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Insulin Action in the Hypothalamus Increases Second-Phase Insulin Secretion in Humans

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Keywords

Hypothalamus · Imaging · Insulin resistance · Insulin secretion · Pancreas

Abstract

Background: Animal studies and initial correlative data in humans indicate that insulin action in the brain may affect pancreatic insulin secretion. An important brain region for this process is the hypothalamus, an area that can develop insulin resistance. ***Methods:*** Fifteen young, healthy men (27 ± 3 years) with a wide BMI spectrum (20–30 kg/m^2) underwent 2 hyperglycemic clamps (target blood glucose: 10 mmol/L). In this double-blind study, subjects received 160 U of insulin or placebo as a nasal spray on 2 days in randomized order. On another day, insulin sensitivity of the hypothalamus was determined by functional magnetic resonance imaging. ***Results:*** Glucose levels were comparable on both study days. In the whole group, C-peptide levels were not significantly different between conditions. Though, there was a significant interaction between insulin sensitivity of the hypothalamus × nasal spray × time on C-peptide levels ($p = 10^{-6}$). The group was therefore divided according to median hypothalamic insulin sensitivity. C-peptide concentrations were higher after intranasal insulin compared to placebo spray in the group with a strong hypothalamic insulin response ($p < 0.0001$, $\beta = 6.00 \pm 1.24$) and lower in the brain insulin-resistant group ($p = 0.005$, $\beta = -2.68 \pm 0.95$). Neither somatostatin nor glucagon kinetics was altered by the nasal spray. ***Conclusions:*** In participants with high hypothalamic insulin sensitivity, insulin action in the brain enhanced second-phase insulin secretion from pancreatic beta cells. This reaction could, for example, contribute to late postprandial glucose regulation by suppressing hepatic glucose production by portal venous insulin.

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Introduction

Human glucose metabolism depends on the tight regulation of insulin secretion from pancreatic beta cells in combination with sufficient action of this secreted insulin. While insulin resistance not necessarily causes type 2 diabetes immediately, it is a well-known risk factor for a number of conditions [1]. Impaired insulin secretion, however, rapidly results in increasing blood glucose concentration and the development of diabetes mellitus [2]. Such disturbances in insulin secretion can either be due to immunological destruction of the beta cell (as in type 1 diabetes) or due to a multifactorial, still not fully understood, process in the pathogenesis of type 2 diabetes [2].

Research in animals and humans indicates that pancreatic insulin secretion is not only regulated in the beta cell itself but also modulated by neuronal inputs that originate in the brain [3–5]. Besides the known pre-ingestive stimulation of insulin secretion, known as cephalic insulin secretion [6], there are little data on brain-derived inputs to the pancreatic islet in humans. In animals, efferents to the pancreas are stimulated by a number of factors in the brain, including glucose [3, 7]. Glucose is sensed by specialized neurons that have been identified in the hypothalamus [8]. In addition to this well-investigated factor, insulin itself was suspected to influence beta cell function via its action in the brain. In dogs, insulin injection directly into the brain promoted the release of insulin from the pancreas into the bloodstream [9, 10]. In line with these findings, results from rodents underscore the possible role of brain insulin for pancreatic insulin secretion: knock down of the insulin receptor in the hypothalamus impaired pancreatic insulin secretion [11]. Specialized neurons in the hypothalamus are believed to be crucial in this regard [5, 12, 13].

Just as in animals, the brain is an insulin-sensitive organ in humans [14]. One technique to study brain insulin effects in humans is the application of insulin as nasal spray [15]. This approach allows the delivery of significant amounts of insulin into the brain, while only very little of the nasally applied peptide enters the bloodstream [16]. Therefore, insulin given as nasal spray allows to distinguish insulin effects in the brain from such in the periphery. Using this technique, insulin effects have been detected in a limited number of brain areas, including the hypothalamus [17]. Of notice, already early studies on insulin effects in the brain discovered individual differences in response to the peptide hormone [14, 18]. A substantial number of individuals display reduced or even absent responsiveness, a condition often called brain insulin resistance [14].

