Hypothalamic and Striatal Insulin Action Suppresses Endogenous Glucose Production and May Stimulate Glucose Uptake During Hyperinsulinemia in Lean but Not in Overweight Men

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Intranasal spray application facilitates insulin delivery to the human brain. Although brain insulin modulates peripheral metabolism, the mechanisms involved remain elusive. Twenty-one men underwent two hyperinsulinemic-euglycemic clamps with D-[6,6-$^2H_2$]glucose infusion to measure endogenous glucose production and glucose disappearance. On two separate days, participants received intranasal insulin or placebo. Insulin spillover into circulation after intranasal insulin application was mimicked by an intravenous insulin bolus on placebo day. On a different day, brain insulin sensitivity was assessed by functional MRI. Glucose infusion rates (GIRs) had to be increased more after nasal insulin than after placebo to maintain euglycemia in lean but not in overweight people. The increase in GIRs was associated with regional brain insulin action in hypothalamus and striatum. Suppression of endogenous glucose production by circulating insulin was more pronounced after administration of nasal insulin than after placebo. Furthermore, glucose uptake into tissue tended to be higher after nasal insulin application. No such effects were detected in overweight participants. By increasing insulin-mediated suppression of endogenous glucose production and stimulating peripheral glucose uptake, brain insulin may improve glucose metabolism during systemic hyperinsulinemia. Obese people appear to lack these mechanisms. Therefore, brain insulin resistance in obesity may have unfavorable consequences for whole-body glucose homeostasis.

Over the past years, the human brain has been variously identified as an insulin-sensitive organ (1). Whereas insulin influences activity in specific brain areas in some individuals, others experience attenuated or even absent responses, with the result that they are considered to be brain insulin resistant (1,2). This phenomenon was first observed in overweight people, who appeared to be resistant to brain insulin action not only in terms of regional brain activity but also with regard to many functional consequences: whereas insulin in the brain influences food intake and body weight (1,3) in lean people, no such effects have been observed in overweight and obese individuals (1,4). However, insulin resistance of the brain does not appear to

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negatively affect all brain functions in overweight subjects since the hormone improves memory consolidation regardless of body weight (4). This might be due to the fact that insulin resistance differentially affects certain brain areas (5).

Many of the more recent studies in this field used intranasal insulin administration to induce brain insulin action in humans. Shortly after nasal application, insulin can be found in the cerebrospinal fluid, where it can be detected for a considerable period of time (6). It probably reaches the brain equally quickly, since effects of nasal insulin have been detected as early as 15 min after spray administration (5). In line with this, research in animals suggests rapid uptake of insulin from the nasal cavity into the brain by mechanisms different from insulin transport at the blood-brain barrier (7).

Animal studies show that insulin action in the brain regulates peripheral metabolism (8). In rodents, brain insulin suppresses hepatic glucose production (9–12). Most (but not all) (11) of these experiments were conducted under systemic hypoinsulinemia. Furthermore, some studies in rodents reported brain insulin action to inhibit lipolysis in adipose tissue (13,14) and to stimulate glucose uptake into skeletal muscle (9,15), and especially the effect on muscle was not seen in all studies (10,11). Furthermore, not all of these findings could be replicated in dogs (16,17). Nonetheless, experimental evidence from animals suggests that the brain orchestrates insulin action in various organs throughout the body to regulate energy fluxes and whole-body metabolism (8).

A number of studies followed up these animal data on the brain's role for whole-body metabolism in humans by combining nasal insulin administration with assessment of peripheral glucose regulation (18–22). While under physiological circumstances brain insulin action can be stimulated only when insulin enters the brain via the bloodstream, i.e., during systemic hyperinsulinemia, some of these studies were conducted under fasting systemic insulin levels (18,21,22). The first of these studies estimated peripheral insulin sensitivity from fasting insulin and glucose levels (18). The results suggested that nasal administration of insulin might indeed improve peripheral insulin sensitivity in humans due to the influence on specific brain areas (18). A further study showed that intranasal application of the insulin analog lispro suppresses endogenous glucose production, albeit only after a substantial delay of approximately 3 h (21). A third study demonstrated an increase in hepatic adenosine triphosphate concentrations and concomitant reduction in liver fat content (22). The latter two studies aimed to experimentally mimic the spillover of small amounts of the nasal insulin into the circulation by administering insulin intravenously coordinated with placebo spray application (21,22).

So far, we are the only group to have investigated the effects of nasal insulin application under systemic hyperinsulinemia (19). After intranasal insulin, higher glucose infusion rates (GIRs) were necessary to maintain euglycemia during a hyperinsulinemic-euglycemic glucose clamp. This is suggestive of improved insulin sensitivity. This response was limited to lean men; insulin sensitivity did not improve in overweight men (19). However, without using a tracer dilution technique to measure endogenous glucose production and glucose disposal to peripheral tissues, the mechanism responsible for the increase in GIR in the previous study remained obscure. Furthermore, one drawback of nasal insulin application was not experimentally taken into account in this study: small amounts of nasally administered insulin are absorbed into the bloodstream (1). This spillover of nasal insulin into the circulation can be measured shortly after insulin spray administration of larger doses of the peptide (21,22). However, the exact kinetics of this phenomenon have not yet been systematically addressed in humans. Although this spillover probably does not present a major obstacle when studying the nasal insulin effects on the brain itself, it might nevertheless interfere in studies that focus on peripheral metabolism.

