



Selective Insulin Resistance in Homeostatic and Cognitive Control Brain Areas in Overweight and Obese Adults

Diabetes Care 2015;38:1044–1050 | DOI: 10.2337/dc14-2319

Stephanie Kullmann,^{1,2,3} Martin Heni,^{1,2,4}
Ralf Veit,^{1,2,3} Klaus Scheffler,^{5,6}
Jürgen Machann,^{1,2}
Hans-Ulrich Häring,^{1,2,4}
Andreas Fritsche,^{1,2,4}
and Hubert Preissl^{1,2}

OBJECTIVE

Impaired brain insulin action has been linked to obesity, type 2 diabetes, and neurodegenerative diseases. To date, the central nervous effects of insulin in obese humans still remain ill defined, and no study thus far has evaluated the specific brain areas affected by insulin resistance.

RESEARCH DESIGN AND METHODS

In 25 healthy lean and 23 overweight/obese participants, we performed magnetic resonance imaging to measure cerebral blood flow (CBF) before and 15 and 30 min after application of intranasal insulin or placebo. Additionally, participants explicitly rated pictures of high-caloric savory and sweet food 60 min after the spray for wanting and liking.

RESULTS

In response to insulin compared with placebo, we found a significant CBF decrease in the hypothalamus in both lean and overweight/obese participants. The magnitude of this response correlated with visceral adipose tissue independent of other fat compartments. Furthermore, we observed a differential response in the lean compared with the overweight/obese group in the prefrontal cortex, resulting in an insulin-induced CBF reduction in lean participants only. This prefrontal cortex response significantly correlated with peripheral insulin sensitivity and eating behavior measures such as disinhibition and food craving. Behaviorally, we were able to observe a significant reduction for the wanting of sweet foods after insulin application in lean men only.

CONCLUSIONS

Brain insulin action was selectively impaired in the prefrontal cortex in overweight and obese adults and in the hypothalamus in participants with high visceral adipose tissue, potentially promoting an altered homeostatic set point and reduced inhibitory control contributing to overeating behavior.

Due to strong associations with numerous conditions, such as type 2 diabetes and cardiovascular disease, obesity has become a major public health concern. Obesity is associated with peripheral insulin resistance in many organs, such as muscle, liver, and adipose tissue. However, only recently was the brain identified as an insulin-sensitive organ regulating food intake (1). In humans, the central nervous effects of insulin still remain ill defined. In search of new insights in the pathogenesis of

¹Institute for Diabetes Research and Metabolic Diseases, Helmholtz Center Munich, University of Tübingen, Tübingen, Germany

²German Center for Diabetes Research, Tübingen, Germany

³Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Tübingen, Germany

⁴Department of Internal Medicine IV, University of Tübingen, Tübingen, Germany

⁵Department of High-Field Magnetic Resonance, Max Planck Institute for Biological Cybernetics, Tübingen, Germany

⁶Department of Biomedical Magnetic Resonance, University of Tübingen, Tübingen, Germany

Corresponding author: Stephanie Kullmann, stephanie.kullmann@med.uni-tuebingen.de.

Received 2 October 2014 and accepted 24 February 2015.

Clinical trial reg. no. NCT01797601, clinicaltrials.gov.

This article contains Supplementary Data online at <http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc14-2319/-/DC1>.

© 2015 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered.

obesity and brain insulin resistance, modern neuroimaging techniques have emerged as valuable tools to investigate insulin action in the human brain.

The clinical relevance of insulin action in the brain was given further merit by discovering cerebral insulin resistance in obese adults by means of magnetoencephalography using a systemic intravenous insulin infusion (2), which was linked to peripheral insulin resistance (2,3). Because the effects of insulin and glucose as well as peripheral versus central insulin actions are difficult to differentiate, intranasal administration has been established as a useful tool in clinical studies in recent years. This technique makes it possible to selectively study cerebral insulin action by delivery of the hormone directly into the brain, without relevant effects on peripheral glucose concentrations (4). After intranasal administration, insulin enters the cerebrospinal fluid compartment and influences brain function, promoting weight loss and demonstrating beneficial effects on memory functions and metabolism in healthy participants as well as patients with diabetes and cognitive impairments (5). Hence, intranasal insulin is a possible therapeutic approach for the treatment of obesity, type 2 diabetes, and neurodegenerative diseases.

