Central insulin administration improves whole-body insulin sensitivity via hypothalamus and parasympathetic outputs in men

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Abstract

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 impacts of brain insulin action on whole-body insulin sensitivity and 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, higher glucose infusion rate was necessary to maintain euglycemia compared to 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.

In summary, 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, our findings provide novel insights into the regulation of insulin sensitivity and the pathogenesis of insulin resistance in humans.
Introduction

Insulin resistance, i.e. the inability of insulin to adequately inhibit glucose production and promote glucose uptake, thereby lowering blood glucose, is one hallmark of type 2 diabetes. Experiments in animals suggest that the brain can rapidly influence insulin sensitivity of the body via the autonomous nervous system (1–5). Particularly, insulin’s action in the brain modulates insulin sensitivity in other organs like liver (2,3,6,7), muscle (8), and adipose tissue (1,9,10). 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 its application as 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). Interestingly, people do not 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 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 now determined the effects of selective insulin delivery into the brain via nasal spray on peripheral insulin sensitivity using a hyperinsulinemic euglycemic clamp. To investigate underlying mechanisms, we assessed activity of the autonomous nervous system and performed functional magnetic resonance imaging (fMRI) to unravel involved brain processes.
Methods

Participants  We studied ten normal weight male (mean age 26±1.3 years, mean weight 76±4 kg, mean BMI 21.8±0.7 kg/m²) and five obese male subjects (mean age 28±1.7 years, mean weight 116±17 kg, mean BMI 33.2±3.7 kg/m²). 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, 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. 90 minutes after initiation of the clamp, nasal spray was administered. Subjects received 160U of insulin (8 puffs in each nostril, 10U per puff over 4 minutes) and placebo on two days in single-blinded randomized order. After spray application, the clamp continued for 120 minutes. Both for intravenous and nasal administration, human insulin (NovoNordisk, Bagsvaerd, Denmark) was used.

Each five minutes, blood glucose was measured and glucose infusion rate (GIR) was adjusted to maintain euglycemia (target glucose 5mmol/l). Additional blood samples were taken at -30,0,75,90,105,120,150,180,195,210 minutes.

For two participants, only data from the insulin spray day insulin were available (in one lean subject steady GIR could not be reached in the designated time before placebo administration, one obese did not show up for the placebo experiment).

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

Heart rate variability  Electrocardiogram was recorded during the steady states before and after nasal spray application for ten subjects (eight lean, two obese). Based on equipment failure recording was not possible for two lean subjects on the insulin day. Recordings were performed with Biopac MP35 (BIOPAC, Goleta, USA). Data were sampled at 1000Hz for 10 minutes. The RR-interval time series were preprocessed by elimination of ectopic beats, detrending and high pass filtering (0.04Hz). We determined frequency based heart rate variability measures by custom made analysis programs (Matlab 12b, Matlab, Natick, USA). We investigated activity in the low (0.04-0.15Hz) and high frequency (0.15-0.40Hz) bands.

Calculations and statistical analyses  The insulin sensitivity indices for steady states before (60 to 90 minutes) and after spray application (180 to 210 minutes) were calculated by dividing mean GIR necessary to maintain euglycemia by mean plasma insulin for t=75 and 90 and t=180 to 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 JMP10 (SAS Institute, Cary, USA) 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 x time) was used to compare time courses. Results with values of p<0.05 were considered statistically significant. Data are given as means±SEM.

**Functional magnetic resonance imaging (fMRI)** Eleven fasted subjects (8 lean, 3 obese) participated in PASL (pulsed arterial spin labeling) measurements to determine cerebral blood flow. After the first measurement, 160U of nasal insulin were applied. Thirty minutes after spray, a second measurement was performed.

