Comparable Sensitivity of Postmenopausal and Young Women to the Effects of Intranasal Insulin on Food Intake and Working Memory

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Context: We have previously shown that enhancing brain insulin signaling by intranasal administration of a single dose of the hormone acutely reduces food intake in young men but not women, whereas its improving effects on spatial and working memory are restricted to young women.

Objective: Against the background of animal studies suggesting that low estrogen concentrations are a prerequisite for the anorexigenic impact of central nervous insulin, we extended our foregoing study by assessing intranasal insulin effects in postmenopausal women with comparatively low estrogen concentrations, expecting them to be more sensitive to the anorexigenic effects of the hormone.

Design, Setting, Participants, and Intervention: In a within-subject, double-blind comparison performed at the University of Lübeck, 14 healthy postmenopausal women (body mass index, 23.71 ± 0.6 kg/m²; age, 57.61 ± 1.14 yr) were intranasally administered 160 IU regular human insulin or vehicle.

Main Outcome Measures: Subjects performed a working memory task (digit span) and a hippocampus-dependent visuospatial memory task. Subsequently, free-choice food intake from an ad libitum breakfast buffet was measured.

Results: Contrary to expectations, results in postmenopausal women mirrored those found in young women (22.44 ± 0.63 yr), i.e., insulin administration did not affect food intake (P > 0.46), but did enhance performance in the prefrontal cortex-dependent working memory task (P < 0.05).

Conclusions: Low estrogen levels as present in postmenopausal women do not modulate the effects of intranasal insulin in females, suggesting that in humans as opposed to rats, estrogen signaling does not critically alter central nervous system sensitivity to the effects of insulin on energy homeostasis and cognition. (J Clin Endocrinol Metab 95: E468–E472, 2010)
ulates central nervous system insulin sensitivity: female rats with postovariectomy estrogen loss reduce food intake in response to icv insulin, whereas estradiol-treated male rats are no longer susceptible to the anorexigenic effect of the hormone (4, 5). Against this background, we assessed acute effects of intranasal insulin in postmenopausal women who display very low estrogen concentrations and compared them to the young women of our previous study who were taking estrogen-dominant contraceptives (9). Applying an identical study design, we hypothesized that postmenopausal women would not display improvements in memory performance but would be sensitive to the anorexigenic effects of enhanced central nervous system insulin signaling.

Subjects and Methods

Subject characteristics

Fourteen healthy women between 51 and 62 yr old (mean age, 57.61 ± 1.14 yr; mean body mass index, 23.71 ± 0.6 kg/m²) participated in the experiments. Their results were compared with those of the 18 young women (age, 22.44 ± 0.63 yr; body mass index, 21.4 ± 0.48 kg/m²) of our previous study (9) who were all taking monophasic ethinyl estradiol-dominant contraceptives, so that biasing influences of estrogen variations during the menstrual cycle were excluded. Hormone concentrations as derived from the (first) baseline measurement of each session and averaged across conditions were as follows: estradiol, postmenopausal women, 41.39 ± 3.46 pmol/liter (all values <110); young women, 68.45 ± 19.96 pmol/liter, P > 0.20; FSH, 83.89 ± 8.44 (all >20) vs. 2.89 ± 0.86 IU/liter, P < 0.001; and testosterone, 0.74 ± 0.14 vs. 1.76 ± 0.25 nmol/liter, P < 0.002. Note that endogenous estradiol but not ethinyl estradiol as contained in the young women’s contraceptives was measured. Average baseline homeostatic model assessment values reflecting insulin resistance were 1.43 ± 0.23 in the postmenopausal women and 1.99 ± 0.27 in the young women (P > 0.13). The 14 postmenopausal women had not had menstrual bleeding for 7.5 ± 1.1 yr and had not received estrogen replacement ever (n = 8), for at least 4 yr (n = 4), and for at least 3 months (n = 2), respectively. All subjects were nonsmokers and free of medication, except as indicated, and gave written informed consent to the study, which was approved by the local ethics committee.

