-B at baseline and changes in the reactivity of selected brain reward regions to food cues (see Table S2 within supplementals). In addition, we also assessed whether there was a correlation between the changes in HOMA-IR and HOMA-B before and after the intervention and changes in the reactivity of selected brain reward areas to food cues (see Table S3 within supplementals). Eventually, no significant associations were found between the peripheral insulin sensitivity indices at baseline and changes in the reactivity of predefined brain reward regions to food cues. Likewise, no significant associations were found between changes in peripheral insulin sensitivity indices and

Table 2 – *Changes in participant characteristics over the 2-year intervention period (N=27)*

	Baseline (CID1) N = 27	**2 Years (CID6) N = 27**	***p****
Male/Female (%)	51.9/48.1		
Weight (kg)	94.0 ± 16.6	85.7 ± 16.0	< 0.000
BMI (kg)	31.8 ± 3.23	29.1 ± 3.81	< 0.000
Waist circumference (cm)	102.9 ± 12.0	98.9 ± 12.8	0.024
Hip circumference (cm)	108.2 ± 7.29	104.1 ± 10.6	0.006
Fat mass (kg)	38.0 ± 8.95	31.5 ± 11.3	< 0.000
Body fat (%)	40.6 ± 6.93	36.6 ± 9.83	< 0.000
Fasting glucose (mmol/L)	6.3 ± 0.63	5.91 ± 0.54	0.001
Fasting insulin (mU/L)	13.8 ± 6.39	10.3 ± 4.49	0.003
HOMA-IR	3.97 ± 1.96	2.78 ± 1.41	0.002
Log-transformed	-	1.55 ± 0.04	
HOMA-B	97.5 ± 42.4	86.4 ± 30.5	0.052
MISI	0.14 ± 0.10	0.15 ± 0.12	0.828
Log-transformed	-0.99 ± 0.42	-0.94 ± 0.37	
HIRI	673.9 ± 323.7	464.4 ± 230.1	0.001

This table shows how the characteristics of the participants have changed over time, from baseline to the end of the intervention. Data are presented as mean ± standard deviations. The total number of participants is 27 (13 women and 14 men), except MISI and HIRI at baseline, which could only be calculated for 25 participants. The MISI and HIRI at the end of the intervention were calculated for 26 participants. This is due to missing OGTT values. * Whether the changes between baseline and the end of the intervention are significantly different was determined using paired samples t-test.

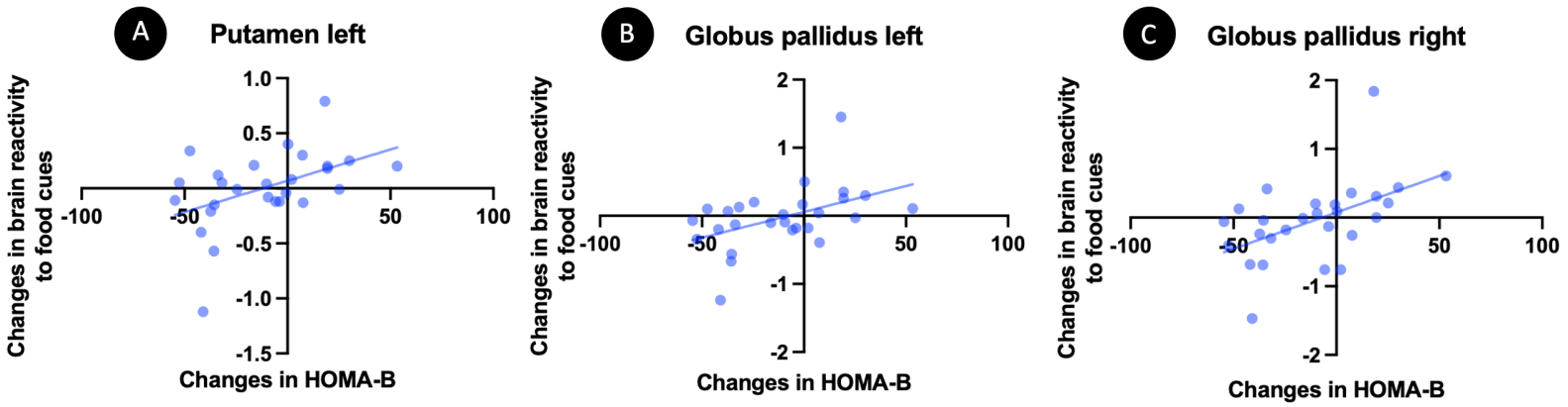

Fig. 1 – These scatterplots show the trends observed for the positive associations between changes in HOMA-B and the changes in the reactivity of the putamen left (A), the globus pallidus left (B), and the globus pallidus right (C) to food cues.

