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Estrogen Replacement Improves Verbal Memory and Executive Control in Oligomenorrheic/Amenorrheic Athletes in a Randomized Controlled Trial

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ABSTRACT

Objective: Both estrogen and exercise may have cognition enhancing benefits; however, young oligomenorrheic/amenorrheic athletes (OA) with estrogen deficiency have not been evaluated for cognitive deficits. Our objective was to determine whether 6 months of estrogen replacement will impact cognitive domains in OA. We hypothesized that estrogen replacement would improve verbal memory and executive control in OA.

Methods: We performed cognitive assessments at baseline and after 6 months in 48 OA (14–25 years) randomized to estrogen (EST+) (oral 30 µg ethinyl estradiol [n = 16] or transdermal 100 µg 17-β-estradiol patch [n = 13]) or no estrogen (EST-) (n = 19) in an ongoing clinical trial. Neurocognitive testing included California Verbal Learning Test—Second Edition (CVLT-II) (for verbal memory) and Delis-Kaplan Executive Function System Color-Word Interference Test (D-KEFS-CWIT) (executive control).

Results: On average, subjects (mean ± SEM age: 19.9 ± 3.1 years, body mass index: 20.6 ± 2.3 kg/m²) participated in 10.3 ± 5.9 hours per week of weight-bearing activities of their lower limbs. The EST+ group performed better for CVLT-II verbal memory scores for immediate recall over 6 months of therapy compared to EST- ($P < .05$) even after controlling for baseline scores and age. Changes in D-KEFS-CWIT scores over 6 months did not differ between the groups. However, the EST+ group had greater improvements in inhibition-switching completion time over 6 months compared with the EST- group after controlling for baseline scores and age ($P = .01$).

Conclusions: OA show improvements in verbal memory and executive control following 6 months of estrogen replacement. These findings in athletes, who are in their prime of neurocognitive development, underscore the need for future studies exploring cognition in OA.

Trial Registration: ClinicalTrials.gov identifier: NCT00946192

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In addition to the known effects of estrogen in regulating reproductive function and bone metabolism, studies suggest that estrogen plays a role in enhancing cognitive performance.^{1–3} Women with Turner syndrome and postmenopausal women, who are characterized by estrogen deficiency, have cognitive deficits including altered memory and executive control, suggesting a role for estrogen in enhancing cognitive skills.^{4–8} Similarly, use of aromatase inhibitors in breast cancer treatment to block estrogen production has been linked to impaired verbal memory and reduced information processing speed.^{9,10}

Consistent with these human studies, research in rodents supports a protective role of estrogen on cognition^{11,12} and provides mechanistic insights into this effect.¹³ Brain-derived neurotrophic factor (BDNF) is a nerve growth factor produced predominantly in the hippocampus, which represents the prime neural correlate of learning and memory.¹⁴ The promoter of the BDNF gene harbors an estrogen response element,¹⁵ suggesting that BDNF may mediate estrogen effects on cognitive function. Additionally, exercise increases BDNF expression, but this effect is evident in ovariectomized rats only after estrogen replacement.¹³ Another postulated mechanism for estrogen is via catecholamine and cholinergic pathways in the prefrontal cortex (PFC).¹⁶ This region is important for mediating executive control, the processes that top-down-regulate information processing to ensure goal achievement.¹⁷ Despite evidence implicating estrogen deficiency in cognitive deficits in humans, studies evaluating cognitive effects of estrogen replacement have yielded equivocal results.^{18–21}

While exercise has a positive impact on cognition,²² excessive exercise can lead to low energy availability and inhibition of the hypothalamic-pituitary-gonadal axis, leading to estrogen deficiency and menstrual dysfunction (which occurs in up to 44% of female athletes²³). Estrogen deficiency is a major component of the female athlete triad, which is characterized by low energy availability, menstrual dysfunction, and low bone density.^{24,25} Although the impact of estrogen deficiency on bone has been widely studied in oligomenorrheic/amenorrheic athletes (OA), its effect on cognitive domains remains to be investigated. Further, female athletes comprise a distinctive population in that the negative impact of estrogen deficiency on cognitive performance may be counteracted by the beneficial effects of physical activity.²² Estrogen replacement in OA may serve as a physiological probe to investigate the cognitive domains that may be altered in these athletes who have estrogen deficiency. Of importance, the PFC matures through the second decade of life, resulting in ongoing

- The impact of estrogen replacement on cognition in athletes who lose their menstrual period due to excessive exercise and are estrogen deficient has not been previously assessed.
- Estrogen replacement may have a role in improving memory and executive function in athletes who develop amenorrhea secondary to exercise.

development of executive control during this period.²⁶ Thus, factors affecting cognitive development during the critical teenage/young adult years of neurobehavioral development merit careful investigation.

Our primary objective was to determine whether estrogen replacement for 6 months could improve cognitive measures of verbal memory and executive control in adolescent and young adult OA (irrespective of route of administration or form). Our secondary objective was to determine whether a more physiologic form of estrogen administration, transdermal estrogen, would be more effective than oral estrogen in improving these cognitive outcomes. We hypothesized that OA randomized to estrogen replacement for 6 months would perform better on tasks evaluating verbal memory and executive control compared to those randomized to no estrogen. Further, we hypothesized that transdermal estrogen will perform better than oral estrogen for these cognitive measures.

