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Clinical Research Article

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Distinct and Convergent Beneficial Effects of Estrogen and Insulin on Cognitive Function in Healthy Young Men

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Abbreviations: ANOVA, analysis of variance; BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; MDBF, Mehrdimensionaler Befindlichkeitsfragebogen (mood scale); SEM, standard error of the mean.

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Abstract

Context: Systematic investigations into the cognitive impact of estradiol and insulin in male individuals are sparse, and it is unclear whether the 2 hormones interact to benefit specific cognitive functions in humans.

Objective: We investigated the acute effect of estradiol and insulin and of their combined administration on divergent (creative) and convergent (arithmetical) thinking as well as short-term and working verbal memory in healthy young men.

Methods: According to a 2 × 2 design, 2 groups of men (each n = 16) received a 3-day transdermal estradiol (100 µg/24 h) or placebo pretreatment and on 2 separate mornings were intranasally administered 160 IU regular human insulin and, respectively, placebo before completing a battery of cognitive tests; we also determined relevant blood parameters.

Results: Estrogen compared with placebo treatment induced a 3.5-fold increase in serum estradiol and suppressed serum testosterone concentrations by 70%. Estrogen in comparison to placebo improved creative performance, that is, verbal fluency and flexibility, but not arithmetical thinking, as well as verbal short-term memory, but not visuospatial memory. The combination of estrogen and insulin enhanced recognition discriminability at delayed verbal memory recall; insulin alone remained without effect.

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Conclusion: Estrogen specifically enhances core aspects of creativity and verbal memory in young male individuals; delayed recognition memory benefits from the combined administration of estradiol and insulin. Our results indicate that insulin's acute cognitive impact in young men is limited and not robustly potentiated by estradiol. Estradiol per se exerts a beneficial acute effect on creative and verbal performance in healthy young men.

Key Words: estrogen, insulin, divergent thinking, creativity, convergent thinking, memory

Beneficial effects on cognitive functions of estradiol, the most potent and prevalent estrogen, have been primarily investigated in women (1) and female animals (2). Cognitive effects of estradiol in men have not received similar attention (3) although estradiol, not least because of the conversion of testosterone by aromatase, circulates throughout the male body in biologically active amounts. Moreover, whereas gonadal production, the main source of estradiol in both sexes, subsides during female menopause, testicular production of testosterone and subsequent aromatization in men maintains estradiol availability throughout older age (4, 5). Estradiol is additionally synthesized in brain areas relevant for memory formation, like the hippocampus, and notably, concentrations of sex steroids are far higher in brain than periphery in men and women (6). Studies in elderly men indicate that cognitive functions such as verbal memory performance are positively associated with estradiol levels (4, 7) and improved by supplementation of testosterone (with subsequent aromatization to estradiol) (8, 9), although absent or negative associations (10) and null effects (11) have likewise been reported. Some longer-term investigations in participants receiving estradiol for male-to-female sex reassignment found improvements in verbal memory function (12, 13) whereas others did not yield effects (14). Assessing the cognitive impact of estradiol in healthy men may deepen our understanding of the neuroendocrine underpinnings of cognitive sex differences, with women outperforming men in tests of verbal fluency and verbal memory and the opposite pattern in results of visuospatial tasks (15, 16). Experiments in healthy young women suggest that performance on tests of creativity (divergent thinking) peaks during the preovulatory phase, when serum estradiol concentrations are highest (17, 18). The assumption that estradiol improves creative thinking is supported by observations that verbal fluency benefits from hormone replacement therapy in postmenopausal women (19) and from male-to-female sex reassignment hormone therapy (20), although results are not unequivocal (14, 21). The contribution of estradiol to divergent thinking in healthy young men is not known.

Insulin, a major regulator of glucose homeostasis, has been demonstrated to improve cognition, particularly memory function, in healthy participants (22, 23) and individuals with cognitive impairments (24) as well as animals (25). In young women and men, 8 weeks of insulin delivery to the brain via the intranasal route enhanced declarative memory retention as assessed by delayed word list recall (23). However, in an acute setting in young participants, the beneficial effect of intranasal insulin on measures of declarative and working memory was restricted to women (22). In conjunction with findings in animals that estradiol modulates the central nervous effect of insulin (26), these results suggest that estrogen and insulin may interact to improve cognitive function in humans. However, the cognitive impact of insulin combined with estradiol has not yet been investigated. We systemically assessed the acute effect on divergent and convergent thinking as well as verbal and visuospatial memory of transdermal estradiol and intranasal insulin in healthy young men. We expected estradiol to improve creative thinking and memory functions and assumed that cognition is unchanged by insulin alone but enhanced by insulin administered in combination with estradiol.

Methods

Participants, Design, and Procedure

Healthy men between 18 and 35 years who were nonsmokers and native German speakers were eligible for participation in the study, which conformed to the Declaration of Helsinki and was approved by the local ethics committee. Relevant illness was excluded by clinical examination and subjects did not take medication. Written informed consent was obtained from all participants prior to inclusion.