changes in the reactivity of predefined brain reward areas to food cues. However, trends could be observed for the positive associations between the changes in HOMA-B and changes in the reactivity of the putamen left (r = 0.465; p = 0.022), globus pallidus left (r = 0.480; p = 0.018), and globus pallidus right (r = 0.510; p = 0.011) after adjusting for BMI, age, and gender (Fig. 1). These trends could not be observed for changes in HOMA-IR, even though HOMA-IR is positively associated with HOMA-B after adjusting for BMI at baseline, age, and gender.

3.2. Association between tissue-specific insulin sensitivity and brain reward reactivity to food cues

In addition to the peripheral insulin sensitivity indices, the role of tissue-specific insulin sensitivity within brain reward reactivity was also examined. Therefore, we investigated whether there was a correlation between HIRI and MISI at baseline and changes in the reactivity of selected brain reward regions to food cues (see Table S2 in supplementals). We also investigated whether there was a correlation between the changes in HIRI and MISI before and after the intervention and changes in the reactivity of predefined brain reward areas to food cues (see Table S3 in supplementals). No significant associations were found between the tissue-specific insulin sensitivity indices at baseline and changes in the reactivity of predefined brain reward regions to food cues. This was the same for changes in tissue-specific insulin sensitivity indices.

3.3. Association between weight change and peripheral insulin sensitivity

Changes in weight were positively associated with changes in fat mass (r = 0.931; p = 0.000). The present study has investigated whether changes in body weight between baseline and the end of the intervention were associated with peripheral insulin sensitivity indices measured at the end of the study. Consequently, a significant positive association was found between body weight change and HOMA-IR (r = 0.703; p = 0.000), and HOMA-B (r = 0.405; p = 0.036) measured at the end of the intervention. After adjusting for BMI at baseline, age, and gender, the association remained significant for HOMA-IR (r = 0.684; p = 0.000) (Fig. 2), but not for HOMA-B (r = 0.340; p = 0.104). Furthermore, it has also been examined whether body weight change is associated with changes in peripheral insulin sensitivity between baseline and the end of the intervention. For the latter, no significant associations were found. Next, the same was assessed for the changes in fat mass between baseline and the end of the intervention. Consistently, a significant positive association was found between changes in fat mass and the HOMA-IR (r = 0.697; p = 0.000), and HOMA-B (r = 0.451; p = 0.018) measured at the end of the intervention. The association with HOMA-IR (r = 0.655; p = 0.001) remained significant after adjusting for BMI at baseline, age, and gender (Fig. 2). However, the

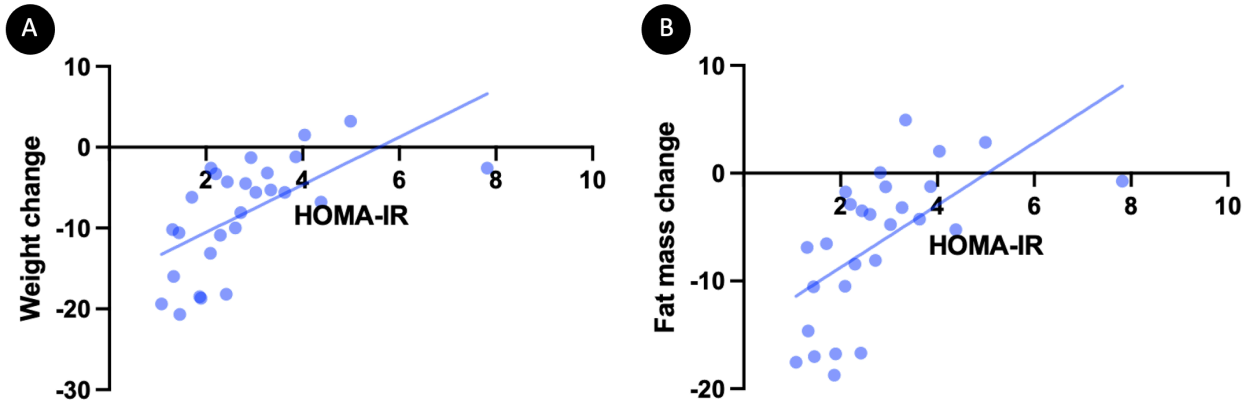

Fig. 2 – Scatterplot A shows the significant positive association between changes in body weight and HOMA-IR measured at the end of the study. Scatterplot B shows the significant positive association between changes in fat mass and HOMA-IR measured at the end of the study. For the Pearson's and partial correlation analyses, HOMA-IR data measured at the end of the study were log-transformed to conform to normality. However, these scatter plots show the non-log transformed HOMA-IR data measured at the end of the study.