**fMRI – data acquisition** A 3T-scanner (Siemens, TimTrio, Erlangen, Germany) with a 12-channel head coil was used. PASL images were obtained with PICORE-Q2TIPS 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: inversion time (TI)ᵰ=700 ms, TIᵴ=1800 ms, TR=3000 ms, TE=19 ms, inplane resolution=3x3 mm², FOV=192 mm, 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 (Wang et al., 2008) and SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK). Functional data were analyzed as described (21). Images were realigned and re-sliced. 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 cerebral blood flow were extracted from the hypothalamus and visual cortex (control area) using the WFU Pickatlas tool (http://www.fmri.wfubmc.edu/download.htm).

**Results**

**Hyperinsulinemic euglycemic clamp**

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

After intranasal insulin spray application, a significantly higher GIR was necessary to maintain euglycemia compared to the placebo (p<0.0015). This difference remained significant after adjustment for age and BMI (p<0.0045). Accordingly, when 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, figure 2). This was independent of age and BMI (p=0.0038).

Neither insulin, nor C-peptide, nor glucose levels differed between conditions (all P_{MANOVA}>0.3, figures 1B-D).

To study factors associated with brain insulin resistance, we additionally examined overweight men (see supplementary figure 1). While insulin sensitivity was significantly higher after insulin compared to placebo spray in lean (+41±8 %, p=0.0077), it did not change in obese participants (-0.7±18 %, p=0.9, figure 2). The difference between lean and obese men in response to intranasal insulin was statistically significant (p=0.0094, figure 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 the high frequency (p=0.0085) but not in the low frequency band (p=0.07) 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, figure 3).

**Functional magnetic resonance imaging – fMRI**

In eleven participants, cerebral blood flow (CBF) was measured by fMRI to assess regional brain activity before and after intranasal insulin application. The change in hypothalamic CBF after nasal insulin 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 (figure 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 autonomous 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 occupying a substantial amount of insulin receptors, thus, possibly blunting effects of intranasal insulin application. Therefore, we decided to use 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, while higher doses caused major effects (17). Furthermore, the lower insulin infusion dose used in the present study does not completely suppress hepatic glucose output (22). 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. Interestingly, the amount of spilled over insulin was too small to further suppress endogenous insulin secretion as 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 less than 10 minutes (23), exogenous nasal insulin was most probably cleared from the circulation before we assessed insulin sensitivity after spray application (150-210 minutes).

Previously, we estimated 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. Our 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 clearly holds true. Of interest, this effect is rapid and corresponds to the time course observed in rodents (3,6,14) but not in dogs (11).

Since obesity is linked to brain insulin resistance (13,14,24), we next investigated if 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 affect these properties. Our results indicate that impaired brain outputs contribute to pathogenesis of whole body insulin resistance in obesity.

To study how the brain communicates with periphery to regulate insulin sensitivity we analyzed autonomous nervous system activity as assessed by frequency based heart rate variability. While the low frequency band is associated mainly with sympathetic activity, the high frequency band is mediated by activity of the parasympathetic nervous system (25). We detected increased high
frequency band, i.e. 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 that regulate 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). Our current finding of a correlation of intranasal insulin’s ability 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 specific brain regions involved. These studies should include elder 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 it difficult to distinguish hypothalamic subregions.

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 via the hypothalamus and parasympathetic outputs.
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Author contributions

MH designed the study, performed experiments, analyzed results and wrote the manuscript. RW performed experiments and discussed the paper. SK performed brain imaging, analyzed results and discussed the paper. RV and HMH analyzed heart rate data and discussed the paper. KL and CB performed experiments and discussed the paper. AP was responsible for laboratory measurements and discussed the paper. NS and HUH discussed the analyses and the discussed the paper. HP analyzed brain imaging and heart rate data and discussed the paper. AF designed the study, supervised the project and discussed the paper.

Conflict of interest

The authors declare that they have no conflict of interest.

