0013-7227/02/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 87(7):3090–3096

Cognitive Effects of Short-Term Manipulation of Serum Sex Steroids in Healthy Young Men

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We examined the effects of sex steroids on cognitive functioning by exogenously manipulating circulating T levels in a group of healthy young men. Thirty-two men were randomized to receive 8 wk of treatment including: 1) im T enanthate 100 mg/wk plus daily oral placebo (T); 2) im placebo/wk plus 125 μg daily oral levonorgestrel (LNG); 3) im T enanthate 100 mg/wk plus 125 μg daily oral LNG (T + LNG); 4) im placebo/wk plus daily oral placebo. Cognitive functions were assessed at baseline and twice during treatment. Serum T and E2 levels were significantly increased in the T and T + LNG groups compared with baseline ($P < 0.01$) and T levels were significantly decreased in the LNG group ($P < 0.05$). Verbal memory significantly decreased in the LNG group ($P < 0.01$) and was maintained by coadministration of T in the T + LNG group. Divided attention was unaffected in the LNG group but improved significantly in the T + LNG group. In summary, decreased serum T levels induced by LNG or direct effects of the progestin, LNG, adversely affects verbal memory in normal young men. These results suggest that short-term changes in sex steroid levels have effects on cognitive function in healthy young men. (*J Clin Endocrinol Metab* 87: 3090–3096, 2002)

IN HEALTHY YOUNG men, significant associations have been found between circulating or endogenous T levels and cognitive abilities. Positive relationships have been reported between endogenous T levels and visuospatial orientation, spatial form comparison, composite visuospatial scores, and tactual spatial tasks (1–6). However, endogenous serum T levels in the low normal range have also been found to be associated with better performance on spatial ability tasks in men (7–10), and some studies have failed to find any relationship between circulating androgen levels and visuospatial abilities (7, 11).

Studies of exogenous T administration have demonstrated mixed results. Several well controlled studies have found improved spatial abilities and spatial memory (12–14), and others have failed to find cognitive improvements in men (15, 16). It has been suggested that beneficial effects of T on cognition may occur within a specified, or optimal range and this may partially account for mixed findings of previous studies. For example, Gouchie and Kimura (8) found that men with low endogenous serum T levels performed better on measures of spatial and mathematical ability compared with men with high endogenous T levels. The opposite pattern was found in women who demonstrated better performance on these tasks with high endogenous T levels compared with low T levels. Moffat and Hampson (9) also found that endogenous total T levels demonstrated a curvilinear relationship with spatial ability. Thus, it is possible that beneficial effects of hormones may occur within an optimal range, and androgen effects may be gender specific. In this study, we examined the effects of sex steroids on cognitive functioning by exogenously increasing or decreasing circulating T levels in a group of healthy young men. T levels were increased using T enanthate and decreased by the administration of levonorgestrel (LNG), a progestin to suppress gonadotropin and T secretion.

In addition, we examined potential changes in cognition in response to a typical contraception regime of T enanthate plus LNG. Cognition was measured with a comprehensive neuropsychological testing battery. We hypothesized that increasing serum T levels would have beneficial effects on spatial memory, whereas decreased serum T levels would have detrimental effects on cognition, and in particular spatial memory.

Materials and Methods

Participants

Participants were healthy young men between the ages of 21 and 46 yr recruited from the community through print advertisement and radio. The study protocol was approved by the University of Washington Human Subjects Review Committee, and approved informed consent procedures were followed. Potential participants who responded to advertisements were asked to attend an informational meeting. Next, potential participants were asked to come to a screening visit that included a thorough physical examination and serum laboratory evaluation to exclude any significant medical illness. Inclusion criteria were absence of major medical illness and normal physical examination; the absence of current use of prescription medications and normal basal serum total T, FSH, and LH levels. Exclusion criteria included any history of significant recent or chronic mental illness, alcohol abuse, anabolic steroid use, or reproductive dysfunction. Thirty-three men were eligible to participate. One participant was subsequently excluded from the analysis due to initiation of CNS active medication (Ritalin) during the study.

Study design

Eligible participants were randomly assigned to one of four treatment groups: 1) im T enanthate 100 mg/wk plus daily oral placebo (T); 2) im

Abbreviations: LNG, Levonorgestrel; PL, placebo; SALT, spatial array learning test.

