ARTICLE

Nasal insulin changes peripheral insulin sensitivity simultaneously with altered activity in homeostatic and reward-related human brain regions

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Abstract

Aims/hypothesis Impaired insulin sensitivity is a major factor leading to type 2 diabetes. Animal studies suggest that the brain is involved in the regulation of insulin sensitivity. We investigated whether insulin action in the human brain regulates peripheral insulin sensitivity and examined which brain areas are involved.

Methods Insulin and placebo were given intranasally. Plasma glucose, insulin and C-peptide were measured in 103 participants at 0, 30 and 60 min. A subgroup ($n=12$) was also studied with functional MRI, and blood sampling at 0, 30 and 120 min. For each time-point, the HOMA of insulin resistance (HOMA-IR) was calculated as an inverse estimate of peripheral insulin sensitivity.

Results Plasma insulin increased and subsequently decreased. This excursion was accompanied by slightly decreased plasma glucose, resulting in an initially increased HOMA-IR. At 1 h after insulin spray, the HOMA-IR subsequently decreased and remained lower up to 120 min. An increase in hypothalamic activity was observed, which correlated with the increased HOMA-IR at 30 min post-spray. Activity in the putamen, right insula and orbitofrontal cortex correlated with the decreased HOMA-IR at 120 min post-spray.

Conclusions/interpretation Central insulin action in specific brain areas, including the hypothalamus, may time-dependently regulate peripheral insulin sensitivity. This introduces a potential novel mechanism for the regulation of

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peripheral insulin sensitivity and underlines the importance of cerebral insulin action for the whole organism.

Abbreviations

fALFF	Fractional amplitude of low-frequency fluctuation
fMRI	Functional MRI
HOMA-IR	HOMA of insulin resistance
HPA axis	Hypothalamic–pituitary–adrenal axis

Introduction

Insulin resistance is a major feature of type 2 diabetes mellitus and precedes the disease by years [1]. Besides the effects on glucose metabolism, insulin resistance is associated with other conditions like cardiovascular disease [2] and cancer [3], and even predicts mortality [4]. The precise mechanisms that regulate peripheral insulin sensitivity in humans are still not fully understood.

Studies in animals have demonstrated that insulin signalling in the brain is pivotal to the regulation of insulin action in major insulin-sensitive organs in the periphery [5]. Thus insulin-dependent hepatic glucose production [6], fat metabolism in adipocytes [7] and muscle glycogen synthesis [8] are all influenced by central actions of insulin.

However, all these data were generated in animals; indeed, studies on insulin's actions in the human brain are much more complex to perform. One tool, the nasal administration of insulin, allows selective investigation of insulin's effects on the human brain without causing relevant peripheral effects [9]. Using this technique, insulin action in the brain has been shown to affect a number of cerebral functions in healthy humans [10], namely declarative memory [11], spontaneous brain activity [12] and food-related activity [13, 14]. Functional MRI (fMRI) in combination with nasal administration of insulin identified insulin-sensitive regions in vivo that may contribute to those functions [13]. Furthermore, long-term nasal application of insulin reduces the activity of the hypothalamic–pituitary–adrenal (HPA) axis, as indicated by decreased serum cortisol levels [11, 15–17]. The nasal delivery of insulin may thus be helpful when specifically studying the influence of insulin action in the human brain on peripheral metabolism and insulin sensitivity.

The aims of this study were: (1) to investigate how nasal insulin delivery affects peripheral metabolism in humans; (2) to examine the brain structures involved; and (3) to look for a possible link between insulin's effects in the brain and peripheral metabolism via the HPA axis.

Methods

Participants There were 103 voluntary participants in the study with nasal insulin. All participants were in good health as ascertained by a physician and did not suffer from any psychiatric, neurological or metabolic illness. The clinical characteristics of these participants are shown in Table 1. Any volunteer with diabetes mellitus or with a family history of diabetes was excluded at screening, as well as anyone being treated for a chronic disease or taking any kind of medication except for oral contraceptive agents. Informed written consent was obtained from all participants and the local ethics committee approved the protocol.

Nasal insulin experiment The participants received either insulin or placebo spray on two different days in randomised order with an interval of at least 7 days between the administrations. The participants were blinded to the order of the sprays. Experiments were conducted after an overnight fast of at least 10 h. A cubital blood vein was cannulated and blood samples were obtained at −30, 0, 30 and 60 min from all participants, and additionally at 90 and 120 min from a subgroup of 12 women.

Intranasal insulin and placebo spray The insulin and placebo sprays were prepared as described earlier [9]. Each puff consisted of 0.1 ml solution containing 40 IU human insulin (400 IU/ml, Insulin Actrapid; Novo Nordisk, Copenhagen, Denmark) or 0.1 ml vehicle as a placebo. At time 0 min, each participant received four puffs of 0.1 ml insulin or placebo spray within 5 min. Two doses were applied to each nostril, resulting in a total insulin dose of 160 IU insulin on the insulin day.

Analytical procedures Blood glucose was determined using a bedside glucose analyser (glucose oxidase method; Yellow Springs Instruments, Yellow Springs, OH, USA). Plasma insulin, C-peptide and cortisol were measured by commercial chemiluminescence assays (ADVIA Centaur; Siemens Medical Solutions, Fernwald, Germany).