Methodological differences and difficulties in study design therefore caused controversies over the interpretation of previous results regarding the role of brain insulin action in whole-body metabolism (1,17,20,23,24). To clarify these issues, we now conducted a placebo- and spillover-controlled randomized study to address the importance of brain insulin action for peripheral glucose metabolism in different tissues under systemic hyperinsulinemia.

RESEARCH DESIGN AND METHODS

Participants

We studied 21 healthy volunteers. The initial intention was to study 20 subjects (10 with BMI <25 kg/m^2 and 10 with BMI >25 kg/m^2); however, since one lean participant showed up only for one clamp experiment, we recruited an additional lean subject. Clinical characteristics are presented in Table 1. All participants underwent a screening visit with medical history, clinical examination, and blood tests to ensure that they were healthy. Written informed consent was provided, and the local ethics committee approved the protocol.

Table 1—Clinical characteristics

	Lean (BMI <25 kg/m^2)	Overweight (BMI >25 kg/m^2)	*P*
Age (years)	26.4 ± 3.4	26.6 ± 2.9	0.9
BMI (kg/m^2)	23.3 ± 1.8	28.3 ± 4.6	0.0050
Body fat content (%)	17 ± 4	23 ± 4	0.0041
Fasting glucose (mmol/L)	4.7 ± 0.4	5.2 ± 0.5	0.0246
Fasting insulin (pmol/L)	58 ± 21	77 ± 34	0.2
HbA_{1c} (%)	5.1 ± 0.4	5.3 ± 0.5	0.4

Data are given as mean ± SD. HbA_{1c}, hemoglobin A_{1c}.

Hyperinsulinemic-Euglycemic Clamp

A summary of the experiments is shown in Supplementary Fig. 1. Experiments commenced at 7:00 A.M. after overnight fast. Participants underwent two hyperinsulinemic-euglycemic clamp experiments in randomized order. A cannula was placed into the dorsal hand vein for blood sampling. The arm was warmed to facilitate arterialized blood sampling. Another cannula was placed into the contralateral antecubital vein for infusions. A primed-continuous intravenous infusion of D-[6,6-$^2H_2$]glucose (98% enriched; Cambridge Isotope Laboratories) with 0.036 mg $\times$ min^{-1} $\times$ kg^{-1} was administered 2 h before initiation of insulin infusion (22). At -90 min, an intravenous insulin bolus of 6.25 mU $\times$ kg^{-1} was administered, after which insulin was infused intravenously at 0.25 mU $\times$ kg^{-1} $\times$ min^{-1} for the rest of the experiment. Nasal spray was administered 1.5 h after initiation of insulin infusion (time point 0 min). On one day, subjects received 160 units of insulin (eight puffs in each nostril over 4 min, 10 units per puff) and vehicle as placebo on the other day, as in our previous study (5). On the placebo day, insulin infusion was increased by 0.17 mU $\times$ kg^{-1} $\times$ min^{-1} for 15 min after the first placebo spray puff (resulting in an intravenous insulin bolus of 2.5 mU $\times$ kg^{-1} over 15 min). The participants were not informed as to whether they had received insulin or placebo spray.

During the experiment, blood samples was taken every 5 min to measure blood glucose, and the GIR of 20% dextrose (2% enriched with D-[6,6-$^2H_2$]glucose) was adjusted to maintain euglycemia with a target glucose of 5 mmol/L. Additional blood samples were taken to determine tracer enrichment, metabolites, and hormones.

In 4 of the 41 hyperinsulinemic-euglycemic clamp experiments, steady GIR could not be reached in the designated time before spray administration (coefficient of variation in GIR for the 20 min before spray >15%). We therefore excluded these experiments from further analyses.

Analytic Procedures

Blood glucose was measured by the glucose oxidase method (EKF Diagnostic, Barleben, Germany). Insulin, C-peptide, and prolactin concentrations were determined by chemiluminescence assays (ADVIA Centaur XPT; Siemens, Eschborn, Germany). On insulin day, the prolactin concentration was more than two SD above the mean in one lean participant. This measurement was therefore excluded from the analysis involving prolactin. Plasma concentrations of nonesterified fatty acid were determined using an enzymatic (acyl-coA synthetase, acyl-coA oxidase) colorimetric method (Wako Chemicals, Neuss, Germany) adapted on the ADVIA 1800 XPT clinical chemistry analyzer. Glucagon was determined as described previously (25).

