Insulin and Estrogen Independently and Differentially Reduce Macronutrient Intake in Healthy Men

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Context: Insulin administration to the central nervous system inhibits food intake, but this effect has been found to be less pronounced in female compared with male organisms. This sex-specific pattern has been suggested to arise from a modulating influence of estrogen signaling on the insulin effect.

Objective: We assessed in healthy young men whether pretreatment with transdermal estradiol interacts with the hypophagic effect of central nervous insulin administration via the intranasal pathway.

Design, Setting, Participants, and Intervention: According to a 2×2 design, two groups of men (n = 16 in each group) received a 3-day transdermal estradiol (100 μg/24 h) or placebo pretreatment and on two separate mornings were intranasally administered 160 IU regular human insulin or placebo.

Main Outcome Measures: We assessed free-choice *ad libitum* calorie intake from a rich breakfast buffet and relevant blood parameters in samples collected before and after breakfast.

Results: Estrogen treatment induced a 3.5-fold increase in serum estradiol concentrations and suppressed serum testosterone concentrations by 70%. Independent of estradiol administration, intranasal insulin reduced the intake of carbohydrates during breakfast, attenuating in particular the consumption of sweet, palatable foods. Estradiol treatment *per se* decreased protein consumption. We did not find indicators of eating-related interactions between both hormones.

Conclusions: Results indicate that, in an acute setting, estrogen does not interact with central nervous insulin signaling in the control of eating behavior in healthy men. Insulin and estradiol rather exert independent inhibiting effects on macronutrient intake. **(*J Clin Endocrinol Metab* 103: 1393–1401, 2018)**

The pancreatic hormone insulin, in addition to its peripheral effects, modulates central nervous functions, including the control of energy metabolism (1, 2). The direct application of insulin to the brain via intracerebroventricular infusion in animals and via intranasal administration in humans (3) has been shown to decrease food intake and body weight in mice (4), rats (5), baboons (6), and men (7, 8). The hypophagic effect of central nervous insulin appears to display a preponderance in male compared with female organisms (7–9). In humans, intranasal insulin administration acutely curbs food intake in men but not in women (7) and during long-term treatment reduces body fat content in male but not age-matched female subjects (8). Similarly, in contrast to male rats, intact female rats do not reduce their food intake upon intracerebroventricular insulin treatment (9).

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Abbreviations: ANOVA, analysis of variance; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SEM, standard error of the mean.

Sensitivity to the anorexigenic effect of intracerebroventricular insulin, however, can be induced in female animals by ovariectomy associated with a reduction in plasma estradiol concentrations; *vice versa*, estrogen-treated male rats are no longer susceptible to the hypophagic effect of the hormone (10), suggesting that sex-related differences in estrogen signaling modulate the impact of central nervous insulin on eating behavior. Nevertheless, although in women intranasal insulin does not affect food intake in the fasted state, it inhibits snack intake when administered postprandially (11), indicating that the peptide can decrease calorie consumption also in female subjects.

Against this background and considering that estrogen has been reported to attenuate energy intake and body weight in animals (12), we investigated whether estrogen and insulin acutely interact in the regulation of eating behavior in humans. We assessed the effect of intranasal insulin on food intake in healthy young men who were pretreated for 3 days with transdermal estradiol or placebo, hypothesizing that increasing the circulating concentrations of estrogen decreases the susceptibility of men to the anorexigenic effect of insulin.

Subjects and Methods

Subjects, design, and procedure

Thirty-two healthy men aged between 18 and 31 years (mean age, 23.94 ± 0.52 years; mean body mass index, 22.80 ± 0.36 kg/m^2) participated in the experiment. Current illness, vegetarianism, and habitual dietary idiosyncrasies (*e.g.*, because of allergy) were excluded by clinical examination. All subjects were free of medication and were nonsmokers. They gave written informed consent to the study, which conformed to the Declaration of Helsinki as revised in 2008 and was approved by the local Ethics Committee on Research Involving Humans.

