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Central Insulin Administration Improves Whole-Body Insulin Sensitivity via Hypothalamus and Parasympathetic Outputs in Men

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Animal studies suggest that insulin action in the brain is involved in the regulation of peripheral insulin sensitivity. Whether this holds true in humans is unknown. Using intranasal application of insulin to the human brain, we studied the impacts of brain insulin action on whole-body insulin sensitivity and the mechanisms involved in this process. Insulin sensitivity was assessed by hyperinsulinemic-euglycemic glucose clamp before and after intranasal application of insulin and placebo in randomized order in lean and obese men. After insulin spray application in lean subjects, a higher glucose infusion rate was necessary to maintain euglycemia compared with placebo. Accordingly, clamp-derived insulin sensitivity index improved after insulin spray. In obese subjects, this insulin-sensitizing effect could not be detected. Change in the high-frequency band of heart rate variability, an estimate of parasympathetic output, correlated positively with change in whole-body insulin sensitivity after intranasal insulin. Improvement in whole-body insulin sensitivity correlated with the change in hypothalamic activity as assessed by functional magnetic resonance imaging. Intranasal insulin improves peripheral insulin sensitivity in lean but not in obese men. Furthermore, brain-derived peripheral insulin sensitization is associated with hypothalamic activity and parasympathetic outputs. Thus, the findings provide novel insights into the regulation of insulin sensitivity and the pathogenesis of insulin resistance in humans.

Insulin resistance, that is, the inability of insulin to adequately inhibit glucose production and promote glucose uptake, thereby lowering blood glucose, is a hallmark of type 2 diabetes. Experiments in animals suggest that the brain can rapidly influence insulin sensitivity of the body through the autonomic nervous system (1–5). Particularly, the action of insulin in the brain modulates insulin sensitivity in other organs such as liver (2,3,6,7), muscle (8), and adipose (1,9,10) tissue. However, at least for the liver, the relevance of this mechanism is still under debate (11,12).

Several studies in humans have clearly indicated that insulin has specific actions in the human brain (13,14). One technique to selectively introduce brain insulin effects is to apply insulin as a nasal spray to bypass the blood-brain barrier and cause significant and sustained elevations of insulin concentrations in the cerebrospinal fluid without major effects on peripheral insulin levels (15). It significantly influences activity in specific brain areas (13,16), including the hypothalamus, the central regulator of metabolism (16). Of note, people do not

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uniformly react to central insulin application. A reduced or even an absent action is called brain insulin resistance, a phenomenon associated with obesity (13,17,18).

Two studies provided the first hints that brain insulin action might influence peripheral insulin sensitivity in humans. They showed lowered postprandial blood insulin levels (19) and lowered blood insulin-to-glucose ratio after intranasal insulin administration (20). However, whether peripheral insulin sensitivity was genuinely altered by central insulin action could not be shown unequivocally in humans.

Therefore, we determined the effects of selective insulin delivery into the brain through nasal spray on peripheral insulin sensitivity using a hyperinsulinemic-euglycemic clamp. To investigate underlying mechanisms, we assessed activity of the autonomic nervous system and performed functional magnetic resonance imaging (fMRI) to unravel the involved brain processes.

RESEARCH DESIGN AND METHODS

Participants

We studied 10 normal weight male (mean age 26 ± 1.3 years, mean weight 76 ± 4 kg, mean BMI 21.8 ± 0.7 kg/m^2) and 5 obese male (mean age 28 ± 1.7 years, mean weight 116 ± 17 kg, mean BMI 33.2 ± 3.7 kg/m^2) participants. Informed written consent was obtained, and the local ethics committee approved the protocol.

Experimental Setup

After overnight fast, subjects participated in two experiments 3–21 days apart. On both occasions, a hyperinsulinemic-euglycemic clamp was performed. A dorsal hand vein was cannulated for blood sampling. This hand and arm were warmed. A contralateral antecubital vein was cannulated for infusion of insulin, glucose, and saline.

Clamps started with an intravenous insulin bolus of 6.25 mU/kg followed by continuous intravenous insulin infusion of 0.25 mU/kg/min. Ninety minutes after initiation of the clamp, nasal spray was administered. Participants received 160 Units insulin (eight puffs in each nostril, 10 Units/puff over 4 min) and placebo on 2 days in single-blinded randomized order. After spray application, the clamp continued for 120 min. Both for intravenous and nasal administration, human insulin (Novo Nordisk, Bagsværd, Denmark) was used.

