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Cognitive Changes Associated With Supplementation of Testosterone or Dihydrotestosterone in Mildly Hypogonadal Men: A Preliminary Report

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ABSTRACT: This study prospectively examined changes in cognition in hypogonadal men given testosterone (T) or older hypogonadal men given dihydrotestosterone (DHT) gel. A battery of cognitive tests assessing verbal and spatial memory, language, and attention was administered at baseline (prior to medication) and again at days 90 and 180 of treatment for men receiving T gel and at baseline and days 30 and 90 of treatment for men receiving DHT gel. For men receiving T gel, circulating total T and estradiol (E_2) were significantly raised compared with baseline, and a significant improvement in verbal memory was observed. For men receiving DHT gel, serum DHT levels increased and T levels decreased significantly compared with baseline, and a significant improvement in spatial memory was observed. The results suggest that beneficial changes in cognition can occur in hypogonadal men using T replacement levels and DHT treatment, and these changes in cognition can be reliably measured during a relative steady-state dose level. Further, our results suggest that aromatization of T to E_2 may regulate verbal memory in men, whereas nonaromatizable androgens may regulate spatial memory.

Key words: Memory, gel, androgens, cognition, spatial.

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Men with idiopathic hypogonadotropic hypogonadism (IHH) have been found to demonstrate impairments in spatial abilities (Buchsbaum and Henkin, 1980; Hier and Crowley, 1982) and memory for both verbal and visual information and spatial attention (Cappa et al, 1988; Kertzman et al, 1990). It has been suggested that these impairments in cognition and other neurological and brain morphological abnormalities observed in men with IHH and Kallmann syndrome (IHH with anosmia) may be due to organizational or brain morphology differences from sex steroids. However, considerable evidence suggests that sex steroids exert dynamic or modulatory effects on cognition throughout the lifespan, and these effects have been termed activational effects. For example, eugonadal young and healthy older men demonstrate beneficial changes in spatial and verbal memory in response to T administration (Janowsky et al, 1994, 2000; Cherrier et al, 2001a; O'Connor et al, 2001).

Hypogonadal men have demonstrated improvements in cognition in response to T supplementation. Alexander et al (1998) examined 33 hypogonadal men receiving testosterone replacement therapy and measured visuospatial ability, verbal fluency, perceptual speed, and verbal memory prior to and following T supplementation. Hypogonadal men were impaired in their verbal fluency compared with eugonadal men at baseline and showed improved verbal fluency following T treatment. We have also reported findings of impaired cognition in an older man with IHH that improved significantly with T supplementation (Cherrier and Craft, 2003). Although the goal of hormonal treatment of male hypogonadism is typically to induce and maintain normal secondary sexual characteristics in adolescents, these studies suggest that improvements in cognition may also occur with T treatment and that these improvements may occur throughout the lifespan.

In this study, we prospectively examined a group of hypogonadal men participating in a phase II/III pharmacokinetic study of T gel and a group of older, mildly hypogonadal men (over age 60) participating in a phase II/III pharmacokinetic study of dihydrotestosterone (DHT) gel. For the T gel study, we hypothesized that increased androgen levels would have a beneficial effect on spatial memory and increased estradiol (E_2) levels secondary to aromatization would have a beneficial effect on verbal memory. Several previous studies in women have shown a potential relationship between E_2 levels and ver-

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bal memory performance (Sherwin and Tulandi, 1996; Duff and Hampson, 2000). For the DHT gel study, we hypothesized that increased DHT would selectively improve spatial memory but not verbal memory. DHT is nonaromatizable; therefore, without an expected increase in E_2 levels, we did not expect to observe a significant increase in verbal memory. We did not expect that T or DHT supplementation would affect language or attention skills.