placebo (sterile sesame oil)/wk plus 125 μg daily oral LNG; 3) im T enanthate 100 mg/wk plus 125 μg daily oral LNG (T + LNG); 4) im placebo (sterile sesame oil)/wk plus daily oral placebo (PL). The treatment groups were expected to reach supraphysiologic T levels in the T and T + LNG groups (at time of testing 24–48 h after injection) and very low or undetectable levels in the LNG group. Medication dose levels used in the current study were determined by prior research in this area from our group. In a contraceptive study, a lower dose LNG and T combination effectively suppressed spermatogenesis and circulating gonadotropin levels with fewer metabolic effects than higher dose combinations (17). In that study, LNG levels were obtained in men taking 125 and 250 μg LNG daily by mouth in addition to 100 mg T ethanate im weekly. The LNG serum levels in the 125 μg group were 0.1 μg/liter before dosing and 0.3 μg/liter 1 h after LNG dosing and serum LH and FSH were suppressed around zero IU/liter. Serum LH and FSH remained suppressed for up to 6 months with combined T + LNG treatment. Although T and E2 were not measured, with LH and FSH effectively suppressed endogenous T and E2 production would likely be suppressed as well. With regard to serum T levels, our group has demonstrated that 24–48 h after injection of 100 mg T ethanate, T levels are just above the upper limit of normal (supraphysiologic), but return to normal at the end of the weekly injection interval (18). The normal range for young men is 8.7–35 nmol/liter. Thus, T and E2 levels were expected to be increased to supraphysiologic levels in the T and T + LNG groups (at time of testing 24–48 h after injection), and significantly decreased or undetectable in the LNG group and in the normal range for PL group.

Thirty-three healthy young men who met screening criteria reported to the Veterans Administration Puget Sound Health Care System for weekly im injections of 100 mg T enanthate (Delatestryl, Manufactured for BTG Pharmaceuticals Corp. by Bristol-Myers Squibb Co., Princeton, NJ) or placebo (sterile sesame oil). LNG was provided as a gift from Wyeth-Ayerst Laboratories, Inc. (Madison, NJ) Participants were an average age of 33 yr (±8 yr) with a mean of 15 yr of education (±2 yr). Assignment for each consecutively enrolled participant was made by a research pharmacist using a predetermined assignment sheet created using a random number generator. Psychometrists, nurses, and investigators were blind to treatment conditions. The groups did not differ on demographic variables (age and education). Cognitive testing was conducted at screening visit (prebaseline), baseline, and repeated at wk 2 and 8 of treatment (Fig. 1). The screening or prebaseline cognitive testing was not used in the analysis and is included in the study design to help reduce practice effects. Testing sessions occurred within 24–48 h following T or placebo injection to capture peak T levels and blood samples were taken at the time of cognitive testing to measure serum T and E2. Therefore both serum T and E2 levels represent peak hormone levels for on treatment time points. Eight participants were randomized into the T group, seven to the LNG group, nine to the LNG+T group, and eight to the P group. Endogenous T levels measured at baseline, before the start of the study were in the normal range for healthy young men and were not significantly different between groups.

Outcome measures

The primary outcome measure was a battery of cognitive tests assessing spatial and verbal memory and attention. Comparable alternative test versions were used for each testing session including the prebaseline screening session. Order of test forms was assigned randomly and counter balanced to ensure comparability across participants. Psychometrists and participants were blind to the treatment condition.

Spatial memory measures

1) Route Test. This test measured the ability to navigate a short route within a room and is based on previous work by Barrash (19–21) and Cherrier (14, 22). Reliability and validity has been established in a population of healthy control and brain damaged subjects (19, 21). A route pattern is indicated with a red ribbon on a large piece of flooring that contains a grid pattern. Once the participant walks the indicated path, the ribbon is removed and the path is recalled from memory. A total of six trials are administered, and the total number of correctly sequenced grids are recorded for a total of 66 possible points.

2) Spatial Array Learning Test (SALT). This measure of spatial memory was adapted from the visual spatial learning test by Malec *et al.* (23). Participants were briefly shown seven unique figures in a particular pattern placed on a grid and asked to recall the correct figure and location. This is repeated for a series of five trials. The total number of figures placed in the correct location was recorded. A total perfect score for immediate recall equals 35 points. Reliability and validity of the VSLT is good (24, 25).

Verbal memory measures

1) Proactive Interference. As adapted from Moscovitch (26), participants listened to a list of 12 words from the same semantic category (*e.g.* articles of clothing), and then recalled as many of these words as possible. The procedure was repeated for a total of 4 trials followed by a trial of words from a new category (*e.g.* fruits). Normal adults recall progressively fewer words across trials 2 through 4 due to the build-up of interference from the semantically similar preceding items. Reliability of the test is generally good, including validity studies conducted with brain damaged patients and controls (27) including alternate versions (28–30). The total number of words recalled for each list was recorded for a total