Study design and experimental procedures are summarized in Fig. 1. According to a 2×2 design, subjects were randomly assigned to two groups of participants (n = 16 in each group) who were treated with either estradiol ("estrogen patch" group; 24.38 ± 0.93 years, 22.62 ± 0.50 kg/m^2) or placebo ("placebo patch" group; 23.50 ± 0.49 years, $P > 0.41$; 22.98 ± 0.54 kg/m^2, $P > 0.61$) each time before participating in two individual experimental sessions where they received intranasal insulin or placebo. Three days before each test session, subjects attended our laboratory at 5:00 PM. In the participants of the estrogen patch group, two transdermal estradiol patches (Estradot 50; Novartis Pharma, Nuremberg, Germany) were applied to the abdomen, delivering a total dose of 100 μg estradiol per 24 hours according to the manufacturer. Participants of the placebo patch group received two patches that looked identical to the estradiol patches but did not contain the hormone. The patches were renewed by the experimenters after 24 and 48 hours (*i.e.*, the third pair of patches was attached on the day before and removed directly after the experiment proper). Subjects and experimenters were blinded to the patches and the intranasal treatment. Experimental sessions were separated by at least 3 weeks, and the order of conditions was balanced across subjects.

The experimental procedure on each test day was similar to our previous experiments on the acute effects of intranasal insulin on food intake (7, 13). All subjects remained fasted and abstained from drinking caloric beverages after 10:00 PM on the evening before testing. After arrival at the laboratory at around 8:00 AM, a venous cannula was inserted into each subject's nondominant arm for the collection of venous blood and the determination of blood glucose (HemoCue B-Glucose-Analyzer; HemoCue AB, Angelholm, Sweden). Sessions started with a 60-minute baseline period, which included blood sampling at 8:15, 8:30, and 8:45 AM and ratings of mood and hunger. At 9:00 AM, subjects were intranasally administered 16 puffs (0.1 mL, eight per nostril) of insulin or placebo at 30-second intervals, amounting to a total dose of 1.6 mL insulin (160 IU) (Insulin Actrapid; Novo Nordisk, Mainz, Germany), or vehicle. At 10:25 AM, after postadministration blood sampling at 10- to 20-minute intervals and further assessments of mood and hunger, a standardized free-choice breakfast buffet was offered comprising a variety of food choices (Table 1) from which subjects ate *ad libitum* during the subsequent 30 minutes. Subjects were not aware that their food intake was measured by weighing buffet components before and after breakfast. This

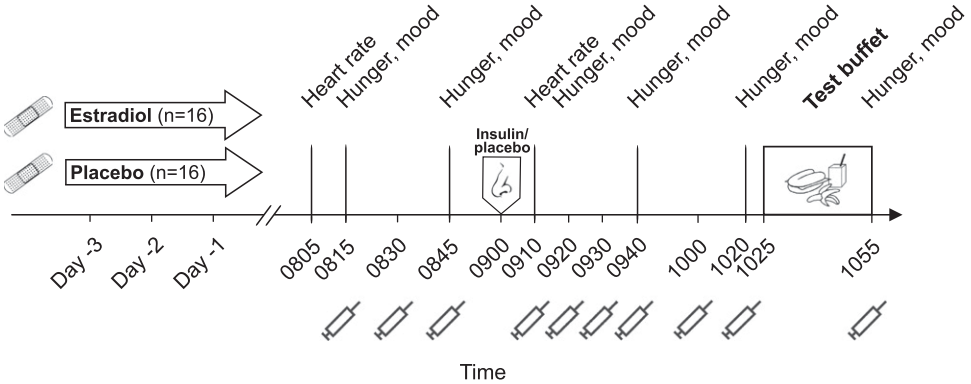

Figure 1. Experimental procedure. Two groups of 16 healthy men who had been pretreated with transdermal estradiol (100 μg/24 h for 3 days) or placebo participated in two experimental sessions. After a baseline period of around 60 minutes, subjects were intranasally administered 160 IU insulin or, in the other condition, placebo at 9:00 AM before a free-choice test breakfast buffet was offered around 85 minutes later. Self-rated hunger, thirst, tiredness, and mood were repeatedly assessed, and blood samples for the determination of glucose and hormone concentrations were obtained (syringe symbols). Heart rate and blood pressure were assessed twice.

Table 1. Composition of the Breakfast Test Buffet

Food	Weight (g)	Energy (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)
Neutral					
Whole wheat bread	165	329	63.9	2	12.1
Wheat rolls	300	857	167.6	5.4	30
White bread	30	73	14.7	0.4	2.5
Butter	75	580	0.5	62.4	0.5
Whole milk	750	495	35.3	26.8	25.4
Condensed milk	40	54	3.9	3	2.6
Sweet					
Jam	50	140	34.2	0	0.1
Hazelnut spread	40	218	22.7	12.4	2.7
Honey	40	123	30	0	0.2
Sugar	24	98	24	0	0
Fruit curd	150	148	24.8	1.2	8.8
Banana	190	167	38.1	0.3	2.2
Apple	120	71	17	0.1	0.4
Pear	190	105	23.5	0.6	0.9
Orange juice	400	173	36	1	4
Savory					
Poultry sausage	40	74	0.1	4.3	8.3
Cervelat sausage	34	138	0.1	11.8	6.9
Sliced cheese	100	198	0	23	20.8
Cream cheese (natural)	33	87	1.1	8.2	1.8
Cream cheese (herbs)	40	84	1.2	7.2	3.2
Total	2811	4312	538	170	133