In addition to regional brain effects recorded by imaging techniques, a number of functional consequences of insulin action in the brain have been discovered in humans so far. Besides cognitive aspects, effects on the processing of food stimuli and eating behavior as well as body weight and body composition have been uncovered [14, 17]. In terms of metabolism, administration of insulin as nasal spray to the brain improves whole-body insulin sensitivity [19] by suppressing endogenous glucose production and stimulating glucose uptake into peripheral tissues [20].

In regard to pancreatic insulin secretion, human data show a statistically significant association between hypothalamic insulin sensitivity and insulin secretion stimulated by an oral glucose load [21]. Though, no clinical study has been reported so far that tested effects of insulin action in the brain on pancreatic insulin secretion in humans using adequate experimental methods that specifically induced central insulin action and measured insulin secretion. We therefore performed this randomized, placebo-controlled, double-blind study using intranasal insulin application and the hyperglycemic clamp technique to test the hypothesis that insulin action in the brain influences pancreatic insulin secretion.

Materials and Methods

Participants

Clinical characteristics are shown in Table 1. All participants underwent a screening visit with medical history, clinical examination, and an oral glucose tolerance test to ensure that they were healthy. None of the participants took any medication.

Intranasal Insulin and Intranasal Placebo Spray

The experiments started after an overnight fast of at least 8 h with the administration of nasal spray (time point –15 min). In a randomized order (online suppl. Fig. 3; for all online suppl. material, see www.karger.com/doi/10.1159/000504551), the subjects received 160 U of insulin (8 puffs in each nostril over 4 min, 10 U per puff) on 1 day and vehicle as placebo on the other day [15]. On the placebo day, 2.5 mU × kg^{-1} of human insulin (Insuman Rapid, Sanofi) was infused intravenously over 15 min starting after the first placebo spray puff [20]. On the insulin day, a matching amount of saline was infused intravenously. Both, the participants and the investigators, were blinded as to whether insulin or placebo spray was given.

Hyperglycemic Glucose Clamp

On both experimental days, participants underwent a hyperglycemic glucose clamp experiment to stimulate insulin secretion. A dorsal hand vein was cannulated for blood sampling. The arm was warmed to enable arterialized blood sampling. The contralateral antecubital vein was cannulated for infusions. The

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Table 1. Clinical characteristics

Number	15
Age, years	27±3 (18–32)
BMI, kg/m^2	24.6±2.4 (20.4–30.0)
Body fat content, %	18.3±5.1 (11.0–29.6)
Fasting glucose, mmol/L	4.73±0.41 (4.05–5.52)
HbA1c, %	5.1±0.2 (4.7–5.5)

Data are given as means ± SD (range). BMI, body mass index; HbA1c, hemoglobin A1c.

clamp procedure started with an intravenous glucose bolus to acutely raise glucose levels to 10 mmol/L. During the experiment, blood was drawn every 2.5–5 min to measure blood glucose, and the infusion rate of 20% glucose was adjusted to maintain hyperglycemia with a target glucose of 10 mmol/L for the entire 90 min of the clamp.

Analytic Procedures

Blood glucose was measured by glucose oxidase method (EKF Diagnostic, Barleben, Germany). Insulin and C-peptide were determined using the ADVIA Centaur XP immunoassay system (Siemens Healthineers, Eschborn, Germany), and intact proinsulin was measured using a commercial ELISA kit (IBL, Hamburg, Germany). Glucagon was measured by radioimmunoassay, as described earlier [22]. Somatostatin was measured by commercially available ELISA (Phoenix Pharmaceuticals, Burlingame, CA, USA).

Functional Magnetic Resonance Imaging

On a different day, all participants underwent whole-brain functional magnetic resonance imaging at a 3.0 T scanner (Siemens MAGNETOM Prisma, Erlangen, Germany) to assess regional insulin sensitivity of the brain. Experiments were conducted after an overnight fast of at least 10 h starting at 7.00 a.m. under basal condition to quantify cerebral blood flow (CBF) with a pulsed arterial spin labeling (PASL) measurement using a PICORE-Q2TIPS sequence (proximal inversion with control for off-resonance effects – quantitative imaging of perfusion using a single subtraction). Following the basal measurement, 160 U of human insulin were administered as nasal spray as previously described [19]. After 30 min, PASL was assessed a second time.