Figure 1 gives an overview of the experimental procedure. According to a 2 × 2 design, participants were randomly assigned to 2 groups that were treated with either estradiol (*estrogen patch* group) or placebo (*placebo patch*) before participating in 2 individual experimental sessions where they received intranasal insulin and, respectively, placebo. Three days before each test session, subjects attended our laboratory at 5 PM. Participants of the estrogen patch group received 2 transdermal 17β-estradiol patches

(Estradot 50, Novartis Pharma, Nuremberg, Germany) that were attached to the abdomen and delivered a total dose of 100 µg estradiol per 24 hours according to the manufacturer. Participants of the placebo patch group received 2 patches of comparable appearance that did not contain the hormone. The patches were renewed after 24 and 48 hours; that is, the third pair of patches was attached on the day before and removed directly after the experiment proper. Participants as well as experimenters were blinded to both study medications. Experimental sessions were separated by at last 3 weeks, and the order of conditions was balanced across subjects.

All participants remained fasted after 10 PM on the evening before testing. After the participant's arrival at the laboratory at around 8 AM, a venous cannula was inserted into his nondominant arm for blood collection. Sessions started with a short mindfulness exercise ("body scan") intended to relax the participant, followed by a 60-minute baseline period including blood samplings at 8:15, 8:30, and 8:45, and mood ratings that were repeated throughout the session. At 9:00 AM, subjects intranasally administered 16 0.1-mL puffs (8 per nostril) of insulin and placebo, respectively, at 30-second intervals, amounting to a total dose of 1.6 mL insulin (160 international units, IU; Insulin Actrapid; Novo Nordisk, Mainz, Germany), or vehicle; this dose and administration protocol has been shown to be effective in previous experiments (22, 27). At 9:20 AM, cognitive testing started with the first run of the digit span task, followed at 9:30 AM by the immediate recall part of the Verbal Learning and Memory Test (VLMT), at 9:45 AM by the visuospatial memory task and at 10:00 AM by the Stroop task; at 10:05 AM, the delayed recall sections of the VLMT and at 10:20 AM, another run of the digit span task took place. After a 30-minute test breakfast from 10:25 to 10:55 h (data reported in 28), a final blood sampling and mood assessment took place, and divergent and convergent thinking were assessed. Two different versions of each task were used in balanced order in the 2 experimental conditions of each participant. At the end of the session, participants were asked which patch (estrogen/placebo) and which spray (insulin/placebo) they thought they had received.

Assessment of Divergent and Convergent Thinking

Divergent thinking (creativity)

The test battery to assess divergent thinking comprised 6 performance tasks adapted from previous work (29, 30) that focused on aspects of verbal fluency and flexibility; participants were asked to write down the respective answers. "Consequences" required the participant to name within 3 minutes as many unusual consequences as possible of a hypothetical situation such as "A new invention makes it unnecessary for people to eat." In the 2-minute "Synonyms" task, synonyms of a presented word (eg, "nice"), and in the 1-minute "Words" task, words with a given first letter (e.g., "B") had to be produced. In the 2-minute "Pattern meaning" task, the participant was shown abstract patterns (eg, straight or curved lines) and asked to note down all associations that came to his mind. The 2-minute "Instances" task required the participant to focus on similarities by writing down, for example, all round objects he could think of. Finally, in the 3-minute "Alternate Uses" task, the participant was asked to list as many unusual uses as possible of common objects like a brick. The order of the tasks was the same in all sessions. Consequences, Pattern meaning, and Alternate Uses probe

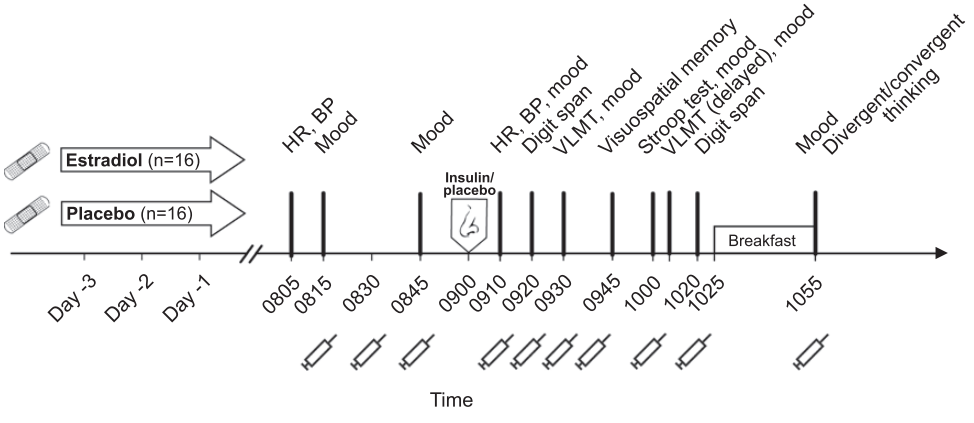

Figure 1. Experimental procedure. Two groups of 16 healthy men who had been pretreated with transdermal estradiol (100 µg/24 h for 3 days) or placebo participated in 2 experimental sessions. Following a baseline period of ~ 60 minutes, they were intranasally administered 160 IU insulin or, in the other condition, placebo at 9:00 AM before a battery of cognitive tests was performed (VLMT, Verbal Learning and Memory Test); a breakfast buffet was offered in between. Mood was repeatedly assessed and blood samples for the determination of glucose and hormone concentrations were obtained (syringe symbols). Heart rate (HR) and blood pressure (BP) were assessed twice.

flexibility while verbal fluency is assessed in the Synonyms, Words, and Instances tasks. Answers were scored according to standard criteria (31), that is, identical responses and responses that were obviously unrelated to the question were discarded; whenever responses reflected a common idea, only 2 respective variations were individually scored. The sum of all valid answers yielded task-specific scores as well as an average score calculated across all 6 tasks.

