Intranasal insulin suppresses systemic but not subcutaneous lipolysis in healthy humans

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Context: Insulin infused into the central nervous system of rats suppresses lipolysis in white adipose tissue, indicating a role of brain insulin in regulating systemic lipid metabolism.

Objective: We investigated whether central nervous insulin delivery suppresses lipolysis in healthy humans.

Design: Placebo-controlled, balanced within-subject comparisons were performed in both a main and an independent corroborative experiment.

Setting/Participants/Intervention: Two groups of healthy volunteers were examined at the German University Clinics of Lübeck and Tübingen, respectively, with molecular analyses taking place at Mount Sinai, USA. The 14 healthy male subjects of the main study and the 22 women and 5 men of the corroborative study each received 160 IU of human insulin intranasally.

Main outcome measures: In the main study, we measured systemic levels of free fatty acids (FFA), triglycerides and glycerol and the rate of appearance of deuterated glycerol (Ra glycerol) as an estimate of lipolysis before and after intranasal insulin administration. We also analyzed expression of key lipolytic enzymes in subcutaneous fat biopsies and measured blood glucose and glucoregulatory hormones. In the corroborative study, FFA concentrations were assessed before and after intranasal insulin administration.

Results: In the main experiment, intranasal insulin suppressed circulating FFA concentrations and lipolysis (Ra glycerol) in the absence of significant changes in circulating insulin levels. Lipolytic protein expression in subcutaneous adipose tissue was not affected. The corroborative study confirmed that intranasal insulin lowers systemic FFA concentrations.

Conclusions: Our findings indicate that brain insulin controls systemic lipolysis in healthy humans by predominantly acting on non-subcutaneous adipose tissue.

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Abbreviations:

Insulin controls white adipose tissue (WAT) metabolism by suppressing lipolysis and inducing lipogenesis, effects which had long been considered to be mediated exclusively by insulin receptors expressed on adipocytes (1). However, mice that lack the insulin receptor in all peripheral tissues including WAT display only a mild reduction of fat mass (2). In contrast, deletion of both peripheral and central insulin receptors in mice leads to severe lipodystrophy (3). We have demonstrated that insulin infused into the hypothalamus of rats rapidly suppresses lipolysis by dampening sympathetic nervous system (SNS) outflow to visceral WAT (4). In these studies we also found that de novo lipogenesis in WAT is enhanced by brain insulin but reduced after the loss of central insulin receptors. Thus, these findings in rodents indicate that following uptake into the brain, insulin regulates WAT metabolism by suppressing lipolysis and inducing lipogenesis, both core metabolic functions of insulin (5). Whether brain insulin serves a similar function in higher mammals is unclear, and there has been no study to examine the impact of brain insulin on lipolysis in humans. Intranasal administration delivers insulin via trigeminal and olfactory nerve fibers to the brain without relevant systemic absorption (6), and has been widely used to investigate central nervous insulin effects (7–9). To study the role of brain insulin in the regulation of lipolysis in adipose tissue, we administered insulin intranasally to lean healthy volunteers and assessed Ra glycerol as an estimate of systemic lipolysis and circulating fatty acid levels.

Materials and Methods

Two placebo-controlled, balanced within-subject studies (main and corroborative) conforming to the Declaration of Helsinki and approved by the local ethics committees were conducted in healthy subjects after obtaining informed consent.

Design and procedure of the main study. Fourteen male volunteers (mean±SEM age, 24.7 ± 1.1 year; BMI 24.4 ± 0.6 kg/m^2; body fat mass, 15.3 ± 1.0 kg, 18.9 ± 1.1%) participated in two conditions (insulin and placebo) at least 4 weeks apart. After a 12-hour fast confirmed by urine ketone bodies (urine test strip, Menarini, Florence, Italy), the experimental protocol (Figure 1A) started at 7:30 AM with the assessment of body composition (BIA 2000-M; Data Input, Frankfurt, Germany; all $P > .37$ for comparisons between conditions). Two intravenous (IV) cannulas were placed, one in a cubital vein and the other in a vein of the dorsal venous plexus of the hand. A microdialysis catheter was placed in abdominal subcutaneous adipose tissue (10). Blood was sampled every 15–30 minutes. At 8:00 AM, a primed bolus of d-[1,1,2,3,3–2H5]glycerol (99% enriched; Cambridge Isotope Laboratories, Andover, MA) was intravenously (IV) administered at a dose of 2 μmol/kg body weight (BW) for 1 minute and continued at 0.2 μmol/kg BW/min. After calibration, microdialysis measurements of glycerol were obtained. Subcutaneous adipose tissue was biopsied by needle aspiration from a lower abdominal quadrant at baseline and from the respective contralateral quadrant 180 minutes post-treatment, and stored at –80°C for Western blot analyses (11). At 9:45 AM, subjects received 160 IU of insulin (1.6 ml Insulin Actrapid; Novo Nordisk, Mainz, Germany) and vehicle (containing all ingredients except for the peptide and zinc chloride), respectively, intranasally [see ref. 8 for further details]. Throughout experiments, participants were monitored as previously described (10, 12).