significant association with HOMA-B (r = 0.391; p = 0.059) disappeared after this correction. Moreover, no significant associations were found between changes in fat mass and changes in peripheral insulin sensitivity before and after the intervention.

3.4. Association between weight change and tissue-specific insulin sensitivity

Similarly, the current study examined whether changes in body weight or fat mass were associated with the tissue-specific insulin sensitivity indices, HIRI and MISI. There were no associations found between the changes in weight or fat mass and the tissue-specific insulin sensitivity indices measured at baseline. This was also true for the changes in tissue-specific insulin sensitivity between baseline and the end of the intervention.

3.5. Association between weight change and brain reward reactivity to food cues

Additionally, the association between body weight change and changes in brain reward reactivity was investigated. However, no significant associations were found between changes in body weight and changes in the reactivity of the selected brain reward regions to food cues after adjusting for BMI at baseline, age, and gender (see Table S4 in supplementals). Accordingly, no associations were found with changes in fat mass (see Table S4 in supplementals).

3.6. Gender-specific differences in brain reward reactivity to food cues

Finally, the effect of gender on brain reward reactivity was examined. The change in body weight (p = 0.010) and fat mass (p = 0.026) differed significantly between men and women (Fig. 3) when adjusted for BMI at baseline and age. The mean changes in weight and fat mass with the associated standard deviations (mean±SD) for both males and females are shown in figure 3. Furthermore, a trend could be observed for the difference in changes in the reactivity of the amygdala left to food cues between males and females (p = 0.033) when adjusted for BMI at baseline and age (Fig. 4). The mean changes in left amygdala reactivity and the corresponding standard deviations (mean±SD) for both males and females can be found in figure 4. For the other selected brain reward regions, no significant differences have been found between genders.

4. DISCUSSION

The present study set out with the aim of assessing the role of tissue-specific insulin sensitivity, weight

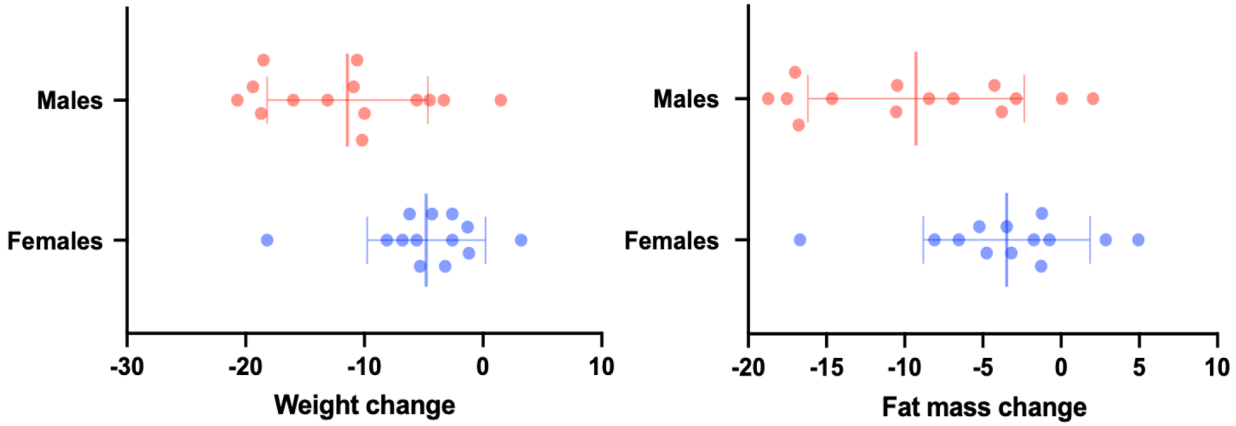

Fig. 3 – This figure shows the significant differences for the change in weight between males (-11.4 ± 6.76) and females (-4.78 ± 4.99). Furthermore, the significant differences for the change in fat mass between males (-9.27 ± 6.92) and females (-3.47 ± 5.33) are also demonstrated.