Convergent thinking (arithmetical thinking)

This test corresponds to the subtest "arithmetical thinking" of the Hamburg-Wechsler Adult Intelligence Scale—Revised 1991 (32) and assesses logical reasoning. The participant was asked to answer a series of 4 questions of comparable difficulty (eg, "Tina has as many sisters as she has brothers, but her brother John has twice as many sisters as brothers. How many sisters and brothers are there?"). For each question the participant was given 3 minutes to write down the solution and the number of correctly answered questions was determined, yielding a sum score of maximally 4 × 4 (ie, 16 points).

Assessment of Memory Function

Short-term memory and recognition

The Verbal Learning and Memory Test (VLMT) (33) was used to assess short-term verbal memory and recognition discriminability. In this task, a list of 15 unrelated nouns (list A) was aurally presented (1 word per 2 seconds) and the participant was asked to immediately recall as many items as possible without feedback on the accuracy of the answer. This procedure was repeated 2 times. An additional list of 15 different nouns (list B) was presented after the third run to induce interference, and the participant again recalled as many items as possible from list A. Subsequently, list A was once again presented and recalled. After a 30-minute delay, recognition discriminability was tested via cued recall, that is, the 15 nouns of list A were aurally presented intermixed with items of list B and with semantically and phonologically similar items (50 nouns in total); the participant was asked to indicate whether or not an item belonged to list A. Corrected performance (the numbers of correctly remembered words minus false alarms; 15 points maximum) was calculated.

Working memory

Participants performed the digit span subtest of the Hamburg-Wechsler Adult Intelligence Scale—Revised 1991 (32) twice to investigate the influence of estrogen and insulin on verbal working memory. In this test, up to 9 digits were read to the subject at a rate of 1 digit per second, for example, "5-8-2." The subject was to repeat the digits chronologically (forward test) or in reverse order (backward test). Starting with 3 digits, the number gradually increased until the participant failed to correctly repeat the digits twice in a row. Correct responses after 1 presentation equaled 2 points, and correct answers after 2 presentations equaled one point. Total performance score (28 points maximum) was the sum of the forward and backward components.

Visuospatial memory

Visuospatial memory was assessed with a computerized version of the game "concentration," a two-dimensional object-location task that consists of 15 card pairs showing pictures of animals and objects (22). Card pairs were randomly distributed on the screen at 30 possible locations displayed as gray squares ("back of the cards") that were geometrically ordered in a checkerboard-like fashion (5 × 6 matrix). During encoding, the first card of each card pair was presented for 1 second followed by the presentation of both cards for 3 seconds. After an interstimulus interval of 3 seconds, the next card pair was presented in the same way. The whole set of card pairs was presented twice in different orders. Immediately after learning, cued recall was tested in one run, for example, the first card of each pair was presented, and the subject was asked to indicate the location of the second card with a computer mouse. Scores reflect the number of correctly identified locations expressed as percentage of the total (ie, 15) of card pairs. Performance on this task relies on temporal lobe structures, including the hippocampus.

Control Parameters

Selective attention

Selective attention was assessed with the Stroop test (34) that includes the word reading test, the color naming test, and the interference test. In the word reading test, the participant was presented a panel with 100 color names (red, green, blue, yellow) printed in black ink. In the color naming test, rows of Xs in different colors were presented and each color had to be named as quickly as possible. In the interference subtest, the participant was shown a series of color names (red, green, blue, yellow) printed in incongruent colors (eg, the word *red* printed in green letters) and had to name as quickly as possible the print color but inhibit the prepotent reading response. For each task, the total number of correct responses within 45 seconds was determined.

Mood

Mood was assessed with 5-point scales covering the categories good/bad mood, alertness/sleepiness, and calmness/agitation (Mehrdimensionaler Befindlichkeitsfragebogen, MDBF (35)). Blood pressure and heart rate were measured before and around 10 minutes after spray administration.

Endocrine Assessments

Blood glucose was measured in whole blood by HemoCue B-Glucose-Analyzer, HemoCue AB, Angelholm, Sweden. The other blood samples were centrifuged, and plasma and serum were stored at –80 °C. Serum concentrations of insulin (all 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 (ie, 17β-estradiol) and testosterone were determined for every other sampling time point by ultra-high-performance liquid chromatography coupled to QTOF mass spectrometry. In brief, plasma samples were diluted with 5% (w/v) phosphoric acid (H_3PO_4) and steroids extracted by solid-phase extraction with an Oasis PRiME HLB SPE cartridge. The extracts were evaporated and reconstituted in methanol before chromatographic separation was performed on an ultra-high-performance liquid chromatography instrument (1290 Infinity UHPLC; Agilent Technologies, Waldbronn, Germany). Analyte detection was carried out on a hyphenated TripleTOF 5600+ mass spectrometer (Sciex, Concord, Ontario, Canada). For quantification, a surrogate calibrant method using $^{13}C_3$-estradiol and $^{13}C_3$-testosterone in true plasma matrix was established. Quantifiable ranges for estradiol and testosterone were 10 to 1000 pg/mL and 20 to 15 000 pg/mL, respectively.