Breakfast was served with coffee or tea as requested by the participant.

procedure has been shown to enable the precise assessment of food intake in the fasted state (7, 13, 14). Throughout the experiments, subjects repeatedly underwent a battery of cognitive tests unrelated to the topic of the current study (data not shown). After final blood sampling and another assessment of mood and hunger, subjects were asked in a short interview which patch (estrogen/placebo) and which spray (insulin/placebo) they thought they had received. In these interviews, none of the subjects reported adverse side effects.

Hormonal and psychometric assessments

Blood samples were centrifuged, and plasma and serum were stored at −80°C. Serum concentrations of insulin, C-peptide, and cortisol (all sampling time points) and of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (first and third baseline time points) were determined by Immulite (DPC, Los Angeles, CA). Plasma concentrations of estradiol (*i.e.*, 17β-estradiol) and testosterone were determined for every other sampling time point (Fig. 2A and 2B) by ultra–high-performance liquid chromatography and subsequent mass spectrometry validated according to FDA guidelines. Samples were precipitated with 5% H_3PO_4, purified, and concentrated in methanol before chromatographic separation was performed on an ultra–high-performance liquid chromatography instrument (1290 UHPL; Agilent Technologies, Waldbronn, Germany). Analyte detection was carried out on a hyphenated TripleTOF 5600+ mass spectrometer (Sciex, Concord, Ontario, Canada) in positive ionization mode. For quantification, a surrogate calibrant method using $^{13}C_3$-estradiol and $^{13}C_3$-testosterone in true plasma matrix was established. Internal standardization was obtained by spiking of d_5-estradiol and d_5-testosterone. Quantifiable ranges for estradiol and testosterone were 10 to 1000 pg/mL and 20 to 15,000 pg/mL, respectively. In a simultaneous mass spectrometric survey scan, we recorded precursor ion data for an untargeted profiling of plasma samples that yielded the relative concentrations of additional steroids of interest [*i.e.*, epitestosterone (17α-testosterone), dihydrotestosterone (androstanolone), androstenedione, dehydroepiandrosterone (androstenolone), progesterone, and hydroxyprogesterone].

Hunger, thirst, and tiredness were rated on nine-point scales twice during baseline, at 20- to 30-minute intervals after spray administration, and after the test breakfast. In parallel, mood was assessed with five-point scales covering the categories good/bad mood, alertness/sleepiness, and calmness/agitation [Mehrdimensionaler Befindlichkeitsfragebogen (MDBF); ref. 15). Blood pressure and heart rate were measured before and ~10 minutes after spray administration.

Statistical analyses

Analyses were performed with SPSS® Statistics Version 21 (IBM, Armonk, NY) and based on repeated-measures analyses of variance (ANOVA) with the between-subjects factor "Group" (estrogen patch vs placebo patch) and the within-subject factors "Treatment" (insulin vs placebo), "Time," "Macronutrient," and "Taste" (*i.e.*, neutral/sweet/savory) as appropriate. Significant ANOVA interactions were specified by Student's *t* tests. All data are presented as means ± standard error of the mean (SEM). A P value <0.05 was considered significant.

Results

Hormonal parameters

Transdermal estrogen in comparison with placebo treatment induced a 3.5-fold increase in baseline plasma

estradiol concentrations [F(1,30) = 75.38, $P < 0.0001$] and a 70% decrease in testosterone [F(1,30) = 88.19, $P < 0.0001$ for Group] (Fig. 2A and 2B). Both estradiol and testosterone displayed a postprandial drop after breakfast intake ($P < 0.0001$ for Time) that was more pronounced in the groups pretreated with estrogen (both $P < 0.002$ for Group × Time). Intranasal insulin did not display a modulatory influence on these parameters (all $P > 0.23$). Concentrations of LH and FSH measured during baseline were strongly suppressed after estrogen treatment (both $P < 0.01$ for Group) (Fig. 2C). Supplemental analyses of steroid hormones (Table 2) indicated that transdermal estrogen administration roughly halved plasma concentrations of epitestosterone and induced 27% and