change, and gender in brain reward reactivity to food cues. For this, 27 overweight or obese subjects with impaired fasting glucose and/or impaired glucose tolerance were enrolled within a dietary intervention period consisting of a 2-month LED followed by a 22-month weight maintenance period with either HP or MP dietary guidelines. The reader should bear in mind that no differences were observed in brain reward reactivity to food cues between the HP and MP dietary intervention groups in both the whole-brain and ROI analysis (72). However, within the PREVIEW study, it was concluded that the increased protein intake in both groups is associated with a decrease in brain reward responsivity to high-calorie food pictures compared to low-calorie pictures (72). As a consequence, these 2 dietary groups were not taken into consideration during the current analysis. To investigate the role of the brain's reward system in feeding behaviour, brain regions that have previously been shown to be activated by food or food-related visual cues were selected (28, 77-83). These brain regions include the insula, which is implicated in processing perceptions of food tastes and their associated hedonic appreciation (84, 85), and the striatum, which plays a role in reward-related motivation and learning processing (43, 86, 87). Furthermore, the amygdala is believed to encode the attractiveness or reward value of food (88, 89), while the anterior cingulate cortex is critical in the general hedonic representation and determines the subjective value of rewards, regardless of their nature (79). In addition to our selected brain regions, other regions, such as the orbitofrontal cortex and nucleus accumbens (90-92), are also known for their role in food reward. Unfortunately, the reactivity of these regions was not monitored in this thesis. Therefore, it may be interesting for future studies to include these additional brain reward regions in their research. Before discussing the results, it should be noted that the current study used Bonferroni correction to tackle the problem of multiple comparisons for the selected brain regions. This is a very strict and conservative method that has already been performed in the PREVIEW study as well. As a consequence, the application of this correction may have been too strict.

4.1. Peripheral insulin sensitivity and brain reward reactivity to food cues

Although HOMA-B did not significantly change over the 2-year intervention (see Table 2), the results of this study indicate that trends could be observed for the positive associations between changes in HOMA-B and changes in the reactivity of the left and right globus pallidus and the left putamen to food cues. These brain regions are part of the posterolateral ventral striatal region, which has been shown to be primarily involved in the motivational salience or "wanting" food, rather

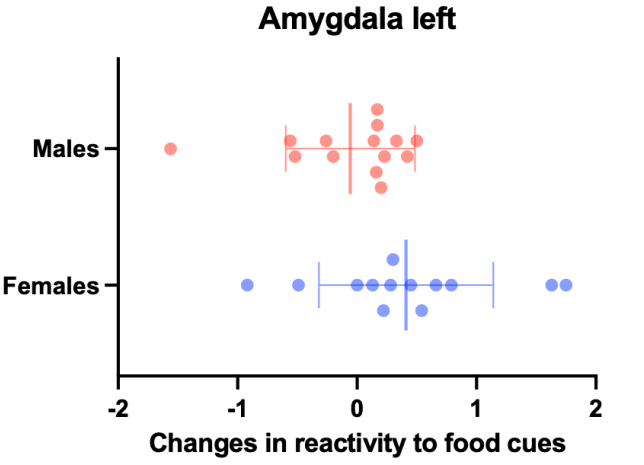

Fig. 4 – This figure shows the difference observed for the change in the reactivity of the left amygdala to food cues between males (-0.05 ± 0.54) and females (0.41 ± 0.73).

than "liking" food (93, 94). Despite that the changes in HOMA-B between baseline and the end of the study were not statistically significant (<0.05), one can argue that the associated *p*-value ($p = 0.052$, see Table 2) is only slightly non-significant. Several studies already demonstrated positive associations between the reactivity of the brain's reward system and indices of peripheral insulin sensitivity in a variety of overweight or obese populations (14, 41, 95, 96). As far as we know, positive associations between HOMA-B, an index that reflects pancreatic ß-cell functioning (97), and brain reward reactivity to food cues have never been demonstrated before. These findings provide support for the importance of insulin signalling within brain reward areas. More specifically, insulin has been shown to play an important role within the brain's reward system, as it regulates the expression or function of the dopamine reuptake transporter (DAT), which ensures the uptake of released dopamine (98, 99). In addition, insulin has the capacity to modify dopamine half-life or action by regulating the expression of the dopamine-degrading enzymes: monoamine oxidases and DAT (100). Several animal and human studies already demonstrated the ability of intranasal insulin administration to reduce feeding behaviour (99, 101-104). Consequently, insulin resistance in the brain is related to diminished dopamine reuptake, causing increased or prolonged synaptic dopamine reactivity, which may ultimately lead to an extreme sensitivity to food stimuli (99).