Methods

Participants

Participants in the T gel study were 12 hypogonadal men between the ages of 34 and 70 years recruited through local endocrinology clinics. The phase II/III pharmacokinetic study was a multicenter study comparing 2 doses of T gel (50 or 100 mg) vs a T patch (Swerdloff et al, 2000; Wang et al, 2000a,b). See Wang et al (2000a,b) and Swerdloff et al (2000) for details of the multicenter study. Participants were required to meet the screening criteria of a single, total T laboratory value of less than 300 ng/dL obtained during an off-treatment period. Participants were not required to meet criteria of a second clinical T value less than 300 ng/dL. Therefore participants represent a group of mildly, rather than severely, hypogonadal men. Participants were required to have either 6 weeks off T ester injections or 4 weeks off transdermal T patches or oral T. Due to the ethical considerations, a placebo or nontreated group in this population was not feasible. Therefore, a comparable nontreatment convenience sample of community-dwelling eugonadal men who were given the identical neuropsychological battery in the same manner was used for the control group. However, this group did not receive any type of treatment such as placebo gel and is purely a testing control group.

Participants in the DHT gel study were 9 older hypogonadal men between the ages of 63 and 87 years recruited from the community through advertisement and flyers and local endocrine clinics. The phase II/III pharmacokinetic study was a multicenter study comparing 2 doses of DHT gel (32 or 64 mg) vs a placebo gel.

For both the T and DHT gel studies, only participants from the Seattle study site participated in additional neuropsychological testing procedures. Study protocols were approved by the University of Washington Institutional Review Board, and approved informed consent procedures were followed.

Procedures

Study Design—Participants in the T gel study ranged in age from 34 to 70 years of age with a mean age of 57 (±9 years). Participants were randomly assigned to 1 of 3 treatment groups for either 2 doses of T gel (50 or 100 mg daily) or a T patch. The original study was double blind and randomized with regard to T gel dose and open label for the T patch group. The T gel was provided by Unimed/Solvay Pharmaceuticals (see Wang et al, 2000b, and Swerdloff et al, 2000, for details of multicenter study). According to the study protocol, dosing regimens at day 90 were adjusted as necessary in some participants to achieve eugonadal levels. Patients with T levels less than 10.4 nmol/L and who were originally assigned to apply 50 mg/d T gel and those with T levels more than 34.7 nmol/L who had received 100 mg/d were then reassigned to administer 75 mg/d T gel for days 91 through 180. Subjects returned to the study center on day 0 (baseline) and days 30, 60, 90, 120, 150, and 180 for a clinical examination, skin irritation, and adverse event assessments. Fasting blood samples were drawn on all days, and 24-hour pharmacokinetics were drawn at baseline and days 30, 90, and 180. Hematology and clinical biochemistry were performed at all clinic visits. Endogenous T levels measured at baseline, prior to the start of the study, were in the hypogonadal range for all participants in both studies with the exception of 1 participant in the T gel study who demonstrated a T level in the hypogonadal range at screening and in the low normal eugonadal range at baseline. However, because this participant met the screening criteria, he was not excluded from these analyses. Cognitive testing was performed at prebaseline, baseline, and days 90 and 180. Psychometrists who performed the cognitive testing were blind to treatment condition.

Participants in the DHT gel study were 9 older hypogonadal men screened and randomly assigned to 1 of 3 treatment groups with either 2 doses of DHT gel (32 or 64 mg daily) or a placebo gel for a total of 3 participants in each treatment group. Participants were older than age 60 with a range of 63 to 87 years, with a mean age of 74 years (±8). Participants returned to the clinic for 24-hour pharmacokinetics at baseline (pretreatment), and days 30 and 90 of treatment involving serial blood samples before and after gel application. Participants were also given a clinical examination and assessment of skin irritation and adverse events during those clinic visits, as well as an additional clinic visit at day 60. Participants, investigators, and psychometrists were blind to treatment conditions. Cognitive testing was performed at baseline and days 30 and 90. T and DHT gel was provided by Unimed/Solvay Pharmaceuticals, Inc (Marietta, Ga).

Neuropsychological Tests—A battery of cognitive tests assessing spatial and verbal memory and selective attention was administered following 24-hour pharmacokinetic procedures to assess changes in cognition. Several precautions were implemented to control for possible practice effects. First, both treatment and control participants were administered the battery of cognitive tests twice prior to onset of hormone treatment. The first cognitive testing session was considered the prebaseline testing session, and the second testing session was used as the true baseline. Second, we used comparable alternate versions of each test (5 total equivalent versions) that were randomly ordered and counterbalanced among participants. Psychometrists and participants were blind to the treatment condition in the DHT gel study. In the T gel study, participants were blind to the dose of T gel and psychometrists were blind to the treatment condition. DHT gel study participants did not receive a prebaseline practice session due to scheduling conflicts.