Microdialysis. Microdialysis was performed using a high-precision pump (CMA 106, CMA Microdialysis, Solna, Sweden) and a microdialysis catheter (CMA 60, cutoff 20000 Da) placed in abdominal subcutaneous adipose tissue. Glycerol concentrations were analyzed using a CMA/600 analyzer (CMA Microdialysis, Solna, Sweden).

Rate of appearance of deuterated glycerol. d-[1,1,2,3,3–2H]glycerol enrichment in plasma was analyzed as previously described (13). Plasma was deproteinized by addition of methanol followed by centrifugation. The fluid fraction was evaporated and reacted with bis(trimethylsilyl)trifluoroacetamide plus 10% trimethylchlorosilane. Isotope enrichment was determined by gas chromatography-mass spectrometry. Rate of appearance of glycerol (Ra glycerol) was calculated using the formula: basal infusion rate of the isotope × [(glycerol infusate enrichment/steady-state basal plasma glycerol enrichment) – 1].

Western blot analyses of subcutaneous adipose tissue. Protein lysates were prepared (14) and subjected to electrophoresis on a 4%–12% NuPAGE gel (Invitrogen, Carlsbad, CA), blotted onto Immobilon-FL PVDF membranes, and incubated with primary antibodies, ie, phospho-hormone-sensitive lipase (Ser563 and Ser660; Cell Signaling Technology, Beverly, MA) and β-actin (Abcam, Cambridge, MA), as well as secondary antibodies (Thermo Fisher Scientific, Waltham, MA). Blots were quantified using LI-COR/Odyssey software 3.0 (LI-COR, Lincoln, NE; ref. 13).

Metabolite and hormone analyses. Serum free fatty acids (FFAs), triglycerides and glycerol were measured via FFA-half micro test (Roche, Prenzberg, Germany), triglycerides assay (Abbott, Wiesbaden, Germany), and 'Freies Glycerin FS kit' (DiaSys, Holzheim, Germany), respectively. Blood glucose concentrations, plasma glucagon and serum leptin were measured with HemoCue Glucose 201 RT Analyzer (HemoCue GmbH, Grossostheim, Germany), glucagon RIA kit (Biotrend, Cologne, Germany) and leptin (human) RIA (IBL, Hamburg, Germany), respectively. Serum concentrations of insulin, C-peptide, thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), cortisol, and plasma concentrations of adrenocorticotropic hormone (ACTH) were measured with Immulite analyzer 1000 (Siemens, Erlangen, Germany) and catecholamines by high-performance liquid chromatography (HPLC) (Chromsystems, Gräfelfing, Gemany).

Corroborative study. FFAs were determined by an enzymatic method (WAKO, Neuss, Germany) in EDTA plasma samples obtained in 27 healthy subjects (22 women; age 26.1 ± 1.2 years; BMI 23.9 ± 0.8 kg/m^2) before and 30 and 60 minutes after intranasal administration of 160 IU insulin and vehicle, respec-