Study design and procedure

Each subject participated in two balanced conditions (insulin and placebo) spaced 28 d apart. The experimental procedure was identical to our previous experiments in young subjects (see Ref. 9 for all details), except that we increased the frequency of blood samplings. In brief, fasted subjects participated in experimental sessions lasting from 0800 to 1100 h and starting with a 60-min baseline period (Fig. 1A). At 0900 h, subjects intranasally administered 16 0.1-ml puffs (eight per nostril) of insulin and placebo, respectively, at 30-sec intervals, amounting to a total dose of 1.6 ml insulin (160 IU; Insulin Actrapid; Novo Nordisk, Mainz, Germany) or vehicle. Thereafter, subjects underwent a battery of cognitive tests (see below). At 1020 h, a standardized breakfast buffet was offered from which subjects were allowed to eat ad libitum during the subsequent 30 min. Blood samples for the determination of plasma glucose and ghrelin as well as serum insulin, C-peptide, and leptin were obtained at baseline (0815, 0830, and 0845 h), after insulin administration (0910 h), at 10- to 20-min intervals thereafter, and after breakfast at 1050 h. Subjects repeatedly rated their hunger on a 9-point scale.

Cognitive tests

Twenty and 75 min after substance administration, i.e. at 0920 and 1015 h, participants performed the digit span subtest of the Hamburg-Wechsler Adult Intelligence Scale-Revised 1991 (10) to assess verbal working memory. This task requires the subject to repeat in chronological (forward test) and in reverse order (backward test), respectively, up to nine digits read by the experimenter. Total performance score is the sum of the forward and backward components (maximum score: 28 points). At 0945 h, subjects performed a visuospatial object location task that relies on hippocampal functions (11). The task is a computerized version of the game “concentration” and consists of 15 card-pairs showing pictures of animals and objects. Subjects had to memorize the respective locations during two runs of presentations and had to recall them immediately thereafter (9).

Statistical analyses

Analyses were based on ANOVA, with the within-subject factors “treatment” (insulin/placebo), “time,” and “macronutrient” as appropriate, and in subsequent analyses, the between-subjects factor “group” (postmenopausal vs. young women). Significant ANOVA interaction effects were specified by Student’s t tests. All data are presented as means ± SEM. A P value ≤0.05 was considered significant.

Results

Food intake and hunger ratings

Insulin administration affected neither total food intake (insulin vs. placebo, 950 ± 82 vs. 927 ± 69 kcal; P > 0.57; Fig. 2B) nor uptake of any specific macronutrient (P > 0.27). In comparisons between postmenopausal and young women, no significant differential insulin effects were found (treatment × group, P > 0.95; treatment × macronutrient × group, P > 0.65; Fig. 2B). Generally, postmenopausal women consumed more calories than young women did (P < 0.05). Hunger ratings did not differ between conditions (P > 0.40 for all comparisons).

Memory performance

Intranasal insulin improved performance on the second run (at 1015 h) of the working memory-related digit span task (insulin vs. placebo, 15.78 ± 1.07 vs. 14.07 ± 0.97 points; P < 0.03; Fig. 2B), whereas performance on the first run (at 0920 h) was not affected (14.35 ± 1.34 vs. 14.64 ± 1.00 points; P > 0.70). Also, intranasal insulin did not alter performance on the visuospatial location task (40.85 ± 4.96 vs. 35.28 ± 4.29% of card-pair locations
recalled correctly; \( P > 0.34 \); Fig. 2B). In both tasks, insulin effects on performance were not statistically different between postmenopausal and young women (treatment \( \times \) group interactions, second run of digit span, \( P > 0.71 \); visuospatial location, \( P > 0.36 \)). Generally, young women in comparison to postmenopausal women displayed improved digit span performance \(( P < 0.01)\) and comparable visuospatial location task performance \(( P > 0.2)\).