Every 5 min, blood glucose was measured and the glucose infusion rate (GIR) adjusted to maintain euglycemia (target glucose 5 mmol/L). Additional blood samples were taken at −30, 0, 75, 90, 105, 120, 150, 180, 195, and 210 min. For two participants, only data from the insulin spray day were available (in one lean participant, steady GIR could not be reached in the designated time before placebo administration, and one obese participant did not show up for the placebo experiment).

Analytic Procedure

Blood glucose level was determined by glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Insulin and C-peptide levels were measured by chemiluminescence assays (ADVIA Centaur; Siemens, Erlangen, Germany).

Heart Rate Variability

Electrocardiograms were recorded during the steady states before and after nasal spray application for 10 participants (8 lean, 2 obese). Because of equipment failure, recording was not possible for two lean participants on the insulin day. Recordings were performed with BIOPAC MP35 (BIOPAC, Goleta, CA). Data were sampled at 1,000 Hz for 10 min. The R-R interval time series were preprocessed by elimination of ectopic beats, detrending, and high-pass filtering (0.04 Hz). We determined frequency-based heart rate variability measures by custom-made analysis programs (MATLAB 2012b; MathWorks, Natick, MA). We investigated activity in the low-frequency (0.04–0.15 Hz) and high-frequency (0.15–0.40 Hz) bands.

Calculations and Statistical Analyses

The insulin sensitivity indices for steady states before (60–90 min) and after spray application (180–210 min) were calculated by dividing the mean GIR necessary to maintain euglycemia by mean plasma insulin for t = 75 and 90 and t = 180–210, respectively. Percent change in GIR, insulin sensitivity, and heart rate variability parameters were calculated based on the values of the steady states after and before spray application.

For all statistical analyses, the software package JMP 10 (SAS Institute, Cary, NC) was used. Groups were compared by unpaired t tests based on missing values. Correlations and adjustments were calculated by multiple linear regression analyses. MANOVA (condition × time) was used to compare time courses. Results with $P < 0.05$ were considered statistically significant. Data are presented as mean ± SEM.

fMRI

Eleven fasted subjects (eight lean, three obese) participated in PASL (pulsed arterial spin labeling) measurements to determine cerebral blood flow (CBF). After the first measurement, 160 Units nasal insulin were applied. Thirty minutes after spray, a second measurement was performed.

fMRI—Data Acquisition

A 3T scanner (Tim Trio; Siemens) with a 12-channel head coil was used. PASL images were obtained with PICORE-Q2TIPS (proximal inversion with control for off-resonance effects quantitative imaging of perfusion using a single subtraction second version with thin-slice inversion time $[TI]_1$ periodic saturation) sequence, using frequency offset–corrected inversion pulse and echo planar imaging readout for acquisition. Sixteen axial slices with slice thickness of 5 mm were acquired. Each measurement consisted of 79 alternating tag and control images with the following imaging parameters: TI_1 = 700 ms, TI_2 = 1,800 ms, resonance time = 3,000 ms, echo time = 19 ms, inplane resolution = 3 × 3 mm^2, field of view = 192 mm, and flip angle = 90°. The

same sequence was used to estimate equilibrium magnetization of the blood (M0) for absolute CBF quantification. A high-resolution T1-weighted anatomical image was acquired.

fMRI—Image Processing

Image preprocessing was performed using ASLtbx (21) and SPM8 (Wellcome Trust Centre for Neuroimaging, London, U.K.). Functional data were analyzed as described (22). Images were realigned and resliced. The M0 images of each session were coregistered separately to the mean image. The functional images were additionally coregistered to the individual anatomical image and smoothed (6 mm). Baseline-corrected relative CBF maps were computed to quantify the CBF changes after spray. Changes in regional CBF were extracted from the hypothalamus and visual cortex (control area) using the Wake Forest University PickAtlas tool (http://www.fmri.wfubmc.edu/download.htm).

