

# Intranasal Insulin Reduces Body Fat in Men but not in Women

Manfred Hallschmid,<sup>1</sup> Christian Benedict,<sup>1</sup> Bernd Schultes,<sup>2</sup> Horst-Lorenz Fehm,<sup>2</sup> Jan Born,<sup>1</sup> and Werner Kern<sup>2</sup>

**Insulin acts in the central nervous system to reduce food intake and body weight and is considered a major adiposity signal. After intranasal administration, insulin enters the cerebrospinal fluid compartment and alters brain functions in the absence of substantial absorption into the blood stream. Here we report the effects of 8 weeks of intranasal administration of insulin (4 × 40 IU/day) or placebo to two groups of healthy human subjects (12 men and 8 women in each group). The insulin-treated men lost 1.28 kg body wt and 1.38 kg of body fat, and their waist circumference decreased by 1.63 cm. Plasma leptin levels dropped by an average of 27%. In contrast, the insulin-treated women did not lose body fat and gained 1.04 kg body wt due to a rise in extracellular water. Our results provide a strong, first confirmation in humans that insulin acts as a negative feedback signal in the regulation of adiposity and point to a differential sensitivity to the catabolic effects of insulin based on sex. *Diabetes* 53:3024–3029, 2004**

**O**besity is a health care problem of global scale, and its increasing prevalence strengthens the need for a thorough understanding of how food intake and body adiposity are regulated (1). Individual body fat content and body weight are of long-term constancy, pointing to a remarkably precise matching of caloric intake and energy expenditure (2). In recent years, research on the neuroendocrine networks maintaining energy homeostasis has made considerable progress in identifying the pivotal messengers that report the level of energy stored as body fat to the brain (3). Systemic insulin and leptin levels are proportional to body adiposity and decrease during fasting, enabling food intake to be triggered (4). Meals increase the concentration of insulin in the cerebrospinal fluid (CSF) and the hypothalamus (5). High concentrations of insulin receptors are found in brain regions important to food intake regulation, suggesting that insulin, independent of its ability to stimulate glucose uptake in peripheral cells, plays an essential role in modulating caloric intake (2). Furthermore, hypothalamic

insulin signaling is necessary for the inhibition of hepatic glucose production (6).

Administration of insulin to the central nervous system (CNS) reduces food intake and body weight (7), whereas its antagonization has reverse effects (8). Accordingly, switching off neuronal insulin receptors increases body weight and susceptibility to diet-induced obesity (9). In animals, the catabolic effects of central insulin are well documented (10), and there are some reports on the disproportional relation between insulin secretion and weight gain in men (11,12); however, to our knowledge, there is no direct evidence for catabolic effects of brain insulin in humans.

Circulating insulin reaches the CNS via a saturable, active transport across the blood-brain barrier (13), with CSF insulin levels increasing during intravenous infusion in humans (14). However, inducing central nervous catabolic effects using long-term intravenous infusion is not practicable, as peripheral insulin is an anabolic factor in energy homeostasis. Intranasal administration has been demonstrated to increase the concentration of insulin in CSF without the insulin being absorbed into the blood stream (15,16). In previous experiments, we found a distinct increase in CSF insulin levels within 40 min after intranasal administration of 40 IU insulin, averaging  $1.82 \pm 0.76 \mu\text{U/ml}$  compared with baseline values before administration. This increase was also significant in comparison with a placebo condition (15). Furthermore, insulin was shown to distinctly alter brain functions after intranasal delivery (17). Thus, the nasal route provides an effective way of conveying insulin to the brain. In the present experiments, we examined the effects of 8 weeks of intranasal insulin administration (4 × 40 IU/day) on body weight, body composition, and plasma hormone levels in healthy male and female subjects. The single dose of 40 IU was chosen based on our previous studies confirming significant CSF accumulations for this dosage level.

## RESEARCH DESIGN AND METHODS

Participants in this study were 40 healthy normal weight subjects (16 women, 24 men). They were all nonsmokers and none were taking medication, except that all women were taking oral contraceptives. All relevant illnesses were excluded by clinical examination and routine laboratory tests. Subjects abstained from alcohol, caffeine, and food for 12 h before the testing. They gave written informed consent before the experiments, and the study protocol was approved by the local ethics committee. Experiments were performed in a double-blind manner.

