Diabetes Publish Ahead of Print, published online September 28, 2010

Intranasal insulin enhances postprandial thermogenesis and lowers postprandial serum insulin levels in healthy men

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Submitted 5 March 2010 and accepted 20 September 2010.

Objective: Animal studies indicate a prominent role of brain insulin signaling in the regulation of peripheral energy metabolism. We determined the effect of intranasal insulin, that directly targets the brain, on glucose metabolism and energy expenditure in humans.

Research Design and Methods: In a double-blind, placebo-controlled, balanced within-subject comparison, 15 healthy normal-weight men (18-26 yrs) were intranasally administered 160 IU human insulin after an overnight fast. Energy expenditure assessed via indirect calorimetry and blood concentrations of glucose, insulin, C-peptide, and free fatty acids (FFAs) were measured before and after insulin administration and the subsequent consumption of a high-calorie liquid meal of 900 kcal.

Results: Intranasal insulin, compared to placebo, increased postprandial energy expenditure, i.e. diet-induced thermogenesis, and decreased postprandial concentrations of circulating insulin and C-peptide while postprandial plasma glucose concentrations did not differ from placebo values. Intranasal insulin also induced a transient decrease in prandial serum FFA levels.

Conclusions: Enhancing brain insulin signaling by means of intranasal insulin administration enhances the acute thermoregulatory and glucoregulatory response to food intake, suggesting that central nervous insulin contributes to the control of whole-body energy homeostasis in humans.

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Animal studies have yielded evidence that the regulation of whole-body energy flux critically depends on intact brain insulin signaling (1;2). Most recent findings have shown that the hypothalamic administration of insulin increases brown adipose tissue thermogenesis by direct inhibitory effects on warm-sensitive neurons (3). Moreover, studies in rodents have demonstrated that in addition to its direct inhibitory effect on hepatic gluconeogenesis, insulin acts in the hypothalamus to decrease glucose production in the liver (4;5), thus establishing an insulin-driven brain-liver axis that controls systemic glucose homeostasis. We examined if insulin acting in the human brain exerts comparable effects on energy homeostasis by administering intranasal insulin that bypasses the BBB and reaches the brain compartment along the olfactory nerve (6, 7), modulating central nervous functions in the absence of relevant peripheral effects (8). Notably, intranasal insulin reduces food intake (9) and body fat content (10) in healthy men, indicating that following intranasal administration, the hormone accesses neuronal networks relevant for energy homeostasis. Against this background, in the present study we assessed the effects of intranasal insulin on the glucoregulatory and thermoregulatory response to food intake in humans.

METHODS

Participants. Nineteen healthy men (mean ± SEM: age, 23.2 ± 0.6 yrs; BMI, 23.5 ± 0.3 kg/m^2) who were free of medication participated in the experiments. They gave written informed consent to the study that conformed to the Declaration of Helsinki and was approved by the local ethics committee.

Experimental Protocol. Each subject participated in two conditions (Insulin, Placebo) spaced apart by at least 4 weeks. The order of conditions was balanced across subjects. Body weight and body composition

(BIA 2000-M; Data Input, Frankfurt, Germany) did not differ between conditions.

After a 12-h fast, experimental sessions started at 7:00 AM with baseline assessments of energy expenditure and blood parameters (Figure 1). Throughout the experiment, subjects rested in bed in a supine position in a quiet room of constant temperature (23°C). At 9:10 AM, sixteen 0.1-ml puffs (8 per nostril) of insulin and placebo, respectively, were intranasally administered in 2-min intervals, amounting to a total dose of 1.6 ml insulin (160 IU; Insulin Actrapid; Novo Nordisk, Mainz, Germany) or vehicle (HOE 31 dilution buffer; Aventis Pharma, Bad Soden, Germany). Insulin and placebo were administered using precision air pumps (Aero Pump, Hochheim, Germany) that fill the nostrils and the nasal cavity with aerosol, thus enabling the solution to effectively target the olfactory epithelium. The dose of intranasal insulin used here has been previously shown to be functionally effective in healthy humans (9;11). Following post-insulin administration measurements (see below), subjects consumed 600 ml of a standard liquid meal (Fresubin® energy drink, Fresenius Kabi, Bad Homburg, Germany) at a dose of 20 ml/min (totaling 900 kcal; 33.6 g protein; 34.8 g fat; 112.8 g carbohydrate) from 10:15-10:45 AM and subsequently, assessments were continued until 4:00 PM.