4.2. Tissue-specific insulin sensitivity and brain reward reactivity to food cues

However, no associations were found between tissue-specific insulin sensitivity and changes in the reactivity of the selected brain reward regions. To the best of our knowledge, no previous research has investigated the association between the tissue-specific insulin sensitivity indices, MISI and HIRI, and brain reward reactivity to food cues. Previously, Drummen *et al.* have already demonstrated a positive association between (changes in) HOMA-IR and (changes in) the reactivity of brain reward regions to food cues within the PREVIEW study (72, 73). Given that HOMA-IR is calculated with fasting glucose and fasting insulin concentrations, it reflects hepatic insulin sensitivity and basal hepatic glucose production (105). Therefore, we expected that an association could be found between HIRI and changes in brain reward reactivity. A potential source of weakness in the present study was the small size of the different insulin resistance groups within the study population. When looking at previously conducted studies examining tissue-specific insulin sensitivity indices, much larger study populations are used (106-108). Therefore, future studies using larger sample sizes could shed more light on the association between tissue-specific insulin resistance and food reward. Since this will ensure that the different insulin resistance groups increase in size and thus create even more representative results.

4.3. Weight loss and brain reward reactivity to food cues

Weight or fat mass change was not associated with changes in the reactivity of selected brain reward regions to food cues. These findings are in accordance with the idea that insulin sensitivity influences the responsiveness of brain reward regions to food stimuli rather than just weight status. For instance, one study conducting a glucose challenge within insulin-sensitive normal-weight subjects demonstrated that elevations of plasma insulin concentrations were associated with reductions in brain reward responsiveness to food

pictures (109). In addition, similar studies reported a decrease in the brain's reward reactivity to food images during a glucose challenge in insulin-sensitive but not insulin-resistant subjects (110, 111). Consistently, another human study examining the brain glucose metabolism reported a difference in the metabolism of brain reward areas between insulin-sensitive and insulin-resistant subjects, with the insulin-resistant individuals having a significantly less metabolic activity (112). These studies indicate that insulin's ability to inhibit brain reactivity to food cues is impaired in the presence of peripheral insulin resistance (110-112). Together with the aforementioned studies, our findings confirm the regulatory role of insulin in feeding behaviour and body weight by signalling the central nervous system (113).

4.4. Weight loss and insulin sensitivity

Moreover, the results within this study emphasize the positive effect of weight loss on insulin sensitivity, given the positive associations between changes in weight and fat mass and HOMA-IR measured at the end of the intervention. Many studies already confirmed the beneficial effect of weight loss on insulin sensitivity (56-59). Additionally, it has even been reported that bariatric surgery within obese T2DM patients improves glycaemic control and reduces brain reward reactivity towards food, compared to similar patients without bariatric surgery and less controlled T2DM (114). However, it is unfortunate that the present study did not include an extra time point. For instance, an additional time point for measuring brain reward reactivity directly after the LED period could potentially provide more insight into the effect of intensive weight loss on brain reward reactivity and insulin sensitivity. Furthermore, it should be noted that the changes in body weight as a result of the 2-year intervention period vary between the participants. Therefore, it could be postulated that a more consistent period of weight change would provide an even better overview of the effect of weight loss.