Each PASL measurement consisted of 79 alternating tag and control images with the following imaging parameters: inversion time (TI), TI1 = 700 ms, TI2 = 1,800 ms, repetition time = 3,000 ms, echo time = 13 ms, in-plane resolution = 3 × 3 mm^2, field of view = 192 mm, matrix size 64 × 64, and flip angle = 90°. In addition, a high-resolution T1-weighted anatomical image was acquired.

As previously reported [23], image preprocessing was performed using the ASLtbx with SPM8 (Wellcome Trust Centre for Neuroimaging). The general kinetic model was used for absolute perfusion quantification. Perfusion images were generated by calculating the control-tag differences by surround subtraction. For accurate CBF quantification (mL × 100 g^{-1} × min^{-1}), we used the M0 map to quantify the perfusion on each voxel. The high-resolu-

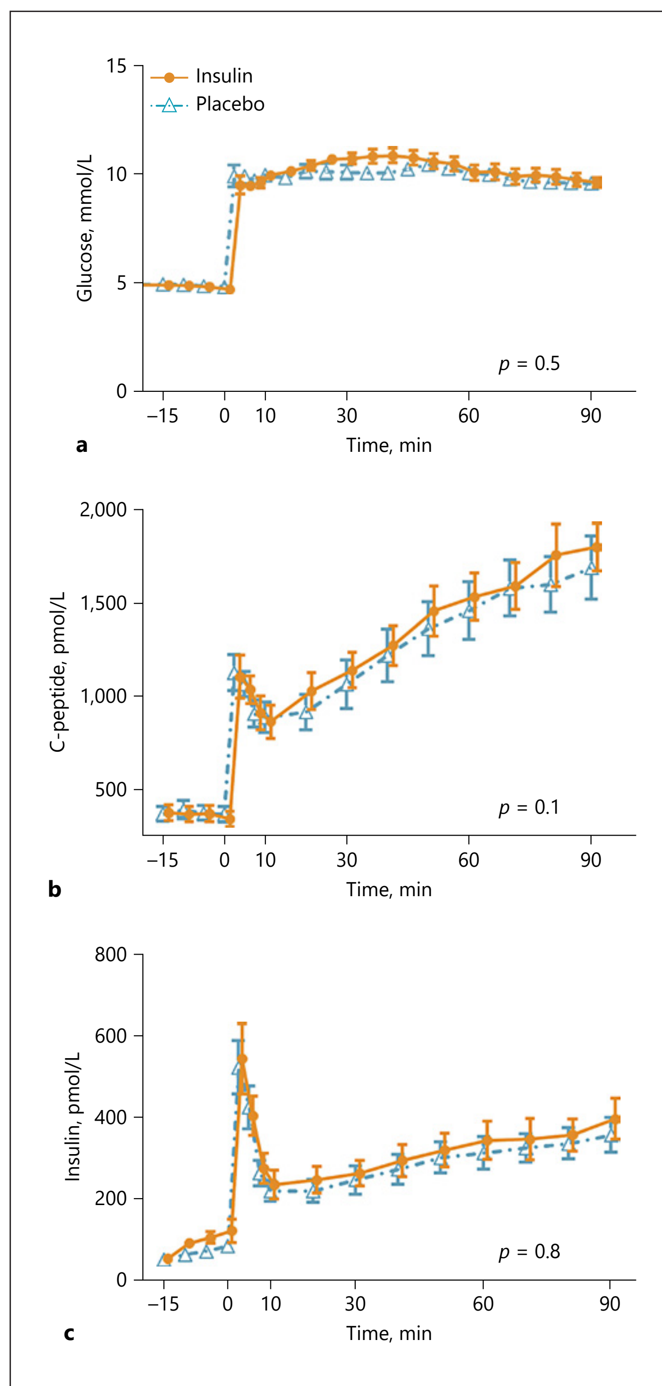

Fig. 1. Effect of nasal insulin versus placebo in the entire cohort. After initiation of the hyperglycemic glucose clamp, plasma glucose was rapidly elevated to 10 mmol/L and remained there for the entire 90 min of the experiment (**a**). There was no significant difference for glucose ($p = 0.75$), C-peptide ($p = 0.1$), or insulin ($p = 0.76$) courses between the 2 conditions (**a–c**). Interactions between condition and time were tested by linear mixed models. $n = 15$; values are mean ± SE; circles represent results after intranasal insulin, triangles after placebo spray, that was administered at –15 min.