Assessments. Energy expenditure (expressed as kcal/24 h) was measured via indirect calorimetry using a ventilated-hood system (Deltatrac II, MBM-200 Metabolic Monitor; Datex-Engström Deutschland, Achim, Germany). Before each use, the device was calibrated with Quick Cale calibration gas to 5% CO2 and 95% O2. Calorimetric measurements took place from 8:30 to 9:00 AM (baseline), from 9:45 to 10:15 AM (to assess effects of intranasal insulin alone) and five times between 10:45 and 3:15 PM, i.e., after liquid food intake (Figure 1). The rise in energy expenditure between the fasting state (baseline measurement from 8:45 to 9:15 AM) and the postprandial state (mean energy expenditure from 10:45 to 3:15 PM) reflects diet-induced thermogenesis, i.e., the energy which is emitted as heat during food metabolization and thus does not contribute to the production of ATP. Postprandial measurements were separated by 30-min breaks during which the ventilation hood was not worn but the subjects remained in bed.

For the assessment of plasma glucose levels as well as serum concentrations of insulin, C-peptide and free fatty acids (FFAs), blood was sampled twice during baseline (8:00 and 9:00 AM), immediately after intranasal insulin administration (9:40 AM), every 10 min during liquid food intake (10:20-10:40 AM) and at 60-min intervals thereafter (11:30 AM-2:30 PM) with a final sample taken at 4:00 PM (Figure 1). Plasma glucose levels were measured in fluoride plasma (hexokinase method, Aeroset, Abbott Diagnostics, North Chicago, IL). Serum concentrations of insulin and C-peptide were measured by Immulite analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). FFA concentrations were measured by enzymatic assays as previously described (12).

Statistical Analysis. Data are presented as *means ± SEM*. Statistical analyses were based on analyses of variance (ANOVA) including the repeated measures factors 'Condition' and 'Time' (referring to the immediate post-treatment and postprandial periods). Postprandial glucose and hormone concentrations (10:20 AM - 4 PM) were expressed as areas under the curve (AUC) calculated according to the trapezoidal rule. Post hoc two-sided t-tests were used for single time point comparisons. A p-value <0.05 was considered significant.

RESULTS

Resting metabolic rates were comparable between conditions during baseline and

immediately after insulin administration (P>0.19 for all comparisons; Figure 2). However, the increase in metabolic rate following liquid food intake was on average ~17 % greater in the Insulin than in the Placebo condition (P<0.05 for the 'Insulin/Placebo' main effect; Figure 2), indicating an increase in postprandial energy expenditure, i.e., diet-induced thermogenesis, due to intranasal insulin.

Plasma glucose and hormonal as well as FFA concentrations did not differ between conditions during baseline (P>0.25). Immediately after intranasal insulin administration at 9:40 AM a slight and transient decrease in fasting plasma glucose was detected (Insulin vs. Placebo, 4.5 ± 0.1 vs. 4.7 ± 0.1 mmol/l; P<0.02, 'Insulin/Placebo' main effect), but the subsequent postprandial increase in glucose concentrations did not differ between conditions ($AUC_{10:20\ AM-4\ PM}$, Insulin vs. Placebo, 1574 ± 23 vs. 1570 ± 20 mmol/l*min; P>0.90; Figure 3A). In parallel with the slight post-insulin administration drop in plasma glucose, a small increase in serum insulin (Insulin vs. Placebo, 40.1 ± 6.3 vs. 22.6 ± 3.0 pmol/l, P<0.01) but not C-peptide concentrations emerged (0.34 ± 0.02 vs. 0.37 ± 0.03 nmol/l, P>0.31). Following liquid food intake, the postprandial increase in both insulin and C-peptide concentrations was reduced by intranasal insulin in comparison with placebo ($AUC_{10:20\ AM-4\ PM}$, serum insulin, 45521 ± 5052 vs. 62315 ± 4973 pmol/l*min; serum C-peptide, 409 ± 30 vs. 487 ± 33 nmol/l*min, P<0.003 and P<0.02, respectively; Figure 3B,C). Serum FFA concentrations showed a transient decrease during food intake in the Insulin compared to the Placebo condition (P<0.01 for 'Condition' x 'Time') but did not differ between conditions during the postprandial period ($AUC_{10:20\ AM-4\ PM}$, Insulin vs. Placebo, 53.7 ± 4.6 vs. 56.6 ± 4.2 mmol/l*min, P<0.60; Figure 3D).