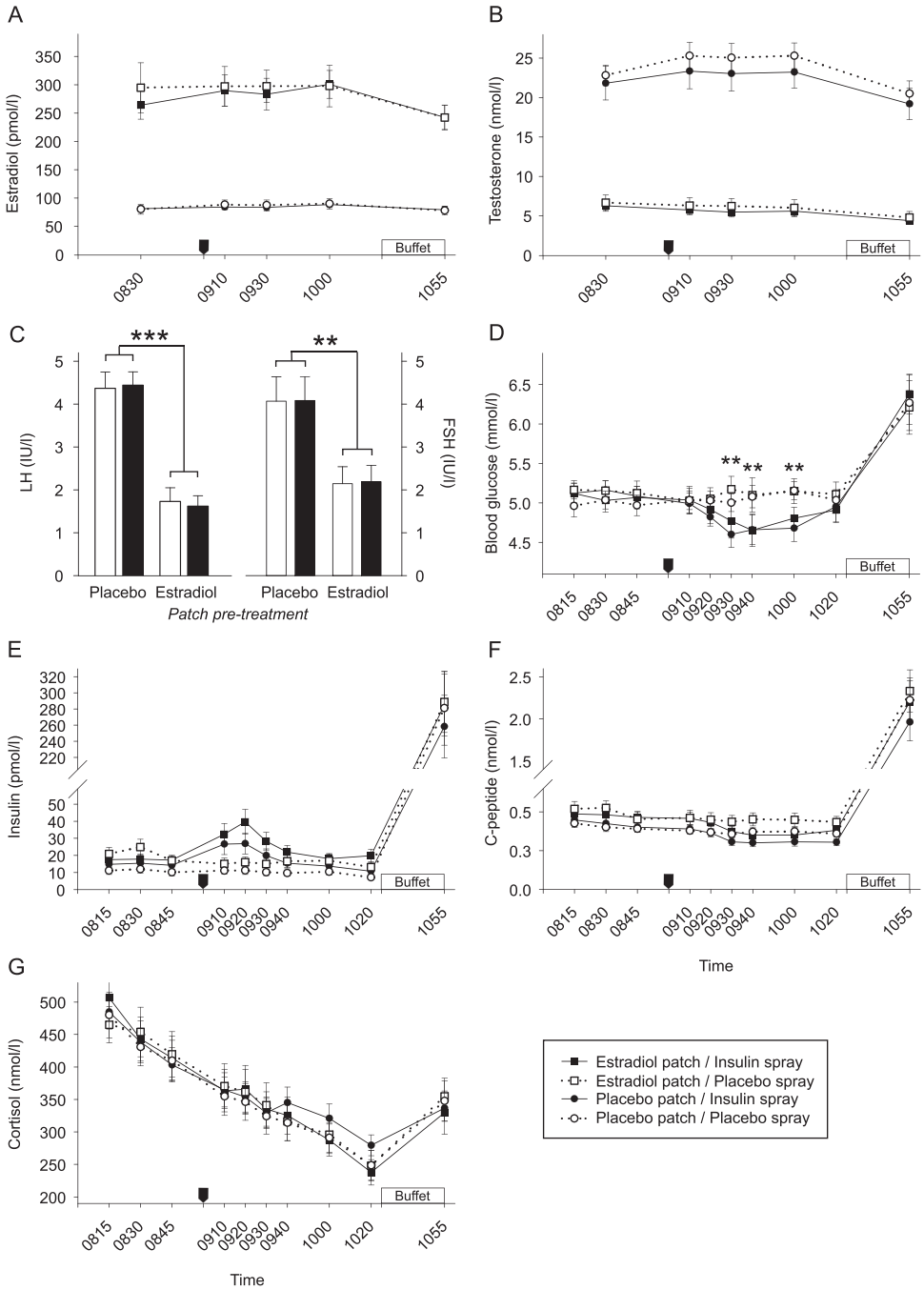

Figure 2. Endocrine parameters. Plasma concentrations of (A) 17β-estradiol and (B) testosterone; (C) serum concentrations of LH and FSH; (D) blood glucose concentrations; and serum concentrations of (E) insulin, (F), C-peptide, and (G) cortisol. Experiments were performed in two groups of 16 men each who had received 3 days of transdermal estradiol (100 μg/24 h; squares) or placebo pretreatment (circles) before participating in experimental sessions starting with baseline measurements followed by the intranasal spray administration of 160 IU insulin (filled symbols, solid lines) or placebo (empty symbols, dashed lines), respectively, at 9:00 AM (arrow mark). LH and FSH represent the average of the 8:15 and 8:45 AM baseline measurements. Values are means ± SEM. $^{**}P < 0.01$ for the ANOVA factor Treatment (D); $^{**}P < 0.01$ and $^{***}P < 0.001$ for the ANOVA factor Group (C).