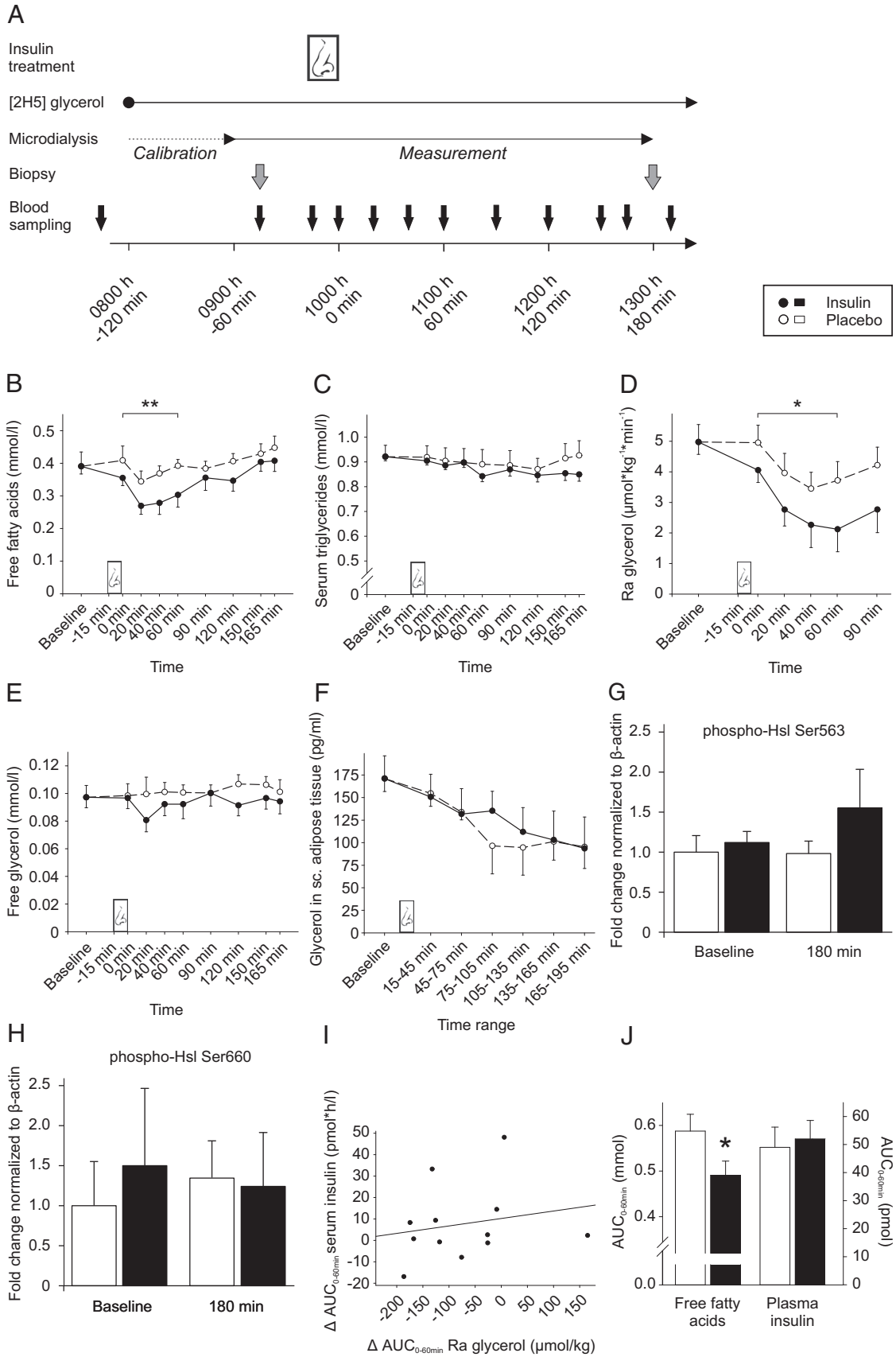

Figure 1. Design of the main study and key results. **(A)** At 8:00 AM, an infusion of d-[1,1,2,3,3–2H5]glycerol and microdialysis started. Baseline blood sampling took place at 7:45 AM, 9:15 AM, and 9:45 AM for blood glucose, C-peptide and insulin, and at 9:15 AM and 9:45 AM for all other blood parameters, along with one baseline collection of subcutaneous adipose tissue via biopsy at 9:15 AM. Intranasal insulin (160 IU) or

tively, at 8:00 AM after an overnight fast. Some of these samples were derived from previously published experiments (15).

Statistical analysis. Data are means ± SEM. Baseline adjustment was achieved by individually subtracting averaged baseline values from post-treatment values. Analyses relied on ANOVA with the repeated-measure factors *Treatment* and *Time*, and Greenhouse-Geisser correction of degrees of freedom. Two-sided pairwise *t* tests were used for comparisons of areas under the curve (AUCs) calculated according to the trapezoidal rule. Four subjects of the main study were excluded from analysis of subcutaneous adipose tissue glycerol and two subjects from the analysis of Ra glycerol due to technical failures. Exploratory biopsy analyses were restricted to 7 subjects. A P value < 0.05 was considered significant.