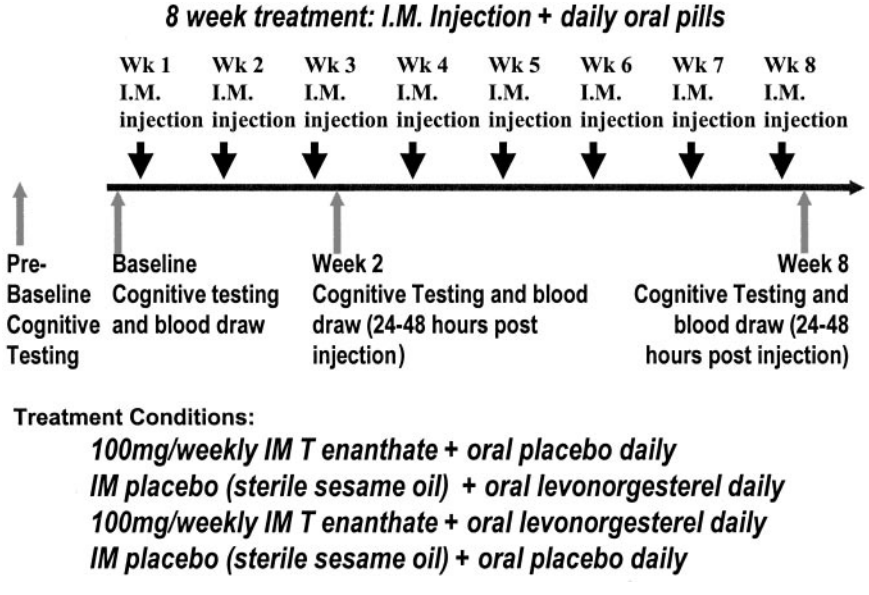

FIG. 1. Outline of study procedures.

possible score of 60. For the present study, total number of words recalled across the first four lists was analyzed. Thus, the amount of words recalled across four successive word lists is a measure of verbal memory rather than a measure of semantic interference.

2) Story Recall. Participants listened to two brief narratives (stories) and were asked to recall as much as possible immediately after hearing each story and following a 20-min delay. This test is based on the Wechster Memory Scale-Revised logical memory subtest and reliability and validity of this modified version are very good (28–32). Number of individual bits of information recalled is recorded for a total possible score of 88 across both stories.

Divided attention measure

Stroop Color Word Interference Task. This task based on the original Stroop test used three trials for which total reading time and errors were recorded (33). In the first condition, participants were asked to read 100 color words (red, green, blue), followed by identification of color blocks (color) followed by (interference) reading the color of the ink and ignoring word (*e.g.* the word "blue" printed in green letters). The test has demonstrated good reliability and validity when examined in closed head injured individuals compared with controls (27) and reliability and validity of this modified version are very good (28–30).

Hormone assays. Cognitive testing was performed 24–48 h following injection and hormone levels were drawn immediately after the testing session. Samples were kept frozen at −70 C until the completion of the study when all samples were assayed. The total T assay was conducted using a standard DELFIA T kit, Wallac, Inc. (Boston, MA) with sensitivity of 0.5nmol/liter and 4.5% coefficient of variation for intra assay variability and 9.5% coefficient of variation for interassay variability. The normal range for young men is 8.7–35 nmol/liter. E2 was measured with a standard DSL-39100 kit from Diagnostics Systems Laboratories, Inc. (Webster, TX) with 0.6 pg/ml sensitivity and 3.6% coefficient of variation for intraassay variability and 6.03% coefficient of variation for interassay variability. Samples from each participant were run in duplicate in the same assay to avoid interassay variability. Hormone levels reported were the hormone values taken at the time of cognitive testing and are the average of the duplicate samples. Cognitive testing sessions were scheduled at numerous time points throughout the day (morning, afternoon, and evening). For subsequent testing sessions (wk 2 and 8) participants were scheduled at the same time of day. Thus, hormone levels are variable with regard to time of day between participants, but consistent within each individual participant across weeks. Serum samples were not obtained for four participants (two participants in the PL group, one in the LNG group and one in the T + LNG group) during their baseline cognitive visit. Baseline serum samples that were obtained the night of their screening physical, approximately 1 wk before baseline cognitive testing session were substituted for these four participants. A subsequent analysis of hormone levels without these substitutions revealed similar findings as the analysis including the substituted data. Therefore, hormone results reported below include substituted baseline values for these four participants. See Table 1, for mean hormone values throughout the study.

Statistical analyses

Neuropsychological measures. The primary study question was to assess whether manipulation of circulating hormone levels in healthy young men resulted in subsequent changes in cognitive functioning as assessed by change from baseline. The primary outcome measure was change over time on a battery of neuropsychological tests measuring verbal and spatial memory and attention. A repeated measures, multivariate ANOVA was used with group as the independent factor (T, LNG, T + LNG, Placebo) and weeks (baseline, wk 2 and 8) as the repeated factor, spatial memory (SALT, route test), verbal memory (proactive interference and story recall) and attention (Stroop) were dependent measures. Planned comparisons of on treatment time points (wk 2 and 8) compared with baseline were performed and subsequent pair-wise mean comparisons (paired *t* tests) were subjected to Bonferroni correction.