In functional magnetic resonance imaging (fMRI) studies using blood oxygen level–dependent contrast, insulin was shown to significantly attenuate resting state activity as well as visual processing of food images in healthy lean adults. Thereby, regions well beyond the homeostatic system of the brain were modulated, especially the occipital and prefrontal brain regions and the hypothalamus (6–8). Compared with blood oxygen level–dependent fMRI, arterial spin labeling offers quantitative cerebral blood flow (CBF) measurements, providing a well-characterized physiological parameter in physiological units (mL/100 g brain tissue/min). Hence, arterial spin labeling measurements have been proposed to be ideally suited for pharmacological MRI studies (9).

No study has evaluated cerebral insulin action in obese adults to our knowledge; therefore, we aimed to identify specific brain regions responsive to intranasal insulin and regions affected by cerebral insulin resistance. We

performed MRI using arterial spin labeling before and 15 and 30 min after application of intranasal insulin in lean and overweight/obese adults. We hypothesized that overweight and obese adults will show cerebral insulin resistance in regions associated with food intake and eating behavior.

RESEARCH DESIGN AND METHODS

Subjects

We recruited 25 healthy lean, 10 overweight, and 13 obese adult participants for this study (BMI 19–46 kg/m²). Overweight and obese participants were required to have a BMI >25 kg/m². Informed written consent was obtained from all participants, and the local ethics committee approved the protocol. All participants were students at the University of Tübingen recruited through broadcast e-mails.

Study Design

Before the experiment, all participants underwent a medical examination to ensure that they did not have psychiatric, neurological, or metabolic diseases. Diabetes was ruled out by a 75-g oral glucose tolerance test (OGTT). Any volunteer treated for chronic disease or taking any kind of medication other than oral contraceptives was excluded. The Patient Health Questionnaire (10) was used to address psychiatric diseases, and to assess eating behavior, subjects took the German Three Factor Eating Questionnaire (11), the eating disorder examination (12), and the trait version of the Food Craving Questionnaire (13).

To assess body fat distribution, whole-body MRI measurements were obtained. Participant characteristics are provided in Table 1.

After the OGTT and whole-body MRI measurement, all subjects participated in an intranasal insulin and placebo experiment (on 2 separate days with a time lag of 7–14 days) with repetitive measurement of CBF by MRI. Participants were blinded to the order of the conditions. Experiments were conducted after an overnight fast of at least 10 h and started at 7:00 A.M. with a resting CBF measurement under basal conditions (CBF 1). After the basal measurement, an insulin/placebo spray was administered intranasally as described next. After 15 and 30 min, the second and third resting

CBF measurements were performed (CBF 2 and 3).

Subjective feelings of hunger were rated on a visual analog scale from 0 to 10 (0: not hungry at all; 10: very hungry) at time points 0, 60, and 120 min. Venous blood samples were obtained at 0, 30, 60, 90, and 120 min, and plasma glucose and insulin concentrations were determined. Pictures of high-caloric savory and sweet food were rated on a scale of 1–9 using a laptop computer outside the scanner 60 min after the application of insulin/placebo (duration ~10 min). Participants rated 100 food pictures in two separate blocks according to explicit liking (i.e., “How much do you like the food item in general?”) and wanting (i.e., “How much would you like to eat the food item right now?”). The study design is shown in Fig. 1.

Application of Intranasal Insulin/Placebo

The insulin and placebo were prepared as nasal sprays. In a randomized fashion, participants received on one day 160 units insulin (Actrapid; Novo Nordisk, Bagsværd, Denmark) and on the other measurement day, vehicle as placebo. Participants were blinded to the order of the conditions.