Statistical Analyses

Sample size was determined based on previous experiments on the cognitive effect of estrogen (29) and insulin (22). Analyses were performed with SPSS Statistics (IBM, Armonk, NY) and based on analyses of variance (ANOVA) with the between-subjects factor *Group* (estrogen patch vs placebo patch) and the within-subject factors *Treatment* (insulin vs placebo spray), *Task* and *Time* as appropriate. Significant ANOVA interactions were specified by Student *t* tests. Note that in the following, if not further specified, group effects refer to estrogen vs placebo comparisons collapsed across insulin and placebo spray conditions and treatment effects refer to insulin vs placebo comparisons collapsed across estrogen and placebo patch groups. All data are presented as means ± standard error of the mean (SEM). A *P* value less than 0.05 was considered significant.

Results

Participants

Thirty-two participants in total were included in the study. They had a mean age of 23.94 ± 0.52 years and a mean body mass index (BMI) of 22.80 ± 0.36 kg/m^2. The mean age and BMI of the participants assigned to the "estrogen patch" and the "placebo patch" groups (each n = 16) were, respectively, 24.38 ± 0.93 years and 23.50 ± 0.49 years ($P > 0.412$); and 22.62 ± 0.50 kg/m^2 and 22.98 ± 0.54 kg/m^2 ($P > 0.619$).

Endocrine Parameters

Transdermal estradiol in comparison to placebo treatment induced 3.5-fold increase in plasma estradiol concentrations ($F(1, 30) = 84.50$; $P < 0.0001$) and a 70% decrease in testosterone ($F(1, 30) = 67.00$; $P < 0.0001$ for Group; Fig. 2A and 2B). Both estradiol and testosterone displayed a postprandial drop after breakfast intake ($P < 0.0001$ for Time). Intranasal insulin did not display any modulatory influence on these parameters (all $P > 0.440$). Concentrations of LH and FSH measured during baseline were strongly suppressed after estrogen treatment (both $P < 0.008$ for Group; Fig. 2C). Parameters of glucose metabolism did not differ between conditions during baseline (all $P > 0.097$) and were generally not affected by estrogen treatment (all $P > 0.345$ for respective interactions). Intranasal insulin administration induced a slight decrease in blood glucose concentrations ($F(3, 98) = 3.81$; $P < 0.011$ for Treatment × Time) that remained within the euglycemic range (Fig. 2D). Corresponding increases in insulin concentrations after intranasal insulin in comparison to placebo administration did not reach statistical significance ($F(1, 37) = 2.42$; $P < 0.123$; Fig. 2E).

Divergent and Convergent Thinking

Estrogen in comparison with placebo treatment generally enhanced divergent thinking ($F(1, 30) = 6.83$; $P = 0.014$ for the factor Group in ANOVA comprising all subtasks; Fig. 3A). Analyses of individual tasks indicated a beneficial effect of estradiol vs placebo on performance in the tasks Synonyms, Instances, and Alternate Uses and a respective trend for Words (Table 1; $F(4, 119) = 1.59$; $P = 0.183$ for Group × Task). In contrast, insulin compared with placebo administration had no effect on divergent thinking ($F(1, 30) = 1.08$; $P > 0.304$ for Treatment; $P > 0.202$ for the individual tasks). We also did not find indicators of an interaction between estrogen and insulin ($P > 0.512$ for respective overarching ANOVA terms; $P > 0.151$ for all individual tasks).

Performance on the arithmetical task reflecting convergent thinking was altered neither by estrogen ($F(1, 30) = 0.38$; $P = 0.531$) nor insulin compared with placebo, respectively ($F(1, 30) = 0.67$; $P = 0.793$; $F(1, 30) = 0.03$; $P = 0.855$ for Group × Treatment; Fig. 3B).