RESULTS

Hyperinsulinemic-Euglycemic Clamp

In normal weight men, stable GIR was rapidly reached and maintained during the steady state before nasal spray application (Fig. 1*A*). Furthermore, stable hyperinsulinemia was reached and C-peptide levels decreased (Fig. 1*C* and *D*). Fifteen minutes after intranasal insulin application, there was a small and nonsignificant increase in serum insulin levels compared with placebo (P = 0.07). This difference in insulin levels was no longer apparent 15 min later (P = 0.3). C-peptide and glucose levels did not differ between the two experiments (Fig. 1*B* and *D*).

After intranasal insulin spray application, a significantly higher GIR was necessary to maintain euglycemia compared with placebo (P_{MANOVA} = 0.0015). This difference remained significant after adjustment for age and BMI (P_{MANOVA} = 0.0045). Accordingly, when the clamp-derived insulin sensitivity index was calculated for both steady states, it improved more after insulin than after placebo spray application (151 ± 9% vs. 111 ± 10%, P = 0.0077) (Fig. 2). This was independent of age and BMI (P = 0.0038). Neither insulin, C-peptide, nor glucose levels differed between conditions (all $P_{MANOVA} > 0.3$) (Fig. 1*B–D*).

To study factors associated with brain insulin resistance, we also examined overweight men (Supplementary Fig. 1). Although insulin sensitivity was significantly higher after insulin compared with placebo spray in lean participants (+41 ± 8%, P = 0.0077), it did not change in obese participants (−0.7 ± 18%, P = 0.9) (Fig. 2). The difference between lean and obese men in response to intranasal insulin was statistically significant (P = 0.0094) (Fig. 2), even after adjustment for age (P = 0.0068).

Heart Rate Variability

To assess potential mechanisms linking brain and peripheral metabolism, we investigated the effect of intranasal insulin versus placebo spray on the autonomic nervous system as assessed by heart rate variability. Changes in