Oral Glucose Tolerance Test

Before the intranasal MRI experiment (7–14 days), participants underwent a 75-g OGTT after an overnight fast. Insulin sensitivity during OGTT was estimated according to Matsuda and DeFronzo (14).

Measurement of Adipose Tissue by MRI Examinations

On the same day as the OGTT, MRI examinations were performed in the early morning on a 1.5T whole-body imager (MAGNETOM Sonata; Siemens Healthcare, Erlangen, Germany). A whole-body imaging protocol was used to record a set of 90–120 parallel transverse slices. This approach enabled quantification of body volume, total adipose tissue (TAT), and total mass of specific fat depots, such as visceral adipose tissue (VAT) (15).

Whole-Brain fMRI Measurement

Data Acquisition

Scanning was performed on a 3T scanner (MAGNETOM Trio, A Tim System; Siemens Healthcare) equipped with a 12-channel transceiver head coil. Pulsed arterial spin labeling images were obtained with a PICORE

Table 1—Participant characteristics

	Lean group	Overweight/obese group	P value
Sex (female/male)	10/15	11/12	0.594
Age (years)	25.88 ± 3.30	26.73 ± 3.55	0.392
BMI (kg/m ²)	22.65 ± 2.01	31.26 ± 4.77	<0.001
Waist-to-hip ratio	0.82 ± 0.08	0.86 ± 0.08	0.066
Lean body mass	54 ± 13.72	62.91 ± 17.01	0.044
Percent body fat	21.75 ± 6.25	34.96 ± 11.32	<0.001
OGTT-derived insulin sensitivity index (AU)	16.0 ± 7.6	10.24 ± 6.72	0.003
HbA _{1c} (%)	5.2 ± 0.3	5.3 ± 0.3	0.155
HbA _{1c} (mmol/mol)	33.15 ± 3.1	34.2 ± 3.04	—
Insulin and glucose values during MRI experiment			
Intranasal spray*			
Fasting plasma insulin (pmol/L)			0.0001
Insulin	38.1 ± 9.1	79.9 ± 42.4	—
Placebo	46.1 ± 14.7	75.0 ± 49.1	—
Fasting plasma glucose (mmol/L)			0.0009
Insulin	4.80 ± 0.30	5.14 ± 0.34	—
Placebo	4.88 ± 0.34	5.07 ± 0.43	—
AUC insulin 0–120 min			0.0025
Insulin	214.2 ± 49	346.25 ± 170.6	—
Placebo	200.3 ± 65.7	276 ± 147.5	—
AUC glucose 0–120 min			0.0017
Insulin	16.9 ± 1.72	19.66 ± 3.4	—
Placebo	17.4 ± 1.7	19 ± 3.1	—
Whole-body MRI (L)			
TAT	17.82 ± 4.41	42.1 ± 12.84	<0.001
VAT	1.55 ± 0.86	3.37 ± 1.71	<0.001
SAT	4.87 ± 1.69	15.44 ± 6.22	0.046
Nonadipose tissue lower extremities	17.92 ± 4.25	20.32 ± 4.2	0.055
Nonadipose tissue upper extremities	10.43 ± 2.53	10.70 ± 1.83	0.675

Data are mean ± SD. P values for comparison of unadjusted log_e-transformed data by ANOVA. AU, arbitrary unit; SAT, subcutaneous adipose tissue. *No significant within-group differences between intranasal placebo and insulin day. P values are lean vs. obese, insulin day.

(proximal inversion with control for off-resonance effects) Q2TIPS (quantitative imaging of perfusion using a single subtraction) sequence by using a frequency offset-corrected inversion pulse and echo planar imaging readout for acquisition (16). Sixteen axial slices with a slice thickness of 5 mm (1.00-mm gap) were acquired in ascending order. Each measurement comprised 79 alternating tag and control images with the following imaging parameters: inversion time (TI) 1 = 700 ms, TI2 = 1,800 ms; repetition time = 3,000 ms; echo time = 19 ms; inplane resolution = 3 × 3 mm²;

field of view = 192 mm; matrix size 64 × 64; and flip angle = 90°. The same sequence was used to estimate the equilibrium magnetization of the blood for absolute CBF quantification with the same parameters as mentioned previously, except that repetition and TI2 were chosen to be 10 and 4 s, respectively (17). In addition, a high-resolution T1-weighted anatomical image was acquired.