METHODS

Study Subjects

The study was approved by the Institutional Review Board of Partners Healthcare System and conducted between 2011 and 2015. Informed consent was obtained from subjects ≥ 18 years of age and parents of subjects < 18 years of age, and informed assent was obtained from subjects < 18 years of age. We performed baseline and 6-month cognitive assessments in 48 OA aged 14–25 years randomized to estrogen or no estrogen in an ongoing clinical trial (ClinicalTrials.gov identifier: NCT00946192). Subjects were informed during the consenting process that questionnaires would be administered over the course of the study, but we did not specify that we would examine the impact of estrogen on cognitive outcomes. All participants had a body mass index (BMI) between the 10th and 90th percentiles. Athletes were required to engage in (1) ≥ 4 hours per week of weight-bearing exercise of their legs or (2) ≥ 20 miles of running per week for ≥ 6 months in the preceding year. Our inclusion criteria for oligomenorrhea/amenorrhea were rigorous and included (1) ≥ 3 missed menstrual periods in the preceding 6 months, and cycle length of > 6 weeks for any menses that occurred during this 6-month period or (2) menstrual onset at > 15 years if subjects were pre-menarchal.²⁷ Subjects with other conditions that can cause amenorrhea such as polycystic ovary syndrome, thyroid dysfunction (unless euthyroid on medications for ≥ 3 months), primary ovarian insufficiency (indicated by an elevated follicle-stimulating

hormone), or hyperprolactinemia and subjects with known contraindications to estrogen were excluded. All athletes with oligomenorrhea/amenorrhea were assessed for active eating disorders by the study psychologist, and if a diagnosis of active anorexia nervosa was made, they were excluded from the study. Menstrual recovery, defined as at least 3 consecutive cycles in the past 6 months, was assessed for the no-therapy group during the follow-up period. Assessing menstrual recovery consequent to lifestyle changes is not possible in groups receiving exogenous estrogen/progesterone, as cyclic menses would be expected on treatment.

Experimental Protocol

Randomization of subjects to estrogen and progesterone versus placebo cannot be adequately blinded as subjects receiving estrogen and progesterone will typically have cyclic menses on treatment. Therefore, a no-treatment arm was utilized instead of a placebo. Subjects determined eligible were randomized to 1 of 3 arms based on a predetermined algorithm: (1) 30 μg ethinyl estradiol and 0.15 mg desogestrel taken orally daily for 21 days and a placebo pill for 7 days (EST ORAL) ($n = 16$), (2) physiologic estrogen replacement as the transdermal estrogen patch (100 μg 17- β -estradiol patch applied twice weekly) with 200 mg of micronized progesterone (taken orally for the first 12 days of every month) (EST PHYSIOL) ($n = 13$), or (3) no estrogen (EST-) ($n = 19$). Participants were asked to maintain a medication calendar that was monitored by our research coordinators at follow-up visits. Participants were also asked to return the remaining medication and empty medication packs at subsequent study visits. Transdermal estradiol administration as 17- β -estradiol in replacement doses is considered a physiologic form of estrogen administration because (1) in contrast to ethinyl estradiol (synthetic derivative of estrogen), 17- β -estradiol is the endogenous form of estrogen and (2) unlike oral estrogen and similar to endogenous estrogen, transdermal estrogen does not undergo first pass hepatic metabolism, known to suppress insulin-like growth factor 1 (IGF-I).²⁸ For this study, we first report data for both estrogen-treated groups together (EST+) and then for EST ORAL and EST PHYSIOL groups separately. Neurocognitive tests were administered at baseline and 6 months following the respective intervention. We did not limit our assessments to times when subjects were on active estrogen only, given that animal data indicate that it is chronic (and not acute) estrogen administration that impacts cognitive parameters.¹²

Neurocognitive Tests

Intelligence: Wechsler Scale of Intelligence. To test for general intellectual ability, we assessed fluid and crystallized intelligence using Wechsler Scale of Intelligence (WASI)²⁹ subtests: (1) Matrix Reasoning: Subjects completed 35 gridded patterns by choosing the correct pattern out of 5 options, indicating fluid intelligence.²⁹ (2) Vocabulary Test: Subjects defined a total of 42 items. Their verbal responses

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Table 1. Participant Characteristics at Baseline Across Treatment Groups^a

| | EST- (n = 19) | EST+ (n = 29) | P (t Test) | EST PHYSIOL (n = 13) | EST ORAL (n = 16) | P (ANOVA) |
|-----------------------------|------------------|------------------|---------------|-------------------------|----------------------|--------------|
| Age at screen, y | 19.0 ± 0.6 | 20.4 ± 0.6 | .12 | 21.04 ± 0.84 | 19.95 ± 0.87 | .19 |
| Race, % white | 90 | 86 | .23 | 77 | 94 | .05 |
| Ethnicity, % non-Hispanic | 100 | 97 | .41 | 100 | 94 | .36 |
| Age at menarche, y | 13.4 ± 0.4 | 13.7 ± 0.5 | .66 | 14.5 ± 0.6 | 13.1 ± 0.6 | .16 |
| Total activity, h/wk | 9.73 ± 1.38 | 10.63 ± 1.15 | .63 | 8.70 ± 0.91 | 12.19 ± 1.88 | .26 |
| BMI, kg/m ² | 20.7 ± 0.5 | 20.5 ± 0.4 | .83 | 20.7 ± 0.6 | 20.3 ± 0.6 | .90 |
| % Ideal BMI | 97.2 ± 2.5 | 96.6 ± 2.5 | .86 | 95.2 ± 3.7 | 97.6 ± 3.5 | .88 |
| Weight change over 6 mo, kg | 1.2 ± 0.6 | 0.5 ± 0.7 | .51 | -0.2 ± 1.1 | 1.1 ± 0.8 | .47 |

^aValues are presented as mean ± SEM if not stated otherwise. Student *t* test and ANOVA were used to compare the means for EST+/EST- groups and EST PHYSIOL/EST ORAL/EST- groups, respectively, and *P* values are shown.