Spatial Memory Measures

Route Test—This test measured the ability to navigate a short route within a room and is based on previous work by Barrash et al (2000) and Cherrier et al (2001b). The task used a 6-foot

by 24-foot piece of black flooring on which a diamond pattern was placed using bright yellow tape. In the nonlandmark version, the examiner created a particular route on the grid using a bright red ribbon. The subject was asked to walk the route as shown. The ribbon was removed, and the subject was asked to immediately retrace the route without the ribbon. Three trials were administered followed by 3 trials of a new route using pictures placed on the floor as landmarks. Then a delayed recall of both routes was assessed after 20 minutes. Performance was assessed by calculating the number of correct sequential units summed across all trials. This test has been shown to have good reliability in both young and old populations and validity when compared with other route tests and in comparison with brain injured individuals and controls (Barrash and Tranel, 1996).

Spatial Array Learning Test (SALT)—This measure of spatial memory was adapted from the visual spatial learning test by Malec et al (1992). Participants were shown 7 unique figures in a particular pattern placed on a grid. The subject was allowed to look at the designs and placement briefly, and then asked to choose the correct designs from 8 distractor designs and to place them in the correct position on the grid. This procedure was repeated for a series of 5 trials and after a 20-minute delay. The number of correct tokens placed in the correct location was recorded for each trial and summed across trials. Reliability and validity assessed in an older adult population was very good (Malec et al, 1992). This measure was administered to participants in the DHT gel group only.

Verbal Memory Measures

Proactive Interference (PI)—As adapted from Moscovitch (1994), participants listened to a list of 10 words from the same semantic category (eg, articles of clothing) and then recalled as many of these words as possible (Moscovitch, 1994). The procedure was repeated for a total of 4 trials, each containing different words drawn from the same semantic category. For the fifth trial 10 words from a new semantic category (eg, types of furniture) were read and participants were asked to recall these words. The total number of words recalled correctly on each trial was recorded. Normal adults recall progressively fewer words across trials 2 through 4 because of 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 (Lezak, 1995).

Story Recall—The story recall task was modeled on the Wechsler Memory Scale Revised (WMS-R) and measured memory for aurally presented contextual material. Participants listened to 2 brief narratives, each containing 25 informational bits, and were asked to recall as much as possible immediately after hearing each story and following a 20-minute delay (Wechsler, 1987). Total number of words recalled from both stories was summed for immediate and delayed recall. Delayed recall was divided by immediate recall to obtain a savings score or a percent of information retained. Reliability and validity of WMS-R, WMS-III Logical Memory, and this modified version are very good (Wechsler, 1987; Craft et al, 1992, 1993, 1994; Wechsler, 1997).

Selective Attention Measure

Stroop Color Word Interference Task—This task, based on the original Stroop test, utilized 3 trials for which total reading time and errors were recorded (Stroop, 1935). The first condition (word reading) required participants to read as quickly as possible 100 color words (red, green, blue) presented in rows on a sheet of paper. The second condition (color naming) required participants to name the color of 100 colored blocks presented in rows on a sheet of paper. In the third condition (color word interference), stimuli consisted of color names that were printed in discordant colors (eg, the word ''blue'' printed in green letters). Participants were asked to name the ink color of the printed words, and were thus required to inhibit the reading of the words themselves. Because only the interference trial is a measure of divided attention, total time for trial 3 was used as the dependent measure. The test has demonstrated good reliability and validity when examined in closed head injured individuals compared with controls (Lezak, 1995).