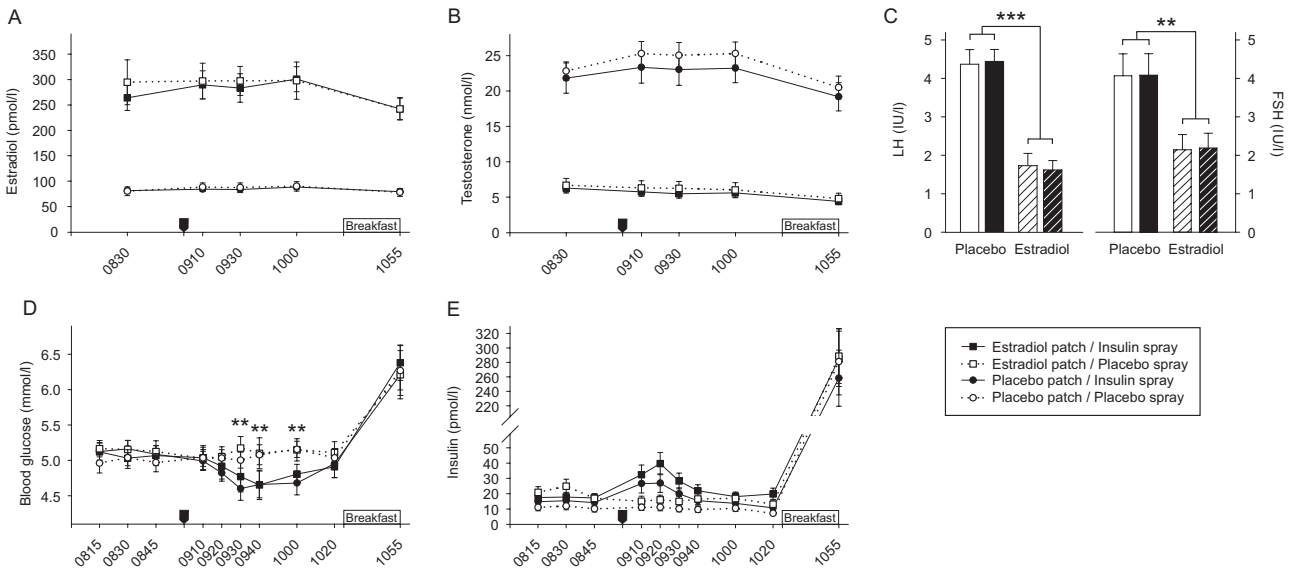

Figure 2. Endocrine parameters. Plasma concentrations of (A) 17β-estradiol, (B) testosterone, serum concentrations of (C) LH and FSH, (D) blood glucose concentrations, and serum concentrations of (E) insulin. Experiments were performed in 2 groups of 16 men each who had received 3 days of transdermal 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 AM and 8:45 AM baseline measurements. Values are means ± SEM. *** $P < 0.001$, ** $P < 0.01$ for the ANOVA factors Group (panel C) and Treatment (D), respectively.

Memory Function

Short-term memory and recognition

Estrogen compared with placebo generally improved immediate recall of words from list A of the VLMT (F (1, 30) = 5.11; $P = 0.032$ for ANOVA factor Group in analyses covering all runs; $P > 0.464$ for Group × Task). Thus, estrogen vs placebo enhanced the first immediate recall (Fig. 3C and Table 2). The second and the third runs of list A likewise yielded slight indicators of an improving effect of estrogen. Recall of word list A after distraction with the interference list B tended to be improved by estrogen. Estrogen treatment significantly enhanced the subsequent recall after re-learning (Table 2). Insulin generally remained without effect on immediate recall (all $P > 0.182$).

Recognition discriminability (correctly remembered items minus false alarms) assessed 30 minutes after the assessment of immediate recall was improved compared to control only in participants who received both estrogen and insulin (F (1, 30) = 4.32; $P < 0.051$ for insulin vs placebo in estrogen-treated participants; F (1, 15) = 3.17; $P < 0.094$ for estrogen vs placebo in insulin-treated participants; Fig. 3D). This additive effect of both agents was confirmed by the significant interaction of Group × Treatment (F (1, 30) = 4.73; $P < 0.030$).

Working memory

Estrogen and insulin, when compared with their respective control conditions, generally did not alter total performance on the digit span test ($P > 0.243$ for all comparisons and interactions regarding the first and second run). In analyses differentiating between the forward and the backward scores of the test, estrogen compared with placebo slightly improved performance on the backward part of the first run and the forward part of the second run (Table 3; $P > 0.504$ for all other comparisons). In contrast, insulin remained without effect also in these analyses ($P > 0.143$; $P > 0.181$ for interactions with estrogen).

Visuospatial memory

In the visuospatial memory task, namely, the two-dimensional object-location task, participants of the estrogen patch group achieved scores of 52.94 ± 4.36 after insulin and 51.25 ± 4.29 after placebo administration; participants who received placebo patches achieved, respectively, 55.94 ± 4.70 and 55.00 ± 4.60. We did not detect significant effects of estrogen (F (1, 30) = 0.51; $P = 0.474$) and insulin (F (1, 30) = 0.91; $P = 0.763$) compared with placebo or a significant statistical interaction (F (1, 30) = 0.08; $P = 0.930$; Fig. 3E).

Control Parameters

Selective attention

Performance on the Stroop test (data not shown) was neither affected by estrogen (F (1, 30) = 0.26; $P = 0.613$ for ANOVA factor Group), nor by insulin (F (1, 30) = 1.16; $P = 0.281$ for Treatment; all $P > 0.092$ for respective interactions).

