Nasal insulin administration does not affect hepatic glucose production at systemic fasting insulin levels

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Abstract

*Aim.* Administration of insulin as nasal spray in humans has demonstrated a modulation of endogenous glucose production via the brain when systemic insulin levels are high. Results are conflicting under fasting insulin concentrations. To evaluate effects of brain insulin on endogenous glucose production in fasting humans with focus on hepatic glucose release, we performed a randomized, placebo controlled, blinded, cross-over experiment.

*Materials and Methods.* On two separate days, 2H2-glucose was infused to nine healthy lean males and blood was sampled from the hepatic vein and a radial artery. On day 1, participants received 160 U human insulin through nasal spray, on day 2 participants received placebo spray together with an intravenous insulin bolus to mimic spill over of nasal insulin to the circulation. Hepatic glucose fluxes and endogenous glucose production were calculated.

*Results.* Plasma insulin concentrations were comparable between study days, and no differences in whole-body endogenous glucose production or hepato-splanchnic glucose turn-over were detected.

*Conclusions.* Nasal administration of insulin does not influence whole-body or hepatic glucose production in fasting humans. In contrast, brain insulin action might modulate insulin effectiveness in glucose-producing tissue when circulating insulin levels are elevated. Therefore, metabolic consequences of brain insulin action appear to dependent on the metabolic prandial status.

1. INTRODUCTION

Research over the last years has demonstrated that the human brain is sensitive to insulin 1. Many studies used nasal insulin as a tool to deliver insulin selectively to the human brain. After nasal administration, insulin rapidly enters the brain and remains there for a considerable time while serum insulin levels hardly change 2. Thus, nasal application enables insulin delivery into the brain with minor effects on peripheral insulin concentrations, and it therefore allows to address the insulin effects on the human brain 1,3.

Several studies have demonstrated the effects of nasal insulin administration on regional brain activity 1. However, this result was not consistent and not present in all subjects, some showed little or no effect, e.g. they appear to be brain insulin resistant 4.

One of the brain areas which responds to nasal insulin is the hypothalamus 5,6. This brain area is crucial for whole body homeostasis - including glucose homeostasis and peripheral insulin sensitivity 1,4,6. In line with this, research has demonstrated that central insulin delivery improves peripheral insulin sensitivity during hyperinsulinemia in lean - but not in obese - men 7,8. This effect was observed approximately 60 min post nasal insulin application and was still detectable after 210 min post spray 7,8. Improved peripheral insulin sensitivity appears to result from combined suppression of endogenous glucose production and stimulation of glucose uptake into peripheral tissues 8. Experiments in animals indicate that brain insulin mainly affects regulation of endogenous glucose production in the liver 9.

However, in the fasting state, data in humans are less clear. Using nasal insulin, we previously did not detect effects on peripheral insulin sensitivity, on endogenous glucose production, or glucose disappearance up to 180 minutes post spray 10. However, Dash et al. reported suppression of endogenous glucose production approximately 180 min after nasal application of the insulin analogue lispro during a pancreatic clamp 11 which is blunted in obese individuals 12. While both these studies applied tracer dilution techniques for the assessment of endogenous glucose production, neither used blood sampling from a hepatic vein to specifically assess hepatic and hepato-splanchnic glucose metabolism.

The aim for the present study is to test if brain action of human insulin has ~~delayed~~ effects on endogenous glucose production in the absence of somatostatin infusion in healthy subjects. This was investigated in a randomized, placebo controlled, blinded, cross-over experiment with blood sampling from a hepatic vein and the periphery.

2. MATERIALS AND METHODS

**2.1 Experimental design**

The protocol was approved by the local ethical committee of The Capital Region, Denmark (H-2-2014-092). After oral and written information and consent, ten healthy males were included. They underwent a medical examination including routine biochemistry to exclude liver and metabolic disease. The subjects participated for two experimental days separated by at least two weeks, randomized to either receiving nasal insulin spray or placebo spray on the first day. One subject was excluded due to increased glucose concentration in the hepatic vein concomitantly with the indocyanin green infusion.