Abbreviations: ANOVA = analysis of variance, BMI = body mass index, EST- = no estrogen replacement, EST+ = estrogen replacement, EST ORAL = oral estrogen, EST PHYSIOL = transdermal estrogen.

were scored for their match with the definition in the manual,²⁹ measuring crystallized intelligence.

Verbal memory: California Verbal Learning Test—Second Edition. The California Verbal Learning Test—Second Edition (CVLT-II)³⁰ assesses verbal memory across several time delays. A 16-word list (List A) was read to the subject 5 times. Subjects were instructed to recall this from memory after each read-through (immediate recall; Trials 1–5). Then, a different (interference) list of words (List B) was read to the subjects and recalled by them (Trial B). Following this, participants were asked to recall words of List A (short-delay recall). Fifteen to 25 minutes later, long-delay recall took place, followed by cued recall, in which the 4 categories to which the words belonged were provided as cues. Finally, in a recognition test, participants indicated whether an item was on the original list or not. From these assessments, the following measures were calculated: (1) semantic clustering, which measures the ability to learn by organizing target words into categorical groups; (2) serial clustering, which is the ability to recall the words in the order presented; (3) repetition errors are words repeatedly recalled; (4) intrusion error scores sum up non-list words; (5) total recognition discriminability index is the ability to correctly identify words from List A relative to total false-positives, ie, recalled words that were not on List A. The test-retest reliability is high, in the range of 0.80–0.84 when testing with the same standard form after 1 month, with minimal practice effects.³¹

Executive control: Delis-Kaplan Executive Function System Color-Word Interference Test. Subjects were provided with a list of words representing color names (eg, blue) or patches, displayed in varying ink colors. They completed 4 test conditions: (1) Color Naming: participants named the color of 50 patches, providing an index of naming speed. (2) Word Reading: subjects read words representing color names (eg, blue) displayed in black, providing a measure of reading speed. (3) Response Inhibition: participants named the color of the ink of words representing colors printed in dissonant ink colors (eg, the word *blue* in red ink). Correctly naming the ink color requires overcoming the automatic response to the meaning of the word and measures the ability to inhibit responses. (4) Inhibition Switching: subjects named the

color of the ink. However, if words were framed by a box, subjects were instructed to read the word instead. This condition adds mental-set shifting demands for successful task completion, interacting with inhibition in exerting executive control. From Conditions 1 and 2, a combined general processing speed measure was calculated (combined naming and word completion time). For Conditions 3 and 4, completion time and accuracy were calculated (inhibition and inhibition-switching completion time and errors,

respectively). Eleven participants did not complete the inhibition-switching condition at the 6-month visit primarily due to time constraints, despite measures being taken to allocate sufficient time for study procedures (from subjects arriving late or technical issues with study procedures). For the Delis-Kaplan Executive Function System Color-Word Interference Test (D-KEFS CWLT),³² the test-retest reliability is high (range, 0.79–0.90) when tested with the same standard form after 9–74 days with minimal practice effects.³²

For both tests, we employed the same version at baseline and 6 months following careful consideration of the test-retest literature on the measures. For all measures, raw scores were first calculated followed by *T* scores using the respective manuals. Higher *T* scores corresponded with better performance.

Statistical Analysis

JMP Pro12 was used for analysis. Student *t* test and ANOVA were used for comparisons of continuous variables (2- and 3-group comparisons, respectively) after excluding occasional outliers that otherwise skewed the distribution considerably. The Tukey Kramer test was used to account for multiple comparisons when comparing > 2 groups. To determine associations of cognitive measures with possible covariates, we used Pearson correlations for all groups taken together (*n* = 48). Furthermore, we performed multivariate analysis to control for confounding variables such as age (as cognitive scores increase with increasing age³⁰) and baseline cognitive scores (to control for baseline functioning) when assessing the impact of estrogen administration on cognitive changes over 6 months. Finally, we performed exploratory analyses to determine whether there was any interaction of age with the treatment group or baseline scores. *P* values ≤ .05 were considered significant.

RESULTS

Participant Characteristics

Overall, subjects had a mean ± SEM age of 19.9 ± 3.1 years, had a mean ± SEM body mass index of 20.6 ± 2.3 kg/m², and participated in a total of 10.3 ± 5.9 hours per week of

Table 2. Baseline WASI, CVLT-II, and D-KEFS Color Word Interference Test Scores by Treatment Groups^a