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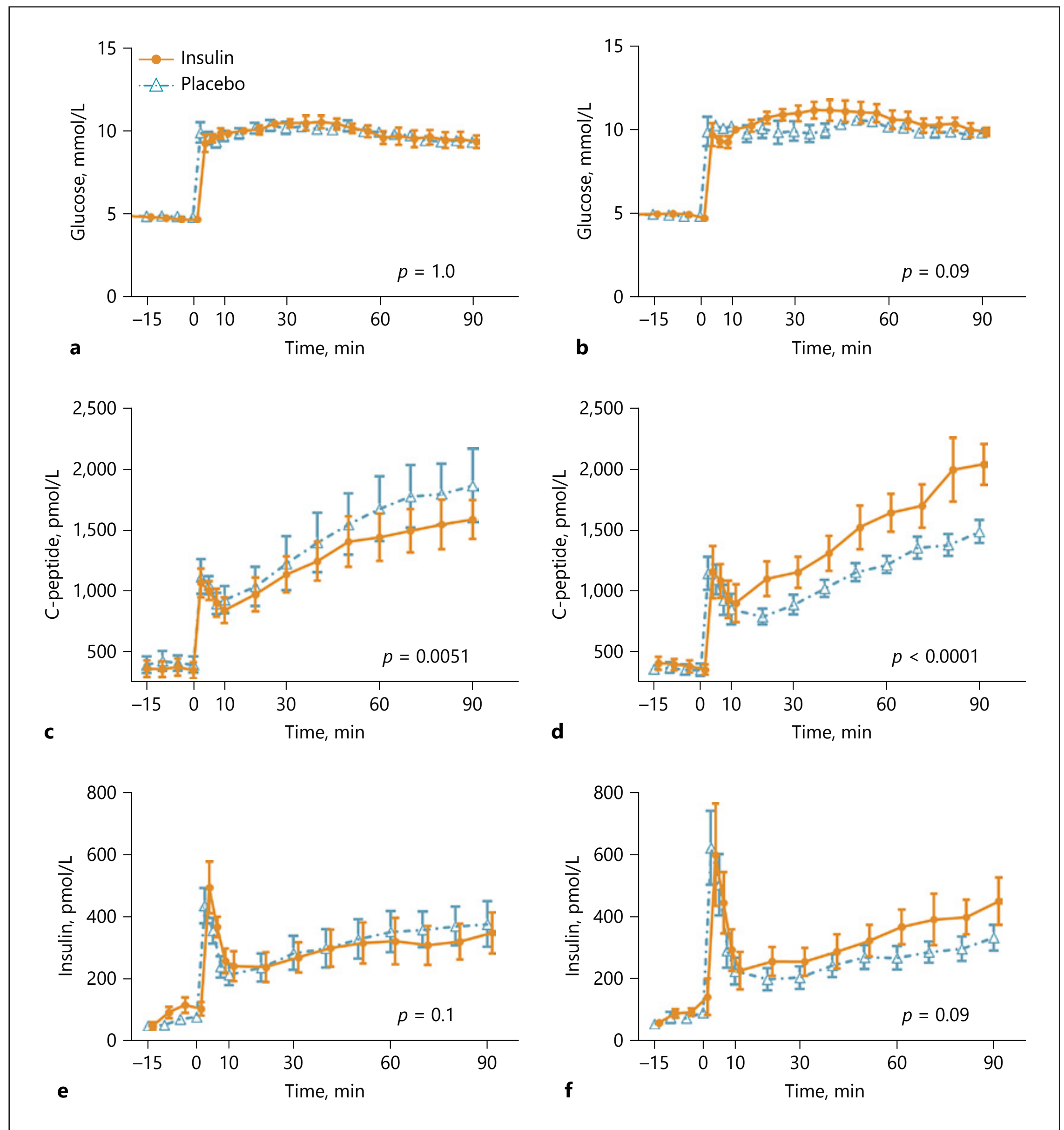