The participants reported at the laboratory after an overnight fast, a catheter was inserted into the antecubital vein and baseline blood samples were drawn. Subsequently, the following tracers were infused: [6,6-2H2]-glucose (prime: 17.6 µmol/kg infusion rate 0.6 µmol/kg/min; Cambridge Isotopes Laboratories, MA, USA). Next, after application of local anesthetic, an introducer (Avanti+, 7F, Cordis, CA, USA) was inserted into the right femoral vein and through this, a catheter (MPA 1, 6F, Cordis, CA, USA) was positioned in a right hepatic vein guided by fluoroscopy. The tip of the catheter was positioned 6 - 10 cm from the inferior caval vein. Finally, a catheter was inserted into the radial artery, allowing for paralleled sampling from the artery and the hepatic vein. After 2 hours of tracer infusion (to reach steady state equilibration), 2 puffs of nasal spray (either human insulin (Actrapid, Novo Nordisk, Bagsværd, Denmark, total dose: 160 U) or placebo substance) were administered into each nostril a total of 4 times. On the placebo day an intravenous infusion of insulin (Actrapid Novo Nordisk) was applied to mimic the spill over from the nasal spray to the circulation (2.5 mU × kg−1 over 15 min) 8. Blood samples were drawn simultaneously from the artery and the hepatic vein every 30 min for the following 5 hours (300 min). The subjects were fasted and in supine position during the whole experimental day, tap water was allowed ad libitum. All subjects were asked not to engaged in any form of exercise 24 h before the experimental day. An outline over the experimental day is presented in Figure S1.

The hepatic blood flow was measured three times during the experiment: 1 hour before administration of the insulin/placebo spray, after 1.5 hours and at the end of the experiment (after 4 hours). The hepatic blood flow was measured by indocyanine green dilution. In brief, immediately prior to the trial, 75 mg ICG (ICG-Pulsion 5 mg/ml, Pulsion medical systems, Feldkirchen, Germany) was mixed with 500 ml of isotonic saline with 2% Human Albumin (CSL Behring, Marburg Germany) and infused at a rate of 2 ml/min. The infusion was started 40 min before the flow measurement with blood drawn 5 times at 5 min intervals, please see supplemental figure S1 for details. Indocyanine green is solely eliminated by the liver and by assessing the arterial to hepatic venous difference of indocyanine green the blood flow was calculated according to Ficks principle, as detailed in 13.

Blood was collected in EDTA-containing tubes, placed on ice and centrifuged at 4 °C. Plasma was stored at –80 oC until analysis. After the last blood sample was drawn, all catheters were removed and the subjects were offered a meal.

**2.2 Plasma analysis**

Insulin and C-peptide were measured with the ADVIA Centaur XPT immunoassay system (Siemens Healthineers, Erlangen, Germany). Glucose concentrations and enrichments were measured as previously described 14.

**2.3 Tracer calculations**

The tracer-to-tracee ratio (TTR) was calculated by subtracting the background enrichment (plasma sample obtained before the tracer infusions were commenced time point -120 min Figure S1) from the enrichment measured in the following samples. Whole body rate of appearance (Ra) and disappearance (Rd) of glucose was calculated using the non-steady state Steele equation 15 adapted for stable isotope labelling where the volume of glucose distribution set to 230 ml/kg 16. To evaluate how much glucose was entering the systemic circulation from the hepato-splanchnic bed, the net balance of glucose over the hepato-splanchnic bed was calculated by multiplying the arterial-to-venous difference with the blood flow. Furthermore, the hepato-splanchnic uptake of glucose was calculated both as a percentage (the fractional extraction of glucose) and in absolute quantity. The release of glucose from the hepato-splanchnic bed accounted for a possible glucose uptake was calculated as the hepato-splanchnic release. Finally, a contribution from non-hepato-splanchnic tissue was assessed by subtracting the whole-body rate of appearance by the hepato-splanchnic release. The time points for the calculations of the hepato-splanchnic glucose turn-over were averaged to match the whole-body non-steady-state time points. The equations used for the calculations are stated in the supplement section.

**2.4 Statistics**

Our current study was sufficiently powered (power [1-beta error probability] > 0.9, alpha = 0.05) to detect the effect of intranasal insulin on the suppression of endogenous glucose production observed under systemic hyperinsulinemia in lean men (Cohen’s dz = 2.34, 8).

Data are presented as mean ± SEM. Differences between groups were evaluated by a two-way repeated measurement ANOVA using the proc mixed procedure. The statistical analyses were performed by SAS 9.4 (Institute Inc., Cary, NC, USA) and a *P* < 0.05 was considered statistically significant. Power calculation was done using the G power software (<http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/>).

3. RESULTS

The participants included were nine healthy males with no medical history and a mean (range) age of 24.1 (19 - 28) years with a normal body mass index of 22.2 (20.5 - 23.2) kg/m2. Before the experiment, the subjects were screened using routine biochemistry which confirmed normal liver and glycaemic parameters and no signs of infection (see Table 1).