Gas Chromatography–Mass Spectrometry

The determination of atom percent enrichment of ^2H in blood glucose was performed after deproteinization using $Ba(OH)_2$-$ZnSO_4$. Measurements were performed on a Hewlett-Packard 6890 gas chromatograph equipped with a 25-m CPSil5CB capillary column (0.2 mm inner diameter, 70.12 μm film thickness; Chrompack/Varian, Middelburg, the Netherlands), interfaced to a Hewlett Packard 5975 mass selective detector. Selected ion monitoring was used to determine enrichments of the fragments C3 to C6, with the average mass units 187 for the endogenous glucose and 189 for the D-[6,6-$^2H_2$]glucose. Intra- and interassay coefficients of variation were 0.6 and 1.0%. Tracer enrichment data are presented in Supplementary Fig. 6.

Functional MRI

On a separate day, participants underwent a pulsed arterial spin labeling measurement to determine cerebral blood flow (CBF). After the first measurement, 160 units of nasal insulin was applied. A second measurement was performed 30 min after administration of the spray.

Functional MRI Data Acquisition

Scanning was performed on a 3T scanner with a 12-channel trans-receiver head coil (Magnetom Prisma; Siemens). Pulsed arterial spin labeling images were obtained with a PICORE-Q2TIPS (proximal inversion with control for off-resonance effects–quantitative imaging of perfusion by using a single subtraction) sequence. Each measurement consisted of 78 alternating tag and control images with the following imaging parameters: inversion time (TI), TI1 = 700 ms, TI2 = 1,800 ms, repetition time (TR) = 3,000 ms, echo time (TE) = 13 ms, inplane resolution = 3 $\times$ 3 mm^2, field of view = 192 mm, matrix size = 64 $\times$ 64, and flip angle = 90°. The same sequence was used to estimate the equilibrium magnetization of the blood (M0B) for absolute CBF quantification. In addition, a high-resolution T1-weighted anatomical image was acquired.

Functional MRI Data Processing

Image preprocessing was performed with the ASLtbx (26) with SPM8 (Wellcome Trust Centre for Neuroimaging). As previously reported (5), we used the general kinetic model for absolute perfusion quantification. Perfusion images were generated by calculating the control-tag differences by surround subtraction. We determined the perfusion on each voxel with an equilibrium magnetization (M0) map to obtain accurate CBF quantification (mL $\times$ 100 g^{-1} $\times$ min^{-1}). Functional images were coregistered to the individual anatomical image and smoothed (full width at half maximum: 6 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 change 30 min after intranasal insulin administration. Change in CBF was extracted from the hypothalamic region of interest based on our recent finding (5). Multiple regression analyses were performed to evaluate the relationship between the increase in GIR and hypothalamic brain insulin action. Furthermore, multiple regression analysis was performed

on a whole-brain level evaluating the relationship between increase in GIR post–insulin spray and change in CBF after intranasal insulin.

Calculations

Two periods of time were prespecified for analysis of spray-induced changes in both GIR and tracer enrichment (from 20 to 0 min before spray administration to 100–120 min and 190–210 min postspray; clinicaltrials.gov NCT02468999). These changes were calculated by dividing the average value of the latter period by the average value of the former period. The result was then multiplied by 100 and is therefore expressed as a change in percent.

Rates of endogenous glucose production were determined from the tracer infusion rate of D-[6,6-$^2H_2$]glucose and its enrichment to the hydrogens bound to carbon 6 divided by the mean percent enrichment of plasma D-[6,6-$^2H_2$]glucose. Steel's single-pool steady-state equations were used to calculate insulin-stimulated glucose rate of disappearance (27).

Statistical Analyses

Pairwise two-tailed Student *t* tests were used to compare conditions. Correlations and adjustments were addressed by multiple linear regression analyses. *P* values ≤ 0.1 were considered as a trend and <0.05 were considered to be significant. The statistical software package JMP (SAS Institute, Cary, NC) was used for statistical analysis.

RESULTS

Hyperinsulinemic-Euglycemic Clamps in the Whole Group

In the whole cohort, onset of the clamp with intravenous insulin infusion resulted in comparable plasma insulin levels on both study days in the first designated time period for analysis, i.e., in the 20 min before spray administration (insulin day: 198 ± 39 pmol/L, placebo day: 204 ± 51 pmol/L, $P = 1.0$). During this period, comparable glucose levels were reached (insulin day: 5.1 ± 0.4 mmol/L, placebo day: 4.9 ± 0.2 mmol/L, $P = 0.08$) by comparable GIRs (insulin day: 2.8 ± 1.2 mg × kg^{-1} × min^{-1}, placebo day: 3.2 ± 1.3 mg × kg^{-1} × min^{-1}, $P = 0.2$).