Fig. 2. Effect of nasal insulin depends on hypothalamic insulin sensitivity. There were statistically significant interactions between hypothalamic insulin sensitivity (analyzed as a continuous variable), treatment, and time on C-peptide and serum insulin ($p = 10^{-6}$ and $p = 0.03$, respectively). Therefore, the cohort was split by the median hypothalamic response into persons with low (**a**, **c**, **e**; $n = 8$) and high hypothalamic insulin sensitivity (**b**, **d**, **f**; $n = 7$). Presented are glucose (**a**, **b**), C-peptide concentrations (**c**, **d**), and serum insulin (**e**, **f**). Interactions between condition and time were tested in the 2 subgroups by linear mixed models. Values are mean ± SE. Circles represent results after intranasal insulin, triangles after placebo spray, that was administered at –15 min.

tion T1-weighted image was normalized in Montreal Neurological Institute space (1 × 1 × 1 mm), and the resulting parameter file was used with the individual co-registered CBF maps in normalized space (3 × 3 × 3 mm).

Based on our recent findings, we extracted CBF of the hypothalamus during basal measurement and in response to insulin (30 min post spray) for further statistical analyses [21, 23].

Statistics

All analytes measured repeatedly during the hyperglycemic clamp were investigated with linear mixed models using the interaction between treatment (placebo or insulin nasal spray) and time with the respective marginal effects as fixed-effect terms. Participant and time were used as random effects in a random-intercept, random-slope model setup. To test interaction with brain insulin sensi-

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tivity, hypothalamus insulin responsiveness defined as nasal insulin-induced change in regional blood flow and its interaction term with all of the above model terms was used as fixed effect. In these models, hypothalamic insulin responsiveness was used as a continuous variable. For additional analysis, participants were stratified by the median hypothalamic insulin response. The models were computed using the lme4 library in R, with Satterthwaite's degrees of freedom method utilized in p-value approximation (lmerTest library). A two-sided alpha level of 0.05 was used to evaluate statistical significance.

Results

During clamps, plasma glucose was rapidly increased to 10 mmol/L after initiation of the hyperglycemic clamp and remained there for the entire experiment. We first tested the effect of intranasal insulin versus placebo spray in the entire cohort. There were no significant differences between the insulin and placebo days ($p = 0.5$; Fig. 1a). In the entire cohort, neither the C-peptide nor the insulin-level excursions differed between the 2 study days ($p \geq 0.1$; Fig. 1b, c).

Our initial hypothesis included hypothalamic insulin sensitivity as a modulator of treatment response [21]. We therefore tested for interactions between hypothalamic insulin sensitivity and the response to nasal spray (i.e., hypothalamic insulin response × treatment × time) on the respective endocrine responses. For glucose, no interaction was present ($p = 0.09$). However, there were significant hypothalamic insulin response × treatment × time interactions for serum C-peptide and insulin ($p = 10^{-6}$ and $p = 0.03$, respectively).

We therefore stratified our cohort by the median hypothalamic insulin response. In both groups, plasma glucose levels during the clamp were comparable between study days ($p \geq 0.09$; Fig. 2a, b).

C-peptide concentrations were higher after intranasal insulin compared to placebo spray in the brain insulin-sensitive group ($p_{\text{time × treatment}} < 0.0001$, $\beta = 6.00 \pm 1.24$; Fig. 2d). This remained statistically significant even after adjustment for age and BMI ($p_{\text{time × treatment}} < 0.0001$). In contrast, C-peptide was lower after nasal insulin than placebo in the brain insulin-resistant subjects ($p_{\text{time × treatment}} = 0.005$, $\beta = -2.68 \pm 0.95$; Fig. 2c). Plasma proinsulin levels were higher after nasal insulin compared to placebo only in the group with high hypothalamic insulin sensitivity but not in those with low hypothalamic insulin sensitivity ($p_{\text{time × treatment}} < 0.0001$ and $p_{\text{time × treatment}} = 0.7$, respectively). Differences in plasma insulin concentrations reached statistical significance in neither of the 2 subgroups ($p_{\text{time × treatment}} \geq 0.09$; Fig. 2e, f).