Results

In the main study, baseline concentrations of endocrine and metabolite parameters were similar in both conditions (all $P > .14$). Intranasal insulin compared to placebo lowered FFA concentrations ($F(1,13) = 7.49$, $P < .02$ for treatment; $P < .01$ for $AUC_{0-60min}$; Figure 1B), while triglyceride concentrations remained unchanged ($P > .24$; Figure 1C). Ra glycerol, an estimate of whole body lipolysis, was decreased after intranasal insulin administration ($F(1,11) = 6.61$, $P < .03$ for Treatment; $P < .04$ for $AUC_{0-60min}$; Figure 1D). This reduction in whole body lipolysis was reflected in lower circulating glycerol concentrations (Figure 1E), although this effect did not reach statistical significance ($F(1,13) = 1.04$, $P = .33$ for Treatment). Local lipolysis rates in subcutaneous abdominal adipose tissue as assessed by microdialysate glycerol levels did not differ between conditions ($P > .29$; Figure 1F). Likewise, the activation state of the key lipolytic enzyme hormone-sensitive lipase (Hsl) as assessed by Western blot analyses using phospho-specific antibodies against the activating phosphorylation sites Ser563 and Ser660, was unaltered by intranasal insulin (Figure 1G/H).

No treatment effects were discernible on blood glucose, serum insulin and C-peptide, as well as the concentrations of glucagon, ACTH, cortisol, and leptin (all $P > .12$ for Treatment and Treatment × Time; see Table 1 for comparisons of AUC). In particular, signs of a slight post-treatment increase in serum insulin were not confirmed by ANOVA ($P > .15$). Concentrations of epinephrine, norepinephrine, and TSH were unchanged, while fT3 levels showed a trend towards lower values in the insulin condition ($P > .064$ for Treatment). Correlational analyses revealed that the 0–60 minutes post-treatment differences between conditions in serum FFA, free glycerol and Ra glycerol were statistically unrelated to respective differences in serum insulin ($P > .17$ for all Pearson's coefficients calculated for single time points and $AUC_{0-60min}$; see Figure 1I for Ra glycerol). The suppressive effect of intranasal insulin on circulating FFA concentrations was confirmed in our independent corroborative study in men and women (Figure 1J).

Discussion

We demonstrate in humans that administering insulin intranasally to raise CNS insulin levels suppresses systemic lipolysis as assessed by Ra glycerol and circulating FFA measurements. These results extend findings in rodents (4) which established brain insulin as a regulator of WAT lipolysis to humans. Systemic concentrations of insulin, C-peptide, glucose, and glucagon were not affected by intranasal insulin, indicating that its effect on lipolysis was not mediated through changes in circulating insulin or glucose, and suggesting that the regulation of lipolysis is more sensitive to changes in CNS insulin signaling than systemic glucose homeostasis. This conclusion is supported by rodent studies where brain insulin suppressed lipolysis earlier and more markedly than hepatic glucose production; moreover, the latter effect was only detectable after the initiation of a somatostatin/insulin infusion (4).

Legend to Figure 1 Continued. . .
vehicle were administered from 9:45–10:00 AM (t = 0; nose symbol) and post-treatment measurements followed as depicted. – Serum concentrations of (**B**) free fatty acids and (**C**) triglycerides (both $n=14$), (**D**) rate of appearance of glycerol (Ra glycerol; $n=12$), (**E**) free glycerol in serum ($n=14$), and (**F**) glycerol in subcutaneous adipose tissue as assessed by microdialysis ($n=10$) measured before (baseline, averaged across pretreatment measurements) and after intranasal administration of insulin (black dots and solid lines) and placebo (white dots and dashed lines), respectively. Mean baseline values of both conditions are averaged to a common baseline. ** $P < .01$, * $P < .05$ for comparisons of $AUC_{0-60min}$ between conditions (pairwise t tests). – Activation state of hormone-sensitive lipase (Hsl) as assessed by Western blot analyses using phospho-specific antibodies detecting the activating phosphorylation sites Ser563 (**G**) and Ser660 (**H**) in subcutaneous adipose tissue biopsied 30 minutes before (baseline) and 180 minutes after intranasal administration of placebo (white bars) and insulin (black bars). Data are expressed as fold change compared to baseline values of placebo-treated individuals and are normalized to β-actin. (**I**) Individual treatment effects (expressed as difference between the insulin and the placebo condition) on Ra glycerol (area under the curve (AUC) from 0–60 minutes, $AUC_{0-60min}$) plotted against the respective effects on serum insulin concentrations, indicating statistical independence of both effects ($n=12$, $r=0.20$, $P > .54$). (**J**) Corroborative study: Plasma concentrations of free fatty acids (left) and insulin (right) expressed as $AUC_{0-60min}$ after intranasal administration of placebo (white bars) and 160 IU insulin (black bars), respectively, adjusted for sex. * $P < .03$ for comparison between conditions (pairwise *t* test). Note that the difference in free fatty acid concentrations was also significant after adjustment for age and BMI ($P < .05$) and plasma insulin ($AUC_{0-60\ minutes}$; $P < .02$).