Calculations of HOMA of insulin resistance The HOMA of insulin resistance (HOMA-IR) was calculated as described in

Table 1 Clinical characteristics of the participants

Characteristic	Value
n	103
Male/female (*n*)	35/68
Age (years)	28±9
BMI (kg/m²)	22.6±2.9
Fasting glucose (mmol/l)	4.86±0.40
Fasting insulin (pmol/l)	40.2±21.4

Data are given as means±SD

[18]. Baseline levels were calculated as the mean of two measurements (at −30 and 0 min) before nasal spray application. Changes in the HOMA-IR at 30 and 120 min after nasal insulin spray application were assessed in the subgroup of participants undergoing fMRI measurements. They were calculated by dividing the HOMA-IR at 30 or 120 min by the HOMA-IR before spray delivery (0 min). Thus, values less than 1 indicate a decrease in HOMA-IR over time. Data that were not normally distributed were logarithmically transformed prior to statistical analysis. For all statistical analyses, the software package JMP 8 (SAS Institute, Cary, NC, USA) was used. Results with values of $p \leq 0.05$ were considered statistically significant.

fMRI data acquisition Whole-brain fMRI data were obtained using a 3.0 T scanner (Siemens Tim Trio, Erlangen, Germany). Participants in the resting state underwent 5-min fMRI acquisitions at time points 0, 30 and 120 min. All participants were instructed not to focus their thoughts on anything in particular and to keep their eyes closed during the resting-state fMRI acquisition. Functional data were collected using an echo-planar imaging sequence as follows: TR=1.8 s, TE=30 ms, FOV=210 mm², matrix 64×64, flip angle 90°, voxel size $3 \times 3 \times 4$ mm^3, slice thickness 3 mm, 1 mm gap, where TR is repetition time, TE is echo time and FOV is field of view, and images were acquired in ascending order. Each brain volume comprised 28 axial slices and each functional run contained 160 image volumes, resulting in a total scan time of 4.52 min. In addition, high-resolution T1-weighted anatomical images (MPRage 176 slices, matrix 256×224, $1 \times 1 \times 1$ mm^3) of the brain were obtained.

Data pre-processing Functional image pre-processing and statistical analysis were carried out using SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). Images were realigned to the first image. Geometrically distorted echo planar images were unwarped using the FieldMap Toolbox that is available for SPM5 to account for susceptibility to movement artefacts. A mean image was created and co-registered to the T1 structural image. The anatomical image was normalised to the Montreal Neurological Institute (MNI) template and the resulting variable file used to normalise the functional images (voxel size $3 \times 3 \times 3$ mm^3). Finally, the normalised images were smoothed with a three-dimensional isotropic Gaussian kernel (full width half maximum 6 mm). fMRI data were high-pass- (cut-off period 128 s) and low-pass-filtered (autoregression model AR [1]).

Fractional amplitude of low-frequency fluctuation We carried out the fractional amplitude of low-frequency fluctuation (fALFF) analysis on the pre-processed functional data using REST (State Key Laboratory of Cognitive Neuroscience and Learning in Beijing Normal University, http://resting-fmri.sourceforge.net, accessed 1 June 2011).

Zang et al. [19] have developed an index for the ALFF by integrating the square root of the power spectrum over the low-frequency range in order to examine the regional intensity of spontaneous BOLD fluctuations. Since the ALFF appeared to be sensitive to physiological noise, Zou et al. [20] have proposed an fALFF approach, which effectively suppressed non-specific signals, and significantly improved the sensitivity and specificity for detection of regional spontaneous brain activity. In brief, after linear trend removal, the resting-state time series was transformed into the frequency domain using the fast Fourier transform (FFT). To obtain the amplitude, the square root of the power at each frequency bin was calculated. The ratio of the averaged amplitudes between 0.009 and 0.08 Hz and over the entire frequency range (0–0.25 Hz) was computed at each voxel. An fALFF map for the whole brain was determined by the following normalisation procedure: fALFF=(fALFF−global mean fALFF)/standard deviation of global mean fALFF.

Statistical analysis For each participant, the fALFF maps of the basal fMRI measurement were subtracted from the fALFF maps of the 30 and 120 min fMRI measurements. The fALFF maps of each participant, corrected for basal fMRI measurement, were entered into a multiple regression analysis in SPM5 using HOMA-IR as a covariate. Only activations exceeding an uncorrected threshold of $p<0.001$ at the whole-brain level were considered to be significant.

Correlation of intrinsic brain activity (fALFF values) with HOMA-IR The regional fALFF of significant activations was extracted using a volume of interest approach in SPM5 (http://web.mit.edu/swg/rex, accessed 1 June 2011). These average z values were correlated with the change in HOMA-IR at 30 and 120 min after insulin application, adjusted for BMI by using least-squares regression analysis in JMP (SAS Institute). A value of $p<0.05$ was considered statistically significant. Non-normally distributed variables were logarithmically transformed.

Results

Effects of nasal insulin on glucose, insulin and C-peptide Plasma glucose significantly decreased by about 0.2 mmol/l (about 4 %) after nasal insulin delivery (Fig. 1a). In the subgroup of 12 women for whom blood samples over 120 min were available glucose levels remained lower for 120 min, but without reaching statistical significance (Fig. 1e).

Insulin levels were significantly higher (about 30 %) at 30 min after nasal insulin spray than after placebo spray (Fig. 1b). This was accompanied by lower C-peptide levels at the same time point (Fig. 1c). By as early as 60 min after spray delivery, plasma insulin levels fell again and returned to