After spray administration, plasma insulin levels were determined in 5-min intervals for 30 min. After nasal insulin administration, plasma insulin levels increased and peaked 15 min postspray (mean increase 51 ± 53 pmol/L), returning to baseline afterward (Fig. 1*B*). On placebo spray day, this was mimicked by infusion of an intravenous insulin bolus over 15 min. The insulin course over the 30 min after spray administration was comparable between study days ($P_{AUC0–30} = 0.6$) (Fig. 1*B*). For the rest of the experiment, insulin levels between study days were comparable (Fig. 1*B*).

However, to maintain euglycemia, glucose infusion had to be more strongly increased after intranasal insulin than after placebo (Fig. 1*A*). The increase in GIR was higher after insulin spray than after placebo in the first designated period of analysis (100–120 min postspray, difference 0.63 ± 0.26 mg × kg^{-1} × min^{-1}, $P = 0.0277$). Whereas glucose levels had been comparable up until this point in time ($P = 0.5$), they were slightly higher thereafter on the insulin day (see Fig. 1*D*). Despite a higher increase in GIR during the second designated period of analysis, i.e., 190–210 min postspray ($P = 0.0408$), glucose levels were again comparable between study days for the whole cohort ($P = 0.1$). In both designated periods of time, C-peptide, glucagon, and free fatty acid concentrations were comparable (all $P > 0.1$) (Fig. 1*C* and Supplementary Table 1).

Analysis of tracer enrichment within the whole group (lean and overweight combined) showed no significant difference between the two sprays in the suppression of endogenous glucose production ($P \geq 0.4$ for both periods). However, the increase in the rate of glucose disappearance was greater after nasal insulin than after placebo spray application ($P = 0.0318$ for the first period and $P = 0.1$ for the second).

The magnitude of suppression of endogenous glucose production was associated with serum prolactin levels only after insulin spray ($P = 0.0204$) (Supplementary Fig. 4*A*) but not after placebo spray ($P = 0.3$) (Supplementary Fig. 4*B*). The increase in glucose disappearance rate was neither after insulin nor after placebo spray associated with prolactin concentrations (both $P > 0.6$) (Supplementary Fig. 4*C* and *D*).

Comparison of Lean and Overweight Participants During Hyperinsulinemic-Euglycemic Clamps

On the basis of our previous results (19), we stratified the group into lean and overweight participants. The absolute increase in GIR after nasal insulin was different between the two weight groups ($P = 0.0092$ for the first period and $P = 0.08$ for the second). In the lean group, the increase in GIR was greater after insulin than after placebo spray application for the two designated periods ($P = 0.0298$ and $P = 0.0413$) (Fig. 2*A* and *B* and Supplementary Fig. 2*A*). In overweight participants, no comparable differences between study days were found ($P \geq 0.3$) (Fig. 2*A* and *B* and Supplementary Fig. 2*B*).

For the lean participants, analysis of tracer enrichment revealed that endogenous glucose production was more strongly suppressed from before to after nasal insulin than after placebo spray for the first designated time period ($P = 0.0015$) (Fig. 2*C*) but not for the second time period ($P = 0.9$) (Fig. 2*D*). However, the rate of glucose disappearance did not differ significantly in the first period ($P = 0.1$) (Fig. 2*E*) but tended to be higher in the second ($P = 0.05$) (Fig. 2*F*).

In the overweight participants, no comparable differences could be detected either for endogenous glucose production or for rate of glucose disappearance (all $P \geq 0.09$) (Fig. 2*C–F*).

Functional MRI

On the basis of our recent findings (5), we assessed the insulin-induced decrease of hypothalamic regional blood