Table 1. Systemic parameters in the main study.

	Insulin	Placebo	*P* value
Blood glucose (mmol × h/liter)	11.5 ± 0.2	11.6 ± 0.2	.37
Insulin (pmol × h/liter)	65.2 ± 6.5	55.7 ± 5.2	.26
C-peptide (nmol × min/liter)	53.1 ± 2.7	56.6 ± 2.2	.43
Glucagon[a] (ng × h/liter)	132.5 ± 3.1	136.7 ± 2.6	.26
Leptin (nmol × min/liter)	39.7 ± 1.3	40.1 ± 1.2	.78
ACTH (pmol × h/liter)	11.4 ± 1.3	11.8 ± 1.8	.88
Cortisol (nmol × h/liter)	648.6 ± 77.0	735.4 ± 46.0	.49
Epinephrine (pmol × h/liter)	212.8 ± 69.4	255.8 ± 53.2	.60
Norepinephrine (nmol × min/liter)	218.9 ± 10.5	219.3 ± 7.8	.98
TSH (mIU × min/liter)	171.8 ± 6.9	189.8 ± 7.9	.18
fT3 (pmol × min/liter)	617.6 ± 25.7	701.6 ± 21.5	0.07

$AUC_{0-150\ min}$ ([a]glucagon, $AUC_{0-60\ min}$) values for serum and plasma concentrations, respectively, after intranasal administration of insulin (160 IU) and placebo. AUC values derived from individually baseline-adjusted data were set to a common baseline averaged across conditions. Right column indicates *P* values for comparisons between conditions (pairwise *t* tests). $n = 14$.

Although we cannot completely rule out that a small amount of intranasal insulin may enter the bloodstream via the nasal mucosa, in both of our cohorts the suppression of lipolysis was statistically independent from any changes in serum insulin levels, supporting the notion that the effect of intranasal insulin was predominantly conveyed via central nervous pathways. In line with this conclusion, we found no significant differences between conditions in circulating fT3, TSH, and stress hormones, which are known to affect systemic lipolysis (16). Also, no effect of intranasal insulin on leptin, an adipokine regulating lipolysis via peripheral and central mechanisms, was observed (5, 17).

As previously demonstrated in our rodent studies, brain insulin reduces SNS outflow to visceral WAT and thereby reduces lipolysis (4). In follow-up studies in additional cohorts of rats, we could not observe a consistent effect of brain insulin on subcutaneous WAT (unpublished observations), although lipolysis in visceral WAT again was reduced. The failure to detect a suppression of Hsl activation in subcutaneous WAT could be attributed to the large variability of Hsl expression between animals in this depot, but it may also indicate that brain insulin regulates lipolysis primarily by controlling visceral WAT metabolism (4, 5). The latter is in agreement with our present finding that intranasal insulin does not alter lipolysis in subcutaneous abdominal tissue as indicated by interstitial glycerol concentrations and activation states of lipolytic enzymes. SNS outflow to adipose tissue is depot-specific (18) which supports the notion that centrally administered insulin may affect primarily visceral, but not subcutaneous WAT tissue (3, 4). In addition, brain insulin may also regulate lipid handling in other organs such as the liver, which our study did not examine.

In summary, we demonstrate that insulin delivered to the CNS by intranasal administration acutely suppresses systemic lipolysis in humans. Notably, the suppression of circulating FFAs by intranasal insulin found in our main study with male participants was corroborated by a study in an independent group of subjects that also included women. Emerging evidence suggests that diabetes and obesity are associated with reduced insulin sensitivity in the CNS of animals and humans (19, 20). Thus, impaired brain insulin action may contribute to the unrestrained lipolysis which may account for the lipotoxicity commonly observed in the obese or diabetic state. Hence we speculate that restoration of brain insulin signaling should improve adipose tissue function in these metabolic disorders.

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