Supplementary analyses revealed that the immediate effects of intranasal insulin on preprandial glucose and insulin levels as well as the prandial decrease in FFA levels were statistically unrelated to the treatment-induced increase in postprandial thermogenesis and the suppression in postprandial serum insulin concentrations (Pearson's correlations; P>0.23 for all coefficients).

DISCUSSION

We demonstrate in humans that acutely enhancing brain insulin signaling by intranasal administration of the hormone increases postprandial thermogenesis. The parallel treatment-induced reduction in postprandial serum insulin concentrations while plasma glucose levels were comparable between conditions indicates that following intranasal insulin administration to the brain, lower circulating levels of the hormone are sufficient to dispose of meal-related increases in plasma glucose. In line with findings in animals (4;5;13), our results support the notion that brain insulin signaling in humans is involved in the control of whole-body energy homeostasis.

In keeping with previous experiments (9;11), intranasal administration of 160 IU insulin induced a transient and mild increase in serum insulin concentrations accompanied by a slight drop in pre-food intake plasma glucose that clearly remained within the euglycemic range. Due to the relatively high dose administered here as compared with previous studies (6;8), a small ratio of the hormone may have entered the circulation via the nasal mucosa. However, the transient nature and limited size of these immediate effects argues against an involvement of systemic uptake of intranasal insulin in its impact on postprandial thermogenesis and glucose metabolism. This conclusion is corroborated by the fact that immediate and postprandial effects were not statistically related.

The balanced regulation of nutrient intake and energy expenditure relies on the hypothalamus as a major integrator of nutritional and hormonal signals from the body periphery, including glucose and insulin (1). Direct injections of insulin into the preoptic area of the hypothalamus induce a dose-dependent increase in core body temperature due to stimulation of brown adipose tissue thermogenesis that is assumed to be mediated by inhibitory insulinergic action on warm-sensitive hypothalamic neurons (3). In our experiments, intranasal administration of the hormone to the brain did not affect resting energy expenditure but evoked a distinct increase in postprandial thermogenesis. Increased postprandial energy expenditure due to enhanced brain insulin signaling adds to the reduction in food intake previously observed after intranasal administration of the hormone (9), suggesting that the catabolic impact of central nervous insulin (10;14) is mediated not only by anorexigenic, but also by thermogenic effects of the hormone. Still, further studies on this issue are needed and should include measurements of body temperature, brown adipose tissue activity, and relevant vital signs like heart rate and blood pressure to elucidate the effect of brain insulin signaling on energy expenditure in humans.

A most remarkable finding of our study is the intranasal insulin-induced reduction in postprandial serum insulin concentrations while the food intake-induced rise in plasma glucose remained unaffected, suggesting that intranasal insulin improves postprandial insulin sensitivity. A regulatory effect of central nervous insulin on hepatic glucose metabolism has been indicated by animal studies showing that a selective decrease in hypothalamic insulin receptors reduces hepatic insulin sensitivity and results in marked increases in hepatic glucose production in the presence of plasma insulin concentrations equaling those of control animals (15). Fittingly, insulin hyperpolarizes glucose-responsive hypothalamic neurons by opening ATP-sensitive potassium channels (16) which triggers a decrease in hepatic glucose production that is mediated by vagal efferences (5;15). This pattern suggests that enhancing brain insulin signaling by intranasal administration of the hormone may act on glucose homeostasis in the body periphery by supporting hepatic insulin action. Nevertheless, given that postprandial liver glucose production accounts for around one fifth to one half of fasting values (17), improved insulin-dependent metabolization of ingested glucose may also have contributed to the intranasal insulin-induced decrease in postprandial serum insulin levels. Such an effect could basically be supported by the observed decrease in prandial FFA levels due to intranasal insulin inasmuch FFAs are known to impair insulin-stimulated muscle uptake of glucose (18). However, FFA effects on peripheral insulin-stimulated glucose uptake slowly develop over some hours (19), which, in conjunction with the lack of a significant correlation between the decreases in prandial FFA and postprandial insulin concentrations, makes this view unlikely. Furthermore, a contribution of enhanced non-insulin-mediated glucose disposal, i.e. glucose effectiveness, to our effects cannot be ruled out.