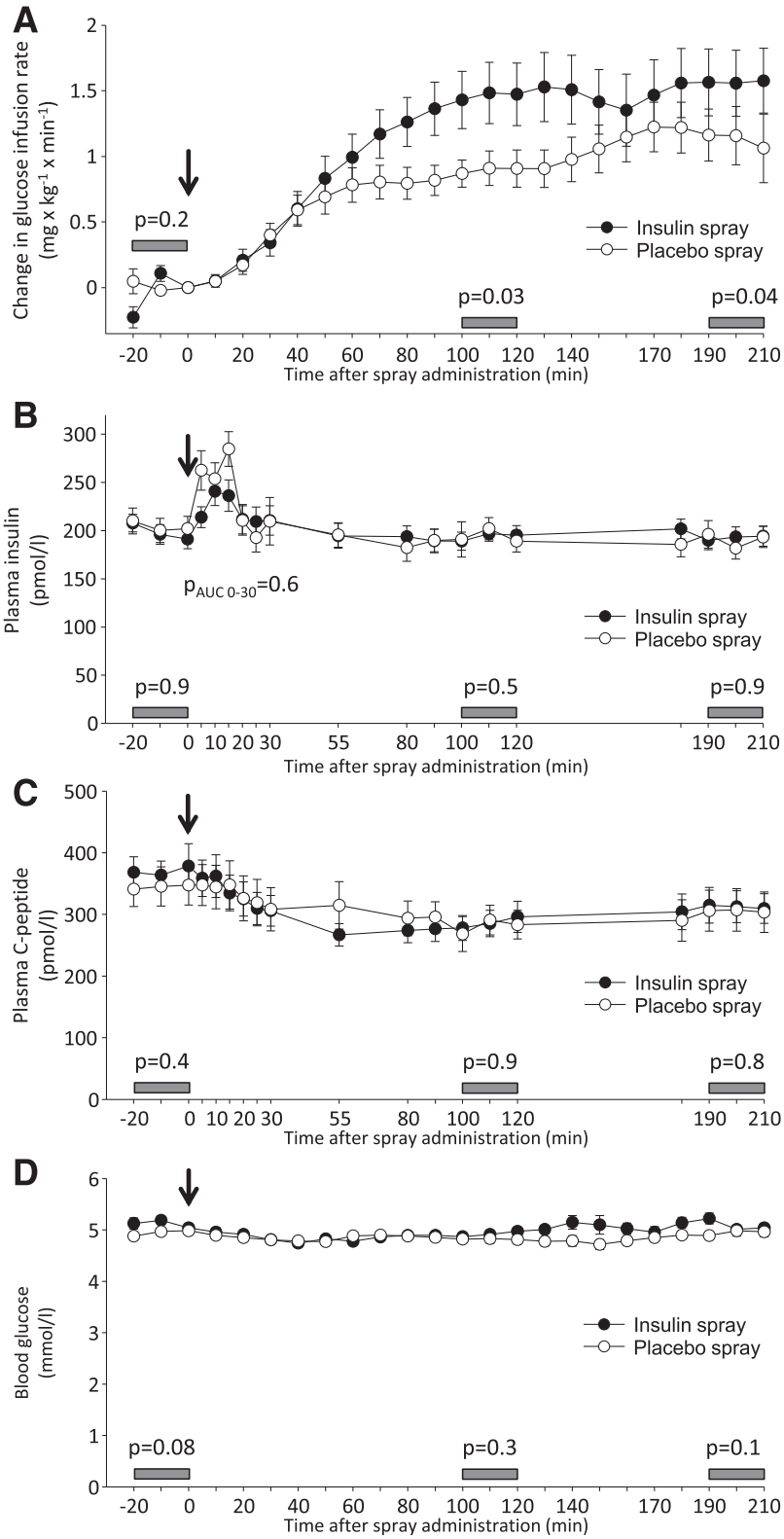

Figure 1—Hyperinsulinemic-euglycemic clamp results in the whole cohort (lean and overweight participants combined). The absolute change in GIR after spray administration at t = 0 min is presented here (*A*). Time courses of plasma insulin (*B*), plasma C-peptide (*C*), and blood glucose (*D*) also begin with the first time period for calculation 20 min before spray application at t = 0 min. Means ± SEM for the whole group (lean and overweight combined) are given. Periods for further assessments (see Fig. 2) are indicated as gray boxes. The black arrows indicate the time of spray application. Differences in means between insulin and placebo spray during these designated periods were tested by pairwise two-tailed Student t tests.