Hormone analyses. A secondary analysis was performed to assess changes in circulating hormone levels in response to treatment. As noted in the methods section, serum samples were taken at the time of testing and therefore represent peak hormone levels. Similar to neuropsychological measures, changes in circulating plasma hormone levels (total T and E2) compared with baseline was assessed using a repeated measures, multivariate ANOVA with group as the independent factor (T, LNG, T + LNG, Placebo), weeks (baseline, wk 2 and 8) as the repeated factor, and total T and total E2 as the dependent measures. The primary outcome measure assessed change from baseline in each treatment group, and a secondary outcome measure compared differences between groups during on treatment time points. Pearson correlation coefficient analyses were also conducted between cognitive measures and hormone levels.

Results

Hormone analyses

Significant change from baseline for both T and E2 was evident in the T [F (4, 22) = 19.1, $P < 0.01$] and T + LNG groups [F(4,22) = 11.1, $P < 0.01$]. In particular, significant increases in total T were observed at wk 8 ($P < 0.01$) and wk 2 ($P < 0.01$) in the T and T + LNG groups from pair-wise comparisons with baseline. The LNG group evidenced a significant decrease in T levels at wk 8 ($P < 0.05$) compared with baseline. Significant increases between baseline and wk 8 ($P < 0.01$) for the T and T + LNG groups and baseline and wk 2 ($P < 0.01$) for the T group (Figs. 2 and 3) were also evident for total E2 from pair-wise mean comparisons with Bonferroni correction. No significant changes from baseline were evident in the PL group. These findings were not due to chance as a significant omnibus multivariate comparison of group [F(6,50) = 2.91, $P < 0.05$], weeks [F (4, 22) = 11.2, $P < 0.01$] and week by group interaction [F (12, 72) = 2.49, $P < 0.01$] was observed. An examination of the multivariate effect of change over time revealed that both T [F (2, 50) = 13.8 $P < 0.01$] and E2 [F (2, 50) = 10.2, $P < 0.01$] changed significantly over time, and this change over time demonstrated an interaction effect with group for T [F (6,50 = 9.08 $P < 0.01$]. This significant interaction effect was due to an increase in T levels in the T and T + LNG groups compared with a decrease in the LNG group as noted above.

TABLE 1. Serum hormone values (mean, SD)

	Treatment groups			
	T	LNG	T + LNG	Placebo
Total T baseline nmol/liter	23.0 (7.1)	18.9 (3.6)	20.0 (5.7)	27.8 (8.1)
Total T wk 2 nmol/liter	43.1[a] (22.2)	13.7 (2.5)	35.5[a] (9.5)	26.9 (9.3)
Total T wk 8 nmol/liter	41.5[a] (10.3)	13.2[b] (4.3)	32.4[a] (5.5)	29.4 (6.5)
Total E2 baseline pmol/liter	107.8 (15.8)	99.5 (17.8)	113.3 (27.5)	129.2 (57.3)
Total E2 wk 2 pmol/liter	138.4[a] (36.4)	82.8 (11.7)	130.2 (32.4)	134.2 (78.7)
Total E2 wk 8 pmol/liter	152.2[a] (28.4)	102.0 (24.5)	146.0[a] (31.3)	145.0 (55.2)

[a] $P < 0.01$ change from baseline.
[b] $P < 0.05$ change from baseline.