Table 2. Supplemental Steroid Measurements

	Placebo Patch		Estradiol Patch		ANOVA Result[a]	
	Placebo Spray	Insulin Spray	Placebo Spray	Insulin Spray	Group	Treatment
Epitestosterone (17α-testosterone)	41.42 ± 2.62	40.81 ± 3.95	18.97 ± 2.23	18.84 ± 2.50	F(1,29) = 43.91, $P < 0.001$	F(1,29) = 0.90, $P = 0.35$
Dihydrotestosterone (Androstanolone)	133.77 ± 9.62	124.55 ± 9.30	100.30 ± 7.71	88.61 ± 6.00	F(1,30) = 15.33, $P < 0.001$	F(1,30) = 1.87, $P = 0.18$
Androstenedione	1216.63 ± 108.87	1113.46 ± 95.55	1033.52 ± 87.05	979.30 ± 97.84	F(1,29) = 1.10, $P = 0.30$	F(1,29) = 6.73, $P < 0.02$
Dehydroepiandrosterone (Androstenolone)	741.41 ± 63.87	694.04 ± 52.62	817.51 ± 81.19	832.87 ± 78.65	F(1,29) = 1.44, $P = 0.24$	F(1,29) = 0.18, $P = 0.67$
Progesterone	619.90 ± 88.90	500.20 ± 94.13	538.26 ± 92.01	432.08 ± 42.90	F(1,29) = 0.43, $P = 0.52$	F(1,29) = 3.02, $P = 0.09$
Hydroxyprogesterone	754.86 ± 69.49	641.86 ± 53.90	242.43 ± 34.47	238.78 ± 34.61	F(1,30) = 53.27, $P < 0.001$	F(1,30) = 3.02, $P = 0.09$

Relative plasma levels as derived from response ratios (peak area of the analyte/peak area of the internal standard) obtained in a mass spectrometric survey scan (relative quantification) and expressed as areas under the curve of the main experimental period (8:30–10:55 AM). Experiments were performed in two groups of men who received 3 days of transdermal estradiol (100 μg/24 h) or placebo before participating in experimental sessions including intranasal treatment with 160 IU insulin or placebo.

[a]Results for the ANOVA factors Group and Treatment; respective interactions were not significant (n = 31 or 32).

66% reductions in, respectively, dihydrotestosterone and hydroxyprogesterone, all independent of insulin or placebo administration. Intranasal insulin compared with placebo induced a mild, estradiol patch–independent decrease in androstenedione.

Parameters of glucose metabolism and cortisol concentrations did not differ between conditions during baseline (all $P > 0.09$) and were generally not affected by estrogen treatment (all $P > 0.10$ for respective interactions). Intranasal insulin administration induced a slight decrease in blood glucose concentrations [$F(3,98) = 3.81$, $P < 0.02$ for Treatment × Time] that remained within the euglycemic range (Fig. 2D). Corresponding changes in serum insulin and C-peptide after intranasal insulin in comparison with placebo administration failed to reach statistical significance in ANOVA [$F(2,37) = 2.42$, $P < 0.12$, and $F(1,33) = 3.01$, $P < 0.09$ for Treatment × Time) (Fig. 2E and 2F). However, supplemental area-under-the-curve analyses covering the time period between the final baseline (8:45 AM) and the final pre-breakfast sample (10:22 AM) indicated significant respective increases in serum insulin [$F(1,30) = 15.15$, $P < 0.01$] and decreases in serum C-peptide [$F(1,30) = 4.91$, $P < 0.04$]. Cortisol concentrations showed the expected circadian decline but were not affected by any of the hormonal interventions ($P > 0.41$) (Fig. 2G).