Image Processing

Image preprocessing was performed by using the ASLtbx (18) program with

SPM8 extension (Wellcome Trust Centre for Neuroimaging). We used the general kinetic model for absolute perfusion quantification, as previously reported (19). Functional images were coregistered to the individual anatomical image and smoothed (full width at half maximum 6 mm). Perfusion images were generated by calculating the control – tag differences by using surround subtraction. For accurate CBF quantification (mL · 100 g⁻¹ · min⁻¹), we used an M0 map instead of a global value to quantify the perfusion on each voxel. The high-resolution T1-weighted image was normalized in Montreal Neurological Institute space (1 × 1 × 1 mm) using SPM8 unified segmentation normalization, and the resulting parameter file was used with the individual coregistered CBF maps in normalized space (3 × 3 × 3 mm). A brain mask was used to exclude extracranial voxels in the normalized CBF images. Baseline-corrected CBF maps were computed to quantify the CBF changes 15 and 30 min after intranasal insulin/placebo administration.

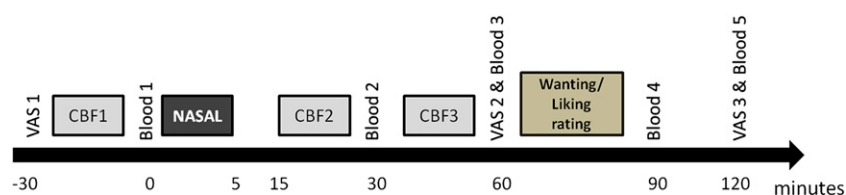


Figure 1—Study design. The order of placebo and insulin day were balanced over participants. NASAL, intranasal application of insulin or placebo; VAS, visual analog scale.

Statistical Analyses

Whole-brain analyses were performed using a voxelwise approach. CBF maps of each subject, corrected for basal measurement (CBF 2 – CBF 1; CBF 3 – CBF 1), were entered into a second-level analysis in SPM8 using a full factorial model to determine the effect of insulin versus placebo (factors: condition and time) 15 and 30 min after applying the spray and the effect of lean versus overweight/obese (factor: group). Additionally, a full factorial model was calculated to evaluate the effect of sex, which was entered as a separate between-subject factor. We first evaluated main effects and interactions using F-contrasts (two-tailed *F* tests) followed by directional T-contrasts if results of F-contrasts were statistically significant. A statistical threshold of $P < 0.05$ familywise error (FWE) corrected for multiple comparisons at a cluster level was applied to all contrasts. Furthermore, we used a region of interest (ROI) approach for the bilateral hypothalamus using a small volume correction for multiple comparisons ($P < 0.05$ FWE corrected). The hypothalamic ROI was based on the Montreal Neurological Institute coordinates of Baroncini et al. (20), including the lateral ($x: \pm 6; y: -10; z: -10 + 3$ mm sphere radius; total of 38 voxels) and medial hypothalamus ($x: \pm 4; y: -2; z: -12 + 2$ mm sphere radius; total of 16 voxels). Additionally, we extracted CBF values of significant clusters of the main effects and interactions to perform partial correlation analyses with TAT, VAT, OGTT-derived insulin sensitivity

index, and eating behavior measurements adjusted for BMI ($P < 0.01$, corrected for number of tests) in SPSS version 20 software (IBM Corporation).

Behavioral and Metabolic Data

Repeated-measures ANOVAs (between-subject factor: group, lean vs. obese, and sex, men vs. women; within-subject factor: condition, insulin vs. placebo) were calculated for wanting and liking of savory and sweet foods and hunger ratings separately.

For each trait questionnaire, a MANOVA was calculated with group (lean vs. obese) and sex as between-subject factors. Results surviving a statistical threshold of $P < 0.05$ were considered statistically significant (using SPSS version 20 software) (data not shown).