We randomly assigned subjects to either the insulin or placebo group (12 men and 8 women in each group). Both groups were comparable in age ( $24.6 \pm 1.3$  and  $25.8 \pm 1.2$  years, respectively) and BMI ( $22.6 \pm 0.3$  and  $22.7 \pm 0.4 \text{ kg/m}^2$ , respectively) in the pretesting examination. During 2 weeks of baseline, all subjects received placebo. During the succeeding 8-week treat-

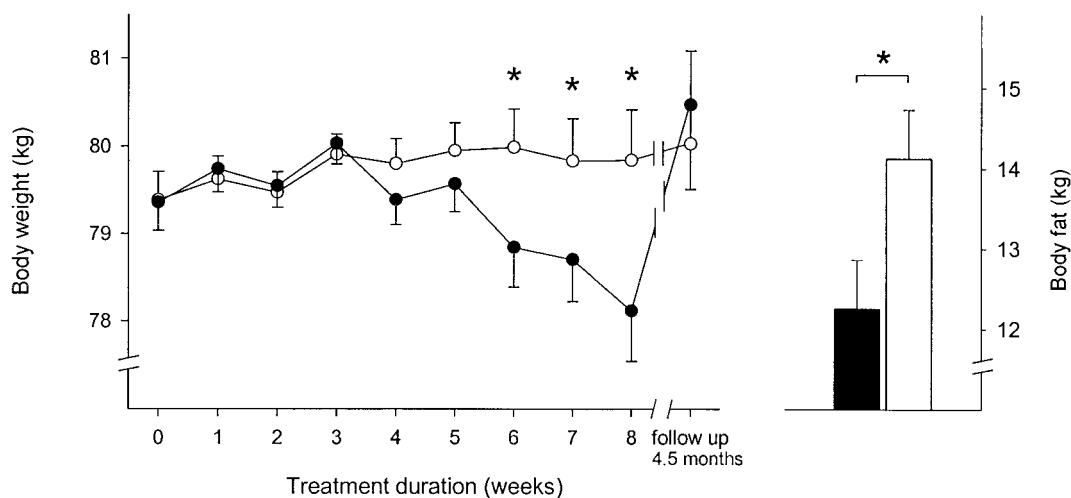
From the <sup>1</sup>Institute of Neuroendocrinology, University of Lübeck, Lübeck, Germany; and the <sup>2</sup>Department of Internal Medicine I, University of Lübeck, Lübeck, Germany.

Address correspondence and reprint requests to Manfred Hallschmid, PhD, Institute of Neuroendocrinology, University of Lübeck, Ratzeburger Allee 160, Haus 23a, 23538 Lübeck, Germany. E-mail: hallschmid@kfg.mu-luebeck.de.

Received for publication 16 June 2004 and accepted in revised form 6 August 2004.

CNS, central nervous system; CSF, cerebrospinal fluid.

© 2004 by the American Diabetes Association.



**FIG. 1. Intranasal insulin reduces body weight and fat in men. Left panel:** Average time course of body weight ( $\pm$ SE) in male subjects during 8 weeks of intranasal insulin administration and in a follow-up examination  $\sim$ 18 weeks after cessation of treatment. **Right panel:** Body fat ( $\pm$ SE) in the same subjects after 8 weeks of treatment. One group of subjects received insulin (●, ■), and the other group received placebo (○, □). Data are baseline adjusted as derived from ANCOVA. \* $P \leq 0.05$  ( $n = 12$  for each group).

ment phase, subjects were administered insulin or placebo intranasally four times a day: in the morning, around noon, and in the evening (each  $\sim$ 30 min before mealtime) and before going to bed. Each dose consisted of 0.4 ml insulin (40 IU; Insulin Actrapid; Novo Nordisk, Mainz, Germany) or vehicle (HOE 31 dilution buffer for H-Insulin; Aventis Pharma, Bad Soden, Germany) administered within four 0.1-ml puffs (two per nostril), amounting to 1.6 ml (160 IU) of insulin or vehicle per day. Sprays were stored at  $\sim$ 5°C and replaced every week. To ensure compliance, subjects kept a diary about their intake routine and were told that irregular intake would be detected via urine sampling.

Test sessions (scheduled between 0700 and 0900) took place at the beginning (session A) and end (session B) of the baseline phase and at the end of the treatment phase (session C). Subjects were kept unaware of the study aims by embedding examinations into the assessment of cognitive parameters, not described here in detail. Posttreatment interviews ensured all subjects had remained unaware of the treatment effects. In the beginning of a session, subjects were administered placebo (session A), insulin or placebo (B), and again placebo (C). Thus, session A was used to familiarize subjects with the intake routine, and sessions B and C allowed for the examination of acute and long-term treatment effects, respectively.

Immediately after substance administration, we weighed subjects and measured their body composition by standard bioelectrical impedance analysis (frequencies of 1, 5, 50, and 100 Hz; BIA 2000-M; Data Input, Frankfurt, Germany) indicating body fat, total body water, intracellular water, extracellular water, lean body mass, and body cell mass (Eurobody software; Data Input). Waist circumference was also measured, and subjects completed a questionnaire on their eating behavior (18). Subjects rated their hunger on a 10-point scale 60 min after insulin administration and at the end of the session. Thus we obtained difference values indicating the gradient of hunger feelings. Between ratings, resting energy expenditure was registered using indirect calorimetry (Deltatrac II, MBM-200 Metabolic Monitor; Datex-Engström Deutschland, Achim, Germany). Heart rate variability was measured simultaneously for an interval of 20 min (12-lead simultaneous electrocardiogram, sampling rate 1,000 Hz; ECG Lab Version 2.0; Meigaoyi, Beijing, China). Weekly, at 0800, we weighed subjects and sampled blood to determine leptin, insulin, and glucose concentrations. Levels of epinephrine and norepinephrine were taken from 12-h (2000–0800) nocturnal urine samples and measured by standard high-performance liquid chromatography.