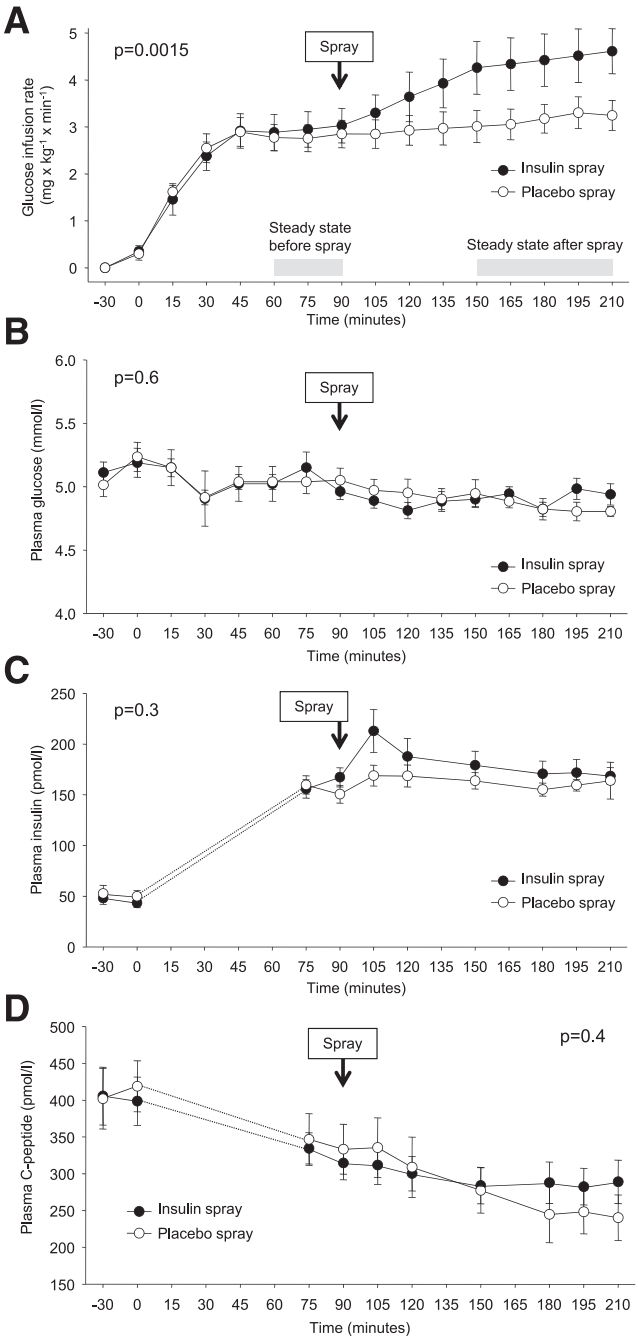

Figure 1—Hyperinsulinemic-euglycemic glucose clamp results. *A*: After 45 min, a stable GIR was reached in all participants. At 90 min, insulin or placebo was administered as nasal spray. After insulin spray, a significantly higher GIR was necessary to maintain euglycemia. *B*: On both study days, plasma glucose was at the target level and did not differ between days. *C*: During the clamp experiment, plasma insulin levels were elevated and did not differ before spray application. Fifteen minutes after spray application, there was a slight increase in insulin that was diminished 15 min later. *D*: During the clamp experiment, C-peptide levels decreased without a difference between study days. Data are mean ± SEM for the 10 lean participants. Differences between insulin and placebo spray application were examined by MANOVA (treatment × time).

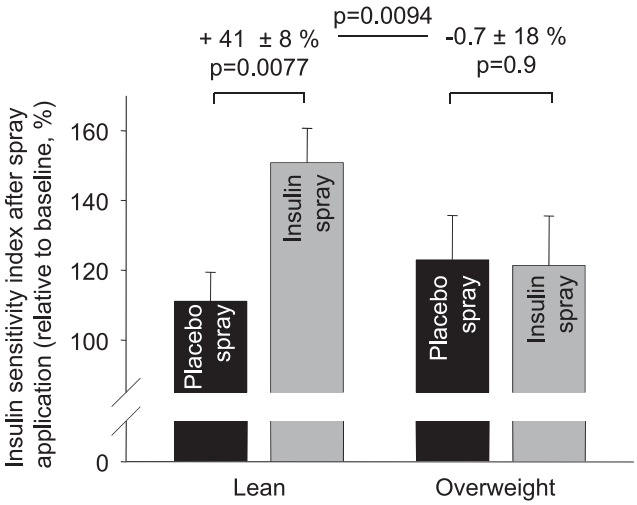

Figure 2—Change in peripheral insulin sensitivity index from before to after spray application. In lean participants, insulin sensitivity improved significantly more after the insulin than after the placebo spray application. In obese participants, there was no difference between insulin and placebo spray. Improvement in the insulin sensitivity index was significantly different between lean and obese participants. Data are mean ± SEM.

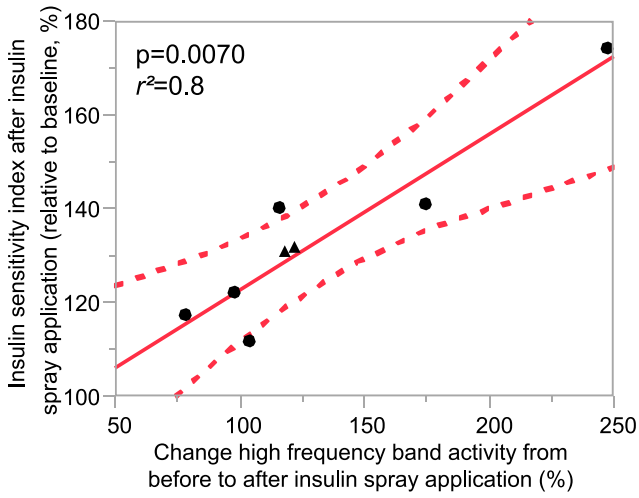

Figure 3—Change in peripheral insulin sensitivity index from before to after insulin spray application is associated with change in high-frequency band activity. Change in insulin sensitivity index from before to after insulin spray application is plotted against change in high-frequency band activity from before to after insulin spray application. ●, lean participants; ▲, obese participants. Lines represent fit line ± CI from a model adjusted for age and BMI. *P* and r^2 values are also from the model adjusted for age and BMI. *n* = 8.

the high-frequency (*P* = 0.0085) but not in the low-frequency (*P* = 0.07) band activity from the steady state of the clamp before to the steady state after spray application were significantly different between insulin and placebo. Differences in the high-frequency band remained significant after adjustment for age and BMI, with an increase after intranasal insulin and a slight decrease after placebo spray application (132 ± 15% vs. 77 ± 13%, *P* = 0.02). The change in high-frequency band activity between the measurements before and after insulin spray correlated positively with the simultaneous change in insulin sensitivity (*P* = 0.0070, adjusted for age and BMI) (Fig. 3).

fMRI

In 11 participants, CBF was measured by fMRI to assess regional brain activity before and after intranasal insulin application. The change in hypothalamic CBF after nasal insulin administration was significantly correlated with the change in insulin sensitivity (*P* = 0.0062, adjusted for age and BMI). Accordingly, there was also a significant correlation with baseline-adjusted absolute hypothalamic CBF after spray application (Fig. 4). As a control region, we analyzed baseline-adjusted absolute CBF of the visual cortex and found no correlation to change in insulin sensitivity (*P* = 0.6, adjusted for age and BMI).