For the metabolic parameters, ANOVAs were calculated to evaluate group differences for peripheral measures. Areas under the curve (AUCs) were calculated for glucose and insulin during the MRI experiment; paired *t* tests were used to test for significant differences between insulin and placebo spray. Results surviving a statistical threshold of $P < 0.05$ were considered statistically significant (using SPSS version 20 software).

RESULTS

MRI Data

Basal CBF

We used the basal measurement before nasal spray application to evaluate intrasubject repeatability and scanner variability. There was no significant

difference in CBF on insulin versus placebo day before nasal spray application ($P = 0.96$); the mean CBF value of insulin day basal measurement (mL/100 g brain tissue/min) was 43.92 (SE 1.22); the mean CBF value of placebo day was 43.97 (SE 1.17). Furthermore, we observed a significant positive correlation between the two baseline CBF measurements ($r_{\text{Spearman } \rho} = 0.509, P < 0.0001$).

Effect of Intranasal Insulin on Regional CBF in Lean and Obese Adults

The whole-brain voxelwise analysis showed a significant main effect of group in the right lingual gyrus ($P_{\text{FWE}} = 0.047$) and a significant interaction between group and condition in the right middle frontal gyrus (MFG) ($P_{\text{FWE}} = 0.006$) (Fig. 2). Post hoc T-contrasts revealed in the right lingual gyrus increased CBF in overweight/obese compared with lean participants after insulin and placebo application and in the right MFG decreased CBF after insulin compared with placebo in lean participants and increased CBF after insulin compared with placebo in overweight/obese participants (Supplementary Table 1). More specifically, 30 min after insulin application, overweight/obese participants showed increased CBF compared with lean participants ($T = 4.59, P = 0.003$) in the right MFG, and 30 min after placebo application, lean participants showed increased CBF compared with overweight/obese participants ($T = 3.44, P = 0.01$) in the right MFG. Within-group T-contrasts showed a

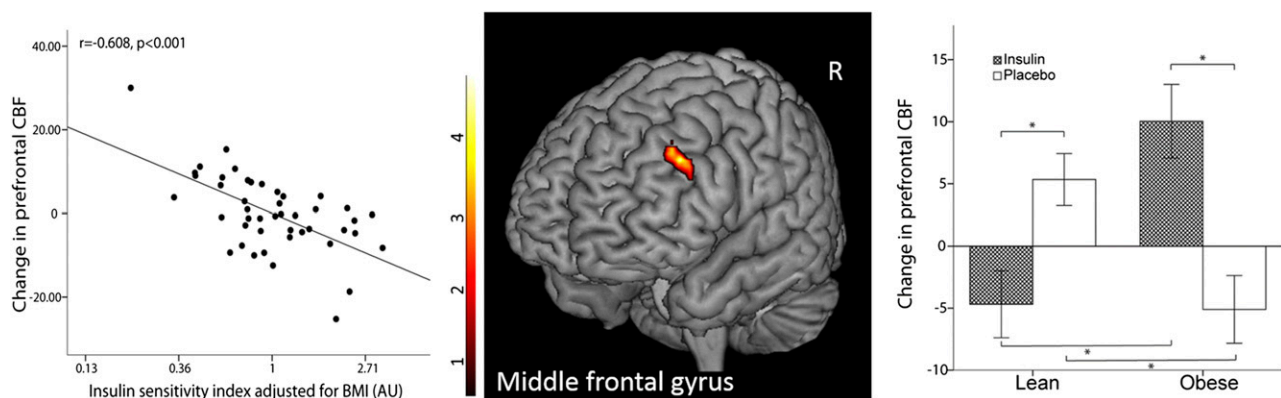


Figure 2—Interaction between group (lean vs. overweight/obese) and condition (insulin vs. placebo) in the prefrontal cortex (color-coded *t* value map; $P < 0.001$, uncorrected for display). Post hoc analyses showed a significant reduction in CBF 15 and 30 min after application of intranasal insulin compared with placebo in lean participants only. Scatter plot on the left shows significant positive correlation between the change in prefrontal CBF after insulin application and OGTT-derived insulin sensitivity index adjusted for BMI ($r = 0.608, P < 0.001$). Bar graphs on the right represent baseline-corrected changes in CBF (mean \pm SE) 30 min after insulin and placebo application in the prefrontal cortex in lean and obese adults. * $P < 0.05$ for post hoc analyses. AU, arbitrary unit; R, right.