To control for possible side effects, we monitored various other parameters in the weekly examinations, including plasma concentrations of ACTH, blood pressure, heart rate, and routine laboratory measurements (serum electrolytes; creatinine; HDL, LDL, and total cholesterol; and triglycerides). In a follow-up examination performed 4–5 months after cessation of treatment, we measured body weight, body composition, and blood parameters of the male subjects.

**Blood hormone concentrations.** Blood samples were centrifuged immediately, and the plasma was stored at  $-20^{\circ}\text{C}$ . Concentrations of leptin, insulin, and ACTH were assessed using standard radioimmunoassays (Human Leptin RIA KIT, Linco Research, St. Charles, MO; Pharmacia Insulin RIA100, Phar-

macia & Upjohn, Uppsala, Sweden; Lumitest ACTH, Brahms Diagnostica, Hennigsdorf, Germany). Glucose was measured in fluoride plasma (Aeroset; Abbott, Wiesbaden, Germany).

**Statistical analyses.** Comparisons between the effects of insulin and placebo were based on ANCOVA, with a group factor representing the treatment condition. In additional analyses, sex was included as a group factor, and treatment effects were evaluated separately for the male and female subgroups. Significant interaction effects were followed by pairwise one-sided contrasts. Respective values of session A (start of the baseline phase) served as covariates for parameters that could be subject to the acute influence of insulin (e.g., heart rate variability); values of session B (start of the treatment phase) were used as covariates in the assessment of long-term influence on body weight and related variables. All data are presented as means  $\pm$  SE.  $P \leq 0.05$  was considered significant.

## RESULTS

**Interactions between insulin effects and sex.** There were no overall differences in body weight, hormonal concentrations of interest, or any other measurements between the two treatment conditions due to the fact that men and women displayed striking differences in their response to intranasal insulin treatment, with only the men losing weight. Statistical analyses confirmed highly significant treatment  $\times$  sex interactions for body weight [ $F(1,35) = 8.91$ ,  $P \leq 0.005$ ], BMI [ $F(1,35) = 9.87$ ,  $P \leq 0.003$ ], and the ratio of extracellular mass to body cell mass [ $F(1,35) = 7.10$ ,  $P \leq 0.02$ ] after 8 weeks of treatment. Consequently, separate analyses were performed for the groups of men and women.

**Prolonged insulin administration induces weight loss and reduces adiposity in men.** In the men, insulin treatment reduced body fat content [ $F(1,21) = 4.65$ ,  $P \leq 0.05$ ], body weight [ $F(1,21) = 4.50$ ,  $P \leq 0.05$ ] (Fig. 1), and BMI [ $F(1,21) = 4.33$ ,  $P \leq 0.05$ ]. Waist circumference was also decreased [ $F(1,21) = 3.98$ ,  $P \leq 0.05$ ] (Table 1). Over the 8-week period, the insulin-treated men lost  $1.28 \pm 0.71$  kg of their body weight (versus a slight gain of  $0.45 \pm 0.49$  kg for the placebo group) and  $1.38 \pm 0.59$  kg of body fat (versus a gain of  $0.57 \pm 0.61$  kg for the placebo group). The BMI of insulin-treated men declined by  $0.38 \pm 0.21$  kg/m<sup>2</sup> (versus a gain of  $0.13 \pm 0.14$  for the placebo group), and their waist circumference decreased by  $1.63 \pm 1.17$  cm

TABLE 1  
Effect of 8 weeks of intranasal insulin administration in men

	Insulin	Placebo	P
Body weight (kg)	78.12 ± 0.57	79.84 ± 0.57	≤0.05
BMI (kg/m <sup>2</sup> )	23.02 ± 0.17	23.51 ± 0.17	≤0.05
Body fat (kg)	12.25 ± 0.61	14.12 ± 0.61	≤0.05
Body cell mass (kg)	37.20 ± 0.45	37.30 ± 0.45	≤0.88
ECM/BCM	0.75 ± 0.01	0.77 ± 0.01	≤0.17
Lean body mass (kg)	65.80 ± 0.72	65.79 ± 0.63	≤0.99
Total body water (kg)	47.92 ± 0.43	48.15 ± 0.43	≤0.71
Extracellular water (kg)	19.42 ± 0.23	19.48 ± 0.23	≤0.84
Waist circumference (cm)	81.06 ± 0.84	83.43 ± 0.84	≤0.05
Leptin (ng/ml)	2.06 ± 0.29	2.86 ± 0.25	≤0.05
Insulin (μU/ml)	6.81 ± 2.81	9.04 ± 2.69	≤0.58
Glucose (mg/dl)	85.99 ± 2.08	88.08 ± 2.08	≤0.48
ACTH (pg/ml)	22.52 ± 2.52	25.02 ± 2.41	≤0.48
Resting energy expenditure (kcal/day)	1,892.67 ± 58.52	1,847.03 ± 53.05	≤0.58
Heart rate (bpm)	67.29 ± 2.91	60.17 ± 2.65	≤0.09
HRV LF/HF	1.55 ± 0.42	2.37 ± 0.54	≤0.25
Hunger FEV	4.28 ± 0.54	5.24 ± 0.51	≤0.22
Hunger gradient	-0.36 ± 0.36	0.99 ± 0.36	≤0.02