| | EST- (n = 19) | EST+ (n = 29) | P (t Test) | EST PHYSIOL (n = 13) | EST ORAL (n = 16) | P (ANOVA) |
|--|------------------|------------------|---------------|-------------------------|----------------------|--------------|
| WASI | | | | | | |
| Matrix Reasoning | 54.47 ± 1.70 | 56.76 ± 1.13 | .25 | 57.77 ± 1.46 | 55.94 ± 1.68 | .40 |
| Vocabulary Test | 61.37 ± 1.83 | 61.24 ± 1.53 | .96 | 62.46 ± 2.03 | 60.25 ± 2.24 | .77 |
| CVLT-II | | | | | | |
| Trial 1 | 49.47 ± 2.62 | 47.93 ± 2.13 | .65 | 45.77 ± 2.52 | 49.69 ± 3.27 | .60 |
| Trial 5 | 51.05 ± 1.93 | 48.28 ± 1.94 | .34 | 48.46 ± 3.02 | 48.13 ± 2.62 | .63 |
| Trials 1–5 total | 55.58 ± 1.86 | 52.69 ± 1.97 | .32 | 53.92 ± 2.95 | 51.69 ± 2.70 | .51 |
| Trial B | 47.37 ± 3.02 | 48.79 ± 1.46 | .64 | 46.92 ± 2.30 | 50.31 ± 1.85 | .61 |
| Short-delay free recall | 52.37 ± 2.04 | 51.72 ± 2.11 | .84 | 55.00 ± 3.20 | 49.06 ± 2.71 | .31 |
| Long-delay free recall | 52.22 ± 2.29 | 51.43 ± 2.02 | .80 | 53.85 ± 2.60 | 49.33 ± 3.00 | .50 |
| Semantic clustering | 57.63 ± 3.23 | 53.28 ± 2.64 | .30 | 58.07 ± 4.75 | 49.38 ± 2.58 | .15 |
| Serial clustering | 49.47 ± 3.13 | 50.52 ± 2.35 | .79 | 44.62 ± 2.56 | 55.31 ± 3.31 | .08 |
| Total repetitions | 49.47 ± 2.91 | 49.83 ± 2.02 | .92 | 48.85 ± 3.36 | 50.63 ± 2.54 | .92 |
| Total intrusions | 49.74 ± 0.89 | 49.14 ± 1.03 | .68 | 49.23 ± 1.59 | 49.06 ± 1.39 | .92 |
| Total recognition discriminability | 52.63 ± 1.64 | 51.72 ± 1.72 | .72 | 51.92 ± 2.86 | 51.56 ± 2.18 | .93 |
| D-KEFS Color Word Interference Test | | | | | | |
| Combined naming + reading time | 54.61 ± 1.14 | 51.76 ± 1.92 | .28 | 51.77 ± 2.75 | 51.75 ± 2.74 | .56 |
| Inhibition completion time | 56.94 ± 1.37 | 52.86 ± 2.10 | .16 | 53.69 ± 2.86 | 52.19 ± 3.10 | .35 |
| Inhibition error analysis | 54.22 ± 1.83 | 52.52 ± 1.19 | .42 | 49.85 ± 1.91 | 54.69 ± 1.30 | .12 |
| Inhibition-switching completion time | 55.15 ± 1.75 | 54.00 ± 1.47 | .63 | 54.27 ± 2.26 | 53.79 ± 2.01 | .88 |
| Inhibition-switching error analysis | 54.00 ± 1.65 | 53.84 ± 1.25 | .94 | 53.18 ± 2.54 | 54.36 ± 1.09 | .89 |

^aValues are presented as mean ± SEM. Student t test and ANOVA were used to compare the means for EST+/EST- groups and EST PHYSIOL/EST ORAL/EST- groups, respectively, and P values are shown. There were no significant differences between the EST+ and EST- groups and the EST-, EST PHYSIOL, and EST ORAL groups at baseline.

Abbreviations: ANOVA = analysis of variance, CVLT-II = California Verbal Learning Test—Second Edition, D-KEFS = Delis Kaplan Executive Function System, EST- = no estrogen replacement, EST+ = estrogen replacement, EST ORAL = oral estrogen, EST PHYSIOL = transdermal estrogen, WASI = Wechsler's Scale of Intelligence.

weight-bearing activities of their lower limbs. EST+ and EST- groups did not differ for age, race, ethnicity, menarcheal age, hours per week of physical activity, BMI, percentage of ideal BMI, or weight change over the 6-month intervention period (Table 1). Six of the 19 OA from the EST- group resumed normal menses with no treatment during follow-up.

Neurocognitive Tests

Baseline scores for WASI Matrix Reasoning and Vocabulary Test, CVLT-II, and D-KEFS-CWIT did not differ between groups (Table 2). When examining associations of these scores with the potential confounding variables of age and BMI, WASI vocabulary test scores ($r = 0.38$, $P = .008$) and CVLT-II total repetition scores ($r = 0.29$, $P = .046$) had significant correlations with age. There were no associations of WASI scores, CVLT-II, or D-KEFS-CWIT with BMI.

Table 3 shows changes in WASI, CVLT-II, and D-KEFS-CWIT scores from baseline to 6 months in EST+ and EST- groups when these measures differed between groups. Changes in WASI Matrix Reasoning and Vocabulary Test scores did not differ across groups (Supplementary eTable 1).

Improvement in immediate recall as assessed by CVLT-II Trial 1 and Trials 1–5, over 6 months of intervention, was greater in EST+ than EST- ($P = .01$) (Table 3). After controlling for (1) baseline scores and (2) baseline scores and age (Figure 1), the improvement in Trial 1 and Trial B scores became more pronounced in the EST+ compared with EST- group ($P < .05$). Serial clustering scores also improved in the EST+ group ($P = .01$) compared with EST- group, but group differences did not persist after controlling for baseline

scores and age. Groups did not differ for short- or long-delay recall, semantic clustering, total intrusions, repetition, and total discriminability scores.

Changes in D-KEFS-CWIT measures over 6 months did not differ between EST+ and EST- groups (Table 3). However, after controlling for baseline measures, changes in inhibition-switching completion time ($P = .06$) and inhibition-switching error analysis scores ($P = .07$) trended toward significance. On inclusion of age and baseline scores in the multivariate model, EST+ had greater improvements in inhibition-switching completion time over 6 months than EST- ($P = .01$) (Figure 1).

Finally, we compared effects of treatment in EST ORAL versus EST PHYSIOL versus EST- groups. Improvements in CVLT-II Trial 1 and Trials 1–5 scores were greater in EST PHYSIOL compared to EST- groups ($P < .05$), and the differences persisted after controlling for baseline scores ± age for Trial 1 scores ($P \leq .05$). D-KEFS-CWIT changes over 6 months did not differ between groups for unadjusted scores and scores adjusted for baseline performance. After controlling for age and baseline scores, EST PHYSIOL had greater improvements in inhibition-switching completion scores than EST- ($P = .04$) with no difference between EST ORAL and EST-.