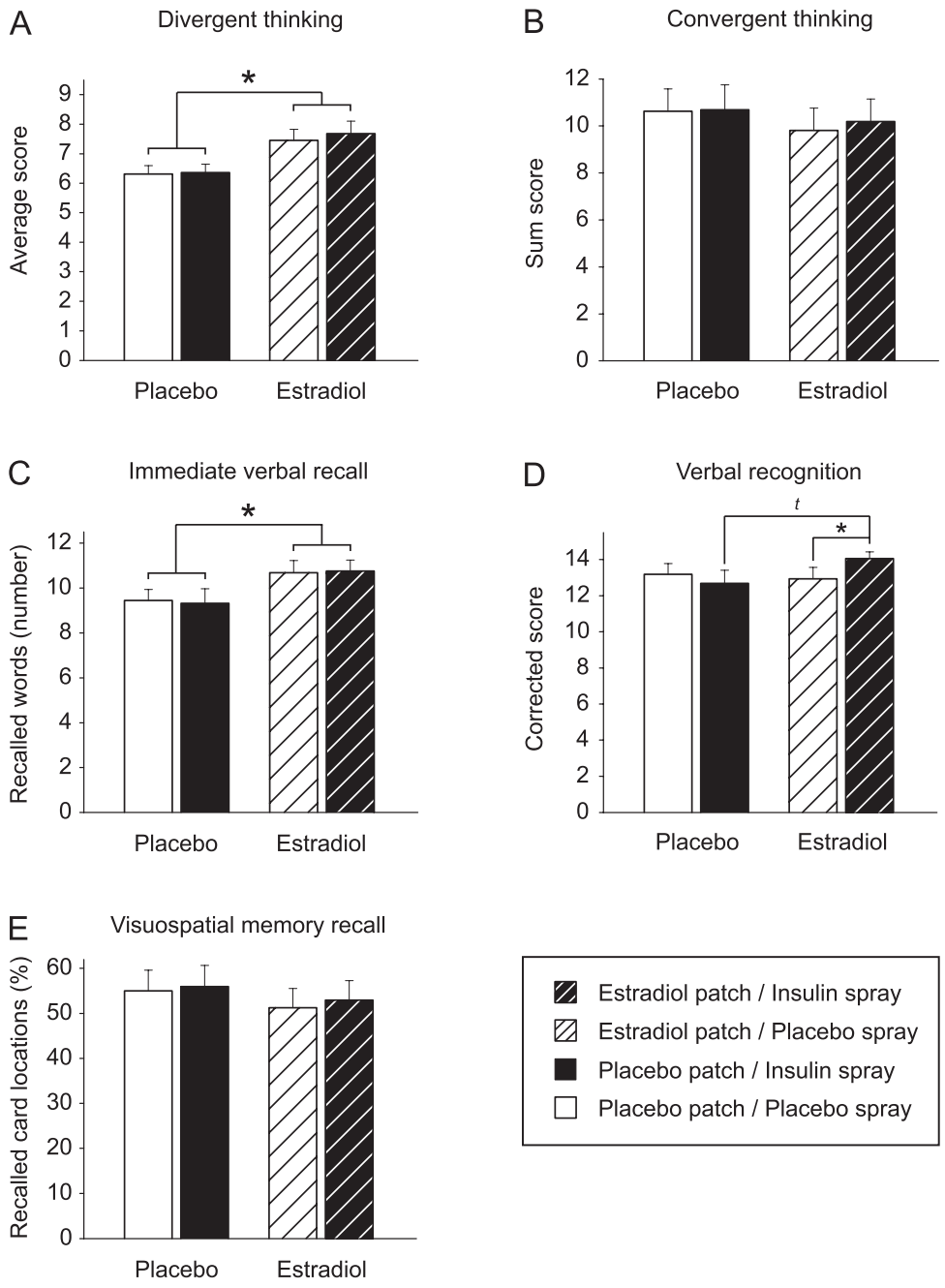

Figure 3. Cognitive parameters. Scores of (A) divergent thinking (average score across 6 tests), (B) convergent thinking (sum score across 4 questions), (C) immediate recall in the first run of the Verbal Learning and Memory Test (VLMT), (D) recognition discriminability in the VLMT, reflecting recognition of previously encoded words (minus false alarms) presented intermixed with previously presented interfering and semantically and phonologically similar words, and (E) visuospatial memory. Experiments were performed in 2 groups of 16 men each who had received 3 days of transdermal estradiol (100 μg/24 h; hatched bars) or placebo pretreatment (nonhatched bars) before participating in experimental sessions starting with baseline measurements followed by the intranasal spray administration of 160 IU insulin (black bars) and, respectively, placebo (white bars). Values are means ± SEM. ^{t} $P < 0.10$, * $P < 0.05$ for the ANOVA factors Group (panels A, C) and Group and, respectively, Treatment (D).

Mood and cardiovascular parameters

Self-rated mood and alertness according to the MDBF adjective scale generally improved during the experiment (both $P < 0.002$ for Time) but, like self-rated calmness, were affected neither by estrogen nor insulin (all $P > 0.113$). Blood pressure and heart rate were comparable between groups and conditions (all $P > 0.214$). In interviews after the experiment, participants were not able to correctly indicate the substances they had received (all $P > 0.531$, χ^2 tests); none of the subjects reported adverse side effects.

Discussion

We demonstrate that estradiol acutely enhances creative thinking and verbal short-term memory in healthy young men; insulin improves verbal recognition discriminability

Table 1. Results of the divergent thinking tasks

	Placebo patch group		Estradiol patch group		ANOVA result (Group)
	Placebo spray	Insulin spray	Placebo spray	Insulin spray	
Consequences	3.50 ± 0.22	3.38 ± 0.34	3.56 ± 0.33	3.63 ± 0.31	F (1, 30) = 0.19; *P* = 0.667
Synonyms	4.81 ± 0.55	5.00 ± 0.47	6.38 ± 0.42	6.94 ± 0.59	F (1, 30) = 9.08; *P* = 0.005
Words	13.56 ± 0.65	13.81 ± 0.63	14.69 ± 0.55	15.56 ± 0.82	F (1, 30) = 2.94; *P* = 0.097
Pattern meaning	5.25 ± 0.49	4.69 ± 0.46	5.75 ± 0.54	6.25 ± 0.54	F (1, 30) = 2.74; *P* = 0.108
Instances	6.13 ± 0.55	6.38 ± 0.46	7.81 ± 0.71	7.31 ± 0.60	F (1, 30) = 4.20; *P* = 0.049
Alternate uses	4.44 ± 0.33	4.75 ± 0.32	6.25 ± 0.64	6.13 ± 0.54	F (1, 30) = 7.00; *P* = 0.013

Mean ± SEM scores achieved in the tasks of the divergent thinking test. Experiments were performed in 2 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, respectively. Right column indicates results for the ANOVA factor *Group* (results for *Treatment* and respective interactions were n.s.). n = 32.