Fig. 1 Metabolic changes after nasal insulin application. At baseline, insulin (black circles) or placebo (white circles) was administered intranasally. **a** The subsequent changes in the whole cohort of 103 participants for glucose, (**b**) insulin and (**c**) C-peptide. **d** Data from the subgroup of 12 volunteers undergoing measurement at 120 min for glucose, (**e**) insulin and (**f**) C-peptide. There were significant differences in the time course of all variables for insulin vs placebo application ($p \leq 0.05$ multivariate ANOVA, treatment × time). *$p < 0.05$ for differences between insulin and placebo application in a post hoc paired t test
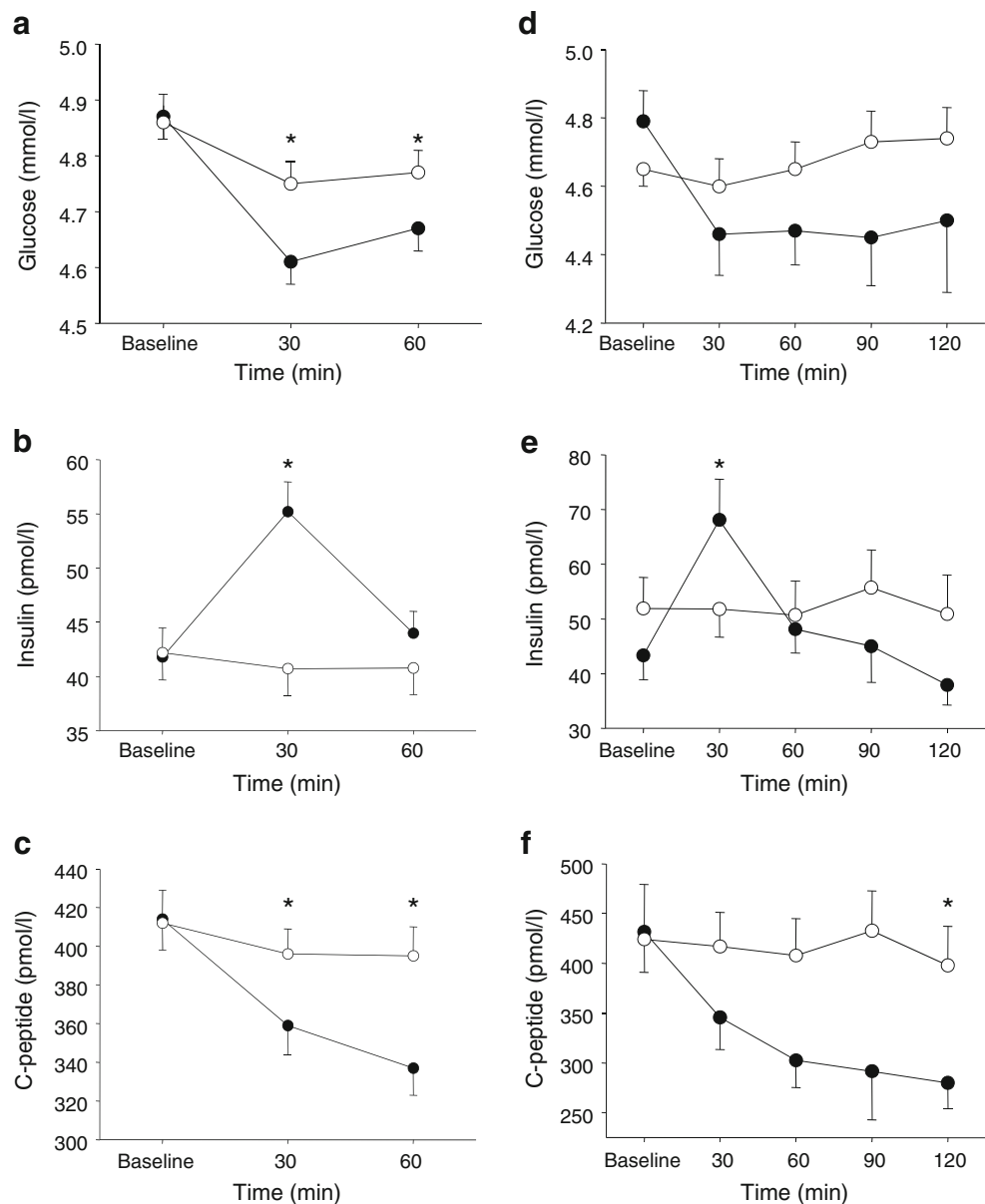

baseline (Fig. 1b), falling even lower at 90 and 120 min post-spray compared with placebo (not significant; Fig. 1f). Over the whole time course, C-peptide levels remained lower after application of nasal insulin than after placebo (Fig. 1c, g).

Effects of nasal insulin on HOMA-IR The HOMA-IR is a widely used index describing the relation between insulin and glucose, and can be seen as an inverse estimation of peripheral insulin sensitivity. At 30 min, the HOMA-IR was significantly higher after insulin spray delivery than after placebo. This was followed by a drop back to baseline levels at 60 min (Fig. 2a, b). In the subgroup followed for 2 h, the HOMA-IR became lower at 60 min after insulin spray compared with placebo. This decrease persisted for the rest of the experiment and reached statistical significance at 120 min after spray application (Fig. 1b).

Effects of nasal insulin on plasma cortisol Plasma cortisol levels decreased over time with no difference between the nasal application of insulin or placebo ($p = 0.3$ multivariate ANOVA; electronic supplementary material [ESM] Fig. 1).

Correlation of change in HOMA-IR with change in regional brain activity We investigated possible interactions between changes in the HOMA-IR and regional resting-state brain activity at 30 and 120 min after insulin spray application. The multiple regression analysis revealed a significant relationship between the HOMA-IR and change in resting-state brain activity in the hypothalamus at 30 min after insulin spray, and in the putamen, orbitofrontal cortex and right insula at 120 min after insulin spray. We observed a significant positive correlation between changes in the HOMA-IR and the hypothalamus at 30 min after insulin spray
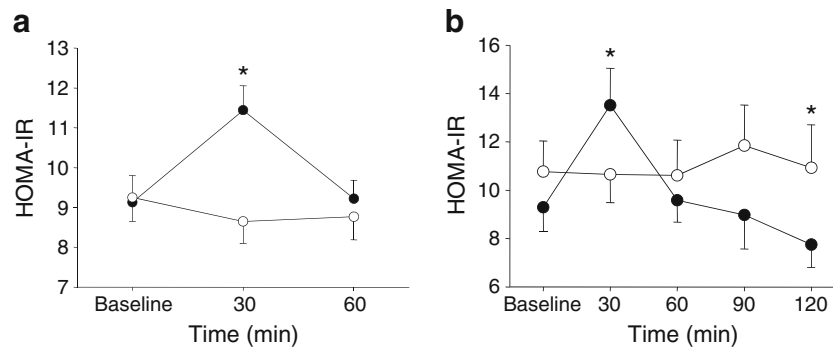
Fig. 2 Changes in HOMA-IR after nasal insulin application. At baseline, insulin (black circles) or placebo (white circles) was administered intranasally. **a** The subsequent HOMA-IR values for each time point in the whole cohort of 103 participants. **b** Data from the subgroup of 12 volunteers undergoing measurement at 120 min. There were significant differences in the time course of all the variables for insulin vs placebo application ($p < 0.05$ multivariate ANOVA, treatment × time). *$p < 0.05$ for differences between insulin and placebo application in a post-hoc paired t test