For most variables, there was no significant interaction of age with treatment group (EST+ and EST-), randomization group (EST PHYSIOL, EST ORAL, EST-), or baseline scores. The only variable that improved with estrogen administration for which there was a significant interaction of age with randomization group was the change in Trials

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Table 3. Changes in CVLT-II and D-KEFS Color Word Interference Test Scores Over 6 Months in Estrogen-Replaced Versus Nonreplaced Groups^{a,b}

| | EST+ (n=29) | EST- (n=19) | Effect Size (95% CI) | P | P ^c | EST PHYSIOL (n=13) | EST ORAL (n=16) | EST PHYSIOL vs EST- Effect Size (95% CI) | EST PHYSIOL vs ORAL Effect Size (95% CI) | EST ORAL vs EST- Effect Size (95% CI) | P | P ^c |
|--------------------------------------|----------------|----------------|-----------------------------|------------|----------------|-----------------------|--------------------|---|---|--|-------------|----------------|
| CVLT-II | | | | | | | | | | | | |
| Immediate recall (Trial 1) | 13.79 ± 2.55 | 3.95 ± 2.77 | 9.85 (2.05 to 17.64) | .01 | .02 | 19.58 ± 3.17 | 11.25 ± 3.31 | 15.64* (4.70 to 26.57) | 8.33 (-2.99 to 19.65) | 7.30 (-2.76 to 17.36) | .005 | .04 |
| Immediate recall (Trial 5) | 3.62 ± 2.13 | 5.26 ± 2.15 | -1.64 (-8.00 to 4.72) | .61 | .24 | 6.25 ± 2.55 | 3.13 ± 3.02 | 0.99 (-8.18 to 10.15) | 3.13 (-6.37 to 12.62) | 2.14 (-6.30 to 10.57) | .70 | .47 |
| Immediate recall (Trials 1-5) | 11.75 ± 1.69 | 5.84 ± 1.13 | 5.91 (1.35 to 10.47) | .01 | .10 | 14.00 ± 2.38 | 10.06 ± 2.34 | 8.16** (1.41 to 14.90) | 3.94 (-3.05 to 10.92) | 4.22 (-1.98 to 10.43) | .02 | .21 |
| Immediate recall (Trial B) | 3.97 ± 1.69 | -1.05 ± 1.93 | 5.02 (-0.24 to 10.27) | .06 | .01 | 5.38 ± 2.23 | 2.81 ± 2.50 | 6.44 (-1.31 to 14.19) | 2.57 (-5.47 to 10.61) | 3.87 (-3.44 to 11.17) | .13 | .04 |
| D-KEFS | | | | | | | | | | | | |
| Inhibition error analysis | 2.19 ± 0.79 | -0.11 ± 1.96 | 2.30 (-1.48 to 6.09) | .23 | .22 | 4.92 ± 1.65 | 0.23 ± 0.51 | 5.03 (-0.55 to 10.62) | 4.69 (-1.33 to 10.71) | 0.34 (-5.25 to 5.93) | .08 | .47 |
| Inhibition-switching completion time | 4.46 ± 0.99 | 1.08 ± 1.40 | 3.38 (-0.16 to 6.92) | .05 | .06 | 3.90 ± 1.07 | 3.92 ± 1.34 | 2.85 (-1.54 to 7.24) | 0.02 (-4.69 to 4.73) | 2.82 (-1.89 to 7.53) | .22 | .14 |
| Inhibition-switching error analysis | 0.39 ± 1.09 | -2.33 ± 1.79 | 2.72 (-1.32 to 6.77) | .18 | .07 | -0.70 ± 1.50 | 1.23 ± 1.56 | 1.63 (-4.27 to 7.54) | 1.93 (-3.87 to 7.73) | 3.56 (-1.95 to 9.08) | .30 | .19 |

^aValues are presented as mean ± SEM. Student *t* test and analysis of variance were used for initial comparisons (unadjusted *P* values reported).

^bBoldface indicates statistical significance.

^cAdjusted for baseline score.

P* = .003, *P* = .01 after Tukey Kramer adjustment for multiple comparisons.

Abbreviations: CVLT-II = California Verbal Learning Test—Second Edition, D-KEFS = Delis Kaplan Executive Function System, EST- = no estrogen replacement, EST+ = estrogen replacement, EST ORAL = oral estrogen groups, EST PHYSIOL = transdermal estrogen.

1-5 scores for CVLT. However, after controlling for baseline scores, changes in this variable over time did not differ among the groups, and adding the interaction term to the model did not alter these results.

DISCUSSION

While studies have examined the effect of estrogen replacement on cognition in other estrogen-deficient states such as postmenopausal women and women with Turner syndrome, OA have not been examined for cognitive deficits with respect to their estrogen status. Our study is the first to assess the impact of estrogen replacement on cognitive measures in young female OA. Estrogen replacement is not the standard of care for athletes with functional hypothalamic amenorrhea.³³ In fact, estrogen replacement is not advised until athletes fail to respond to nonpharmacologic therapy for at least 1 year or until they develop significant fractures with low bone density.³³ In our study, we used estrogen as a physiological probe to investigate the cognitive domains that may be altered in athletes who have estrogen deficiency. We show that immediate free recall, a verbal memory measure, improved significantly following estrogen replacement in OA compared to no treatment.