As brain insulin sensitivity is known to be linked to body weight [14], we additionally tested for interaction between BMI × treatment × time on C-peptide. Indeed, there was a statistically significant interaction ($p_{\text{time × treatment}} = 0.03$). Nasal insulin significantly stimulated C-peptide release in the leaner half of the group ($p_{\text{time × treatment}} = 0.0078$), while no significant effect was present in the heavier half ($p_{\text{time × treatment}} = 0.7$).

Neither somatostatin (online suppl. Fig. 1a) nor glucagon (online suppl. Fig. 2a) courses were different between the 2 study days ($p_{\text{time × treatment}} \geq 0.5$), and there was also no significant interaction between hypothalamic insulin sensitivity and both parameters ($p \geq 0.1$). Accordingly, no significant differences between study days were detected in the subgroups (online suppl. Fig. 1b, c and 2b, c).

To address possible effects of nasal insulin versus placebo at fasting plasma glucose and insulin concentrations, we additionally analyzed glucagon in samples from 13 lean and 10 overweight/obese participants from an earlier study [24]. The glucagon courses from baseline to 30 and 60 min post spray were not significantly different between insulin and placebo spray, neither in the entire cohort ($p_{\text{time × treatment}} = 0.4$) nor in the 2 weight groups ($p_{\text{time × treatment}} \geq 0.5$).

Discussion

In this randomized controlled trial, we found that insulin administration to the human brain selectively promoted glucose-stimulated insulin secretion from pancreatic islets, while glucagon and somatostatin release remained unaffected. Importantly, this response was dependent on insulin responsiveness of the hypothalamus.

Elevation of blood glucose is the strongest physiological stimulator of pancreatic insulin secretion. It has been known for a long time that a number of factors can modulate this process to either attenuate or strengthen insulin release from beta cells [25]. In line with early results in animals [9–11], our current results demonstrate that insulin action in the brain contributes to insulin secretion in humans. In this regard, brain-derived signals most likely reach the pancreas via the parasympathetic branch of the autonomic nervous system, as insulin delivery to the human brain is known to promote parasympathetic tone [16, 19] and parasympathetic innervation is known to promote insulin release from the pancreas by activating muscarinergic acetylcholine receptors [26, 27]. Indeed, subdiaphragmal cutting of the vagal nerve,

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the major parasympathetic nerve, abolished brain insulin's ability to propagate pancreatic insulin release in dogs [10].

Insulin release following a glucose stimulus has a specific pattern with an immediate first peak in insulin secretion, followed by a second phase of insulin secretion, which, by definition, starts after 10 min. When glucose concentrations are kept elevated during a hyperglycemic glucose clamp, insulin secretion is steadily rising during this second phase [28]. Of notice, we detected the effect of insulin action in the brain selectively in the second phase of insulin secretion, while the first phase was unaffected. To our knowledge, this is the first example of an intervention that is able to selectively modulate the second phase of insulin secretion. The different types of insulin granula that are released from beta cells during first and second phase of glucose-stimulated insulin secretion [29] could contribute to this phenomenon; the cellular processes that underlie the second phase are thought to be specifically responsive to parasympathetic innervation [30]. Also in terms of timing, a modulation of later insulin secretion is plausible: under physiological circumstances, insulin is released in response to food intake. Insulin secreted during the first phase will take a couple of minutes to reach the brain. Only thereafter, insulin action in the brain will be able to initiate effects in the periphery and enhance pancreatic insulin secretion.

As the effect of nasal insulin on pancreatic insulin secretion depended on the individual's hypothalamic insulin responsiveness, this classical insulin-sensitive brain area [31] seems to be crucial for the modulation of pancreatic insulin secretion. The central role of the hypothalamus in the coordination of beta cell function is well described in animals [3, 5, 7, 11, 32] and supported by evidence from humans [33]. Our current first-in-man study causally links insulin action in the brain to insulin secretion and shows that this link depends on central nervous insulin sensitivity. In participants with high hypothalamic insulin sensitivity, nasal insulin administration promoted insulin secretion from the pancreas. Interestingly in subjects with low hypothalamic insulin sensitivity, nasal insulin seemed to have an opposite effect, albeit with a considerably smaller effect size.