Data are means ± SE (baseline adjusted, as derived from ANCOVA). ECM/BCM, ratio of extracellular mass (ECM) to body cell mass (BCM); HRV LF/HF, heart rate variability (HRV) expressed as the ratio of low-frequency (LF) to high-frequency (HF) power; hunger FEV, hunger score as derived from eating behavior questionnaire FEV ( $n = 12$  for each group).

(versus a gain of  $0.62 \pm 0.88$  for the placebo group). During the final examination after 8 weeks of treatment, hunger ratings were decreased [ $F(1,18) = 6.99$ ,  $P \leq 0.02$ ]. Heart rate appeared to be enhanced, but this effect was not significant [ $F(1,19) = 3.15$ ,  $P = 0.09$ ].

Leptin levels in the male subjects of the insulin group dropped during treatment, reaching significance compared with the placebo group after 8 weeks [ $2.06 \pm 0.29$  vs.  $2.86 \pm 0.25$  ng/ml;  $F(1,18) = 4.33$ ,  $P \leq 0.05$ ]. Insulin and glucose levels did not differ between the groups after 8 weeks of treatment (Table 1) or when average values during the entire treatment period were compared [ $8.87 \pm 1.65$  vs.  $10.85 \pm 1.65$  μU/ml,  $F(1,21) = 0.70$ ,  $P > 0.41$ ;  $86.69 \pm 0.92$  vs.  $88.22 \pm 0.84$  mg/dl;  $F(1,19) = 1.50$ ,  $P > 0.24$ ]. Also, epinephrine and norepinephrine levels were not affected [ $8.93 \pm 1.05$  vs.  $6.76 \pm 1.00$  μg/l,  $F(1,18) =$

$2.06$ ,  $P > 0.17$ ;  $22.11 \pm 2.17$  vs.  $23.28 \pm 2.17$  μg/l,  $F(1,17) = 0.13$ ,  $P > 0.72$ , respectively], excluding pronounced increases in sympathetic tone after insulin treatment. Comparisons of all other parameters, including resting energy expenditure and heart rate variability (expressed as the ratio of low- to high-frequency power) failed to reveal significant effects of long-term insulin administration in the male subjects (Table 1). The acute effects of 40 IU of insulin were restricted to an increase in diastolic blood pressure [ $70.96 \pm 2.35$  vs.  $63.96 \pm 2.35$  mmHg,  $F(1,21) = 4.43$ ,  $P \leq 0.05$ ].

**Insulin promotes weight gain via extracellular water storage in women.** In contrast to the effects in men, in the insulin-treated women, body weight increased as soon as 1 week after initiation of treatment [insulin,  $61.85 \pm 0.27$  and placebo,  $61.00 \pm 0.27$  kg;  $F(1,13) = 4.92$ ,  $P \leq 0.05$ ] and