DISCUSSION

In the current study, we found intranasal insulin spray application to improve whole-body insulin sensitivity in lean men as assessed by the hyperinsulinemic-euglycemic clamp. The magnitude of this effect was reduced in obese participants. Furthermore, we showed that this insulin-sensitizing action is correlated to changes in heart rate variability, an estimate of autonomic nervous system activity, and to changes in hypothalamic activity.

Because the clamp technique requires intravenous insulin infusion, we were concerned about the possibility that the intravenously infused insulin with the commonly used dosage of 1 mU/kg/min reaches the brain and occupies a substantial number of insulin receptors, thus possibly blunting the effects of intranasal insulin application. Therefore, we used a lower insulin dose (0.25 mU/kg/min) in a modified hyperinsulinemic-euglycemic clamp. In previous clamp experiments, we showed that this lower insulin dose did not alter human brain activity, whereas higher doses caused major effects (17). Furthermore, the lower insulin infusion dose used in the current study does not completely suppress hepatic glucose output (23). Therefore, we assume that this modified low-dose hyperinsulinemic-euglycemic clamp allows reliable determination of the effect of intranasal insulin on peripheral insulin action.

In agreement with previous studies (20), there was a slight increase in plasma insulin levels after intranasal insulin administration. This is a result of spillover of exogenous intranasal insulin to the vascular system. Of note, the amount of spilled over insulin was too small to further suppress endogenous insulin secretion because C-peptide levels during intranasal insulin application were not altered. However, minor acute effects of spillover insulin on metabolism cannot be excluded. Given the insulin half-life of <10 min (24), exogenous nasal insulin was probably cleared from the circulation before we assessed insulin sensitivity after spray application (150–210 min).

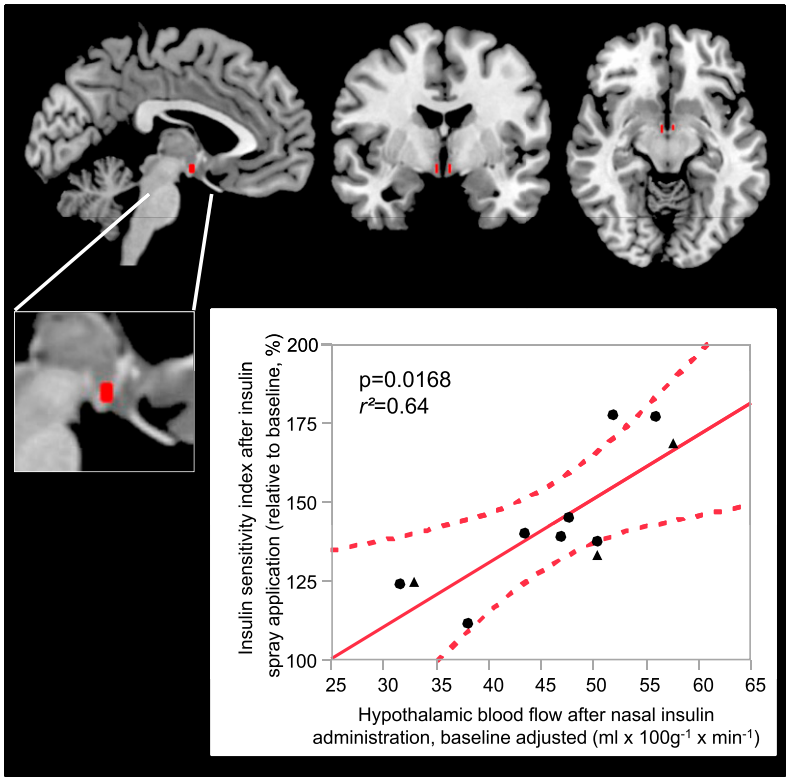

Figure 4—Change in peripheral insulin sensitivity index from before to after insulin spray application is associated with hypothalamic activity after nasal insulin administration. Shown is the hypothalamic region of interest marked in red on a sagittal (left), a coronal (middle), and an axial (right) section plane. Also shown is the change in insulin sensitivity index from before to after insulin spray application plotted against absolute hypothalamic CBF after insulin spray application adjusted for blood flow before spray application. ●, lean participants; ▲, obese participants. Lines represent fit line ± CI from a model adjusted for age and BMI. *P* and r^2 values are also from the model adjusted for age and BMI. n = 11.