Food intake

Across groups, insulin in comparison with placebo specifically reduced the intake of carbohydrates from the test buffet [$F(1,30) = 5.60$, $P < 0.03$; $F(2,48) = 4.39$, $P < 0.03$ for Treatment × Macronutrient] (Fig. 3A) against the

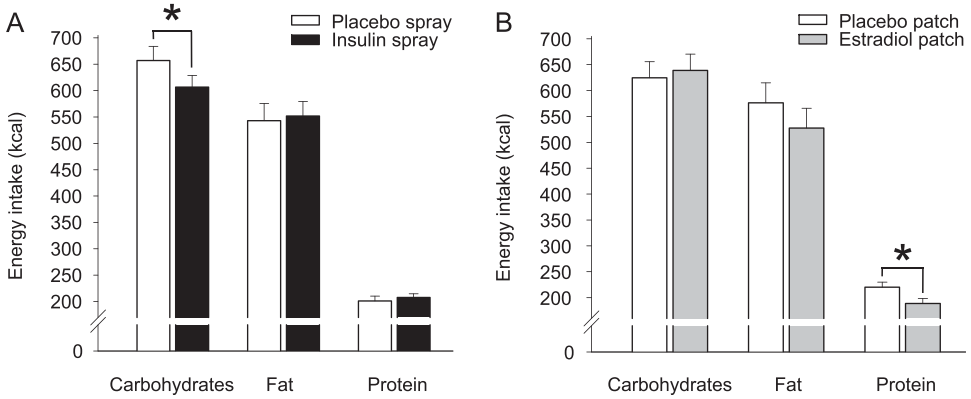

Figure 3. Food intake from the test buffet. Intake of macronutrients (kcal) from a standardized free-choice breakfast buffet presented 85 minutes after intranasal spray administration of 160 IU insulin or placebo in healthy men who had received 3 days of transdermal estradiol (100 μg/24 h) or placebo pretreatment (n = 16 in each group) before the experimental day. (A) Macronutrient intake in the insulin (black bars) and the placebo spray conditions (white bars) collapsed across the estrogen and placebo patch groups. (B) Macronutrient intake in the estradiol (gray bars) and the placebo patch (white bars) groups collapsed across the insulin and placebo spray conditions. Values are means ± SEM. *$P < 0.05$ for the ANOVA factors Treatment (A) and Group (B).

background of comparable total food intake in both conditions [F(1,30) = 0.58, $P > 0.45$] (Table 3), whereas the intake of fat and protein was not affected by insulin (all $P > 0.48$) (Fig. 3A). The suppressive effect of intranasal insulin on carbohydrate intake was confirmed in covariance analyses correcting for the difference between conditions in pre-breakfast concentrations of blood glucose [F(1,29) = 5.38, $P < 0.03$; F(2,47) = 3.91, $P < 0.04$ for Treatment × Macronutrient] and serum insulin [F(1,29) = 6.87, $P < 0.02$ and F(2,48) = 7.08, $P < 0.01$; values expressed as areas under the curve as defined previously]. The reduction in the consumption of carbohydrates was also reflected by slight decreases and increases in the consumption of food items with sweet and savory taste, respectively [F(2,54) = 3.88, $P < 0.04$ for Treatment × Taste] (Table 3). Estrogen administration in the 3 days preceding the experiment did not modulate the effect of intranasal insulin on food intake regarding carbohydrate consumption [F(1,30) = 0.03, $P > 0.87$ for Treatment × Group] or total intake and consumption of fat, protein, and sweet vs savory foods (all $P > 0.52$).

Independent of the intranasal treatment estrogen *per se* attenuated the intake of protein from the test buffet [F(1,30) = 5.12, $P = 0.03$ for Group] (Fig. 3B). Total intake and the intake of fat and carbohydrate remained unaffected by estrogen (all $P > 0.47$). Although protein specificity of estrogen's anorexigenic effect was not statistically confirmed [F(2,48 = 0.72, $P > 0.46$ for Group × Macronutrient], estrogen treatment in particular reduced the intake of savory food items [estrogen vs placebo patch, 297.45 ± 22.28 vs 381.95 ± 22.28 kcal; F(1,30) = 7.19, $P < 0.02$], such as sliced and natural cream cheese [138.72 ± 16.11 vs 184.93 ± 16.11 kcal, F(1,30) = 4.11, $P < 0.06$; 22.87 ± 7.97 vs 44.91 ± 7.97 kcal, F(1,30) = 3.83, $P < 0.06$, respectively] in favor of an increase in the intake of items like hazelnut spread [100.11 ± 17.26 vs 50.31 ± 17.26 kcal; F(1,30) = 4.18, $P = 0.05$]. Hunger and thirst ratings were not affected by estrogen treatment or insulin administration (all $P > 0.13$).