In our previous cross-sectional study, we detected insulin resistance of the hypothalamus to be associated with insulin hypersecretion in response to oral glucose load (without acute insulin delivery to the brain) [21]. In cross-sectional association studies, causality cannot be proven, as multiple confounding biological processes will be present. In light of our current interventional results, one explanation for this previous finding could be that the chronic impairment of hypothalamus-derived signals, as in case of obesity and hypothalamic insulin resistance, shifts the ratio of inputs to the beta cell and causes an imbalance with a predominance of non-neuronal signals that reinforce insulin secretion [21]. Such an effect is known in patients with neurosurgical lesions in the hypothalamus [34] and might also occur in case of hypothalamic insulin resistance.

One very interesting and unexpected finding of our current study is the mismatch of effects of nasal insulin on C-peptide versus plasma insulin. In brain insulin-sensitive subjects, nasal insulin enhanced C-peptide levels, while plasma insulin concentrations were only marginally changed. For the interpretation of this result, it is important to keep in mind that the pancreas drains toward the portal system that reaches the liver first. While around 50% of the secreted insulin is cleared in the liver [25], the co-secreted C-peptide passes the hepatic bed unaffected [35]. As insulin and C-peptide are released from pancreatic beta cells in equimolar fashion, a discrepancy in both indicates altered insulin clearance in the liver. This would mean that besides stimulating insulin secretion from the pancreas, brain insulin might additionally increase hepatic insulin clearance. Even though it is long known that insulin clearance rate is considerably different between persons and reduced, for example, in obesity [36], regulating mechanisms are still largely unknown. Our current results suggest that insulin action in the brain could contribute here, thereby potentially preventing peripheral hyperinsulinemia by increasing postprandial insulin clearance. Though, appropriate clinical intervention studies are needed to test the role of insulin clearance as a potential downstream effect of insulin signaling in brain cells.

Besides stimulating insulin release, cholinergic signals to pancreatic islets also promote somatostatin secretion, at least under specific circumstances [27]. Though, our current results demonstrate that in the context of elevated blood glucose, somatostatin release is not triggered by nasal insulin.

The third major hormone secreted from pancreatic islets, glucagon, markedly decreased during the hyperglycemic clamp experiment. This response to elevated glucose levels is well known and thought mainly to be due to the glucose-stimulated rise in insulin that suppresses glucagon secretion in a paracrine fashion [37]. Our current results argue against an additional effect of brain insulin under hyperglycemia, that is, to postprandial glucagon kinetics. As experimental data from rodents suggest a role

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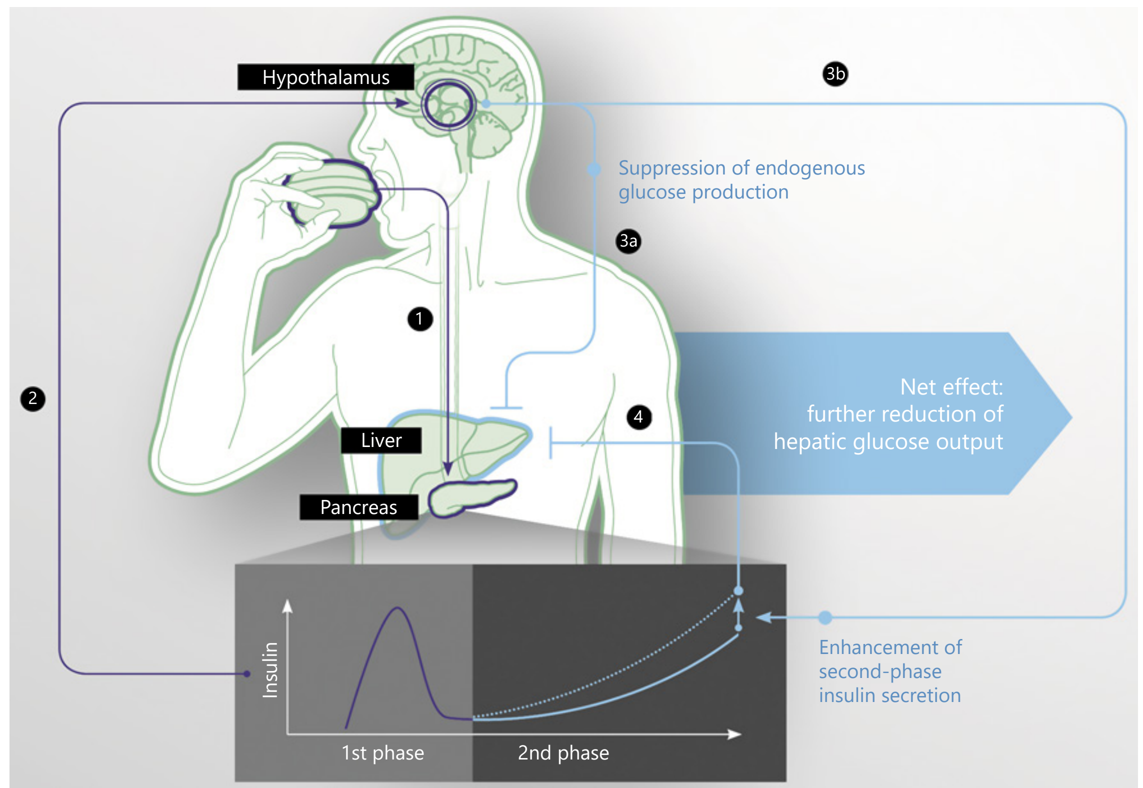