significant difference in the right MFG between insulin and placebo 30 min postspray in the lean group ($T = 4.34$, $P = 0.012$) and overweight/obese group ($T = 5.42$, $P < 0.001$) (Fig. 2, bar graph). Furthermore, the post-insulin response in the right MFG correlated negatively with the OGTT-derived insulin sensitivity index adjusted for BMI ($r_{\text{adj}} = -0.608$, $P < 0.001$) and correlated positively with the disinhibition scale of the German Three Factor Eating Questionnaire ($r = 0.442$, $P = 0.004$) and subscale for cues that may trigger food cravings of the Food Craving Questionnaire ($r = 0.393$, $P = 0.007$), also adjusting for BMI ($r_{\text{adj}} = 0.339$ [$P = 0.03$] and 0.319 [$P = 0.04$], respectively). Additionally, we performed an ROI analysis of the hypothalamus, revealing a significant main effect of condition ($P_{\text{FWE}} = 0.028$) (Fig. 3) resulting in a CBF decrease after insulin compared with placebo application (Supplementary Table 1). The hypothalamic CBF response showed no significant correlation with the OGTT-derived insulin sensitivity index, TAT, or eating behavior measurements ($P > 0.05$). However, we observed 15 min after insulin spray a significant positive relationship between the hypothalamic CBF response and VAT after adjusting for TAT, subcutaneous adipose tissue, and nonadipose tissue ($r_{\text{adj}} = 0.371$, $P < 0.015$, $df = 42$) (Fig. 3, scatter plot). Moreover, we observed a significant interaction between group and VAT for the hypothalamic CBF response (ANCOVA $F = 5.56$, $P = 0.02$). Further subgroup analyses revealed in the

overweight/obese group a significant positive correlation between VAT and the hypothalamic response to insulin ($r_{\text{adj}} = 0.609$, $P = 0.003$, $df = 17$); no such correlation was observed in lean participants ($r_{\text{adj}} = 0.245$, $P = 0.299$, $df = 19$) (Supplementary Fig. 1). Hence, overweight/obese participants with lower VAT showed a stronger reduction in hypothalamus CBF 15 min after insulin application. No main effect of sex, time (15 vs. 30 min), group-by-time interaction, condition-by-time interaction, or interactions with sex were observed.

Behavioral Data

Wanting/Liking Results

Explicit rating for wanting and liking of high-caloric savory and sweet foods were evaluated 60 min after insulin and placebo application. No main effects or interactions for group (lean vs. obese), sex, or condition (insulin vs. placebo) were observed for liking. For wanting of sweet foods, we observed a significant group-by-sex-by-condition interaction ($F = 8.57$, $P = 0.006$) (Supplementary Fig. 2). Post hoc analyses showed in men only a significant difference between lean and obese participants; thereby, lean men showed a significant reduction after insulin compared with placebo for the wanting of sweet foods ($P = 0.01$, using Tukey honestly significant difference correction) (Fig. 4). No main effect of sex, group, or condition and interactions between condition and group or sex were observed for the wanting of sweet foods.

For wanting of savory foods, we observed a main effect of sex ($F = 8.5$, $P = 0.006$), revealing an increased wanting for savory foods in men compared with women. Furthermore, we observed a significant sex-by-condition interaction ($F = 4.68$, $P = 0.036$) and group-by-sex-by-condition interaction ($F = 5.86$, $P = 0.02$). Post hoc analyses revealed in lean participants a significant difference between men and women ($P = 0.03$, using Tukey honestly significant difference correction), revealing a decrease in women and an increase in men after insulin compared with placebo for the wanting of savory foods. No main effect of group or condition and no further interactions were observed for the wanting of savory foods.