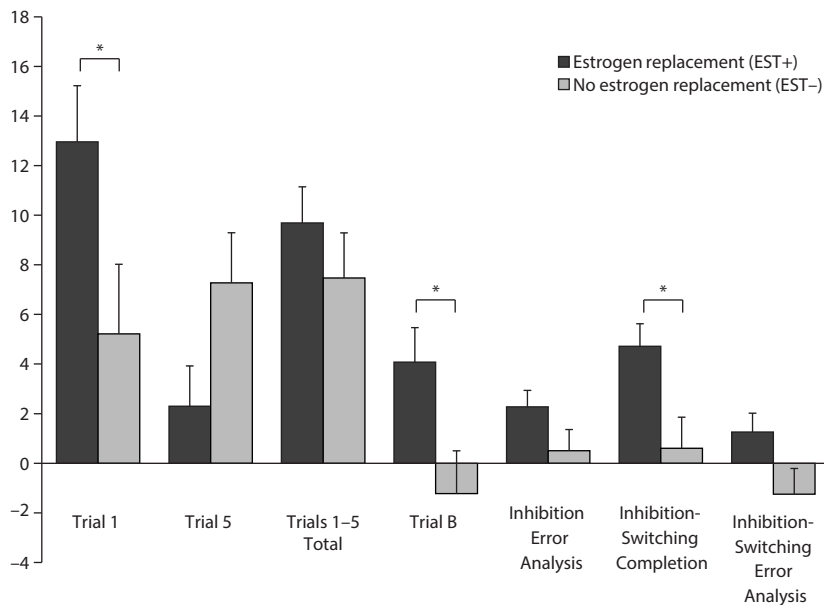
Female athletes in a state of relative energy deficit may develop hypothalamic amenorrhea from suppression of GnRH pulsatility subsequent to changes in hormones such as ghrelin, leptin, and cortisol.³⁴⁻³⁶ The consequence of estrogen replacement on cognitive performance of OA (who have the mitigating influence of exercise activity) has never been studied. Additionally, a study in adult women with adolescent-onset anorexia nervosa, another model of hypothalamic amenorrhea, has reported cognitive dysfunction, which improves with menstrual recovery or oral estrogen replacement, but not with weight recovery alone.³⁷ Similar to this study, we demonstrate that athletes with hypothalamic amenorrhea have improved immediate free recall after 6 months of estrogen replacement compared to no treatment.

The major setback to estrogen replacement came from the National Institutes of Health-initiated Women's Health Initiative study in postmenopausal women that showed no improvement in global cognitive decline with conjugated equine estrogen administration. Furthermore, in women > 65 years, cognitive function worsened with estrogen replacement.^{38,39} In retrospect, the age of the participants and use of conjugated equine estrogen compared to natural estrogens such as 17- β -estradiol used in more recent trials⁴⁰ were thought to have impacted study results. With more promising results from recent studies,⁴⁰⁻⁴² estrogen has reemerged as a potential positive modulator of cognitive function.

Our findings are consistent with the cognition-enhancing effects of estrogen demonstrated previously in animal and human models of estrogen deficiency. Ovariectomized rats treated with estradiol made more correct choices in the water maze test, suggesting that estrogen has a protective effect of working memory.^{1,11,12} In our analysis, verbal memory, assessed by the CVLT-II, improved for immediate-recall scores (Trial 1 and Trial B) after 6 months of estrogen replacement, even after controlling for baseline scores. Girls with Turner syndrome (similar to our population for age and estrogen-deficiency) have decreased intelligence and deficits in executive function, nonverbal memory, and attention, but verbal performance is preserved.^{4,6} It is unclear whether these deficits in Turner syndrome are consequent to the underlying genetic defect or estrogen deficiency. However, estrogen replacement in these subjects results in improved motor speed of nonverbal processing.^{2,43} Of note, in

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Figure 1. Changes in Cognitive Scores in Estrogen-Replaced and No-Therapy Groups After Adjusting for Age and Baseline Scores^a



^aThe figure shows the changes in CVLT-II and D-KEFS Color Word Interference test scores over 6 months adjusted for age and baseline value by treatment group. Immediate recall scores (Trial 1 and Trial B) and scores for cognitive flexibility assessed by inhibition-switching completion time were significantly improved in the EST+ compared with the EST- groups.

* $P < .05$.

Abbreviations: CVLT-II = California Verbal Learning Test—Second Edition, D-KEFS = Delis Kaplan Executive Function System, EST- = no estrogen replacement, EST+ = estrogen replacement.

our study, intelligence did not differ across treatment groups and cannot account for observed changes in memory and executive control following estrogen replacement.

In an older population, women aged 49–68 years who received 17- β -estradiol treatment for a year demonstrated an improvement in verbal memory.⁴⁰ Despite multiple positive studies^{3,40–42} supporting the role of estrogen in augmenting verbal memory, one study in 200 postmenopausal women randomized to testosterone, estrogen, or placebo found no effects of estrogen replacement on this cognitive measure.⁴⁴ However, the very short duration of estrogen replacement in this study (just 4 weeks) may have resulted in these negative findings. In fact, Luine et al¹² showed in a rodent study that only chronic estrogen replacement (3 vs 12 days) leads to improved performance in the radial maze test with a higher number of correct choices, indicating that duration of treatment may impact the cognitive effects of estrogen. In our study, we evaluated cognitive outcomes after 6 months (thus a longer duration) of estrogen replacement than in the study by Kocoska-Maras et al.⁴⁴ Of note, studies such as those by Asthana et al³ and Berent-Spillon et al⁴¹ have been able to demonstrate significant improvements in cognition in humans with estrogen replacement for a duration of 8 weeks and 3 months, respectively. While our data are consistent with animal studies, it is still not clear why effects are evident only after chronic use. Future studies may be necessary to determine the shortest duration of estrogen therapy that will result in changes in cognitive tests.