Previously, we estimated the effects of brain insulin on peripheral insulin sensitivity by fasting insulin-to-glucose ratios (20). These data suggested that intranasal insulin might immediately cause peripheral insulin resistance followed by an insulin-sensitizing effect. The current experiments using the much more reliable clamp technique rules out the first assumption; we observed no decrease in GIR directly after intranasal insulin spray, and thus, no immediate insulin resistance occurred. However, the second observation of enhanced whole-body insulin sensitivity after intranasal insulin application holds true. Of note, this effect is rapid and corresponds to the time course observed in rodents (3,6,14) but not in dogs (11).

Because obesity is linked to brain insulin resistance (13,14,25), we next investigated whether the peripheral insulin-sensitizing ability of brain insulin action is altered in obesity. Indeed, this mechanism was reduced in obese participants, suggesting that brain insulin resistance also affects these properties. The current results indicate that impaired brain outputs contribute to the pathogenesis of whole-body insulin resistance in obesity.

To study how the brain communicates with the periphery to regulate insulin sensitivity, we analyzed autonomic nervous system activity as assessed by frequency-based heart rate variability. Although the low-frequency band is associated mainly with sympathetic activity, the high-frequency band is mediated by activity of the parasympathetic nervous system (26). We detected an increased high-frequency band, that is, parasympathetic activity to be associated with brain-derived peripheral insulin sensitization, indicating that vagal outputs are involved. This interpretation is well in line with animal data, where brain outputs regulating peripheral insulin sensitivity depend on the vagus nerve, the major parasympathetic nerve (2,3).

The hypothalamus is the central brain regulator of metabolism. Previously, we demonstrated that intranasal insulin regulates hypothalamic activity in lean women (16). In animals, specific hypothalamic neurons are crucial for the control of peripheral metabolism by insulin (1,4,5,7). Reduced insulin receptor expression causes peripheral insulin resistance (4,7). The current finding of a correlation of the ability of intranasal insulin to improve peripheral insulin sensitivity with intranasal insulin-induced change in hypothalamic activity indicates that this function of the hypothalamus holds true in men as well.

Further studies could provide additional information on conditions associated with brain insulin resistance as well as on the specific brain regions involved. These studies should include elderly participants and women. To

determine which organs are involved and to assess glucose kinetics, tracer infusion should be applied. Another limitation is the resolution of fMRI, making the hypothalamic subregions difficult to distinguish.

In conclusion, we found central insulin action to improve peripheral insulin sensitivity in men. This reaction is reduced in obese people, thereby possibly contributing to whole-body insulin resistance. Furthermore, we propose that insulin action in the brain promotes peripheral insulin sensitization through hypothalamus and parasympathetic outputs.

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Author Contributions. M.H. designed the study, performed experiments, analyzed results, and wrote the manuscript. R.W., K.L., and C.B. performed experiments and contributed to the discussion. S.K. performed brain imaging, analyzed results, and contributed to the discussion. R.V. and H.M.H. analyzed heart rate data and contributed to the discussion. A.P. was responsible for laboratory measurements and contributed to the discussion. N.S. and H.-U.H. contributed to the analyses and discussion. H.P. analyzed brain imaging and heart rate data and contributed to the discussion. A.F. designed the study, supervised the project, and contributed to the discussion. A.F. is the guarantor for this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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References