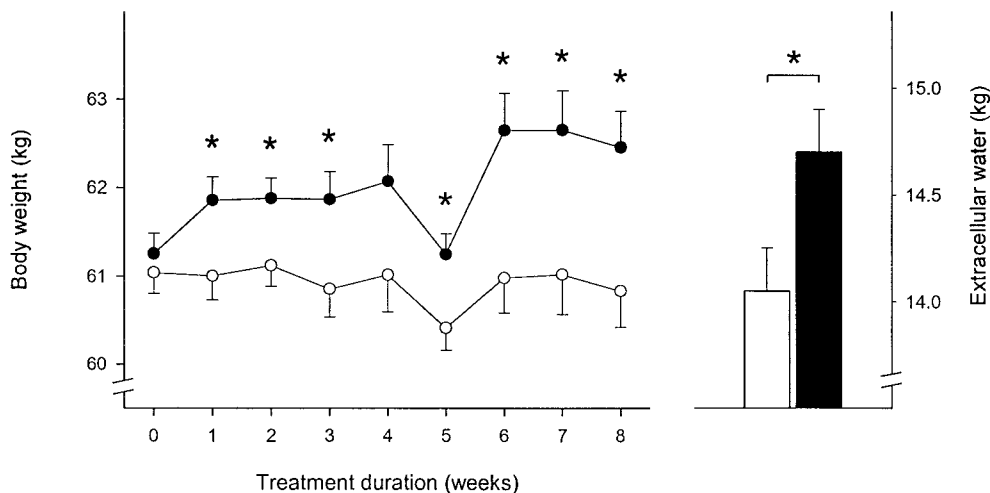


FIG. 2. Intranasal insulin increases body weight and extracellular water in women. *Left panel:* Average time course of body weight ( $\pm$ SE) in female subjects during 8 weeks of intranasal insulin treatment. *Right panel:* Extracellular water ( $\pm$ SE) in the same subjects after 8 weeks of treatment. One group of subjects received insulin ( $\bullet$ ,  $\blacksquare$ ) and the other group received placebo ( $\circ$ ,  $\square$ ). Data are baseline adjusted as derived from ANCOVA. \* $P \leq 0.05$  ( $n = 8$  for each group).

TABLE 2  
Effects of 8 weeks of intranasal insulin administration in women

	Insulin	Placebo	<i>P</i>
Body weight (kg)	62.45 ± 0.41	60.83 ± 0.41	≤0.02
BMI (kg/m <sup>2</sup> )	21.69 ± 0.14	21.11 ± 0.14	≤0.01
Body fat (kg)	16.16 ± 0.49	16.10 ± 0.49	≤0.93
Body cell mass (kg)	23.19 ± 0.36	23.39 ± 0.36	≤0.70
ECM/BCM	0.99 ± 0.02	0.93 ± 0.02	≤0.05
Lean body mass (kg)	46.12 ± 0.46	44.89 ± 0.46	≤0.09
Total body water (kg)	33.78 ± 0.33	32.84 ± 0.33	≤0.08
Extracellular water (kg)	14.70 ± 0.20	14.05 ± 0.20	≤0.04
Waist circumference (cm)	70.85 ± 1.03	69.67 ± 1.03	≤0.47
Leptin (ng/ml)	12.15 ± 1.14	10.85 ± 1.07	≤0.43
Insulin (μU/ml)	14.00 ± 3.91	8.60 ± 4.18	≤0.36
Glucose (mg/dl)	82.11 ± 2.54	83.14 ± 2.54	≤0.78
ACTH (pg/ml)	25.36 ± 5.08	16.49 ± 4.73	≤0.24
Resting energy expenditure (kcal/day)	1,381.74 ± 52.88	1,339.26 ± 52.88	≤0.58
Heart rate (bpm)	74.38 ± 5.12	73.67 ± 4.78	≤0.92
HRV LF/HF	1.24 ± 0.35	1.06 ± 0.32	≤0.71
Hunger FEV	5.26 ± 0.92	5.24 ± 0.92	≤0.99
Hunger gradient	0.27 ± 0.38	0.59 ± 0.38	≤0.56

Data are means ± SE (baseline adjusted, as derived from ANCOVA). ECM/BCM, ratio of extracellular mass (ECM) to body cell mass (BCM); HRV LF/HF, heart rate variability (HRV) expressed as the ratio of low-frequency (LF) to high-frequency (HF) power; hunger FEV, hunger score as derived from eating behavior questionnaire FEV (*n* = 8 for each group).

remained constantly elevated thereafter (Fig. 2). The increase in body weight [ $F(1,13) = 7.49, P \leq 0.02$ ] and BMI [ $F(1,13) = 8.74, P \leq 0.01$ ] after 8 weeks appeared to be due to a rise in extracellular water [ $F(1,13) = 5.10, P \leq 0.04$ ] (Table 2, Fig. 2), in conjunction with an increased ratio of extracellular mass to body cell mass [ $F(1,13) = 4.49, P \leq 0.05$ ] and tendencies toward augmented total body water [ $F(1,13) = 3.65, P = 0.08$ ] and lean body mass [ $F(1,13) = 3.28, P = 0.09$ ]. The supplementary analysis of plasma sodium indicated a trend toward increased levels in the insulin-treated women over the 8-week period [ $139.41 \pm 0.34$  vs.  $138.46 \pm 0.34$  mmol/l;  $F(1,13) = 3.57, P = 0.08$ ], whereas corresponding values in men remained unaffected [ $139.87 \pm 0.23$  vs.  $139.96 \pm 0.23$  mmol/l,  $F(1,21) = 0.08, P > 0.78$ ;  $F(1,35) = 5.69, P \leq 0.05$  for treatment × sex]. There were no treatment effects on plasma hormone and glucose concentrations after 8 weeks of treatment (Table 2) or when average values during the treatment period were compared [leptin:  $12.16 \pm 0.57$  vs.  $11.96 \pm 0.57$  ng/ml,  $F(1,13) = 0.06, P > 0.81$ ; insulin:  $13.70 \pm 1.82$  vs.  $11.54 \pm 1.95$  μU/ml,  $F(1,12) = 0.66, P > 0.43$ ; glucose:  $80.15 \pm 1.46$  vs.  $83.90 \pm 1.36$  mg/dl,  $F(1,12) = 3.44, P > 0.09$ ]. Also, average catecholamine levels were comparable [epinephrine:  $6.50 \pm 0.96$  vs.  $4.83 \pm 0.96$  μg/l,  $F(1,13) = 1.52, P > 0.24$ ; norepinephrine:  $20.00 \pm 1.95$  vs.  $15.47 \pm 1.95$  μg/l,  $F(1,13) = 2.70, P > 0.13$ ]. All other parameters, including those after acute insulin administration, remained unchanged in the female subjects.