application ($r^2 = 0.53$, $p = 0.003$; Fig. 3). A significant positive correlation was found between changes in the HOMA-IR and in the left and right putamen at 120 min post-insulin ($r^2 = 0.44$, $p = 0.01$ and $r^2 = 0.46$, $p = 0.009$, respectively; Fig. 4). A significant positive correlation was also found between changes in the HOMA-IR at 120 min post-insulin and changes in the orbitofrontal cortex ($r^2 = 0.36$, $p = 0.02$; Fig. 5a). A significant negative correlation was found between changes in the HOMA-IR and in the right insula at 120 min post-insulin ($r^2 = 0.49$, $p = 0.006$; Fig. 5b).

Discussion

In this study, we investigated the influence of central insulin action on peripheral metabolism. We detected time-dependent changes. Thus, after nasal insulin delivery, there was an initial marked rise in plasma insulin, followed by a decrease to below the values observed after placebo administration. Interestingly, despite these insulin excursions, we observed only a slight initial decrease in plasma glucose (about 4 %) without further alteration. We calculated the HOMA-IR to describe the relation between insulin and glucose. This index can be seen as an inverse estimation of peripheral insulin sensitivity.

While peripheral insulin sensitivity decreased immediately after delivery of nasal insulin to the brain, it subsequently increased, starting at 1 h after spray application. Peripheral insulin sensitivity remained higher for the following final 60 min of our experiment. We investigated brain areas associated with these reactions and found an increase in hypothalamic activity that correlated with a reduction of peripheral insulin sensitivity at 30 min after nasal insulin application. The enhanced insulin sensitivity at 120 min after the insulin spray correlated with altered activity in the putamen, the orbitofrontal cortex and the right insula. No
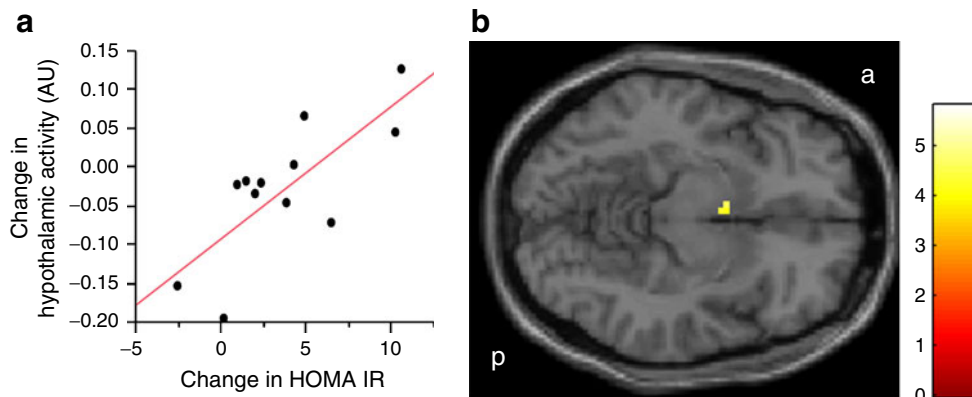
Fig. 3 Correlation of change in HOMA-IR in the first 30 min after nasal spray application with change in hypothalamic activity in the same time period. **a** Plot showing positive correlation between change in HOMA-IR and the corresponding change in hypothalamic activity. Data are adjusted for BMI; $r^2 = 0.53$; $p = 0.003$. **b** Colour-coded T-value map showing significant ($p < 0.001$, uncorrected) correlation between change in HOMA-IR and change in resting-state brain activity. a, anterior; AU, arbitrary unit; p, posterior
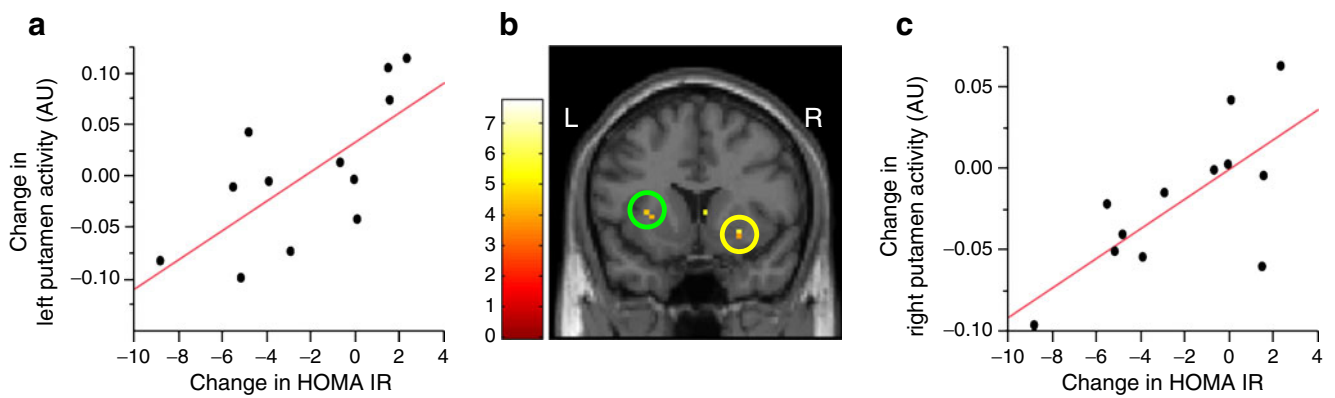
Fig. 4 Correlation of change in HOMA-IR from baseline to 120 min after nasal spray with change in putamen activity in the same time period. **a** Positive correlations between change in HOMA-IR and the corresponding change in activity of the left and (**c**) right putamen. Data are adjusted for BMI; (**a**) $r^2=0.44$; $p=0.01$; (**b**) $r^2=0.46$; $p=0.009$.