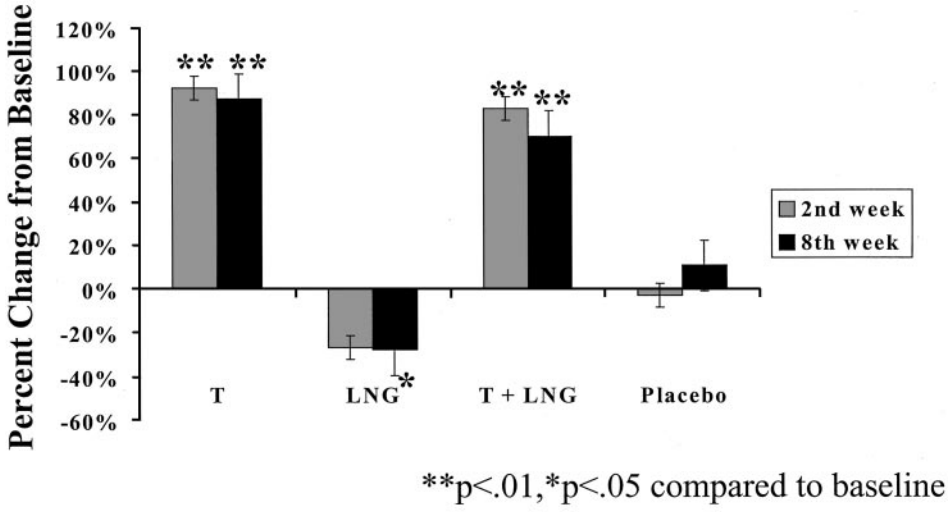

FIG. 2. Percent change in serum total T levels in treatment groups [T enanthate 100 mg/wk im injection + placebo pills (lactose-filled capsule) = (T); Placebo (sterile sesame oil) weekly im injection +125 μg daily oral LNG = (LNG); T enanthate 100 mg/wk im injection + 125 μg daily oral LNG = (T + LNG); Placebo injection + Placebo pill (lactose filled capsule) = (PL)] at baseline and wk 2 and 8 of treatment. SE *bars* represent SE of measurement. *Light bars* represent percent change from baseline at wk 2, and *dark bars* represent percent change from baseline at wk 8.

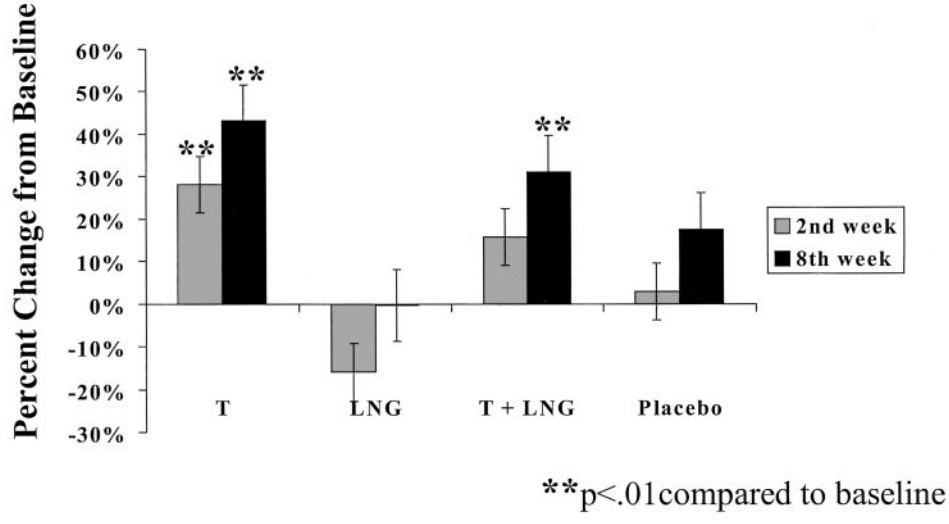

FIG. 3. Percent change in serum total E2 levels in treatment groups [T enanthate 100 mg/wk im injection + placebo pills (lactose-filled capsule) = (T); Placebo (sterile sesame oil) weekly im injection +125 μg daily oral LNG = (LNG); T enanthate 100 mg/wk im injection + 125 μg daily oral LNG = (T + LNG); Placebo injection + Placebo pill (lactose-filled capsule) = (PL)] at baseline and wk 2 and 8 of treatment. SE bars represent SE of measurement. *Light bars* represent percent change from baseline at wk 2, and *dark bars* represent percent change from baseline at wk 8.

Omnibus between group analysis revealed significant differences between groups for T levels at wk 2 [F (3, 22) = 6.1, $P < 0.01$] and wk 8 [F (3, 22) = 17.8, $P < 0.01$] and a trend for total E2 levels at wk 8 ($P < 0.08$). Pair-wise comparisons indicated significant differences between the LNG and T, T + LNG groups at wk 2 ($P < 0.05$) and the LNG and T, T + LNG, P groups ($P < 0.01$), and T, T + LNG and PL ($P < 0.05$) groups at wk 8 for total T. No significant differences between groups were evident for baseline measures. Omnibus interaction effects between conditions and weeks were observed for total T levels [F (6, 50) = 9.0, $P < 0.01$] with a trend toward significance for E2 levels ($P < 0.07$). The interaction effects were due to increases in total T and E2 levels in the T and T + LNG groups and decreased levels of T and E2 in the LNG group. The PL group evidenced no significant changes in T or E2 levels across weeks.

Neuropsychological measures

Only the LNG group evidenced a significant change over time on cognitive measures combined [F (10, 19) = 4.96 $P < 0.01$]. This was due to a significant decrease in verbal memory from wk 8 compared with wk 2 as measured by the proactive interference test (pair-wise comparison of means with Bonferroni correction $P < 0.01$). In contrast, the T and T + LNG groups evidenced no decline at wk 8, which resulted in a significant interaction effect [F (6, 56) = 2.59, $P < 0.05$] (Fig. 4).