**Posttreatment leveling of insulin effects.** In the follow-up examination of the men, conducted  $17.6 \pm 0.52$  weeks after cessation of treatment, the differences between the insulin and placebo groups had vanished: body fat [ $15.24 \pm 0.63$  vs.  $16.11 \pm 0.70$  kg;  $F(1,17) = 0.86, P > 0.32$ ], body weight [ $80.47 \pm 0.96$  vs.  $80.03 \pm 1.06$  kg;  $F(1,17) = 0.10, P > 0.76$ ] and BMI [ $23.82 \pm 0.30$  vs.  $23.75 \pm 0.33$  kg/m<sup>2</sup>;  $F(1,17) = 0.03, P > 0.87$ ] were comparable in the insulin and placebo groups, respectively. There seemed to be enhanced levels in plasma leptin and insulin

levels in the insulin group, but this difference failed to reach significance [plasma leptin:  $4.00 \pm 0.35$  vs.  $3.13 \pm 0.40$  ng/ml,  $F(1,11) = 2.59, P = 0.14$ ; plasma insulin:  $6.09 \pm 0.79$  vs.  $4.15 \pm 0.83$  μU/ml,  $F(1,16) = 2.77, P = 0.12$ ]. None of the other parameters displayed any significant difference [glucose:  $89.04 \pm 2.45$  vs.  $87.95 \pm 2.57$  mg/dl,  $F(1,14) = 0.09, P > 0.76$ ]. Interviews conducted after the final examinations did not reveal any hints at side effects of the treatment. In all, four subjects of the (total) insulin group and five subjects of the placebo group believed that they had been treated with an active agent.

## DISCUSSION

Prolonged intranasal administration of insulin in healthy humans reduced body weight, body adiposity, and leptin levels in the group of men compared with placebo treatment, whereas it induced weight gain due to enhanced water storage in the women. The weight loss in the insulin-treated men vanished 4–5 months after treatment ended. Our results corroborate the importance of CNS insulin signaling in the control of body weight, and confirm reports from animal experiments indicating a fundamental difference in central insulin sensitivity between men and women (19). It could be argued that the lack of body weight reduction in the women was due to a hampered nose-to-brain insulin transport, so that the substance did not reach the target sites in female subjects. However, in our previous studies assessing CSF accumulations after intranasal administration of 40 IU insulin, a distinct increase in CSF insulin concentrations after substance administration was observed in all three women of that study (averaging  $1.84 \pm 0.67$  μU/ml within the first 40 min) (15). This increase also appeared to be comparable with that seen in the men (averaging  $1.81 \pm 1.02$  μU/ml), although increases appeared to be rather variable, independent of the subject's sex. Also, measurements of declarative memory conducted in the present study revealed beneficial

effects of 8 weeks of insulin administration in all treated subjects (20). This outcome likewise supports the view that brain uptake of intranasally administered insulin in the women was as efficient as in the men. Sex differences were, in fact, restricted to insulin effects on body weight regulation.

In animal studies, male rats decreased food intake after intracerebroventricular insulin infusion and had lost substantial weight after only 24 h of treatment, whereas age- and weight-matched female rats remained unaffected. Leptin infusion yielded a reverse pattern, with a greater impact being seen on the female rats (19). Our finding of different central nervous sensitivity to insulin also fits with this picture, as insulin treatment reduced waist circumference in men; visceral fat content is correlated with endogenous insulin secretion and is more prevalent in obese men than women, who carry more subcutaneous fat (21). The mechanism underlying the observed sex difference is unclear. No such difference has been found for downstream neuronal mediators of the catabolic effects of insulin and leptin (19,22). Thus, the observed sex difference may have its source in differentially regulated insulin and leptin signaling, an explanation that appears plausible given that in mouse brain, insulin signaling is essential for reproductive function (9).

In the women, insulin surprisingly increased extracellular water, as reflected by an increased ratio of extracellular mass to body cell mass, associated with rapidly increased total body weight and augmented BMI. A confounding effect of the menstrual cycle can be safely ruled out as all women were taking oral contraceptives and each woman was examined at identical times in the cycle. Body water distribution may have been altered by renal sodium reabsorption, which is known to be modulated by peripheral insulin sensitivity (23). A trend toward increased plasma sodium levels was indeed revealed in the insulin-treated women. Intranasal insulin may have mediated the effects on water storage by enhancing systemic insulin sensitivity, which depends on intact hypothalamic insulin signaling (24). It is also noteworthy that with reference to body weight, the women received a higher dosage of intranasal insulin than the men, which could have enhanced this type of effect.