Control parameters

Self-rated mood and alertness according to the MDBF adjective scale generally improved during the experiment (both $P < 0.002$ for Time) but were not affected by estrogen treatment or insulin administration (all $P > 0.11$). Accordingly, tiredness rated on nine-point scales decreased between morning and noon ($P < 0.001$). Neither tiredness nor calmness/agitation ratings showed differences between conditions or groups (all $P > 0.10$). Cardiovascular parameters were not affected by estrogen or insulin administration (all $P > 0.21$). In the estrogen patch group, systolic/diastolic blood pressure measured 10 minutes after intranasal insulin in comparison with placebo administration was 118.33 ± 3.45/72.40 ± 2.36 vs 123.88 ± 2.71/72.44 ± 2.59 mm Hg and 124.56 ± 2.83/71.06 ± 2.94 vs 120.13 ± 3.69/72.25 ± 2.32 mm Hg in the placebo patch group. Heart rate in the estrogen patch group was (insulin vs placebo) 59.67 ± 1.80 vs 59.06 ± 2.14 beats per minute and 58.63 ± 2.24 vs 59.88 ± 2.71 beats per minute in the placebo patch group. In the post-experimental interviews, subjects were not able to correctly indicate whether they had received estrogen or placebo patches ($P > 0.53$) and insulin or placebo sprays ($P > 0.71$; χ^2 tests).

Discussion

Central nervous insulin administration exerts stronger acute (7) and long-term (8) catabolic effects in male subjects than in female subjects; here we investigated whether estrogen signaling contributes to this sex-specific pattern. We found that strongly increasing circulating

Table 3. Food Intake From the Test Buffet

Food Intake (kcal)	Placebo	Insulin	*P* Value[a]
Total	1401.29 ± 55.84	1365.82 ± 45.61	0.45
Neutral food	743.34 ± 40.59	696.78 ± 38.33	0.18
Wheat rolls	361.24 ± 27.81	301.79 ± 29.24	0.06
Sweet food	343.50 ± 25.58	304.08 ± 23.66	0.09
Jam	37.38 ± 6.82	25.76 ± 7.44	0.03
Hazelnut spread	89.20 ± 14.01	61.23 ± 13.01	0.03
Honey	17.32 ± 5.17	8.85 ± 3.61	0.09
Sugar	10.69 ± 2.46	6.74 ± 2.38	0.07
Savory food	314.45 ± 21.02	364.95 ± 18.44	0.04
Cervelat sausage	67.95 ± 8.37	83.03 ± 8.17	0.10
Sliced cheese	147.46 ± 13.94	176.19 ± 11.96	0.03

Total food intake, food intake according to taste, and consumption of specific food items (all in kcal). All neutral, savory, and sweet foods contained in the test buffet are listed in Table 1. Values are means ± SEM calculated across experimental groups (placebo and estrogen patch) for the experimental conditions (placebo and insulin spray).

[a] *P* values for the ANOVA factor Treatment (n = 32).

estrogen concentrations in healthy young men by means of transdermal estradiol patches does not alter the suppressive effect on carbohydrate intake of intranasal insulin. This outcome stands in some contrast to findings in animals indicating that estrogen action interferes with the anorexigenic effect of brain insulin (9, 10). Estrogen administration *per se* was revealed to induce a small but discernible reduction in protein consumption, indicating that both insulin and estrogen, but in an independent fashion, induce restraining effects on the intake of macronutrients in men.

The pretreatment of our subjects with estradiol patches worn for 3 consecutive days proved to be highly effective, as evidenced by the 3.5-fold increase in circulating estrogen, whereas the concentrations of LH and FSH were roughly halved and serum testosterone dropped by about 70%. Estradiol administration markedly reduced plasma levels of epitestosterone, dihydrotestosterone, and hydroxyprogesterone, further indicating a pronounced impact of our intervention on steroid signaling. The estrogen-induced reduction in protein intake from the test breakfast buffet fits with animal experiments, indicating that centrally administered estrogen, similar to the adiposity signal leptin (16), inhibits food intake (17, 18). This effect is likely mediated via estrogen receptors expressed in the hypothalamic arcuate and ventromedial nuclei and the nucleus of the solitary tract in the hindbrain (19) but also in the reward-processing ventral tegmental area (20). Daily food intake in naturally cycling women reaches its nadir during the peri-ovulatory phase when estradiol concentrations are maximal (21, 22). Protein consumption has been found to be less pronounced during this phase as compared with the mid-luteal phase (23), although findings on cycle-dependent fluctuations in the intake of specific macronutrients are not unanimous (24, 25). Estrogen-induced reductions in the concentrations of testosterone and dihydrotestosterone might have contributed to decreased calorie consumption (26–28). Most recently, 17α-estradiol, an enantiomer of 17β-estradiol, has been suggested to induce centrally mediated catabolic effects (29). Although in our study the estrogen effect was evident for protein rather than fat or carbohydrate intake and appeared to focus on savory foods, it underlines the potential of estrogen delivery to restrain food intake (30).