4.5. Gender-specific differences in food rewards

Existing research has already suggested that reward processing brain regions appear to be more responsive to hedonic food cues in females compared to males, when in a fasting state (115). Consequently, the present study aimed to investigate gender-related differences in the changes of brain reward responses to food cues. A trend was observed only for the amygdala left, which has been shown to be involved in mediating differences in emotional behaviour between the sexes (116). Results indicated that females had a mean increase in amygdala reactivity over the intervention, while males had a mean decrease. Surprisingly, this outcome is contrary to that of Killgore *et al.* who found that men have a greater reactivity in the amygdala to high-calorie food images compared to women (117). Since the amygdala is involved in encoding the appetitive value of food (88, 89), this greater reactivity in men may be consistent with the idea that men process food cues in a less complicated hedonic manner and therefore experience less guilt about their eating habits compared to women, who are thought to process food stimuli in a more intricate cognitive manner (117-119). However, it should be noted that Killgore *et al.* did not include any dietary intervention. While in the current study, males lost significantly more weight than females, which may have affected this difference in left amygdala reactivity. Interestingly, studies in females examining brain reward responsiveness to food stimuli during different phases of the menstrual cycle, found that amygdala reactivity was greater during the late follicular phase compared to the early follicular or luteal phase (120, 121). These different phases are dominated by different hormones which influence the response to food stimuli (120). Given that sex hormones have been shown to affect food intake, it is of great importance that the menstrual cycle and the use of contraceptives are taken into account when investigating brain reward reactivity to food cues (122). Unfortunately, the current study has not been able to do this. Eventually, it should be noted that this outcome is considered exploratory since the study population does not provide gender groups with sufficient statistical power.

5. CONCLUSION

In summary, from the present study, we can conclude that peripheral insulin sensitivity is positively associated with the brain reward

reactivity to food stimuli when we exclude the strict Bonferroni correction. This indicates that increasing insulin sensitivity is associated with reductions in brain reward reactivity to food stimuli. Furthermore, no associations could be found between changes in weight or fat mass and brain reward reactivity to food cues. These findings emphasize the importance of insulin sensitivity in food reward. Next, the positive effect of weight loss on peripheral insulin sensitivity has been demonstrated. Lastly, changes in the reactivity of the left amygdala to food cues have been shown to differ significantly between males and females when we again exclude the Bonferroni correction. So far, the association between tissue-specific insulin sensitivity and brain reward reactivity to food cues remains to be elucidated. Therefore, future research with larger study populations is necessary to provide more clarity. The use of a consistent weight-loss period and sufficient time points to assess changes in (tissue-specific) insulin sensitivity and brain reward reactivity to food cues could help with this. The current study also highlights the importance of research that focuses on gender differences. Within the existing literature, studies that take into account sex hormones, the menstrual cycle, and the use of contraceptives are very scarce. As a result, more accurate future studies with larger sample sizes should further specify gender-related differences in food rewards. This will create a better understanding of the gender-specific prevalence of obesity. Ultimately, examining the associations between weight change, insulin sensitivity, and food reward, will provide more insights into the role of the brain's reward system in gaining excess weight through overeating. By specifically investigating how insulin regulates neuronal responses to food stimuli in reward areas of the brain, it could potentially serve as a target in the treatment of overweight or obesity. In general, with this study, we aim to contribute to public health and raise consciousness about the development, treatment, and prevention of obesity and its associated non-communicable diseases.

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Author contributions – **Prof. dr. Adam and dr. Drummen conceived and designed the research. Student Cloosen and Mr. Bastings performed data analysis. Prof. dr. Adam provided assistance. Student Cloosen wrote the paper. Mr. Bastings and Prof. Dr. Adam carefully edited the manuscript.**