Although the present results suggest that insulin administration to the human brain enhances the efficiency of the glucoregulatory brain-liver axis in response to nutrient intake, our observations should be corroborated in future studies that rely on more refined measurements of insulin sensitivity like, e.g., euglycemic hyperinsulinemic clamps. It is also noteworthy that most recent animal data hint at divergent effects of hypothalamic insulinergic signaling on peripheral glucose homeostasis and energy expenditure depending on the involvement of agouti-related protein (AgRP) or

proopiomelanocortin (POMC) neuronal pathways (13). In this regard, general enhancements in brain insulin signaling as performed in our study do not permit differentiations. Taken together, our findings indicate that intranasal insulin acutely increases postprandial thermogenesis and improves the glucoregulatory response to food intake, suggesting that boosting brain insulin signaling in humans enhances the body's ability to cope with calorie consumption (20;21). Against the background of studies indicating that obesity and peripheral insulin resistance are associated with reduced central nervous insulin sensitivity (22-24), enhancing brain insulin signaling may emerge to be a useful approach in the therapeutic management of disorders hallmarked by disturbed glucose homeostasis (25).

Author Contributions: CB, JB and MH designed the study. CB and MH analyzed the data. SB enrolled patients. CB, HS, BS, JB, HL and MH contributed to writing the paper. CB and SB collected data or did experiments for the study. All authors had full access to all of the data and take responsibility for the integrity and accuracy of the data analysis.

ACKNOWLEDGMENTS

We thank I. von Lützau, M. Grohs, and H. Ruf (Department of Neuroendocrinology, University of Lübeck, Germany) for their expert and invaluable laboratory work. Aero Pump, Hochheim, Germany, generously provided us with precision nasal air pumps. Supported by Deutsche Forschungsgemeinschaft (KFO126/B5), Germany, and Tore Nilsons Foundation for Medical Research, Sweden. The funding sources had no input in the design and conduct of this study; in the collection, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript.

Duality of Interest: The authors have no potential conflicts of interest relevant to this article.

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Figure 1. *Experimental schedule*. Nineteen healthy subjects who had fasted overnight spent the experimental day sitting in bed in a supine position. Measurements of energy expenditure by 30-min periods of indirect calorimetry were performed during baseline (8:30-9:00 AM), immediately after intranasal insulin administration (9:45-10:15 AM, 1.6 ml (160 IU) insulin and placebo, respectively; nose symbol), and five times following the standardized consumption of a predefined liquid meal of 900 kcal (cup symbol). Blood samplings for the determination of plasma glucose, serum insulin, C-peptide, and free fatty acids concentrations are indicated by syringe symbols.