The decline in body weight and adiposity after insulin administration in the male subjects may have stemmed mainly from reduced daily food intake. Insulin's reducing influence on hunger has been inferred from previous studies where, under euglycemic conditions, rated hunger rose more slowly during infusion of higher versus lower dosages of insulin (25). Studies of long-term central nervous insulin administration in several species have indicated a rather slowly evolving attenuation of 24-h food ingestion, extending up to several weeks (7,26). These observations would fit the assumption that even a small decrease in hunger motivation, of which an individual may not necessarily be aware, can lead to reduced caloric intake. The effect of intranasal insulin on body weight in the men emerged after several weeks of treatment, a finding that supports the concept of a gradual impact of elevated brain insulin levels on the regulation of actual hunger and satiety. Correspondingly, rated hunger was not affected by acute insulin administration but decreased

after 8 weeks of treatment. This observation does not exclude the possibility that effects on body weight could occur even sooner with higher CSF concentrations of the compound, although this might be difficult to achieve with intranasal insulin administration in humans. Central nervous insulin administration in animals is reported to not only inhibit food intake but also stimulate thermogenesis via enhanced sympathetic neuronal outflow to thermogenic tissue (5,27). The signs of increased sympathetic tone found in our study (i.e., acutely increased diastolic blood pressure and a trend toward elevated heart rate after long-term treatment) were too weak to justify corresponding conclusions. Likewise, catecholamine levels, resting energy expenditure, and heart rate variability were not elevated after insulin administration.

The central nervous pathways between hypothalamic nuclei and the caudal brainstem, where neural, endocrine, and duodenal nutrient and adiposity signals converge to terminate single meals, have been extensively reviewed (2–4,10). Insulin and leptin regulate body weight through joint downstream signaling via melanocortins, establishing a tonic catabolic output to avert excessive weight gain (28). In our group of men, weight loss was associated with decreased leptin concentrations. After the treatment period, the discontinued insulin in conjunction with diminished leptin signaling presumably permitted prevailing activation of anabolic pathways so that body weight and fat returned to pretreatment levels. Regaining weight might ultimately lead to counterregulation, as was expressed in the slight increase in insulin and leptin levels in the follow-up examination.

In conclusion, the present data strongly support the assumption that in humans insulin is a prominent adiposity signal in the brain and exerts catabolic effects on body weight and adiposity. Because insulin is pharmaceutically produced in large quantities, its intranasal administration may represent an economic but effective therapeutic means of reducing body weight in obese men. Besides impaired central nervous sensitivity to insulin and leptin (29), decreased blood-to-brain transport of both messengers possibly plays a crucial role in the pathogenesis of obesity (30). Reduced CSF levels of insulin in obese animals have been repeatedly reported (31,32), and data gathered in our laboratory point to a similarly degraded level of insulin in the CSF of obese men (33). Thus, enhancing central nervous insulin concentrations by intranasal administration appears to be a promising approach in the campaign against obesity.

#### ACKNOWLEDGMENTS

Aero Pump GmbH (Hochheim, Germany) generously provided us with precision nasal air pumps.

We thank A. Hatke for her expert technical assistance and C. Otten and H. Ruf for their invaluable laboratory work.

#### REFERENCES

1. Kopelman PG: Obesity as a medical problem. *Nature* 404:635–643, 2000
2. Porte D Jr, Seeley RJ, Woods SC, Baskin DG, Figlewicz DP, Schwartz MW: Obesity, diabetes and the central nervous system. *Diabetologia* 41:863–881, 1998
3. Seeley RJ, Woods SC: Monitoring of stored and available fuel by the CNS: implications for obesity. *Nat Rev Neurosci* 4:901–909, 2003