Irrespective of estrogen pretreatment, intranasal insulin reduced carbohydrate consumption. This finding supports previous observations that intranasal insulin acutely decreases free-choice breakfast intake in healthy young men (7), although overall calorie intake was not reduced in the present experiments. *Post hoc* analyses of intense eaters displaying total calorie intake above the median of the respective placebo spray conditions revealed an insulin-driven, estrogen-independent decrease also in overall food intake (n = 8 per estrogen and placebo patch group, respectively; data not shown). These results support the notion that insulin transported to the central nervous system acts as a negative feedback signal in the control of eating behavior (2, 6). It appears unlikely that the moderate insulin-induced reduction in androstenedione levels was involved in this effect (31). Intranasal insulin also induced a slight increase in serum insulin and a euglycemic decrease in blood glucose concentrations that presumably stemmed from a small ratio of exogenous insulin entering the circulation via the nasal mucosa (7, 13, 32). Insulin's effect on food intake was confirmed in analyses corrected for these subtle changes in glucoregulation, so that a peripheral mediation of the decrease in carbohydrate consumption may be excluded.

Our observation that insulin restrains carbohydrate intake and the ingestion of sweet food items ties in with previous findings of reduced calorie and, in particular, carbohydrate intake after pre-sleep intranasal insulin administration (33) and of an insulin-induced decrease in the intake of chocolate cookies in nonfasted women (11). Studies in rats, in contrast, have indicated that central insulin administration predominantly reduces fat intake (34) but have also found respective reductions in sucrose self-administration (35). In humans, intranasal insulin acutely reduces the responsiveness to food stimuli of the ventral tegmentum and nucleus accumbens of the brain reward circuit as well as rated food palatability in men and women (36). Although animal experiments in general confirm that insulin inhibits the reward-related consumption of palatable foods (37, 38), conflicting data exist on the effect of insulin on dopaminergic signaling (39, 40).

Contrary to our expectation, estrogen pretreatment did not modulate the reduction in carbohydrate intake induced by intranasal insulin. In male rats, the peripheral administration of estradiol at a dosage of 2 μg administered every fourth day for 1 month completely blunted the reduction in 24-hour food intake and body weight observed in control animals after insulin injection into the third cerebral ventricle (10). In these animals, estradiol treatment increased plasma estradiol concentrations by 60%, averaging peri-ovulatory peak concentrations of female animals (41), whereas increasing serum estradiol concentrations by a factor of 3.5 in our male subjects yielded concentrations of around 300 pmol/L; women typically achieve ovulatory concentrations of 100 to 600 pmol/L. In principle, extended administration periods and higher dosages of estradiol might modulate the hypophagic effect of insulin, but estrogen's impact on eating behavior in the present paradigm is clearly indicated

by its suppressive effect on protein intake. Thus, it seems safe to conclude that inducing peripheral estrogen concentrations in healthy young men that approximate the situation regularly found in women and that induce strong reductions in the concentration of testosterone and related steroid hormones does not alter the brain's sensitivity to the anorexigenic impact of insulin. Different expression patterns of estrogen receptors in the male and female brain (42, 43) or basic genetic sex differences might contribute to altered estrogen-insulin interactions in men compared with women. However, in previous experiments restricted to women (13), the difference in estrogen levels between young women receiving ethinyl estradiol–dominant contraceptives and postmenopausal women, in accordance with the present data, was not associated with differences in the response to intranasal insulin.

Conclusion

In healthy men, central nervous insulin delivery via the intranasal route decreases carbohydrate consumption regardless of whether concurrent circulating estrogen concentrations are normal or elevated. This pattern indicates that estrogen, which displays moderate suppressive effects on protein intake, and insulin do not acutely interact in the regulation of eating behavior in humans. Further investigations, which should also cover longer time scales, are needed to gain insight into neurobiological mechanisms underlying the stronger anorexigenic effect of central insulin in male than female subjects reported in animals (9) and humans (7, 8). Such studies might contribute to the development of sex-specific, individually tailored approaches in the treatment of eating disorders.

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