Registration

This study was registered at Clinicaltrials.gov as trial number NCT01847456.
References


Figure legends

Figure 1
Hyperinsulinemic euglycemic glucose clamp results

(A) After 45 minutes, a stable glucose infusion rate (GIR) was reached in all participants. At 90 minutes, insulin (closed circles) or placebo (open circles) 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 target 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 already 15 minutes later. (D) During the clamp experiment, C-peptide levels decreased without a difference between study days. Given are means ± SEM for the ten lean participants. Differences between insulin and placebo spray application were examined by MANOVA (treatment x time).

Figure 2
Change in peripheral insulin sensitivity index from before to after spray application.

In lean participants (left two lanes), insulin sensitivity improved significantly more after insulin (black bar) then after placebo spray application (grey bar). In obese participants (right two lanes), there was no difference between insulin (black bar) and placebo spray (grey bar). Improvement in the insulin sensitivity index was significantly different between lean and obese participants. Presented are means ± 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. Dots represent lean participants, triangles are obese persons. Lines represents fit line ± Confidence Interval from a model adjusted for age and BMI, p- and r²-values are also from the model adjusted for age and BMI. N=8.
Figure 4
Change in peripheral insulin sensitivity index from before to after insulin spray application is
associated with hypothalamic activity after nasal insulin.

The upper part shows the hypothalamic region of interest marked in red on a sagittal (left), a coronal
(middle), and an axial section plane (right). In the lower part, change in insulin sensitivity index from
before to after insulin spray application is plotted against absolute hypothalamic cerebral blood flow
after insulin spray application adjusted for blood flow before spray application. Dots represent lean
participants, triangles are obese persons. Lines represent fit line ± Confidence Interval from a model
adjusted for age and BMI, p- and r²-values are also from the model adjusted for age and BMI. N=11.
Figure 1

A

Plasma glucose (mmol/l)

-30 0 15 30 45 60 75 90 105 120 135 150 165 180 195 210

Steady state before spray
Steady state after spray

B

Plasma glucose (mmol/l)

-30 0 15 30 45 60 75 90 105 120 135 150 165 180 195 210

C

Plasma insulin (pmol/l)

-30 0 15 30 45 60 75 90 105 120 135 150 165 180 195 210
Figure 1

![Graph showing plasma C-peptide levels over time for Insulin spray and Placebo spray. The graph indicates a trend with a p-value of 0.4.](image)

- **Insulin spray**
- **Placebo spray**

**Axes:**
- **Y-axis:** Plasma C-peptide (pmol/l)
- **X-axis:** Time (minutes)

**Legend:**
- Black circle = Insulin spray
- White circle = Placebo spray

**Statistical Note:**
- p=0.4
Insulin sensitivity index after spray application (relative to baseline, %)

Lean

Placebo spray

Insulin spray

Overweight

Placebo spray

Insulin spray

+ 41 ± 8 %

p=0.0077

-0.7 ± 18 %

p=0.9

p=0.0094
Figure 3

Insulin sensitivity index after insulin spray application (relative to baseline, %)

Change high frequency band activity from before to after insulin spray application (%)

$p=0.0070$

$r^2=0.8$
Figure 4

Insulin sensitivity index after insulin spray application (relative to baseline, %)

Hypothalamic blood flow after nasal insulin administration, baseline adjusted (ml x 100g⁻¹ x min⁻¹)

p=0.0168

$r^2=0.64$
Supplementary figure 1

A

Steady state before spray

Steady state after spray

B

C

Diabetes
Supplementary figure 1 - Hyperinsulinemic euglycemic glucose clamp results in obese subjects.

(A) After 45 minutes, a stable glucose infusion rate (GIR) was reached in all participants. At 90 minutes, insulin (closed circles) or placebo (open circles) was administered as nasal spray. (B) On both study days, plasma glucose was at target and did not significantly differ between days. (C) During the clamp experiment, plasma insulin levels were elevated. Fifteen minutes after spray application, there was an increase in insulin that was diminished already 15 minutes later. (D) During the clamp experiment, C-peptide levels decreased without a difference between study days. Given are means ± SEM for the obese participants. Differences between insulin and placebo spray application were examined by MANOVA (treatment x time).