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7. SUPPLEMENTALS

7.1. Experimental procedures

7.1.1. Study population

For the recruitment, announcements in newspapers, newsletters, and social media were made in the region of Maastricht/Limburg (NL). Additionally, contacts with national and local obesity and diabetes association or primary and occupational health care providers were used as well. Furthermore, female participants needed to use contraceptive methods and not wish or plan to become pregnant during the intervention study. Several general exclusion criteria were established, when any of these criteria were met, the subject was excluded from participation in the study. These exclusion criteria were the following: fluctuations in weight (increase or decrease) of more than 5% during the past 2 months prior to the study, blood donation within the month prior to the study, changes in smoking habits during the month prior to the study, regularly drinking habit (men: >21 alcoholic units per week; women: >14 alcoholic units per week), engagement in competitive sports, self-reported eating disorders, following a special diet (e.g. vegan, Atkins) within the 2 months prior to the study (Lacto-vegetarian diets are allowed), intolerance and allergies expected to interfere with the study, abuse of drugs within the previous 12 months prior to the study, no access to either (mobile) phone or internet, insufficient communication with national language, being physically or mentally unable to comply with the required procedures in the study protocol. Specifically for women, pregnancy and lactation were exclusion criteria as well. Furthermore, the following medical conditions were included in the exclusion criteria: surgical treatment of obesity, diagnosed with diabetes mellitus, medical history of cardiovascular diseases (e.g. current angina, myocardial infarction or stroke within the past 6 months, heart failure, symptomatic peripheral vascular disease), systolic blood pressure above 160 mmHg and/or diastolic blood pressure above 100 mmHg whether on or off treatment for hypertension, advanced chronic renal dysfunction, significant liver diseases (however, fatty liver disease is allowed), a currently active malignancy or in remission for less than five years after last treatment (however, local basal and squamous cell skin cancer are allowed), disorders potentially causing malabsorption (e.g. active inflammatory bowel disease, celiac disease, chronic pancreatitis), chronic respiratory, neurological, musculoskeletal or other disorders that would give unacceptable risk or difficulty to comply to the study protocol, transmissible blood-borne diseases (e.g. hepatitis B, HIV), recent surgical procedure until after full recovery, psychiatric illness (e.g. major depression, bipolar disorders). Additionally, participants could be excluded based on the concomitant medications they were taking: current use or within the previous 3 months of prescription medication that potentially can affect body weight or glucose metabolism such as glucocorticoids (except for inhaled and topical steroids). Furthermore, the use of low-dose antidepressants and Levothyroxine treatment for hypothyroidism on a stable dose for at least 3 months was allowed. All the aforementioned criteria were checked during the telephone pre-screenings and subsequent laboratory screenings. Ultimately, specialized fMRI-related exclusion criteria were constructed for the fMRI scans: subjects that contain metal objects such as implants present within the body (e.g. electronic implants, pacemakers, metal fragments in eyes, skin, or body); permanent make-up (e.g. eyeliners, eyebrows); subjects with tattoos on their head, shoulders, breast, or neck; subjects who do not want to be informed about accidental findings.

7.1.2. Diet interventions

The LED consisted out of 800-1000 kcal per day and macronutrient composition of 15-20 E% from fat, 35-40 E% from protein, and 45-50 E% from carbohydrate. All participants were provided with the Cambridge Weight Plan powder sachets and were instructed to consume 4 sachets (4 x 55 g, each consisting out of 200 kcal) per day. All of the sachets had to be dissolved in water (4 x 250 mL water), except for 1 sachet that had to be dissolved in 250 mL low-fat milk. In addition to the 4 sachets, participants were allowed to consume calorie-free drinks and eat less than 400 grams of non-starchy and low-carbohydrate vegetables

per day. Furthermore, for the weight maintenance period, both dietary groups focused on protein intake, with one comprising a moderate protein (MP) group with 15/55/30% of energy from protein/carbohydrate/fat and a moderate dietary glycaemic index (GI) (≥56), while the other comprised a high protein (HP) group with 25/45/30% of energy from protein/carbohydrate/fat, and a low dietary GI (≤50). Furthermore, participants were given examples of daily eating plans according to the macronutrient and GI requirements of the two diet groups.

8. SUPPLEMENTARY TABLES

Table S1 – *Summary of the partial correlation analysis to determine the association between the different (tissue-specific) insulin sensitivity indices measured at different time points.*

	CID1		CID6		Changes	
	r	*p*	r	*p*	r	*p*
HOMA-IR and HOMA-B	0.772	0.000	0.721	0.000	0.824	< 0.000
HOMA-IR and HIRI	0.590	0.004	0.247	0.255	0.340	0.132
HOMA-IR and MISI	-0.544	0.009	0.189	0.388	-0.202	0.379
HOMA-B and HIRI	0.788	0.000	0.397	0.061	0,333	0.141
HOMA-B and MISI	-0.625	0.002	0.005	0.982	-0.290	0.203

Partial correlation coefficients along with the corresponding *p*-values are presented between the different (tissue-specific) insulin sensitivity indices. All these correlations were adjusted for BMI at baseline, age, and gender.