b Colour-coded T-value map showing significant ($p<0.001$, uncorrected) correlation between change in HOMA-IR and change in resting-state brain activity. Green circle, left putamen; yellow circle, right putamen. AU, arbitrary unit; L, left; R, right

effect of nasal insulin on HPA axis activity as assessed by cortisol levels was observed.

We observed decreased insulin sensitivity in the first phase of our experiment. The HOMA-IR index increased because the approximately 30 % increase in plasma insulin

was not accompanied by an adequate decrease in glucose (glucose decreases only about 4 %). This increase in plasma insulin is most likely to be due to the absorption of small quantities of the nasal insulin into the systemic circulation, and not to enhanced release from beta cells, since the

Fig. 5 Correlation of change in HOMA-IR from baseline to 120 min after nasal spray with change in activity in the orbitofrontal cortex (**a**, **b**) and the insula (**c**, **d**) in the same time period. **b**, **d** Colour-coded T-value maps showing significant ($p<0.001$, uncorrected) correlation between change in HOMA-IR and change in resting-state brain activity. **a** Plot showing positive correlation between change in HOMA-IR and the corresponding changes in the orbitofrontal cortex and (**c**) insula activity. Data are adjusted for BMI; (**a**) $r^2=0.36$; $p=0.02$; (**c**) $r^2=0.49$; $p=0.006$. AU, arbitrary unit
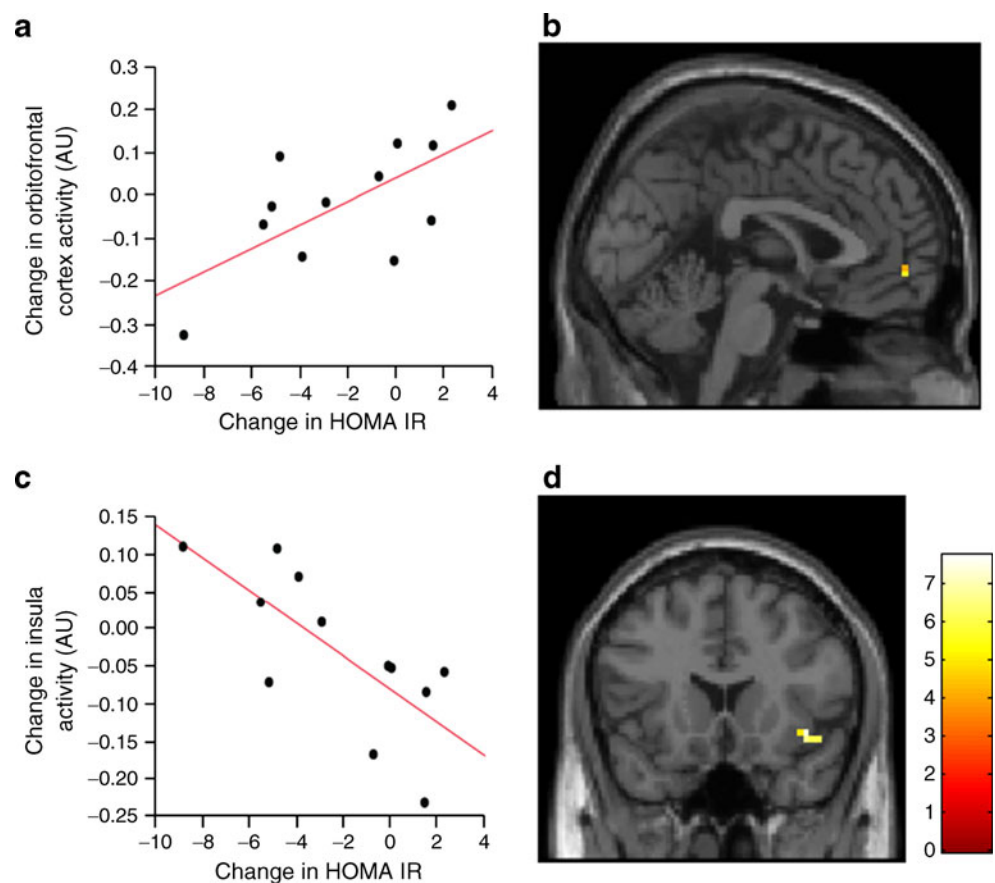
increase in plasma insulin was accompanied by a decrease in C-peptide. The absorption of small amounts of nasally delivered insulin into the peripheral circulation was not unexpected, having been noted in other studies [11, 15, 21]. A transient 20 % increase in plasma insulin as observed in our study at 30 min should be biologically active [22, 23], regardless of whether it is of endogenous or exogenous origin. The drop in plasma glucose, however, was only slight, indicating that the effectiveness of insulin was impaired at the periphery. This is also indicated by the increased HOMA-IR. It is unlikely that the slight decrease in plasma glucose was accompanied by counter-regulatory mechanisms, since these are activated only at much lower glucose levels [24]. Accordingly, one major counter-regulatory hormone, cortisol, was unaffected in our study.