Another finding that merits discussion is the improvement in executive control (assessed by the inhibition-switching completion time) following estrogen replacement. Although the EST+ group performed better for inhibition-switching completion times and inhibition-switching error, this finding was not statistically significant even after controlling for baseline performance. Nevertheless, when adjusted for age, the inhibition-switching completion time was shortened in the EST+ group. The small number of subjects in the groups and missing entries for the inhibition-switching task might have contributed to this lack of significance. Although it is well recognized that verbal memory is the single most common domain altered with hormonal replacement,²⁰ our study opens an interesting area of executive control to be explored in future studies. Consistent with our results, ovariectomized monkeys treated with estrogen performed better on learning to shift to a new cognitive set in the Wisconsin Card Sort Task, a measure of the cognitive flexibility

feature of executive control.⁴⁵ Furthermore, Colzato et al⁴⁶ demonstrated that the inhibition of return effect, which reflects the ability to delay attention from returning to a previously attended location, hence a measure of cognitive flexibility, was high in the late follicular phase in women, when estradiol levels are higher compared with luteal and menstrual phases.

Variables known to affect results of estrogen replacement in previous studies in postmenopausal women were the type of estrogen used, timing of initiation of estrogen replacement after menopause, and duration of estrogen replacement. Studies that used 17- β -estradiol reported better results than those using conjugated equine estrogen.^{20,40} Further, the longer the duration of estrogen deficiency, the less was the response of neural tissues to estrogen replacement.^{47–49} Our study provided estrogen replacement for a period of 6 months, a reasonable duration to demonstrate significant effects.^{41,50} We used oral ethinyl estradiol with progesterone or the 17- β -estradiol transdermal patch with progesterone to reproduce a more physiologic form of estrogen replacement. For immediate recall and cognitive flexibility, subjects randomized to transdermal estradiol performed better than those who received no treatment after controlling for baseline scores and age. These associations merit further investigation in a larger group of subjects. Of note, a study conducted in 9 academic centers across the United States on 693 postmenopausal women using transdermal estradiol ($n = 211$) did not show significant changes for verbal memory

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(using CVLT-II) and executive control (using a Stroop color interference test) when compared with oral conjugated estradiol or placebo.²¹ Of studies that used transdermal estrogen replacement, Asthana et al³ and Joffe et al⁴² showed positive cognitive responses with estradiol, while Dunkin et al¹⁸ showed neutral findings.

Of note, the 2 modes of estrogen administration utilized different estrogen molecules. Although we attempted to minimize the difference by providing similar dose equivalents of estrogen (100 µg of estradiol patch ~30 µg of ethinyl estradiol), oral contraceptives typically have ethinyl estradiol, a synthetically derived estrogen, whereas 17-β estradiol used in the patch is the physiological form of estrogen. The 30 µg of ethinyl estradiol used in our study in the oral estrogen arm is the most common form of estrogen used in oral contraceptives, and the study was designed to use an oral estrogen preparation that is commonly used in adolescents and young adults and is also commonly prescribed by practitioners to regulate menstrual cycles in athletes.⁵¹ However, there could be differences stemming from the variation in the type of estrogen molecule as well as the route of administration. Further, for this study, we used a monophasic estrogen preparation (that does not mimic estrogen changes across a menstrual cycle), and future studies are necessary to determine whether the use of a biphasic or triphasic estrogen preparation that more closely mimics cyclic changes in estrogen levels across a menstrual cycle results in a different outcome.

Our sample size was based on other studies that have used neurocognitive testing in subjects with estrogen deficient states, such as girls with Turner syndrome and postmenopausal women.^{3,4} Although our sample size is not large, it compares well with other studies such as that of Asthana et al,³ which reported a significant improvement in verbal memory in 12 postmenopausal women following estrogen replacement. A limitation of the study is that we do not have data for the total duration of estrogen deficiency, because athletes may have periods of amenorrhea interspersed with periods of oligomenorrhea/amenorrhea or eumenorrhea depending on the training season and kind of athletic activity. Also, it was difficult to accurately assess the degree of adherence to study medications given that ethinyl estradiol (in oral pills) cannot be assessed with standard assays. However, we did use tools such as a

medication calendar and returned pill packs and backings of patches to determine adherence. Although subjects with active anorexia nervosa were excluded, subjects with a past history of anorexia nervosa or active bulimia were not excluded, and we acknowledge this as a study limitation. Further, the impact on cognitive function of local estrogen produced in the brain versus estrogen produced in the ovaries remains unclear. Estradiol is known to cross the blood-brain barrier,⁵² and changes in systemic estradiol levels (as observed in postmenopausal women and those with hypogonadal conditions such as Turner syndrome known to impact cognition) may alter local estradiol production in the brain through their effect on GnRH neurons.⁵³ Studies are needed to delineate the effects of peripheral versus locally produced estradiol on cognitive indices and to assess the impact of gonadal steroids on mood outcomes, as changes in mood can affect cognitive indices.⁵⁴

Data regarding effects of estrogen on cognition and memory in humans have thus far been inconclusive and are lacking in adolescents and young adults with estrogen deficiency, except for a few studies in Turner syndrome. Our investigation supports the cognition-enhancing role of estrogens in another population that presents with estrogen deficiency early in life. In this study, we have used exogenous estrogen as a physiologic probe rather than as a treatment strategy. Importantly, our data provide the first clue that estrogen replacement may improve cognitive function in oligomenorrheic/amenorrheic athletes. However, further studies exploring the differences in cognitive indices following estrogen replacement in athletes, particularly focusing on verbal memory and executive control, are warranted.

CONCLUSION

Female oligomenorrheic/amenorrheic athletes show improvements in immediate-recall memory and cognitive flexibility with estrogen replacement, which supports the role of gonadal steroids in regulation of higher cognitive functions. These youth are in the prime of their neural and behavioral development, and the impact of estrogen deficiency on cognition in this population needs further exploration.

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Supplementary material: See accompanying pages.

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Editor's Note: We encourage authors to submit papers for consideration as a part of our Focus on Women's Mental Health section. Please contact Marlene P. Freeman, MD, at mfreeman@psychiatrist.com.

Supplementary material follows this article.