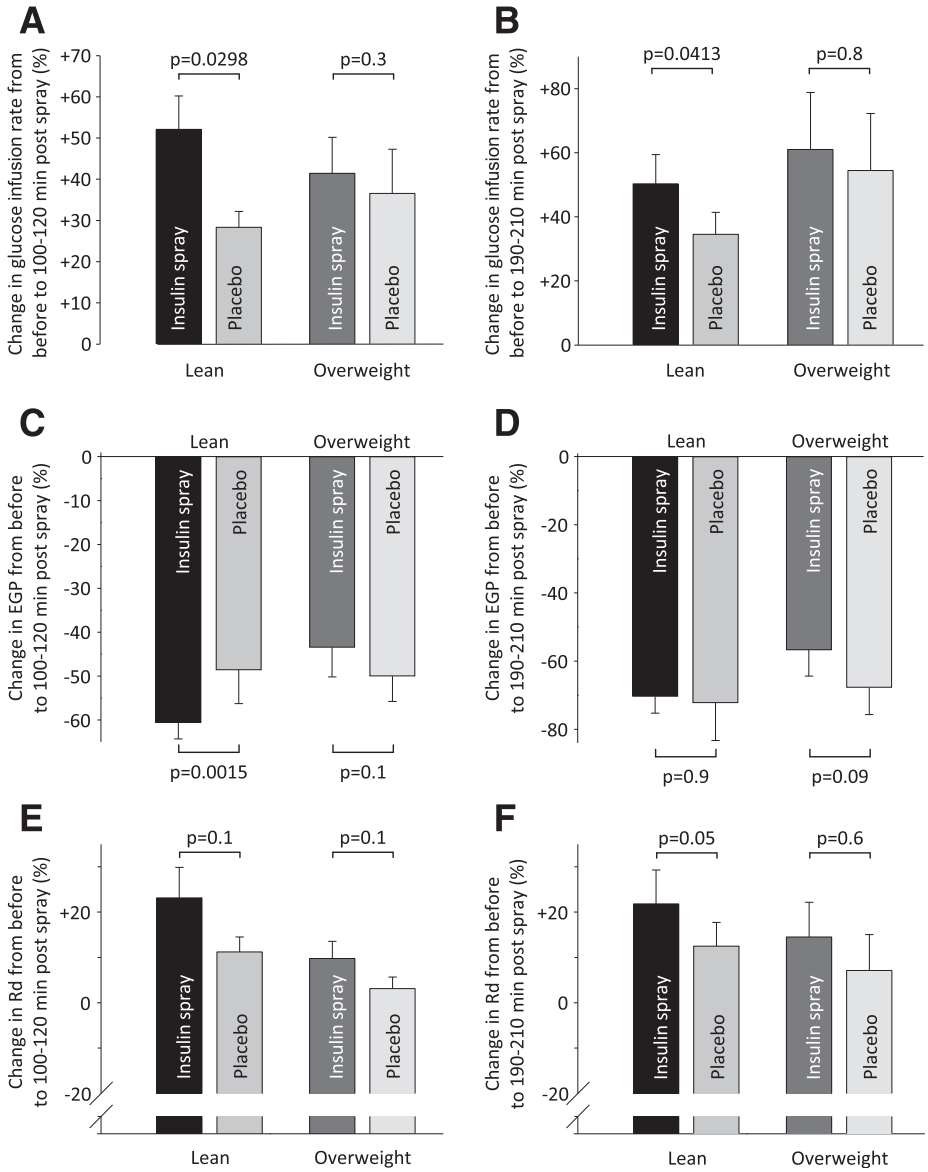

Figure 2—Effects on GIR, endogenous glucose production (EGP), and glucose disappearance rate (Rd) in lean and overweight participants. Changes from the 20 min before spray application to the first predefined time period after spray, i.e., 100–120 min postspray (*A*, *C*, and *E*), and to the second predefined time period after spray, i.e., 190–210 min postspray (*B*, *D*, and *F*), in lean (left two bars) and overweight (right two bars) participants are indicated. *A* and *B* represent changes in GIR, *C* and *D* show changes in EGP, and *E* and *F* are changes in Rd. Means ± SEM. Differences between insulin and placebo spray were tested by pairwise two-tailed Student *t* tests.

flow as a readout for hypothalamic insulin action. After adjustment for BMI, a significant correlation was observed between hypothalamic insulin sensitivity and the increase in GIR from before insulin nasal spray to the first designated period after spray ($P = 0.0314$, $r^2 = 0.29$). This association remained significant after additional adjustment for age ($P = 0.0249$, $r^2 = 0.33$).

To enlarge the sample size, we next pooled the data of our current study with those of our earlier study (19). For two subjects who participated in both studies, only the functional MRI recordings of the current study were included. In the resulting 28 subjects, we analyzed associations between the increase in GIR from before to 100–120 min after insulin spray and insulin-induced changes in regional brain activity on the whole-brain level, i.e., in a hypothesis-free approach ($P < 0.001$, uncorrected). We found a significant association with the caudate nucleus (MNI coordinates $x = -9$, $y = 20$, and $z = 4$), part of the striatum (Fig. 3, left panel). The change in striatal regional blood flow after intranasal insulin correlated with the increase in GIR from before to 100–120 min after insulin nasal spray ($P = 0.0026$, $r^2 = 0.30$) (Fig. 3). This association remained significant after adjustment for age and BMI ($P = 0.0101$, $r^2 = 0.26$) and after limiting the analysis to the subjects of the current study ($P = 0.0311$, $r^2 = 0.29$).

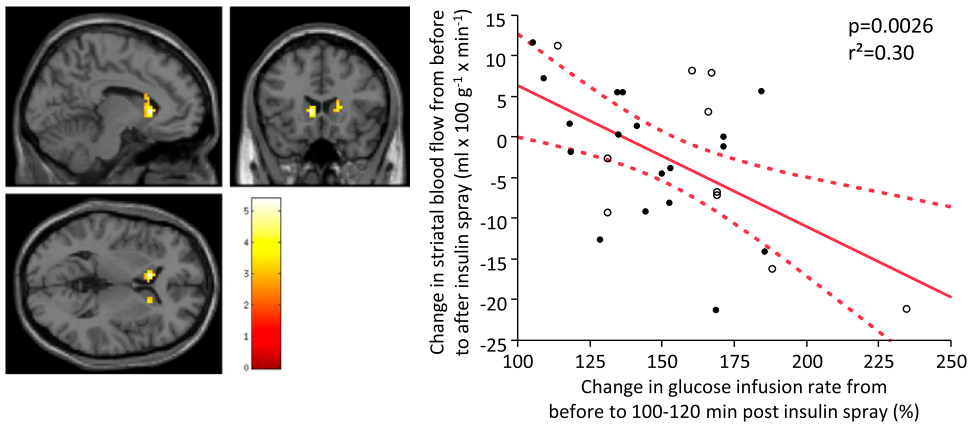

Figure 3—Change in GIR from before to 100–120 min after insulin spray application is associated with insulin effects on the striatum. The left panel shows the striatal regions for which significant associations were detected on a sagittal (left), coronal (right), and axial section plane (bottom). In the right panel, change in GIR is plotted against absolute change in striatal CBF after insulin spray application. Filled dots represent participants from the current study, and open dots are participants from the previous study. Lines represent fit line ± CI from an unadjusted model.