Table 2. Results of the short-term memory and recognition test (VLMT)

	Placebo patch group		Estradiol patch group		ANOVA result (Group)
	Placebo spray	Insulin spray	Placebo spray	Insulin spray	
Immediate recall					
First run	9.44 ± 0.49	9.31 ± 0.65	10.68 ± 0.54	10.75 ± 0.49	F (1, 30) = 4,28, *P* = 0.047
Second run	12.10 ± 0.57	12.12 ± 0.61	13.37 ± 0.48	13.41 ± 0.41	F (1, 30) = 3.95, *P* = 0.056
Third run	13.63 ± 0.39	13.56 ± 0.35	14.31 ± 0.20	14.13 ± 0.18	F (1, 30) = 4.06, *P* = 0.053
Recall after distraction	12.25 ± 0.55	11.69 ± 0.85	13.50 ± 0.33	13.31 ± 0.38	F (1, 30) = 3.93, *P* = 0.057
Recall after re-learning	11.88 ± 0.65	12.06 ± 0.79	13.31 ± 0.33	13.88 ± 0.34	F (1, 30) = 4.37, *P* = 0.045
Recognition	13.19 ± 0.59	12.69 ± 0.72	12.94 ± 0.63	14.06 ± 0.36	F (1, 30) = 0.56, *P* = 0.45*

Mean ± SEM scores achieved in the short-term memory and recognition test (VLMT). Experiments were performed in 2 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, respectively. Right column indicates results for the ANOVA factor *Group* (results for *Treatment* and respective interactions were n.s.).
* F (1, 30) = 4.73; *P* < 0.03 for Group × Treatment; n = 32.

only in combination with estradiol but, in accordance with previous findings (22), does not acutely benefit other cognitive measures in young men without cognitive impairments. Against the background of previous work on the role of estrogen for cognitive function that largely focused on women as well as, to a lesser extent, elderly men (eg, 1, 7) and individuals undergoing male-to-female sex reassignment (12, 13, 20), our findings pinpoint the acute contribution of estrogen to cognitive function in healthy young men and highlight the convergent impact of estradiol and insulin. The 3-day transdermal estrogen treatment in comparison to placebo induced a 3.5-fold increase in circulating estradiol and a decrease in testosterone levels of around 70%; LH and FSH concentrations were likewise decreased. This pattern confirms the compliance of our participants and, because estradiol readily passes the blood-brain barrier, suggests sufficient brain permeation of transdermal estradiol. Intranasal insulin is known to change brain activity within 45 minutes after administration (36). The negligible impact of insulin on peripheral glucose metabolism in the present experiments largely excludes systemic mediation of the cognitive outcomes.

Estrogen compared with placebo improved performance on tests of divergent thinking, namely, verbal fluency but also flexibility. Divergent thinking reflects the activation of mental representations that have only a weak associative connection to the stimulus (29). In contrast, we did not find effects on convergent (arithmetical) thinking that draws on strongly associated representations (30); this pattern indicates that estrogen exerted a specific enhancing effect on creative in contrast to arithmetical thinking. Improvements in verbal fluency have been likewise, albeit not unanimously (21), observed upon individuals receiving estrogen for male-to-female sex reassignment (20). Hormone therapy in postmenopausal women has been consistently found to benefit verbal fluency (19, 37). However, the same 3-day transdermal estradiol regimen as in the present study impaired divergent but enhanced convergent thinking in postmenopausal women with a mean age of 58 years (29), suggesting a critical influence of treatment timing and duration. During the menstrual cycle, performance on the same

Table 3. Results of the working memory test (digit span)

	Placebo patch group		Estradiol patch group		ANOVA result (Group)
	Placebo spray	Insulin spray	Placebo spray	Insulin spray	
First run					
Forward	8.75 ± 0.46	8.56 ± 0.38	8.56 ± 0.56	8.75 ± 0.41	F (1, 30) = 0.01; *P* = 0.94
Backward	7.13 ± 0.38	8.25 ± 0.49	8.63 ± 0.53	8.69 ± 0.47	F (1, 30) = 3.27; *P* = 0.09
Total	15.88 ± 0.64	16.81 ± 0.73	17.19 ± 0.78	17.44 ± 0.75	F (1, 30) = 1.41; *P* = 0.24
Second run					
Forward	8.44 ± 0.46	8.75 ± 0.56	9.38 ± 0.43	9.69 ± 0.49	F (1, 30) = 3.04; *P* = 0.09
Backward	8.81 ± 0.53	8.81 ± 0.56	8.38 ± 0.71	8.31 ± 0.39	F (1, 30) = 0.46; *P* = 0.50
Total	17.25 ± 0.81	17.56 ± 0.99	17.75 ± 0.92	18.00 ± 0.71	F (1, 30) = 0.18; *P* = 0.67

Mean ± SEM scores achieved in the first run (~ 0920 h) and second run (~ 1020 h) of the digit span test that included a forward and a backward recall task. Experiments were performed in 2 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, respectively. Right column indicates results for the ANOVA factor *Group* (results for *Treatment* and respective interactions were n.s.). n = 32.

and comparable tasks of verbal fluency and flexibility, but not of convergent thinking, peaks during the preovulatory phase, when endogenous estrogen levels are highest (18). Verbal fluency recruits the inferior prefrontal cortex (38), where insulin receptors are abundant (39), but we did not detect insulin effects on divergent thinking in our healthy young men. Although the impact of insulin on creativity is yet to be assessed in women, this outcome implies that the acute cognitive effect of insulin centers on memory performance.