The T + LNG demonstrated a trend toward significant change over time on cognitive measures combined [F (10, 19) = 2.31 $P < 0.056$]. This was due to a significant improvement (*i.e.* decreased duration to complete the trial) on the Stroop Test, a measure of selective attention (pair wise comparison of means with Bonferroni correction $P < 0.05$). This was due to significantly improved performance at wk 8 compared with baseline (pair wise comparison of means with Bonferroni correction $P < 0.05$). A trend toward significance was observed at wk 2 compared with baseline (pair-wise comparison of means with Bonferroni correction $P < 0.08$). In contrast to the T + LNG group, the LNG group demonstrated a decline or worse performance (*i.e.* increased duration to complete the trial) on the Stroop (Fig. 5) which resulted in a significant interaction effect [F (6, 56) = 2.39 $P < 0.05$].

All groups demonstrated a significant improvement or

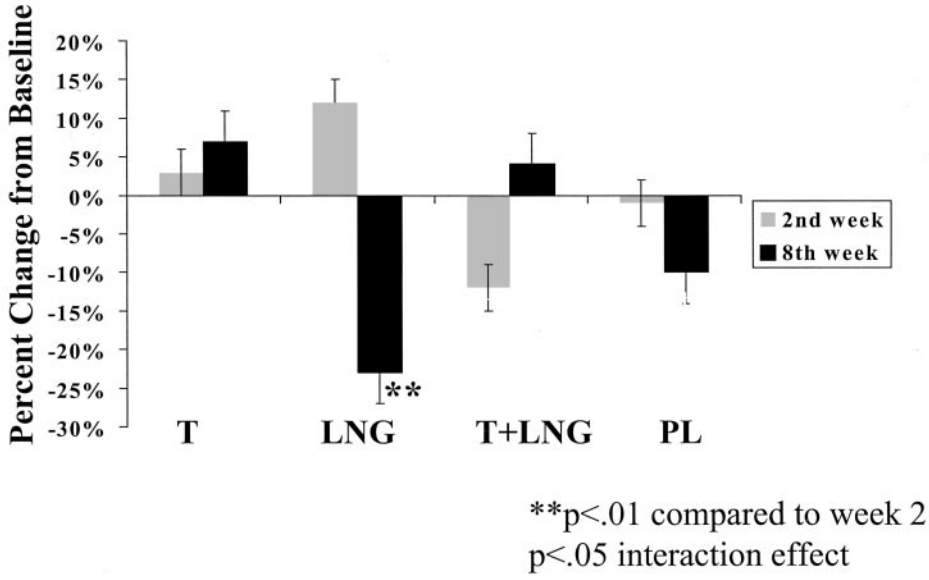

FIG. 4. Mean percent change from baseline on the proactive interference test a measure of verbal memory. Percent change is calculated by total number of words recalled summed across four trials at wk 2 or 8 minus total number of words recalled at baseline divided by baseline total recall score. Treatment groups include [T enanthate 100 mg/wk im injection + placebo pills (lactose-filled capsule) = (T); Placebo (sterile sesame oil) weekly im injection +125 μg daily oral LNG = (LNG); T enanthate 100 mg/wk im injection + 125 μg daily oral LNG = (T + LNG); Placebo injection + Placebo pill (lactose-filled capsule) = (PL)] at baseline and wk 2 and 8 of treatment. SE bars represent SE of measurement. *Light bars* represent percent change from baseline at wk 2, and *dark bars* represent percent change from baseline at wk 8.