Fig. 3. Schematic overview of the proposed brain-derived second phase of glucose allocation. (**1**) After food intake, insulin secretion from pancreatic beta cells is initiated. This follows a biphasic pattern with an initial peak during the first phase of insulin secretion. (**2**) This insulin reaches the hypothalamus via the bloodstream. Specific hypothalamic neurons sense insulin and trigger outflows to the periphery. (**3a**) Brain-derived signals reach the liver to suppress endogenous glucose production. (**3b**) Projections to the pancreas enhance second-phase insulin secretion into the portal vein. As portal insulin is the strongest suppressor of hepatic glucose production, (**4**) enhanced insulin secretion will potently suppress endogenous glucose production. Thus, brain-derived signals that are activated by initial insulin secretion help to reduce hepatic glucose output in the later postprandial state.

of brain insulin for glucagon secretion also at fasting glycemia [38], we additionally analyzed the effect of brain insulin delivery on glucagon levels under fasting condition, but detected no effect. These data suggest that the modulation of glucagon release via brain insulin sensitivity is not relevant under normo- and hyperglycemic conditions. However, it plays a crucial role during hypoglycemia [8].

Limitations of the current work include the limited sample size and the fact that only male healthy volunteers were included. Further and larger studies should include both sexes, older persons, and also patients with metabolic alterations.

Taken together, our current results demonstrate that insulin action in the human brain is able to modulate pancreatic insulin secretion depending on insulin sensitivity of the hypothalamus. In lean persons with high hypothalamic insulin sensitivity, brain insulin delivery stimulates second-phase pancreatic insulin secretion. Together with brain insulin's ability to enhance hepatic insulin sensitivity [20], higher portal insulin concentrations due to this response might contribute to the postprandial suppression of endogenous glucose production and represent a second phase of postprandial glucose allocation (Fig. 3). In heavier persons with hypothalamic insulin resistance, this reaction is absent or even reversed with a possibly negative impact on postprandial metabolic control. Further research is needed to delineate the relative contribution of brain insulin-derived modulation of postprandial glucose metabolism and the long-term impact of brain insulin resistance for the development of type 2 diabetes.

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Statement of Ethics

Informed written consent was obtained from study participants prior to inclusion in the study and the Ethics Committee of the University of Tübingen approved the protocol (086/2016BO1).

Disclosure Statement

The authors declare no conflicts of interest with direct relevance to the content of this work.

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Author Contributions

M.H.: designed the experiment, researched and analyzed data, and drafted the manuscript. R.W. and S.K.: researched and analyzed data and contributed to discussion. C.W., B.A.J., A.V., and C.K.: researched data and contributed to discussion. V.H.: prepared the nasal sprays and contributed to discussion. K.S.: supervised MRI measurements and contributed to discussion. A.P. was responsible for hormone measurements and contributed to discussion. H.-U.H., H.P., and A.F.: contributed to the design of the study and to discussion. All authors approved the final version of the manuscript prior to submission. A.F. is the guarantor for this work.

Registration

This study was registered at Clinicaltrials.gov as trial number NCT02870361.

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