At least two explanations for the transient decrease in peripheral insulin sensitivity are possible. First, the insulin absorbed into the circulation could have caused peripheral insulin resistance [25]. Second, insulin's actions in the brain could be responsible for this reaction. We cannot rule out the possibility that the spill-over from intranasal insulin directly affects peripheral insulin sensitivity. However, the second possibility is supported by our observations in the hypothalamus, since participants with a decrease in peripheral insulin sensitivity at 30 min post-spray exhibited a simultaneous increase in hypothalamic activity. Therefore, the effects of insulin in the hypothalamus could be involved in the reaction in the periphery. This hypothesis is supported by studies in animals, which have revealed that the hypothalamus is crucial to the regulation of peripheral insulin action, especially in the liver [6, 26, 27], as well as pointing to the well-known abundance of insulin receptors in this area [28, 29].

The second half of our experiment showed a very different result. Starting at 60 min post-spray, insulin sensitivity was even better after intranasal insulin than it was after placebo. Thus, in the long run, central insulin action seems to promote insulin's actions in the periphery, a finding that is consistent with a recent report [21]. In that study, nasal insulin was administered more than 1 h before participants drank a liquid high-energy meal. During this meal, insulin levels were significantly lower after the insulin spray than after placebo, while glucose excursions remained similar.

The phenomenon described by Benedict et al. [20], namely that only smaller amounts of plasma insulin were required for postprandial glucose control, could be explained on the basis of our findings as being due to brain-triggered enhancement of peripheral insulin sensitivity. This enhancement may occur mainly in the classical insulin-sensitive tissues liver [6], adipocytes [7] and muscle [8], as suggested by animal studies.

Our results suggest that insulin's actions in the brain may have a biphasic effect on peripheral insulin sensitivity, which fits well with insulin kinetics after a meal. Thus

directly after food intake, insulin levels increase sharply. During this first phase, the brain makes the periphery less insulin-sensitive, thereby possibly blunting the effects of the hormone. Later, when insulin levels decrease, peripheral insulin actions are still crucial to the control of glucose and fat metabolism. Thus, augmented insulin sensitivity can promote insulin's effects in the later postprandial state. Physiologically, this biphasic response is reasonable and we therefore propose that, apart from the many other effects of insulin on the brain [11–13, 30], the central action of the hormone regulates its own efficiency in peripheral tissues in a time-dependent manner.

Our study found that the hypothalamus may be involved in the first phase of this reaction. We also explored brain areas with altered activity concordant to the change in peripheral insulin sensitivity in the second phase, i.e. at 120 min post-spray. Three brain regions revealed such associations: the putamen, the right insula and the orbitofrontal cortex.

The putamen is part of the basal ganglia and well-known for its role in movement and motor coordination. It has also been found to be involved in appetite and feeding behaviour in humans [31]. Studies in animals indicate that this area may be involved in the regulation of energy homeostasis and in sensing of changes in energy balance [32, 33]. The major neurotransmitter input to the putamen is dopamine. Interestingly, the dopamine agonist bromocriptine enhances peripheral insulin sensitivity [34, 35]. Of course, other very important brain areas could also be involved in the dopaminergic regulation of insulin sensitivity, but our results certainly allow speculation on involvement of the putamen. Interestingly, we recently found fasting insulin to correlate with basal brain activity in the putamen such that higher fasting insulin was accompanied by higher activity in this area [36]. Therefore, this brain area might sense insulin, either directly or via input from other regions. Our results can be interpreted as an indication that insulin's effects on the putamen can help to regulate peripheral insulin sensitivity.

The second region identified, the insula, is part of the limbic system and important for processes of high-level cognitive control and attention [37, 38]. It also links the processing of various stimuli (e.g. gustatory and visceral information) with brain regions monitoring the internal milieu [38]. Interestingly, the insular cortex itself was recently shown to be responsive to insulin at the neuronal level [39]; moreover, postprandial changes in plasma insulin correlate with altered blood flow in this region [40]. Therefore, the insula may monitor the feeding state by sensing postprandial insulin levels and one of its outputs might contribute to the central regulation of insulin sensitivity.

In addition, the insula is connected to the orbitofrontal cortex, the third region identified to respond to intranasal

insulin at 120 min after application [41]. The orbitofrontal cortex receives information from many different sensory systems, e.g. the gustatory, olfactory and visceral sensory systems [41, 42], and is one of the brain regions for which we had previously found a significant correlation between fasting insulin and basal brain activity [36]. Furthermore, postprandial changes in plasma insulin have been found to correlate with altered cerebral blood flow in this region [40, 43]. Therefore, this area might directly or indirectly sense insulin as a further input. The orbitofrontal cortex is known to be involved in decision-making, emotional processes, and reward, including food reward [42]. A functional influence of this region on more homeostatic functions like insulin sensitivity has not, to our knowledge, been reported to date. Since the region is highly interconnected with many other areas [42, 44], it has many outputs that may directly or indirectly influence peripheral insulin sensitivity.

The associations identified in our study indicate that all of the brain regions discussed above may contribute to insulin-induced central regulation of peripheral metabolism and peripheral insulin sensitivity. Which brain area contributes most remains open; probably, there is an interplay between all of them.

We next addressed the question of how the brain communicates with the periphery to regulate insulin sensitivity. Our first hypothesis was that the HPA axis may be the linker. However, cortisol levels were not affected by the application of nasal insulin. By contrast, Bohringer et al. [45] found the cortisol response to psychosocial stress to be reduced by nasal insulin. Thus, a stress-induced increase in activity of the HPA axis may be a response to high insulin levels in the brain, while in a non-stressed milieu (as in our experiment) this neuroendocrine system is not affected. It is therefore unlikely that the HPA axis is the linker for regulation of peripheral insulin sensitivity by the brain.