1. Koch L, Wunderlich FT, Seibler J, et al. Central insulin action regulates peripheral glucose and fat metabolism in mice. J Clin Invest 2008;118:2132–2147
2. Macedo MP, Lima IS, Gaspar JM, et al. Risk of postprandial insulin resistance: the liver/vagus rapport. Rev Endocr Metab Disord 2014;15:67–77
3. Filippi BM, Yang CS, Tang C, Lam TKT. Insulin activates Erk1/2 signaling in the dorsal vagal complex to inhibit glucose production. Cell Metab 2012;16:500–510
4. Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. Nat Neurosci 2002;5:566–572
5. Belgardt BF, Brüning JC. CNS leptin and insulin action in the control of energy homeostasis. Ann N Y Acad Sci 2010;1212:97–113
6. Obici S, Zhang BB, Karkanias G, Rossetti L. Hypothalamic insulin signaling is required for inhibition of glucose production. Nat Med 2002;8:1376–1382
7. Pocai A, Obici S, Schwartz GJ, Rossetti L. A brain-liver circuit regulates glucose homeostasis. Cell Metab 2005;1:53–61
8. Coomans CP, Biermasz NR, Geerling JJ, et al. Stimulatory effect of insulin on glucose uptake by muscle involves the central nervous system in insulin-sensitive mice. Diabetes 2011;60:3132–3140
9. Scherer T, O'Hare J, Diggs-Andrews K, et al. Brain insulin controls adipose tissue lipolysis and lipogenesis. Cell Metab 2011;13:183–194
10. Iwen KA, Scherer T, Heni M, et al. Intranasal insulin suppresses systemic but not subcutaneous lipolysis in healthy humans. J Clin Endocrinol Metab 2014; 99:E246–E251
11. Ramnanan CJ, Edgerton DS, Cherrington AD. Evidence against a physiologic role for acute changes in CNS insulin action in the rapid regulation of hepatic glucose production. Cell Metab 2012;15:656–664
12. Girard J. Insulin's effect on the liver: "direct or indirect?" continues to be the question. J Clin Invest 2006;116:302–304
13. Ketterer C, Tschritter O, Preissl H, Heni M, Häring H-U, Fritsche A. Insulin sensitivity of the human brain. Diabetes Res Clin Pract 2011;93(Suppl. 1):S47–S51
14. Filippi BM, Mighiu PI, Lam TKT. Is insulin action in the brain clinically relevant? Diabetes 2012;61:773–775
15. Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL. Sniffing neuropeptides: a transnasal approach to the human brain. Nat Neurosci 2002;5:514–516
16. Kullmann S, Frank S, Heni M, et al. Intranasal insulin modulates intrinsic reward and prefrontal circuitry of the human brain in lean women. Neuroendocrinology 2013;97:176–182
17. Tschritter O, Preissl H, Hennige AM, et al. The cerebrocortical response to hyperinsulinemia is reduced in overweight humans: a magnetoencephalographic study. Proc Natl Acad Sci U S A 2006;103:12103–12108
18. Kullmann S, Heni M, Veit R, et al. The obese brain: association of body mass index and insulin sensitivity with resting state network functional connectivity. Hum Brain Mapp 2012;33:1052–1061
19. Benedict C, Brede S, Schiöth HB, et al. Intranasal insulin enhances postprandial thermogenesis and lowers postprandial serum insulin levels in healthy men. Diabetes 2011;60:114–118
20. Heni M, Kullmann S, Ketterer C, et al. Nasal insulin changes peripheral insulin sensitivity simultaneously with altered activity in homeostatic and reward-related human brain regions. Diabetologia 2012;55:1773–1782
21. Wang Z, Aquirre GK, Rao H, et al. Empirical optimization of ASL data analysis using an ASL data processing toolbox: ASLtbx. Magn Reson Imaging 2008;26:261–269
22. Frank S, Linder K, Kullmann S, et al. Fat intake modulates cerebral blood flow in homeostatic and gustatory brain areas in humans. Am J Clin Nutr 2012; 95:1342–1349
23. Ader M, Bergman RN. Peripheral effects of insulin dominate suppression of fasting hepatic glucose production. Am J Physiol 1990;258:E1020–E1032
24. Tomasi T, Sledz D, Wales JK, Recant L. Insulin half-life in normal and diabetic subjects. Rev Neuropsychiatr Infant 1966;14:315–317
25. Heni M, Kullmann S, Ketterer C, et al. Differential effect of glucose ingestion on the neural processing of food stimuli in lean and overweight adults. Hum Brain Mapp 2014;35:918–928
26. Rajendra Acharya U, Paul Joseph K, Kannathal N, Lim CM, Suri JS. Heart rate variability: a review. Med Biol Eng Comput 2006;44:1031–1051