**3.1 Insulin and hepatic blood flow**

As previously observed 3, a small increase in the arterial insulin concentration was detected during the minutes after administration of the nasal insulin spray. The insulin concentration returned to fasting levels after 30 min. This spill-over of nasal insulin to the circulation was mimicked on the placebo day by an intravenous infusion of insulin from 0 - 15 min which increased the arterial insulin concentration similarly (Figure 1A). No corresponding increase in C-peptide was observed on the insulin or on the placebo day (Figure 1B). The hepatic blood flow was assessed three times during the experiment: before (-60 min) and after (90 and 240 min) nasal insulin administration. No statistically significant changes in blood flow were detected on either the absolute values (Figure 1C) or the relative change form timepoint “0” (data not shown).

**3.2 Whole body glucose turnover**

The arterial glucose concentration decreased similarly in both trials throughout the experimental day (Figure 2A). The rate of glucose entering the circulation, i.e. rate of appearance (Ra), did not reveal any change after administration of nasal insulin or placebo, (Figure 2B). Likewise, calculating the rate of glucose leaving the circulation, rate of disappearance (Rd) did not reveal any effect of nasal insulin or placebo (Figure 2C).

**3.3 Hepato-splanchnic glucose turnover**

The experimental design allowed a regional assessment of the glucose fluxes over the hepato- splanchnic bed. Calculation of the net glucose concentration balance revealed that glucose was released from the hepato-splanchnic bed into the systemic circulation, however no effect of nasal insulin on the net glucose release was detected (Figure 3A). Furthermore, the fraction of glucose extracted by the hepato-splanchnic bed (e.g. the liver) was negligible, meaning that hepatic glucose uptake by the hepato-splanchnic bed was around zero, during both study conditions (Figure 3B and C). When the net balance was corrected for the hepato-splanchnic glucose uptake, the hepato-splanchnic glucose release did not reveal any effect of either nasal insulin administration or placebo (Figure 3D). Finally, the contribution of the non-splanchnic glucose release was minimal in our experiment (Figure 3E).

4. DISCUSSION

In this study we tested whether brain insulin action under low systemic fasting insulin levels has delayed effects on hepatic glucose production. No differences were detected for hepato-splanchnic glucose uptake or production. In addition, total body endogenous glucose production was not affected either. Together, this suggests that nasal insulin does not affect endogenous glucose production in the fasted state in healthy young individuals.

As circulating insulin is a major regulator for endogenous glucose production 17,18, it is crucial to have comparable concentrations of the hormone in the blood on both measurement days. In the current study this was accomplished by a tiny intravenous insulin administration together with placebo spray administrations using a recently developed protocol 8. Thereby, comparable plasma insulin levels were accomplished, however, the statistical analysis revealed a significant group effect, which was due to difference in peak shape between nasal insulin and placebo from 0 – 30 minutes. A slight reduction of circulating C-peptide following intranasal insulin administration has been observed before in studies that did not control for spill over of the nasal spray into the circulation 19,20. Eventhough, there are hints towards the regulation of β-cell function by brain insulin action 21,22, the different kinetics of insulin and C-peptide detected in the current project might indicate an additional effect on insulin clearance. However, further research is needed to test this hypothesis.

In the present study the experimental design allows estimation of the non-hepato-splanchnic glucose contribution by subtracting the whole-body rate of appearance of glucose by the hepato-splanchnic glucose release, which was fluctuating around zero. The kidney is suggested to account for the majority of the non-hepato-splanchnic glucose release and a net renal release of glucose has been reported in the range of 0.6 – 2 µmol/min/kg using a direct measurement by arterial-to-renal vein catheterization 23-25. This small non-hepato-splanchnic release is not detected with the indirectly estimate applied in the present study without direct sampling from the renal vein.

In contrast to our results, Dash et al. reported a marked suppression of endogenous glucose production which started around 180 min post nasal insulin administration 11. One possible explanation for the difference to our present results can be the type of insulin spray used. While we applied human insulin, Dash et al. used the fast-acting insulin analogue Lispro. Differences of insulin analogues from regular human insulin regarding the modulation of brain activity have been reported before 26-28. However – to the best of our knowledge - no direct comparison of human insulin with insulin lispro has been conducted before. Another explanation for the results obtained by Dash et al. can be the infusion of somatostatin in their experiments. Somatostatin is used to block the endogenous production of insulin from pancreatic beta cells to avoid differences in circulating insulin levels. The low peripheral plasma insulin concentrations during our current experiments were comparable to the insulin levels in the study of Dash et al. 11, demonstrating that no somatostatin is needed to achieve stable low peripheral insulin concentrations. Somatostatin not only blocks the release of insulin from pancreatic β-cells which results in a relative portal hypoinsulinemia estimated to be 3-fold lower compared to the fasting state 29, but also impairs the release of glucagon from the α-cell. As the current study did not use somatostatin, both these processes might explain the different results obtained by Dash et al. 11.