DISCUSSION

In this study, glucose infusion had to be increased more to keep blood glucose stable when intranasal insulin instead of a placebo was administered to the brain of our subjects during a hyperinsulinemic-euglycemic clamp. However, this effect was restricted to lean participants. The higher amount of glucose required in this group was due to improved suppression of endogenous glucose production, and partly by higher glucose uptake into peripheral tissues. In overweight participants, who are known to be brain insulin resistant (1,28), these effects were absent.

We had predefined two periods of time for the assessment of changes in peripheral metabolism (100–120 min and 190–210 min postspray). In both time intervals, considerably more glucose had to be infused to maintain euglycemia after nasal insulin application, indicating improved peripheral insulin sensitivity. This effect was more pronounced during the first but was also detectable in the later period. Thus, we hypothesize that the brain-derived modulation of peripheral metabolism occurs around 60 min after spray administration and persists for a considerable length of time, probably up to 3.5 h postspray, when our experiments ended.

However, the underlying mechanisms appear not to be the same for the rapid and the delayed effects. During the earlier period, i.e., 100–120 min postspray, glucose infusion had to be increased more after insulin spray since endogenous glucose production was more strongly reduced. This suppression by brain insulin is well in line with studies in rodents in which the hormone's actions in specific brain areas are shown to regulate hepatic glucose production (9–12). Since the present results were obtained at systemic hyperinsulinemia, the mechanism detected in our study might involve insulin sensitization of the liver itself. By contrast, no immediate effects on endogenous glucose production could be detected in two earlier studies under fasting systemic insulin levels (21,22). In line with rodent data at fasting insulin levels (12), the study by Dash et al. (21) found such effects with marked delay, i.e., 3–6 h after spray application. After this length of time, a comparable reaction could no longer be detected in our study. Of note, research in rodents with systemic hyperinsulinemia detected effects of brain insulin action on endogenous glucose production in a time frame comparable to that of our study (11). Brain insulin action might thus regulate hepatic glucose output more rapidly and without major delayed effects when occurring simultaneously in the presence of systemic hyperinsulinemia, as it takes place under physiological circumstances, i.e., after a meal. To unravel underlying mechanisms, more experiments in animals are needed that directly assess insulin signaling in the liver in the context of brain insulin action.

Another major difference between our present study and the work by Dash et al. (21) is that they used somatostatin. Since this substance is known to affect the central nervous system (29), this may complicate the interpretation of their results (24). Furthermore, the type of insulin used as nasal spray seems to be critical for the interpretation of the data. Whereas Dash et al. (21) used the rapid-acting insulin analog lispro, we applied regular human insulin. Previous results derived with other insulin analogs showed that when administered as a nasal spray, these analogs appear to act in a different way than regular human insulin (30,31), which might eventually also be relevant for insulin lispro. However, up to now, no information about a direct comparison with human insulin is available for this analog insulin.

In addition to the effects on endogenous glucose production, an increased rate of glucose disappearance was detected after nasal insulin application. Central insulin action might therefore also improve insulin sensitivity in peripheral tissues in addition to the liver. This action may

involve an elevation of glucose uptake. Findings from rodent studies suggest that skeletal muscle in particular could play a part in this respect (9,15). However, not all animal studies showed effects on glucose uptake into tissue (10,11). Besides species differences, a differential time course of brain insulin action on endogenous glucose production and glucose uptake into tissue may have contributed.

In sum, our current results indicate that brain insulin action may improve peripheral insulin sensitivity by suppressing endogenous glucose production and stimulating glucose uptake into tissue. This reaction might help to control whole-body metabolism after food intake when insulin levels rise quickly.

Earlier work has already demonstrated that higher body weight associates with insulin resistance of the human brain (1,28). Although this phenomenon does not affect all brain areas equally (5) or all insulin-regulated brain functions (4), our results indicate that it may be of importance for systemic metabolism. In line with previous work (19), insulin administration to the brain did not alter peripheral metabolism in the overweight participants of our study. In this group, neither endogenous glucose production nor glucose uptake was modified by nasal insulin spray. A lack of brain-derived modulation of peripheral metabolism could thus contribute to the pathogenesis of whole-body insulin resistance, which is often found in obesity, thereby increasing the risk for type 2 diabetes. Hence, the development of strategies that improve brain insulin resistance in humans will be one major goal for further research.