Hunger Ratings

We observed a main effect of time, such that the subjective feeling of hunger increased with time (60 vs. 120 min postspray) for both insulin and placebo day ($F = 8.44$, $P = 0.006$). No main effect of group, sex, or condition (insulin vs. placebo) and no interactions were found.

Metabolic Parameters

No significant differences were observed for AUC insulin and AUC glucose 120 min after intranasal insulin compared with placebo in either lean or obese participants ($P > 0.05$). However,

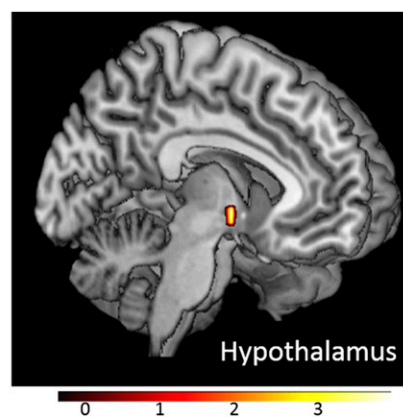
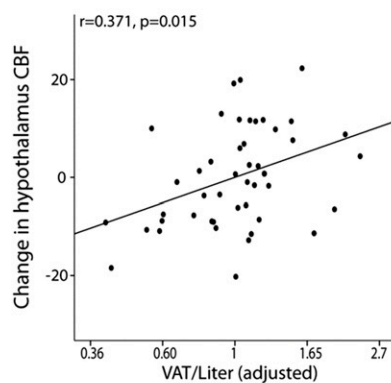


Figure 3—Sagittal view of hypothalamus showing a significant reduction in CBF 15 and 30 min after application of intranasal insulin compared with placebo in the lean and overweight/obese group (color-coded t value map; $P < 0.001$, uncorrected for display). Scatter plot on the left shows significant correlation between the change in hypothalamic CBF 15 min after insulin application and VAT adjusted for other fat compartments ($r = 0.371$, $P_{\text{adj}} = 0.015$).



Figure 4—Differential rating (mean \pm SE) for wanting of high-caloric sweet foods between insulin and placebo day. Repeated-measures ANOVA revealed significant group-by-sex-by-condition interaction for the rating of high-caloric sweet food stimuli ($P = 0.006$). Post hoc analyses showed only in men a significant difference between the lean and obese group after insulin application compared with placebo ($*P = 0.01$), revealing a significant decrease for the wanting of high-caloric sweet food after insulin application in lean men.

as expected, significant differences were found between lean and obese participants in fasting and postspray insulin and glucose concentrations ($P < 0.05$) (Table 1).

CONCLUSIONS

In this study, we aimed to evaluate central insulin action in lean, overweight, and obese adults to identify brain regions affected by insulin resistance. Although both lean and overweight/obese participants revealed a significant CBF decrease in the hypothalamus after insulin compared with placebo application, the prefrontal cortex responded only in the lean group with a decrease in CBF, pointing to selective insulin resistance. Of note, the magnitude of hypothalamic response correlated with the amount of VAT independent of other fat compartments in the overweight/obese group, whereas the prefrontal response to insulin was associated with peripheral insulin sensitivity and eating behavior (e.g., disinhibition, food craving). Regarding behavior, we were able to observe a significant reduction for the wanting of sweet foods after insulin application in lean men only.

The hypothalamus belongs to the brain's homeostatic system that controls whole-body energy balance and is fundamental in the regulation of peripheral homeostasis. Both lean and overweight/obese participants notably showed a marked insulin-induced hypothalamic CBF decrease. The inhibition of the hypothalamus could lead to an increase in satiety and potentially to an attenuated response to food. Furthermore, this change in CBF probably contributes to the homeostatic control of whole-body metabolism (21). Concomitantly, we found in lean individuals that nasal insulin administration correlates with peripheral insulin sensitivity (7) and improves peripheral insulin sensitivity through hypothalamic and parasympathetic outputs in lean but not in obese men (22), providing further evidence that peripheral and central insulin sensitivity are highly linked processes. Additionally, the insulin-induced reduction in hypothalamic CBF in the current study correlates significantly with the amount of VAT independent of the individual's other fat compartments. Thus, participants with higher visceral fat content reveal a diminished hypothalamic response to insulin,