In animals, there is evidence that the effects of central insulin action on hepatic gluconeogenesis are mediated by the autonomous nervous system [27, 46]. For humans, the issue is not yet clear. Recently, nasal insulin application was demonstrated to influence noradrenaline (norepinephrine) levels, an indicator of sympathetic nervous system activity [47]. However, a smaller study found no effect of intranasal insulin on muscular sympathetic nervous activity [48]. Given these data, the autonomous nervous system might be involved in the insulin-induced central regulation of peripheral insulin sensitivity. This hypothesis is supported by the brain regions identified in our study, since at least three of them contribute to regulation of the autonomous nervous system. The hypothalamus has outputs to the autonomous nervous system that are regulated by many different neuropeptides [49]. The orbitofrontal cortex has pathways that also influence this system [50]. Furthermore, the insula was found to contribute to autonomic regulation

of the gastrointestinal tract and the heart [41]. Of course, further larger studies with more direct assessment of sympathetic activity will be necessary to further clarify the role of the autonomous nervous system in the central regulation of peripheral insulin sensitivity.

Our interpretation of the data is based on the observation of simultaneous and statistically correlated alterations in brain activity and peripheral metabolism following the application of nasal insulin. Additional studies would be required to confirm the role of the hypothalamic versus limbic brain regions with regard to changes in peripheral insulin sensitivity. It would also be very interesting to replicate our findings with another measure of peripheral insulin sensitivity before and after nasal insulin application. However, the classic gold standard, the hyperinsulinaemic–euglycaemic clamp, can hardly serve as such a measurement, since it requires intravenous application of larger amounts of insulin. This exogenous insulin would also reach the brain, occupying a substantial amount of insulin receptors there, which would no longer be available for the additional effects of nasal insulin. All participants in our study were healthy and had normal glucose tolerance. The important question of whether the reaction detected by us would be the same in patients with impaired glucose tolerance or type 2 diabetes needs to be addressed by further studies in such populations.

In conclusion, central insulin delivery resulted in a biphasic response and effect on peripheral metabolism, which may reflect effects on peripheral insulin sensitivity. Initially, peripheral insulin sensitivity decreased after insulin delivery to the brain, a reaction that may prevent excessive effects of the hormone. Later a sustained improvement in peripheral insulin sensitivity occurred. This reaction may promote insulin actions in the later postprandial state. We also detected associations between hypothalamic activity and the initial reaction, as well as between the later response and three other brain areas: the putamen, the insula and the orbitofrontal cortex. Whether these regions are directly involved and which of them make the greatest contribution remains open.

Our findings introduce a novel mechanism that may contribute to the regulation of peripheral insulin sensitivity in humans, while underlining the importance of insulin action in the brain for the whole organism.

References

1. Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR (1992) Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. Lancet 340:925–929

2. DeFronzo RA, Ferrannini E (1991) Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173–194

3. Godsland IF (2010) Insulin resistance and hyperinsulinaemia in the development and progression of cancer. Clin Sci (Lond) 118:315–332

4. Ausk KJ, Boyko EJ, Ioannou GN (2010) Insulin resistance predicts mortality in nondiabetic individuals in the US. Diabetes Care 33:1179–1185

5. Pagotto U (2009) Where does insulin resistance start? The brain. Diabetes Care 32(Suppl 2):S174–S177

6. Obici S, Zhang BB, Karkanias G, Rossetti L (2002) Hypothalamic insulin signaling is required for inhibition of glucose production. Nat Med 8:1376–1382

7. Koch L, Wunderlich FT, Seibler J et al (2008) Central insulin action regulates peripheral glucose and fat metabolism in mice. J Clin Invest 118:2132–2147

8. Perrin C, Knauf C, Burcelin R (2004) Intracerebroventricular infusion of glucose, insulin, and the adenosine monophosphate-activated kinase activator, 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside, controls muscle glycogen synthesis. Endocrinology 145:4025–4033

9. Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL (2002) Sniffing neuropeptides: a transnasal approach to the human brain. Nat Neurosci 5:514–516

10. Ketterer C, Tschritter O, Preissl H, Heni M, Haring HU, Fritsche A (2011) Insulin sensitivity of the human brain. Diabetes Res Clin Pract 93(Suppl 1):S47–S51

11. Hallschmid M, Schultes B (2009) Central nervous insulin resistance: a promising target in the treatment of metabolic and cognitive disorders? Diabetologia 52:2264–2269

12. Stingl KT, Kullmann S, Guthoff M, Heni M, Fritsche A, Preissl H (2010) Insulin modulation of magnetoencephalographic resting state dynamics in lean and obese subjects. Front Syst Neurosci 4:157

13. Guthoff M, Grichisch Y, Canova C et al (2010) Insulin modulates food-related activity in the central nervous system. J Clin Endocrinol Metab 95:748–755

14. Guthoff M, Stingl KT, Tschritter O et al (2011) The insulin-mediated modulation of visually evoked magnetic fields is reduced in obese subjects. PLoS One 6:e19482

15. Benedict C, Hallschmid M, Hatke A et al (2004) Intranasal insulin improves memory in humans. Psychoneuroendocrinology 29:1326–1334

16. Reger MA, Watson GS, Green PS et al (2008) Intranasal insulin improves cognition and modulates beta-amyloid in early AD. Neurology 70:440–448

17. Baker LD, Frank LL, Foster-Schubert K et al (2010) Effects of aerobic exercise on mild cognitive impairment: a controlled trial. Arch Neurol 67:71–79

18. Herzberg-Schafer SA, Staiger H, Heni M et al (2010) Evaluation of fasting state-/oral glucose tolerance test-derived measures of insulin release for the detection of genetically impaired beta-cell function. PLoS One 5:e14194