Our study indicates that speculations about a relevant major metabolic effect of the spillover of intranasal insulin into the circulation (17,20,24) are unwarranted. Since circulating insulin levels were similar on the two study days, the current study clearly demonstrates that brain insulin has additional effects to modulate peripheral glucose metabolism in humans.

Although the kinetics of spillover of nasally administered rapid-acting insulin analog have been reported before (21), our present study is the first to provide a precise description of the kinetics of this spillover of nasal human insulin application into the systemic circulation. Therefore, we used a specific protocol to accurately mimic this phenomenon by intravenous insulin infusion. It is worth mentioning that the spillover of intranasal human insulin into the circulation appears to differ from the insulin analog lispro in both magnitude and duration. Whereas intravenous administration of 2.5 mU $\times$ kg^{-1} human insulin was sufficient to mimic spillover after 160 units of intranasal human insulin, twice as much insulin lispro was necessary to mimic spillover after 40 units of intranasal lispro (21). Furthermore, plasma insulin peaked around 15 min post–human insulin spray and returned to prespray levels 15 min later, as observed in some (19,22), but not all (18), previous studies. This is well in line with insulin's half-life, which is very short in the blood circulation (32) but seems to prevail considerably longer in the brain (6,33). In contrast to human insulin, the peak venous insulin lispro concentration was not reached until 30 min after spray administration (21). Furthermore, the amount of intranasal insulin lispro absorbed into the bloodstream seems not to be strictly dose dependent (21). Such a dose dependency has not yet been tested for human insulin. Due to their increased and prolonged reabsorption into circulation, intranasal insulin analogs such as lispro might have stronger systemic side effects than intranasal human insulin. However, an appropriate randomized study with a direct comparison of human insulin and insulin analogs (including lispro) administered as nasal spray has not been reported yet.

To gain a better comprehension of the underlying physiology, it is important to investigate the brain areas in which the efferent outputs originate. Using an improved functional MRI approach (5), we now verified the involvement of the hypothalamus that had already been hypothesized by earlier studies in humans (18,19) and animals (14,34,35). This brain area plays a key role in the control of whole-body homeostasis. Reduced expression of insulin receptors in the hypothalamus causes peripheral insulin resistance (34,35). This well-known hypothalamic response to food intake (36,37) might contribute to the modulation of peripheral metabolism detected in our study.

The larger sample size, resulting from inclusion of comparable data from our previous study (19), enabled us to use a hypothesis-free approach to investigate any brain areas that might additionally be involved in this process. After rigorous correction for multiple comparisons, a specific brain area (the striatum) was detected. This part of the basal ganglia is a critical component of the reward system. It appears to respond to the postprandial rise in endogenous insulin concentrations (37,38) as well as to nasal insulin application (39). It is worth bearing in mind that the functional connection between the striatum and the hypothalamus was enhanced after glucose ingestion (38), suggesting that the interplay between these two crucial brain areas is regulated postprandially. Insulin action in this specific area was already suspected to contribute to the modulation of peripheral metabolism (18,40), as is supported by our current study. One major striatal neurotransmitter is dopamine. The striatal dopamine receptor availability is associated with whole-body insulin sensitivity (41). Central dopaminergic tone (as assessed by blood prolactin concentrations) is age-dependently associated with peripheral insulin sensitivity (42). In our current study, serum prolactin concentrations were associated with the magnitude of suppression in endogenous glucose production after nasal insulin. Since no such correlation was detected for glucose uptake into tissue, it is tempting to speculate that distinct mechanisms underlie these two effects of nasal insulin, with striatal dopamine being important especially for endogenous glucose production. However, in line with experimental evidence

for a molecular interaction between insulin and dopamine transporter in the striatum (43), insulin action in the human brain might modulate striatal dopamine signaling, which might, in turn, affect outflows that control endogenous glucose production. However, additional neurotransmitters in the striatum may contribute as well.

The effects of nasal insulin during a hyperinsulinemic-euglycemic clamp appear to be consistent in young men (see also Supplementary Fig. 5). Further studies are needed, though, to elucidate this effect in other groups, especially women, older people, and participants with another ethnic background. Furthermore, it is still not sufficiently studied how much of the nasally administered insulin reaches the brain. Further research should also assess what insulin concentrations are reached at the neuronal level after nasal insulin spray administration. Despite comparable C-peptide and glucagon concentrations, we cannot exclude that subtle alterations in portal insulin or glucagon concentrations may have potentially been present in our study. Whether distinct central processes underlie the differential time course of brain insulin action on endogenous glucose production versus glucose uptake into tissue will also be an important question for further research.

In sum, we provide novel evidence in support of the theory that brain insulin action may improve whole-body glucose metabolism during systemic hyperinsulinemia by enhancing insulin-mediated suppression of endogenous glucose production as well as by possibly stimulating glucose uptake into tissue. Besides the hypothalamus, the striatum might be an important brain area involved in this response. In overweight subjects, these mechanisms are not detectable. Brain insulin resistance might therefore contribute to the pathogenesis of whole-body insulin resistance in obesity.

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