Estradiol compared with placebo treatment enhanced short-term verbal memory as reflected by the immediate recall of words and recall after distraction and re-learning. Signs of respective improvements also emerged in the digit span task, an indicator of working memory, which corroborates observations in elderly women (5) and individuals undergoing male-to-female transition (13) receiving estrogen. In some contrast, hippocampus-dependent visuospatial declarative memory did not benefit from estrogen, suggesting that the hormone primarily enhances declarative memory functions that do not heavily depend on temporal lobes including the hippocampal formation, but rather prefrontal cortical regions (40). The acute estrogen-induced improvement in verbal memory is in line with observations of better verbal memory performance in elderly men with high compared to those with lower concentrations of circulating estradiol (7) and during long-term estrogen treatment of male-to-female transsexual individuals (12), although the latter findings were not replicated (14). In postmenopausal women, 3-day transdermal estradiol administration improved memory for a word list (29). While endogenous estradiol levels have been found to be positively associated with attention in older men (41), selective attention assessed with the Stroop task was not changed in our experiments. Estrogen-triggered improvements in cognitive functions including verbal short-term memory and divergent thinking may be assumed to involve changes in the activity of neurotransmitter systems (42), dendritic spine and synapse morphology (43) as well as synaptic plasticity (44). Considering that our participants received estrogen for 3 days prior to testing, classical estrogen effects mediated via gene transcription might have added to rapid effects that depend on cell-signaling cascades and can emerge within minutes.

We also investigated delayed verbal recognition discriminability by asking our participants to identify words encoded 30 minutes before that were presented along with interfering and distractor items. Interestingly, we found this measure to be improved only after the combined administration of estradiol and insulin, but not of the individual hormones. This result is a partial confirmation of our hypothesis that young healthy men who—in contrast to women of young (22) and older age (27)—do not acutely respond to the memory-improving effects of intranasal insulin (22), would show better memory performance after pretreatment with estradiol. Thus, insulin acts as an acute conditional neuroenhancer in healthy young men by improving delayed recognition memory when estradiol levels are elevated. Recognition memory engages hippocampal-prefrontal networks as well as thalamic nuclei (45) and has been shown to benefit from de novo hippocampal estradiol synthesis via aromatase-induced conversion of testosterone (46) (for review see 2). The hippocampus harbors high densities of receptors for insulin (47), which is known to contribute to activity-dependent synaptic long-term potentiation and depression (48). In contrast to the estradiol-dependent effect of insulin on recognition memory, but in line with our previous findings in young men (22), insulin did not acutely alter visuospatial declarative memory or verbal working and short-term memory recall, nor selective attention. This outcome argues against the assumption that high estradiol concentrations, as found in the young

women taking estrogen-dominant contraceptives of our previous studies (22, 27), are a prerequisite for insulin's verbal and visuospatial memory effect, and it leaves open the question whether progesterone might play a role in this context (49). Mood and cardiovascular parameters were not affected by our hormonal interventions, ruling out respective mediating or biasing effects on cognitive measures.

We applied a rigorous experimental design to assess the acute cognitive effect of estradiol and insulin in a well-characterized, homogenous sample of healthy young men; our choice of dosing and administration routes relied on established paradigms (22, 27, 29). The acute nature of our experiment sets it apart from the majority of clinical investigations (12-14, 23, 24) but further studies should assess whether chronic estrogen interventions improve divergent thinking, and as was previously shown for 8-week intranasal insulin administration (23), verbal memory. The fact that we did not test different doses of estradiol and insulin and the overall moderate magnitude of the observed effects represent limitations of our study that are of particular relevance when it comes to potential clinical applications. In this context, the strong suppressive effect of transdermal estradiol on the circulating concentrations of testosterone and gonadotropins moreover precludes an immediate translation into application.

In sum, our results indicate that in healthy young men estradiol acutely benefits creative rather than arithmetic thinking and verbal rather than visuospatial memory and, moreover, enables insulin-induced improvements in recognition memory. In men, around 80% of circulating estradiol stems from aromatization of testosterone, which has been shown to mediate enhancing testosterone effects on verbal, but not spatial memory (8). The present data highlight the relevance of estradiol for specific cognitive functions, that is, divergent thinking and verbal memory, in young, healthy men. They also shed new light on the role of estradiol in cognitive sex differences (16), its impact on creative performance during hormonal male-to-female sex reassignment (14, 20, 21) and respective ramifications for psychiatric disorders (50). Considering the negative cognitive outcomes of large-scale clinical trials of testosterone replacement regimens in elderly men (11) and notwithstanding the caveats summarized above, our findings raise the question whether tailored interventions that target estradiol signaling might, in principle, be a suitable means to support or restore creative thinking and verbal memory function in men of older age.

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