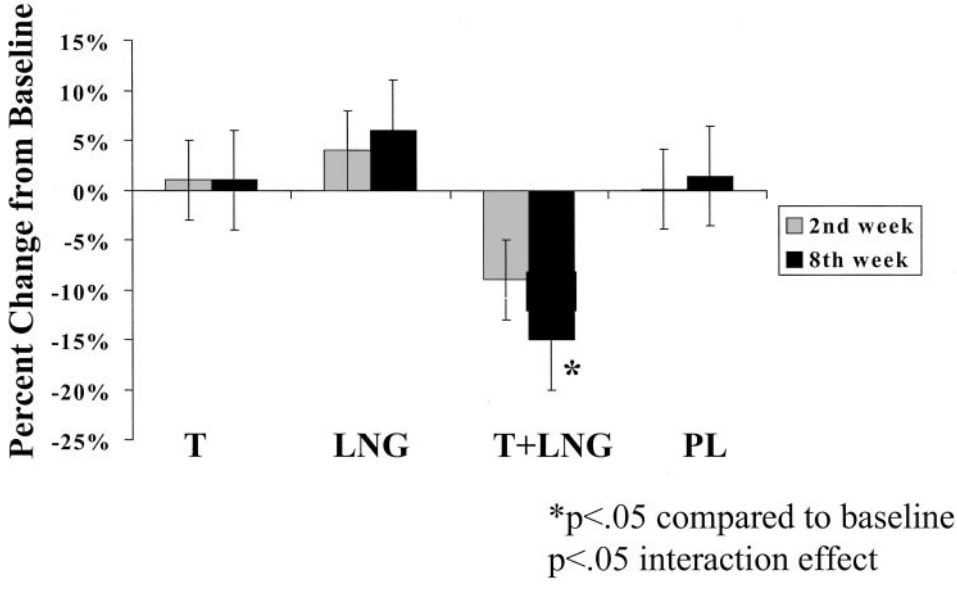

FIG. 5. Mean percent change from baseline on the Stroop test, a measure of divided attention. Percent change is calculated by total time in seconds to complete the interference trial of the Stroop test at wk 2 or 8 minus total time for interference trial at baseline divided by total time at baseline. Lower time to complete trial indicates better performance and higher times to complete trial indicate worse performance. Treatment groups include [T enanthate 100 mg/wk im injection + placebo pills (lactose-filled capsule) = (T); Placebo (sterile sesame oil) weekly im injection +125 μg daily oral LNG = (LNG); T enanthate 100 mg/wk im injection + 125 μg daily oral LNG = (T + LNG); Placebo injection + Placebo pill (lactose-filled capsule) = (PL)] at baseline and wk 2 and 8 of treatment. SE *bars* represent SE of measurement. *Light bars* represent percent change from baseline at wk 2, and *dark bars* represent percent change from baseline at wk 8.

trend toward significant improvement on the route test a measure of spatial memory. Because significant improvement was also observed in the PL group ($P < 0.05$), these results are difficult to interpret beyond practice effects. The placebo group did not demonstrate significant change on any other cognitive measures. We did not find significant changes compared with baseline on other tests in our battery including the SALT, a measure of visual-spatial memory and story recall, a measure of verbal memory. Significant findings observed for the cognitive data are not due to the effects of multiple comparisons as a significant omnibus effect for weeks [$F(10,106) = 3.48$, $P < 0.01$] and week by condition interaction [$F(30,280) = 1.62$, $P < 0.05$] were observed.

Between group comparisons revealed a significant difference between groups on the Route test [$F(3,28) = 4.52$, $P < 0.01$]. Subsequent pair wise comparisons revealed a significant difference between the T and T + LNG groups at wk 2 ($P < 0.01$) with the T + LNG group demonstrated a higher score compared with the T group. This significant difference between groups was not evident at baseline or wk 8 of treatment. Pearson correlation coefficient analyses revealed no significant correlations between hormone levels and cognitive measures.

Discussion

Our results indicate that healthy young men with decreased T levels induced by LNG treatment demonstrated a significant decline in verbal memory. LNG treatment significantly decreased E2 levels at wk 2 but not wk 8 of treatment. In contrast, maintenance of verbal memory was observed in men who were treated with T + LNG and who demonstrated significantly increased T and E2 levels and suggests that changes in verbal memory may be related to changes in T, E2, or both. Beneficial changes in verbal memory and verbal fluency have been observed in a transsexual population of

men receiving estrogen treatment (34, 35) and in a group of healthy older men receiving T supplementation (14). However, T administration in healthy older men has also blocked observed practice effects (*i.e.* improvement) on a verbal fluency task (16). Our findings suggest that changes in cognition may be more readily evident for a rapid decrease but not increase in T and E2 levels in healthy young men. Thus, robust changes in cognition may be more readily evident in populations whereby the change in hormone levels represents a more dramatic shift from the basal range as observed in populations of transsexuals, hypogonadal, or elderly men.

T enanthate and LNG demonstrate different pharmacokinetic properties. T enanthate (100 mg im weekly) reaches a peak level within 24–48 h after injection and returns to a baseline level in approximately 7 d (18). Although little is known about the pharmacokinetics of LNG in men, we have shown in a male contraceptive study that 125 μg LNG taken daily by mouth effectively suppresses serum LH and FSH to undetectable levels. The mean half-life of a similar LNG dose in women is approximately 22 h and steady-state levels of progesterone are achieved after a few days (36). Although progesterone levels were not measured, daily administration of LNG at the dose in the present study (125 μg/d oral LNG) resulted in a significant decrease in T levels during treatment. E2 levels were also lowered although not significantly.