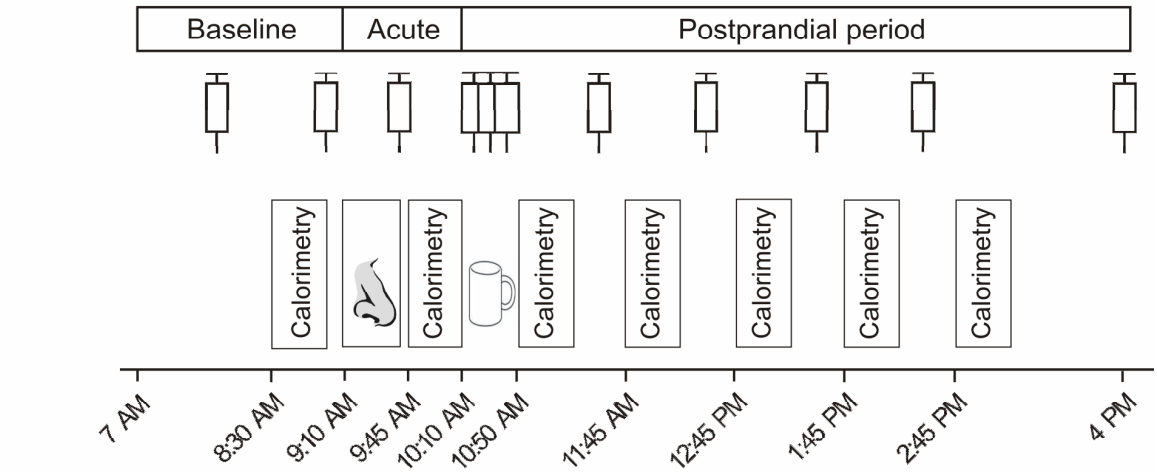

Figure 2. Intranasal insulin enhances postprandial energy expenditure. Following baseline assessment of energy expenditure (EE; expressed per kcal/min), acute effects of intranasal administration (nose symbol) of insulin (black bars; 160 IU) and placebo (white bars), respectively, on EE were frequently measured before and after ingestion of liquid food (900 kcal; cup symbol) for a total of 6.5 h (left panel). The rise in energy expenditure between baseline (8:30-9:45 AM) and the postprandial state (10:45 AM-3:15 PM) reflects the energy emitted mainly as heat during food metabolization (diet-induced thermogenesis, DIT; right panel). Data are means ± SEM; N=19; * P<0.05.

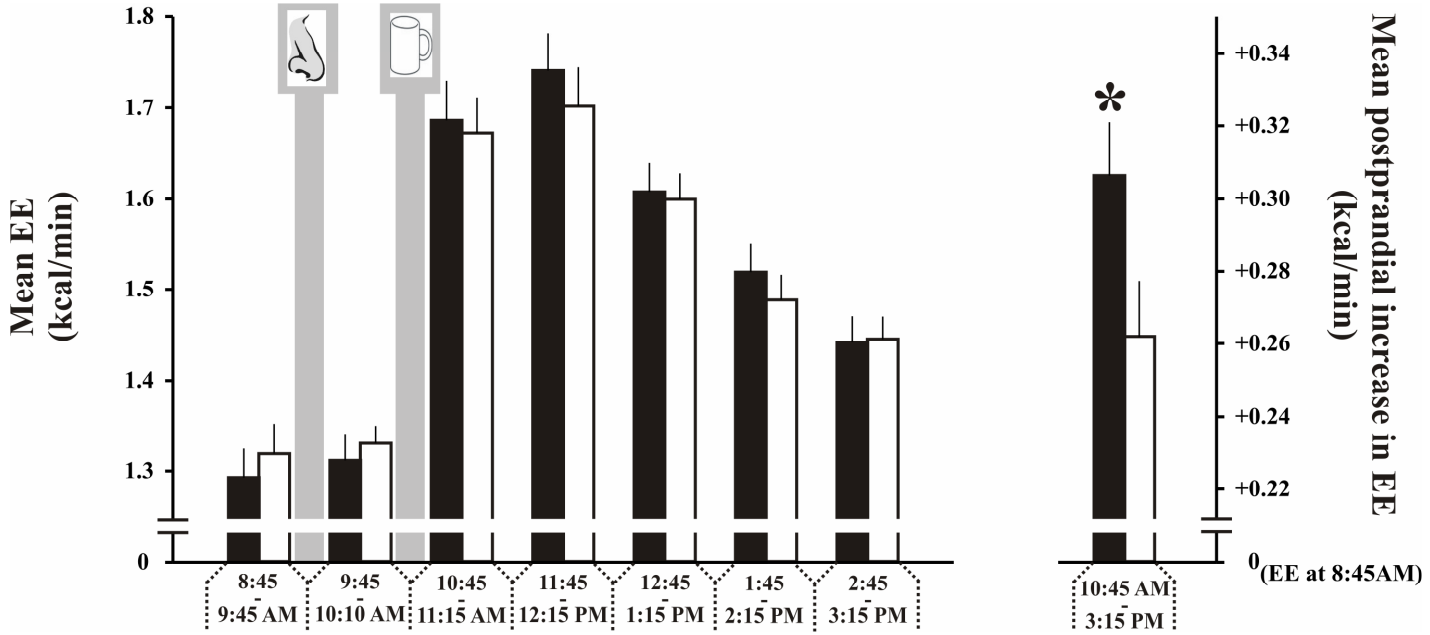

Figure 3. *Intranasal insulin lowers postprandial serum insulin levels.* Concentrations of plasma glucose (A), serum insulin (B), serum C-peptide (C), and serum free fatty acids (D) before and after acute intranasal administration (nose symbol) of intranasal insulin (160 IU; solid lines and black bars) and placebo (dashed lines and white bars) followed by the standardized ingestion of 900 kcal of liquid food (cup symbol). Postprandial levels (10:20 AM-4 PM) were also expressed as areas under the curve (AUC; right panels). All values are presented as means ± SEM. N= 19; * P<0.05; ** P<0.01.