Table S2 – *Summary of the partial correlation analysis to determine the association between the tissue-specific insulin sensitivity indices at baseline and changes in the reactivity of selected brain reward regions*

	HOMA-IR		HOMA-B		HIRI		MISI	
	r	***p***	**r**	***p***	**r**	***p***	**r**	***p***
Insula right	0.250	0.239	-0.039	0.858	0.042	0.854	-0.107	0.635
Insula left	0.179	0.404	-0.040	0.852	-0.057	0.801	-0.049	0.829
Caudate right	0.026	0.906	-0.208	0.330	-0.143	0.525	-0.037	0.871
Caudate left	0.108	0.616	-0.119	0.579	-0.030	0.896	-0.119	0.597
Putamen right	0.147	0.493	-0.108	0.617	0.009	0.969	-0.072	0.750
Putamen left	-0.007	0.975	-0.255	0.229	-0.147	0.513	0.038	0.865
Globus pallidus right	-0.017	0.938	-0.224	0.293	-0.058	0.796	0.091	0.687
Globus pallidus left	-0.032	0.882	-0.312	0.138	-0.118	0.601	0.056	0.803
Anterior cingulate right	0.247	0.245	0.053	0.806	0.143	0.527	-0.158	0.484
Anterior cingulate left	0.281	0.184	0.055	0.799	0.197	0.379	-0.146	0.517
Amygdala right	0.004	0.985	-0.053	0.804	0.075	0.742	0.100	0.656
Amygdala left	-0.291	0.168	-0.081	0.707	-0.089	0.692	0.302	0.172

Partial correlation coefficients along with the corresponding *p*-values are presented for the changes of each selected brain reward region in association with the 4 different tissue-specific insulin indices measured at baseline. These partial correlations were adjusted for BMI at baseline, age, and gender. A $p < 0{,}004$ was considered significant to correct for multiple comparisons.

Table S3 – *Summary of the partial correlation analysis to determine the association between the changes tissue-specific insulin sensitivity indices and changes in the reactivity of selected brain reward regions*

	HOMA-IR		HOMA-B		HIRI		MISI	
	r	***p***	**r**	***p***	**r**	***p***	**r**	***p***
Insula right	-0.193	0.367	0.188	0.380	-0.018	0.937	-0.172	0.456
Insula left	-0.095	0.658	0.226	0.288	0.125	0.588	-0.383	0.087
Caudate right	0.010	0.964	0.385	0.063	0.143	0.535	-0.335	0.138
Caudate left	-0.015	0.943	0.340	0.105	0.016	0.946	-0.414	0.062
Putamen right	-0.035	0.870	0.356	0.087	-0.052	0.822	-0.244	0.287
Putamen left	0.077	0.722	0.465	0.022	0.017	0.940	-0.191	0.407
Globus pallidus right	0.153	0.476	0.510	0.011	-0.111	0.631	-0.177	0.443
Globus pallidus left	0.122	0.569	0.480	0.018	0.108	0.641	-0.201	0.383
Anterior cingulate right	-0.136	0.528	0.148	0.491	-0.087	0.707	-0.434	0.050
Anterior cingulate left	-0.170	0.428	0.118	0.584	-0.168	0.465	-0.357	0.112
Amygdala right	0.076	0.723	0.315	0.133	-0.057	0.806	0.037	0.873
Amygdala left	0.248	0.242	0.268	0.205	0.126	0.586	-0.140	0.544

Partial correlation coefficients along with the corresponding *p*-values are presented for the changes of each selected brain reward region in association with the changes of the 4 different tissue-specific insulin indices before and after the intervention. These partial correlations were adjusted for BMI at baseline, age, and gender. A $p < 0{,}004$ was considered significant to correct for multiple comparisons.

Table S4 – *Summary of the partial correlation analysis to determine the association between the changes in weight or the changes in fat mass and changes in the reactivity of selected brain reward regions*

	Weight change		Fat mass change	
	r	***p***	**r**	***p***
Insula right	-0.227	0.287	-0.257	0.226
Insula left	-0.180	0.401	-0.189	0.375
Caudate right	0.019	0.929	0.007	0.973
Caudate left	-0.003	0.987	-0.015	0.944
Putamen right	-0.013	0.952	-0.034	0.874
Putamen left	0.017	0.936	0.000	0.998
Globus pallidus right	0.105	0.626	0.110	0.610
Globus pallidus left	-0.024	0.912	-0.022	0.919
Anterior cingulate right	-0.060	0.780	-0.019	0.929
Anterior cingulate left	-0.152	0.478	-0.128	0.552
Amygdala right	-0.074	0.733	-0.094	0.661
Amygdala left	-0.116	0.590	-0.129	0.548

Partial correlation coefficients along with the corresponding *p*-values are presented for the changes of each selected brain reward region in association with the changes in weight and fat mass before and after the intervention. These partial correlations were adjusted for BMI at baseline, age, and gender. A $p < 0{,}004$ was considered significant to correct for multiple comparisons.