**Blood glucose and hormones**

Blood parameters did not differ during baseline \(( P > 0.27)\). Signs of a slight decrease in plasma glucose concentrations after insulin administration failed to be statistically confirmed \(( treatment \times time interaction, P > 0.24; \text{Fig. 2})\). Also, introducing postinsulin administration glucose values as covariates in the analyses of food intake and memory performance did not essentially alter the results. Concentrations of circulating insulin, C-peptide, ghrelin, and leptin were not affected by intranasal insulin \(( all P > 0.26; \text{Fig. 2})\).

**Discussion**

We have previously shown that intranasal insulin acutely decreases food intake in young men but not women, whereas its acute enhancing effects on spatial and working memory were restricted to young women \((9)\). Assuming that estrogen concentrations modulate the central nervous system impact of intranasal insulin, in the present study postmenopausal women were tested according to the same study design \((9)\). Contrary to expectations, postmenopausal women with low estrogen levels responded to intranasal insulin like the young women of our previous study \((9)\), i.e. they did not reduce food intake but displayed improved memory performance. Results indicate that the different estrogen levels of both groups did not modulate the central nervous sys-
tem effects of insulin. In line with previous studies (6–9), intranasal insulin did not critically alter blood glucose and insulin levels, indicating that after intranasal administration, the hormone affects brain functions but does not enter the circulation in substantial amounts.

It has to be noted that the postmenopausal women and the young women displayed comparably low endogenous estrogen concentrations, but that the young women were all taking estrogen-dominant contraceptives resulting in constantly high levels of ethinyl estradiol and also of gestagen (12). Ethinyl estradiol compared with endogenous estradiol is known to induce slightly more pronounced effects on fat and carbohydrate metabolism (13), so that biasing influences on central nervous system insulin sensitivity, although unlikely, cannot be ruled out. However, because the main hormonal effects of ethinyl estradiol and endogenous estradiol are largely congruent, our data strongly suggest that the brain impact of insulin in women is not modulated by estrogen. Also, neither increased FSH nor decreased testosterone levels in postmenopausal compared with young women altered the effect of intranasal insulin.

In some contrast to animal studies showing that anorexigenic effects of icv insulin administration are found in female rats after ovariectomy (4, 5), intranasal insulin did not alter calorie intake, rated hunger, and ghrelin as well as leptin concentrations in both postmenopausal and young women. However, whereas respective animal data derive from observations in young adult rats, we compared young women with a sample of older women who display not only decreased estrogen concentrations but generally reduced central nervous system plasticity. Thus, although artificial and, in particular, more rapid and severe manipulations of estrogen status may affect central nervous system insulin sensitivity in animals, the present results provide evidence for the notion that in contrast to men, women do not respond to the anorexigenic effects of enhanced brain insulin signaling, regardless of their age and physiological estrogen status. In both female groups, intranasal insulin improved performance on the digit span task, indicating an age- and estrogen status-independent improving effect of insulin on working memory. Verbal working memory in humans relies on the frontal cortex, which displays a high density of insulin receptors (14). Our data suggest that in women in contrast to men, intranasal insulin acutely enhances the function of the prefrontal-hippocampal loop that enables encoding and retrieval of declarative memory (15). Spatial memory performance benefited from insulin only in young women, although there was no statistically significant difference in treatment effects between both female groups. This memory test requires encoding of information under time pressure, an ability declining with advancing age (16), which might explain the larger performance variance of postmenopausal compared with...
young women that may have prevented the emergence of a clear-cut insulin-induced improvement.

In summary, our data indicate that the marked functional gender difference in central nervous system insulin sensitivity in humans, which implies a preferential anorexigenic effect in men compared with women, is not essentially mediated by differences in estrogen concentrations but may be due to other physiological factors that differentiate men from women. The beneficial effect on memory functions observed here and in previous studies (8, 9, 17, 18) supports the notion that intranasal insulin might be a helpful option in the prevention and treatment of memory dysfunctions in older as well as middle-aged subjects (19).

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