19. Zang YF, He Y, Zhu CZ et al (2007) Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. Brain Dev 29:83–91

20. Zou QH, Zhu CZ, Yang Y et al (2008) An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. J Neurosci Methods 172:137–141

21. Benedict C, Brede S, Schioth HB et al (2011) Intranasal insulin enhances postprandial thermogenesis and lowers postprandial serum insulin levels in healthy men. Diabetes 60:114–118

22. Natali A, Gastaldelli A, Camastra S et al (2000) Dose–response characteristics of insulin action on glucose metabolism: a non-steady-state approach. Am J Physiol Endocrinol Metab 278:E794–E801

23. Rizza RA, Mandarino LJ, Gerich JE (1981) Dose–response characteristics for effects of insulin on production and utilization of glucose in man. Am J Physiol 240:E630–E639

24. Gerich JE, Mokan M, Veneman T, Korytkowski M, Mitrakou A (1991) Hypoglycemia unawareness. Endocr Rev 12:356–371

25. Flores-Riveros JR, McLenithan JC, Ezaki O, Lane MD (1993) Insulin down-regulates expression of the insulin-responsive glucose transporter (GLUT4) gene: effects on transcription and mRNA turnover. Proc Natl Acad Sci U S A 90:512–516

26. Rother E, Konner AC, Bruning JC (2008) Neurocircuits integrating hormone and nutrient signaling in control of glucose metabolism. Am J Physiol Endocrinol Metab 294:E810–E816

27. Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L (2002) Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. Nat Neurosci 5:566–572

28. Marks JL, King MG, Baskin DG (1991) Localization of insulin and type 1 IGF receptors in rat brain by in vitro autoradiography and in situ hybridization. Adv Exp Med Biol 293:459–470

29. Unger JW, Livingston JN, Moss AM (1991) Insulin receptors in the central nervous system: localization, signalling mechanisms and functional aspects. Prog Neurobiol 36:343–362

30. Tschritter O, Preissl H, Hennige AM et al (2006) The cerebrocortical response to hyperinsulinemia is reduced in overweight humans: a magnetoencephalographic study. Proc Natl Acad Sci U S A 103:12103–12108

31. Porubska K, Veit R, Preissl H, Fritsche A, Birbaumer N (2006) Subjective feeling of appetite modulates brain activity: an fMRI study. Neuroimage 32:1273–1280

32. Szczypka MS, Kwok K, Brot MD et al (2001) Dopamine production in the caudate putamen restores feeding in dopamine-deficient mice. Neuron 30:819–828

33. Barsh GS, Schwartz MW (2002) Genetic approaches to studying energy balance: perception and integration. Nat Rev Genet 3:589–600

34. Holt RI, Barnett AH, Bailey CJ (2010) Bromocriptine: old drug, new formulation and new indication. Diabetes Obes Metab 12:1048–1057

35. DeFronzo RA (2011) Bromocriptine: a sympatholytic, d2-dopamine agonist for the treatment of type 2 diabetes. Diabetes Care 34:789–794

36. Kullmann S, Heni M, Veit R et al (2011) The obese brain: association of body mass index and insulin sensitivity with resting state

network functional connectivity. Hum Brain Mapp. doi:10.1002/hbm.21268

37. Menon V, Uddin LQ (2010) Saliency, switching, attention and control: a network model of insula function. Brain Struct Funct 214:655–667

38. Craig AD (2009) How do you feel—now? The anterior insula and human awareness. Nat Rev Neurosci 10:59–70

39. Takei H, Fujita S, Shirakawa T, Koshikawa N, Kobayashi M (2010) Insulin facilitates repetitive spike firing in rat insular cortex via phosphoinositide 3-kinase but not mitogen activated protein kinase cascade. Neuroscience 170:1199–1208

40. Tataranni PA, Gautier JF, Chen K et al (1999) Neuroanatomical correlates of hunger and satiation in humans using positron emission tomography. Proc Natl Acad Sci U S A 96:4569–4574

41. Augustine JR (1996) Circuitry and functional aspects of the insular lobe in primates including humans. Brain Res Brain Res Rev 22:229–244

42. Kringelbach ML (2005) The human orbitofrontal cortex: linking reward to hedonic experience. Nat Rev Neurosci 6:691–702

43. Del Parigi A, Gautier JF, Chen K et al (2002) Neuroimaging and obesity: mapping the brain responses to hunger and satiation in humans using positron emission tomography. Ann N Y Acad Sci 967:389–397

44. Kringelbach ML, Rolls ET (2004) The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. Prog Neurobiol 72:341–372

45. Bohringer A, Schwabe L, Richter S, Schachinger H (2008) Intranasal insulin attenuates the hypothalamic-pituitary-adrenal axis response to psychosocial stress. Psychoneuroendocrinology 33:1394–1400

46. Pocai A, Lam TK, Gutierrez-Juarez R et al (2005) Hypothalamic K (ATP) channels control hepatic glucose production. Nature 434:1026–1031

47. Stockhorst U, de Fries D, Steingrueber HJ, Scherbaum WA (2011) Unconditioned and conditioned effects of intranasally administered insulin vs placebo in healthy men: a randomised controlled trial. Diabetologia 54:1502–1506

48. Benedict C, Dodt C, Hallschmid M et al (2005) Immediate but not long-term intranasal administration of insulin raises blood pressure in human beings. Metabolism 54:1356–1361

49. Leibowitz SF, Wortley KE (2004) Hypothalamic control of energy balance: different peptides, different functions. Peptides 25:473–504

50. Barbas H, Saha S, Rempel-Clower N, Ghashghaei T (2003) Serial pathways from primate prefrontal cortex to autonomic areas may influence emotional expression. BMC Neurosci 4:25