Divided attention as measured by the Stroop test (Fig. 5) was unaffected in the LNG group but significantly improved in the T + LNG group. The cognitive task of divided attention requires simultaneous processing of two different types of information and is considered an executive function task, typically mediated by the frontal and prefrontal cortex (37). Improved performance on the Stroop test has previously been reported in a group of healthy older women and female AD patients receiving estrogen treatment, suggesting that increased E2 may play a role in mediating executive function tasks (38, 39). Several animal and human studies support a specific role of E2 in modulating executive functions (40–42). Both the T and T + LNG groups evidenced a significant increase in E2 levels (Fig. 2) compared with baseline but only the T + LNG group demonstrated a significant improvement on the Stroop test. Thus, it is possible that LNG, a progesterone-like hormone (progestin), may have had a potentiating effect on E2 in the T + LNG group. Progesterone increases acetylcholine levels in the hippocampus and helps potentiate (*i.e.* make more effective) E2 effects on choline acetyltransferase messenger RNA in the hippocampus (43, 44). Thus, the additional effects of progestin in the T + LNG group may have further enhanced positive benefits of increased E2 on complex divided attention. A facilitory effect of progesterone in combination with E2 on attention and memory has been reported in one study of healthy older women receiving estrogen alone *vs.* estrogen combined with a progestin (39).

Observed effects on cognition may be due to changes in circulating T or E2 levels or from direct effects of LNG. LNG is a synthetic steroid that has potent progestational and androgenic effects. LNG and/or progestin administration leads to a decrease in gonadotropins and a subsequent decrease in total T and E2. Studies of *in vitro* metabolism indicate that LNG has a strong affinity for the PR and AR but does not bind strongly to the ER (45). Progesterone is produced in the gonads, adrenal gland and cerebral glial cells. It modulates hypothalamic-pituitary axis activity and has known anxiolytic and analgesic effects (46). The effects of progestins on cognition, however, are not well understood. Freeman *et al.* (47) examined cognitive and behavioral effects of a single (1200 mg) dose of progesterone in a group of healthy women. Four hours after the oral dose administration, during the peak level of serum progesterone, significant increases in confusion, and fatigue and a decline in verbal memory as measured by a verbal list learning task were evident. A more recent study examining a single dose (300 mg) of progesterone in healthy young men found no evidence of changes in cognition measured 11 h following dose administration (48). However, progesterone levels had returned to a baseline levels at the time of cognitive assessment. The mechanism by which progesterone exerts its effects in the CNS are likely complex. The progesterone receptor is heavily concentrated dorsally in the cortex, and in the preoptic area, and hypothalamus regions of the rat that are typically associated with reproductive functions. However, these same regions also have a role in cognition and connections to the hippocampus which mediates memory. Because LNG has both androgenic and progestin effects, the observed maintenance of verbal memory in the T and T + LNG groups and the negative effects in the LNG group suggest that detrimental effects on verbal memory were likely due to progestational effects consistent with findings of impaired verbal memory in healthy young women (47).

A significant increase in spatial memory compared with baseline was observed in the T and both LNG groups (LNG and T + LNG). However, an increase in spatial memory was also observed in the placebo group. Thus, improvement in both the treatment and placebo groups likely represents an observed practice effect. It is unusual that we did not find a significant increase in spatial memory on either spatial memory task included in the cognitive battery, as improved spatial memory from T administration has been observed in healthy older men and transgendered individuals (12–14, 49). However other studies have failed to find beneficial increases in cognition and spatial memory in both hypogonadal (15) and healthy older men (16). Study participants were healthy young men with baseline T levels in the normal range. Increasing T levels in a healthy young male population beyond the normal range may not result in further benefits on certain tasks such as spatial memory. Men with low endogenous T levels have been reported to outperform men with high endogenous T levels on measures of spatial ability, suggesting a nonlinear relationship between T levels and spatial abilities (8, 9). Thus, beneficial effects of T on spatial ability tasks may only be observed when T is within an optimal range.

In summary, our study is the first to examine the effects of exogenous hormone manipulation on cognition in a group of healthy young men. Our results indicate that decreased serum T levels induced by LNG or that the progestational effects of LNG adversely effected verbal memory in normal young men. Increased T and/or E2 levels by coadministration of T + LNG maintained verbal memory and significantly improved divided attention in these men. We did not observe meaningful changes on measures of spatial memory. Due to the limited sample size of our study, these results should be interpreted with caution and will need to be replicated and

confirmed in studies incorporating a larger sample size. Nonetheless, these results are consistent with findings of impaired verbal memory in response to LNG administration in healthy young women and suggest that short-term changes in sex steroid levels effects cognitive function in healthy young men.

Acknowledgments

We thank Karla Grimwood, Jody Lowry, Allison Weston, Maria Gonzales, Connie Nosbisch, Arlen Sarkissian, Rebecca Brotman, and Dorothy McGuinness for their excellent technical assistance. We thank Wyeth-Ayerst Laboratories, Inc. for their gift of LNG.

Received August 29, 2001. Accepted March 7, 2002.

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This research was supported in part by The Mellon Foundation and Veterans Affairs Merit Award. M.M.C. is supported by NIA Grant K0100858.

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