Supplementary Material

Article Title: Estrogen Replacement Improves Verbal Memory and Executive Control in Oligo-Amenorrheic Athletes in a Randomized Controlled Trial

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List of Supplementary Material for the article

1. [eTable 1](#) Changes in WASI, CVLT-II, and D-KEFS Color Word Interference Test Scores by Treatment Group

Disclaimer

This Supplementary Material has been provided by the author(s) as an enhancement to the published article. It has been approved by peer review; however, it has undergone neither editing nor formatting by in-house editorial staff. The material is presented in the manner supplied by the author.

Supplementary eTable 1. Changes in WASI, CVLT-II, and D-KEFS Color Word Interference Test Scores by Treatment Group

| T-score | EST- (n=19) | EST+ (n=29) | Effect Size EST+ vs. EST- | P | P* | EST PHYSIOL (n=13) | EST ORAL (n=16) | Effect size PHYSIOL vs. EST- | Effect size PHYSIOL vs. ORAL | Effect size ORAL vs. EST- | P | P* |
|------------------------------------|----------------|----------------|---------------------------------|-------------|------|--------------------------|--------------------|------------------------------------|------------------------------------|---------------------------------|------|------|
| WASI | | | | | | | | | | | | |
| Vocabulary | -0.21±1.44 | -0.52±0.84 | -0.31 (-3.45,2.83) | 0.85 | 0.80 | 0.00±1.15 | -0.94±1.24 | 0.21 (-4.44,4.86) | 0.93 (-3.89, 5.76) | 0.73 (-3.66, 5.11) | 0.88 | 0.61 |
| Matrix Reasoning | 1.21±1.21 | 0.07±0.90 | -1.14 (-4.13, 1.84) | 0.45 | 0.60 | 0.23±0.99 | -0.06±1.45 | 0.98 (-3.45,5.41) | 0.29 (-4.30, 4.88) | 1.27 (-2.9,5.45) | 0.74 | 0.86 |
| CVLT-II | | | | | | | | | | | | |
| Short-Delay Free Recall | 4.21±1.68 | 5.00±1.66 | 0.78 (-4.18, 5.75) | 0.74 | 0.81 | 2.69±2.44 | 6.88±2.23 | 1.52 (5.70, 8.74) | 4.18 (-3.31,11.67) | 2.66 (-4.15,9.48) | 0.39 | 0.80 |
| Long-Delay Free Recall | 2.35±1.22 | 4.44±1.42 | 2.09 (-2.02,6.21) | 0.31 | 0.57 | 4.23±2.03 | 4.64±2.06 | 1.88 (-4.09,7.85) | 0.41 (-5.83, 6.65) | 2.29 (-3.56,8.14) | 0.59 | .80 |
| Semantic Clustering | 8.42±3.15 | 11.55±3.14 | 3.13 (-6.22,12.49) | 0.50 | 0.66 | 11.54±4.21 | 11.56±4.67 | 3.12 (-10.77,17.0) | 0.02 (-14.38,14.43) | 3.14 (-9.95,16.23) | 0.80 | .86 |
| Serial Clustering Bidirectional | -4.72±1.37 | 1.40±1.65 | 6.12 (1.55, 10.69) | 0.01 | 0.28 | -0.42±2.17 | -3.44±4.07 | 4.31 (-5.63, 14.24) | 3.02 (7.16,13.20) | 1.28 (-7.87,10.44) | 0.57 | 0.51 |
| Total Repetitions | 2.78±2.11 | 4.14±2.18 | 1.36 (-5.15, 7.87) | 0.68 | 0.61 | 4.62±3.65 | 5.00±2.58 | 1.84 (-7.53,11.21) | 0.38 (-9.37,10.14) | 2.22 (-6.78,11.22) | 0.81 | 0.88 |
| Total Intrusions | -0.26±1.85 | -0.34±1.29 | -0.08 (-4.48,4.32) | 0.97 | 0.85 | -1.15±2.48 | 0.31±1.25 | 0.89 (-5.62,7.40) | 1.47 (-5.29,8.22) | 0.58 (-5.56,6.71) | 0.87 | 0.86 |
| Total Recognition Discriminability | 0.79±1.88 | -0.77±1.17 | -1.56 (-5.81,2.69) | 0.46 | 0.87 | 0.91±0.91 | 0.00±2.29 | 0.12 (-6.84,7.08) | 0.91 (-6.39,8.21) | 0.79 (-5.56,7.14) | 0.94 | 0.89 |
| D-KEFS | | | | | | | | | | | | |
| Combined Naming Reading | 0.72±1.04 | 0.76±0.95 | 0.04 (-2.91,2.99) | 0.98 | 0.78 | 1.23±1.19 | 0.38±1.46 | 0.51 (-3.84,4.86) | 0.86 (-3.61,5.32) | 0.35 (-3.76,4.45) | 0.90 | 0.86 |
| Inhibition Completion Time | 1.28±1.08 | 3.27±0.89 | 1.99 (-0.83,4.82) | 0.16 | 0.40 | 3.00±1.66 | 3.50±0.92 | 1.72 (-2.46,5.90) | 0.50 (-3.91,4.91) | 2.22 (-1.78,6.22) | 0.37 | 0.54 |

Supplementary eTable 1 provides the changes in the WASI, CVLT-II and D-KEFS scores at 6 months shown by treatment group. Student t test and ANOVA were used for initial comparisons (unadjusted p values reported). P*: p-value adjusted for baseline scores.

Abbreviations: CVLT-II: California Verbal Learning Test, Edition II, D-KEFS: Delis Kaplan Executive Function System, EST-: No estrogen replacement, EST+: Estrogen replacement, EST ORAL: Oral estrogen groups and EST PHYSIOL: Transdermal estrogen, WASI: Wechsler's Scale of Intelligence.