4. Gerozissis K: Brain insulin: regulation, mechanisms of action and functions. *Cell Mol Neurobiol* 23:1–25, 2003
5. Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte D Jr: Insulin in the brain: a hormonal regulator of energy balance. *Endocr Rev* 13:387–414, 1992
6. Obici S, Zhang BB, Karkanias G, Rossetti L: Hypothalamic insulin signaling is required for inhibition of glucose production. *Nat Med* 8:1376–1382, 2002
7. Woods SC, Lotter EC, McKay LD, Porte D Jr: Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature* 282:503–505, 1979
8. McGowan MK, Andrews KM, Kelly J, Grossman SP: Effects of chronic intrahypothalamic infusion of insulin on food intake and diurnal meal patterning in the rat. *Behav Neurosci* 104:373–385, 1990
9. Brüning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, Kahn CR: Role of brain insulin receptor in control of body weight and reproduction. *Science* 289:2122–2125, 2000
10. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG: Central nervous system control of food intake. *Nature* 404:661–671, 2000
11. Schwartz MW, Boyko EJ, Kahn SE, Ravussin E, Bogardus C: Reduced insulin secretion: an independent predictor of body weight gain. *J Clin Endocrinol Metab* 80:1571–1576, 1995
12. Boyko EJ, Leonetti DL, Bergstrom RW, Newell-Morris L, Fujimoto WY: Low insulin secretion and high fasting insulin and C-peptide levels predict increased visceral adiposity: 5-year follow-up among initially nondiabetic Japanese-American men. *Diabetes* 45:1010–1015, 1996
13. Baura GD, Foster DM, Porte D Jr, Kahn SE, Bergman RN, Cobelli C, Schwartz MW: Saturable transport of insulin from plasma into the central nervous system of dogs in vivo: a mechanism for regulated insulin delivery to the brain. *J Clin Invest* 92:1824–1830, 1993
14. Wallum BJ, Taborsky GJ Jr, Porte D Jr, Figlewicz DP, Jacobson L, Beard JC, Ward WK, Dorsa D: Cerebrospinal fluid insulin levels increase during intravenous insulin infusions in man. *J Clin Endocrinol Metab* 64:190–194, 1987
15. Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL: Sniffing neuropeptides: a transnasal approach to the human brain. *Nat Neurosci* 5:514–516, 2002
16. Lawrence D: Intranasal delivery could be used to administer drugs directly to the brain. *Lancet* 359:1674, 2002
17. Kern W, Born J, Schreiber H, Fehm HL: Central nervous system effects of intranasally administered insulin during euglycemia in men. *Diabetes* 48:557–563, 1999
18. Pudiel V, Westenhöfer J: *Fragebogen zum Essverhalten: Handanweisung*. Göttingen, Germany, Hogrefe, 1989
19. Clegg DJ, Riedy CA, Smith KA, Benoit SC, Woods SC: Differential sensitivity to central leptin and insulin in male and female rats. *Diabetes* 52:682–687, 2003
20. Benedict C, Hallschmid M, Hatke A, Schultes B, Fehm HL, Born J, Kern W: Intranasal insulin improves memory in humans. *Psychoneuroendocrinology* 29:1326–1334, 2004
21. Wajchenberg BL: Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* 21:697–738, 2000
22. Fehm HL, Smolnik R, Kern W, McGregor GP, Bickel U, Born J: The melanocortin melanocyte-stimulating hormone/adrenocorticotropin(4–10) decreases body fat in humans. *J Clin Endocrinol Metab* 86:1144–1148, 2001
23. ter Maaten JC, Bakker SJ, Serne EH, ter Wee PM, Donker AJ, Gans RO: Insulin's acute effects on glomerular filtration rate correlate with insulin sensitivity whereas insulin's acute effects on proximal tubular sodium reabsorption correlation with salt sensitivity in normal subjects. *Nephrol Dial Transplant* 14:2357–2363, 1999
24. Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L: Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nat Neurosci* 5:566–572, 2002
25. Kern W, Peters A, Fruehwald-Schultes B, Deininger E, Born J, Fehm HL: Improving influence of insulin on cognitive functions in humans. *Neuroendocrinology* 74:270–280, 2001
26. Foster LA, Ames NK, Emery RS: Food intake and serum insulin responses to intraventricular infusions of insulin and IGF-I. *Physiol Behav* 50:745–749, 1991
27. Menendez JA, Atrens DM: Insulin and the paraventricular hypothalamus: modulation of energy balance. *Brain Res* 555:193–201, 1991
28. Schwartz MW, Woods SC, Seeley RJ, Barsh GS, Baskin DG, Leibel RL: Is the energy homeostasis system inherently biased toward weight gain? *Diabetes* 52:232–238, 2003
29. Seeley RJ, Van Dijk G, Campfield LA, Smith FJ, Burn P, Nelligan JA, Bell SM, Baskin DG, Woods SC, Schwartz MW: Intraventricular leptin reduces food intake and body weight of lean rats but not obese Zucker rats. *Horm Metab Res* 28:664–668, 1996
30. Nam SY, Kratzsch J, Kim KW, Kim KR, Lim SK, Marcus C: Cerebrospinal fluid and plasma concentrations of leptin, NPY, and alpha-MSH in obese women and their relationship to negative energy balance. *J Clin Endocrinol Metab* 86:4849–4853, 2001
31. Kaiyala KJ, Prigeon RL, Kahn SE, Woods SC, Schwartz MW: Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. *Diabetes* 49:1525–1533, 2000
32. Baskin DG, Stein LJ, Ikeda H, Woods SC, Figlewicz DP, Porte D Jr, Greenwood MR, Dorsa DM: Genetically obese Zucker rats have abnormally low brain insulin content. *Life Sci* 36:627–633, 1985
33. Kern W, Hallschmid M, Born J, Fehm HL: Reduced transport of insulin across the blood/brain barrier in obese humans. *Diabetes* 52:A559, 2003