~~One disadvantage of somatostatin is the well-known central nervous action of this peptide, which in some brain regions might interact with insulin action, thereby altering insulin responsiveness. One further disadvantage of somatostatin is that it not only blocks insulin release, it also inhibits other endocrine systems which must then be replaced by infusion of the appropriate hormones. This replacement will hardly be able to precisely mimic physiologic levels of e.g. glucagon or growth hormone. For some hormones, it is not possible at all and might therefore also have potential effects on the brain or on systemic glucose metabolism.~~

It has previously been demonstrated that during hyperinsulinemic euglycemic clamp experiments, i.e. in the presence of elevated circulating insulin concentrations, brain insulin action introduced by nasal insulin improved peripheral insulin sensitivity 7,8 and suppressed endogenous glucose production 8. This reaction started around 60 min post spray administration. However, under systemic fasting insulin levels, we did not detect such effects. This is in line with earlier results in the presence of fasting insulin levels where brain insulin modulated hepatic energy metabolism, but not endogenous glucose production 10. Therefore, the ability of brain insulin action to modulate peripheral glucose metabolism appears to rely on the concomitant presence of elevated insulin levels at peripheral organs, as physiologically present in the postprandial state. It is therefore likely that brain insulin action itself is not sufficient to modulate endogenous glucose production but can improve insulin sensitivity in peripheral organs (including the liver) thereby enhancing circulating insulin’s effect in the suppression of endogenous glucose production.

As only young men were included in the study, we cannot rule out that different effects of brain insulin might be present in women or older persons. Therefore, further studies are clearly needed to generalize our current findings to other groups. Furthermore, we cannot rule out that the used amount of intranasal insulin has introduced supra-physiological insulin levels in the brain.

Taken together (as summarized in Figure 4), we hypothesise that under physiological circumstances food intake will trigger the release of insulin from the pancreas into the systemic circulation. Insulin will with some delay subsequently enter the brain 30,31. The peptide hormone will act in specific brain areas 1 thereby modulating the autonomic nervous system 7,19,32 to communicate with peripheral organs in order to improve peripheral insulin sensitivity in the postprandial state 7. In the liver, for instance, improved insulin sensitivity appears to augment the suppression of endogenous glucose production 8, which is primarily introduced by elevated circulating insulin concentrations. As the human metabolism is in a postprandial state most of the day time, these effects can be very relevant for the modulation of human metabolism. Thus, reduced brain insulin action (e.g. brain insulin resistance) and thereby lack of this modulation may potentially have an impact on glucose metabolism and could contribute to the pathogenesis of type 2 diabetes.

By contrast, the body depends on the steady production of glucose in the fasting state. To support this, fasting plasma insulin concentrations do not suppress endogenous glucose production. Hence, our current results in combination with previous results 8 indicate that brain insulin action does not regulate endogenous glucose production directly but might modulate insulin sensitivity in glucose producing tissue. Thus, outputs from the brain reach the periphery via the parasympathetic branch of the autonomic nervous system 7,19,32 to increase peripheral insulin sensitivity, thereby augmenting the suppression of endogenous glucose production by peripheral insulin.

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**Conflict of interest**

The authors declare that no conflict of interest exists.

**Author contributions**

P.P. designed the study, performed experiments, analyzed and researched data, and wrote the manuscript. J.S.H. designed the study, performed experiments, analyzed and researched data, contributed to the discussion, and edited the manuscript. B.I. designed the study, performed experiments, and edited the manuscript. J.O.H. performed experiments and edited the manuscript. N.H.S. performed experiments, contributed to the discussion, and edited the manuscript. G.vH. analyzed and researched data, contributed to the discussion, and edited the manuscript. A.F. designed the study, contributed to the discussion and edited the manuscript. C.W. designed the study, contributed to the discussion, and edited the manuscript. R.L. designed the study, analyzed and researched data, contributed to the discussion, and edited the manuscript. H.U.H. designed the study, contributed to the discussion, and edited the manuscript. M.H. designed the study, analyzed and researched data, and wrote the manuscript.