indicating a relationship between cerebral insulin resistance and metabolically unfavorable abdominal adiposity (23). Indeed, during the course of a lifestyle intervention study, individuals with high cerebral insulin sensitivity displayed more loss of visceral fat than those who were brain insulin resistant (24). According to these findings, we speculate that soluble factors like fatty acids derived from visceral fat may cause cerebral insulin resistance, which then may aggravate hypothalamic dysfunction, resulting in a vicious cycle. Furthermore, considerable evidence has been generated in rodent studies indicating the pivotal role of the hypothalamus in insulin action and regulating hepatic glucose production (25) and lipid metabolism (26,27). Alternatively, impaired hypothalamic insulin signaling could cause increased accumulation of visceral fat due to altered response of the autonomic nervous system.

The prefrontal cortex plays a crucial role in cognitive control and decision-making, including inhibitory control of feeding. Studies in successful dieters and weight loss maintainers revealed increased neural activation in the prefrontal cortex (28,29), which could be a possible mechanism in making healthy choices. Specifically, when told to resist cravings, heightened prefrontal activity was observed in successful weight loss maintainers after gastric bypass surgery (30). Furthermore, individuals' endogenous serum insulin levels determined the reactivity of limbic regions and the prefrontal cortex to food cues after glucose ingestion (31,32). Exogenous intranasal insulin had comparable effects and reduced resting state prefrontal cortex activity in lean women (8). Concurrently, we found intranasal insulin to induce a differential pattern, reducing prefrontal cortex CBF in lean participants and increasing it in obese participants. This prefrontal response significantly correlates with peripheral insulin sensitivity. As a result, participants with higher peripheral insulin sensitivity revealed a stronger decrease, whereas those with insulin resistance showed an increase in prefrontal activity in response to intranasal insulin. Additionally, the insulin-induced activation pattern correlated positively with behavioral measurements such as disinhibition and food craving. The eating disinhibition scale has also been described as the

susceptibility for eating problems or disinhibited eating, which occurs when a person loses control and overeats in response to a stimulus (33). Hence, the participants more susceptible to disinhibited eating along with food craving failed to respond to insulin with a reduction in prefrontal CBF. Concomitantly, peripheral insulin resistance has been linked to food cue-induced food craving in obese individuals (34). Cerebral insulin resistance of the prefrontal cortex may promote reduced inhibitory control toward food cues after food intake and could thereby contribute to overeating behavior.

Independent of condition (insulin or placebo), overweight/obese participants showed an increase in CBF in the visual cortex after nasal spray application. This is in line with our previous neuroimaging studies showing altered food cue activity and functional connectivity in the visual cortex in obese individuals (35–37).

Additionally, we evaluated the explicit wanting and liking of high-caloric foods 60 min after insulin or placebo application. Although liking is a hedonic or affective reaction, wanting is important for the motivational aspects (incentive salience) of food reward (38). Even though we observed no sex effects on brain insulin action, we found in lean men only a significant reduction for the wanting of sweet foods after insulin application. Concomitantly, 8 weeks of intranasal insulin administration significantly reduced body fat in men but not in women, pointing to a differential sensitivity to the catabolic effects of nasal insulin based on sex (39). However, in the postprandial state, intranasal insulin reduced food intake and appetite in women 2 h after administration, indicating that insulin also affects hedonic eating in women (40). Taken together, intranasal insulin has the potential to reduce the motivational aspects for food, which can decrease food intake by intensifying satiety. However, whether these sex-specific behavioral effects are reflected by central alterations still needs to be investigated.

In summary, central insulin action was selectively impaired in the prefrontal cortex in overweight and obese adults and in the hypothalamus in adults with high VAT. The successful inhibition of the hypothalamus and prefrontal cortex could lead to an increase in satiety and potentially to an attenuated response to food, which could explain