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TABLE 1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Mean | Range (min – max) | | | Reference interval |
| *Anthropometric measures:* |  |  |  |  |  |
| Age (years) | 24.1 | (19 | - | 28) |  |
| Height (m) | 1.81 | (1.70 | - | 1.92) |  |
| Weight (kg) | 72.7 | (62.9 | - | 82.1) |  |
| BMI (kg/m2) | 22.2 | (20.5 | - | 23.2) |  |
|  |  |  |  |  |  |
| *Red blood cells:* |  |  |  |  |  |
| Hemoglobin (mmol/l) | 9.17 | (8.1 | - | 10.7) | 8.3 - 10.5 mmol/l |
|  |  |  |  |  |  |
| *Infection:* |  |  |  |  |  |
| White blood cell (x109/l) | 4.9 | (2.6 | - | 8.2) | 3.5 - 8.8 x 109/l |
| CRP (mg/l) | 1.5 | (<1 | - | 2) | < 10 mg/l |
|  |  |  |  |  |  |
| *Liver function:* |  |  |  |  |  |
| ALAT (U/l) | 18.7 | (13 | - | 31) | 10 – 70 U/l |
| ASAT (U/l) | 23.3 | (16 | - | 27) | 15 – 45 U/l |
| Albumin (g/l) | 41.2 | (36 | - | 46) | 36 – 48 g/l |
| GT (U/l) | 19.0 | (10 | - | 53) | 15 – 115 U/l |
| Coagulation factor II+VII+X (A.U/l) | 0.72 | (0.53 | - | 0.94) | >0.6 A.U/l |
| Bilirubins (µmol/l) | 12.4 | (5.0 | - | 30.0) | 5 – 25 µmol/l |
|  |  |  |  |  |  |
| *Glucose metabolism:* |  |  |  |  |  |
| HbA1c (mmol/mol) | 30 | (27 | - | 32) | <48 mmol/mol |
| Fasting glucose (mmol/l) | 4.7 | (4.0 | - | 5.3) | 4.2 – 6.3 mmol/l |
| Insulin (pmol/l) | 46 | (19 | - | 82) | 10 – 125 pmol/l |
| C-peptide (pmol/l) | 524 | (366 | - | 702) | 379 – 1630 pmol/l |

Subject characterization (n = 9). BMI: Body Mass Index. CRP: C-Reactive Protein. ALAT: Alanine Aminotransferase. ASAT: Aspartate Aminotransferase. -GT: Gamma-Glutamyltransferase.

FIGURE LEGENDS

Figure 1. Arterial insulin (A) and C-peptide (B) concentration (pmol/l) mean ± SEM. The arterial insulin concentration increased during the minutes after administration (time point 0) of the nasal insulin spray, grey line. At the placebo trial human recombinant insulin was infused from 0 – 15 min to mimic the spill-over observed after administration of nasal insulin spray. (C) Hepatic plasma flow assessed three times during the experimental day: before (-60 min) and after (90 and 240 min) administration of nasal insulin/placebo. Grey bars represent the nasal insulin trial, black bars the placebo trial. The result of the 2-way repeated measurement ANOVA is inserted. P < 0.05 is considered significant.

Figure 2. Whole body glucose turnover. Panel A: The arterial glucose concentration (mmol/l) mean ± SEM. Panel B: Rate of appearance (Ra) for glucose. Panel C: Rate of disappearance for glucose (Rd). The grey line is the nasal insulin day, the black line the placebo day. The nasal insulin spray was administered at time point 0. The result of the 2-way repeated measurement ANOVA is inserted. P < 0.05 is considered significant.

Figure 3. Hepato-splanchnic glucose flux. Panel A: The net balance of glucose over the hepato-splanchnic bed. Panel B: The fractional extraction of glucose by the hepato-splanchnic bed. Panels C and D: The hepato-splanchnic uptake and release of glucose respectively. Panel E: The non-splanchnic glucose release. The grey line represents the nasal insulin trial, the black line the placebo trial. The result of the 2-way repeated measurement ANOVA is inserted. P < 0.05 is considered significant.

Figure 4. Proposed effects of brain insulin in the fasting and postprandial states. During both states, brain insulin action modulates autonomic nervous system activity. While fasting, this does not affect hepatic glucose production, because insulin levels at the hepatocyte are low and do not inhibit gluconeogenesis. In the postprandial state, circulating insulin levels are high and suppress endogenous glucose production. This suppression is further propagated by brain insulin action via the autonomic nervous system. Figure modified from Servier Medical Art. EGP: endogenous glucose production.

FIGURE 1



FIGURE 2



FIGURE 3



FIGURE 4.