Hormone Assays

Blood samples were drawn at each clinic visit, and 24-hour samples were drawn during several clinic visits. For both the T and DHT gel studies, the baseline morning sample presented was drawn at approximated 8:00 AM. For the T gel study, the serum T and E_2 levels are for the treatment group participants only (T gel and T patch combined in 1 treatment group, n = 12), as no hormone levels were available for the testing-only control group. For the DHT study, hormone assays were conducted with all participants (treated and placebo). Except for the screening serum T concentration processed by the local clinical lab, all hormone assays were performed at the endocrine research laboratory of the Harbor–University of California, Los Angeles, Medical Center. Serum T levels were measured after extraction with ethyl acetate and hexane by a specific radio immuno-assay using reagents from ICN Biomedicals Inc (Costa Mesa, Calif). See Swerdloff et al (2000) for further T assay details. DHT levels were measured by RIA after potassium permanganate treatment of the sample followed by extraction. The methods and reagents of the DHT assays were provided by DSL (Webster, Tex). Serum E_2 levels were measured by a direct assay without extraction with reagents from ICN. The intraassay and interassay coefficients of variation of the E_2 assay were 7% and 9%, respectively, for normal adult male range (E_2 63 to 169 pmol/L). The lower limit of quantitation of the E_2 was 46 pmol/L. All values below this value were reported as less than 46 pmol/L. See Wang et al (1998) for details of DHT and E_2 assay methods.

Statistical Analyses

Cognitive tests and hormone measures were analyzed using a mixed-model, repeated measures, multivariate analysis of variance (MANOVA) with group as the independent factor (treatment vs placebo) and time (baseline and days 90 and 180) as the repeated factor, and cognitive tests or hormone levels were dependent measures for T and DHT gel results separately. Planned comparisons of on-treatment time points (days 90 and 180 for T gel and days 30 and 90 for DHT gel study) compared with baseline were performed and post hoc pairwise comparisons between time points (as noted above) were subjected to

Mean serum hormone values for the testosterone (T) gel study and dihydrotestosterone (DHT) gel study

	Baseline	Day 90	Day 180
T-treated (n = 12)†			
T (ng/dL)	278 (40)	647 (87)*	528 (83)**
DHT (ng/dL)	42 (5)	156 (30)*	160 (33)*
E_2 (pg/mL)	25 (2)	35 (3)**	41 (4)*
	Baseline	Day 30	Day 90
DHT gel (n = 6)			
T (ng/dL)	358 (90)	86 (14)*	108 (45)**
DHT (ng/dL)	67 (17)	618 (233)*	602 (213)*
E_2 (pg/mL)	25 (5)	18 (2)	20 (2)
Placebo gel (n = 3)			
T (ng/dL)	237 (6)	295 (12)	299 (21)
DHT (ng/dL)	51 (1)	55 (15)	86 (20)
E_2 (pg/mL)	20 (1)	22 (3)	22 (5)

† Combined T gel and T patch. Standard error of measurements (SEM) are in parentheses.

* $P < .01$ compared with baseline; ** $P < .05$ compared with baseline

Bonferroni correction. Prior to analysis, serum T and DHT levels were subjected to transformations (square root) to reduce skewness and improve the normality, linearity, and homoscedacity of the data. For the T gel study, all hypogonadal men (n = 12) were included in the treatment group regardless of initial T dose or method (gel vs patch), as T levels reached a therapeutic level in all men. For the DHT gel study, all treated participants (n = 6, 32- and 64-mg dose groups) were combined into 1 treatment group as DHT levels reached a therapeutic level in all men, and they were compared with the participants in the placebo gel group (n = 3).

Results

Hormone Measures

There were no significant differences between the treated and placebo groups for T, DHT, or E_2 at baseline. See the Table for mean serum hormone values for raw or nontransformed data. For the T gel study, a repeated measures MANOVA with time (baseline and days 90 and 180) as the repeated factor, and T, DHT, and E_2 as dependent measures was conducted for the treatment group only. The testing-only control group did not undergo treatment, and therefore hormone analysis was conducted in the treatment group only. Omnibus multivariate comparison of time was significant [$F(6,42) = 5.01$, $P < .01$]. Planned comparisons revealed a significant increase between day 90 and baseline for T [$F(1,11) = 13.1$, $P < .01$]; DHT [$F(1,11) = 14.3$, $P < .01$]; and E_2 [$F(1,11) = 11.1$, $P < .01$] and between day 180 and baseline for T [$F(1,11) = 4.98$, $P < .05$]; DHT [$F(1,11) = 10.8$, $P < .01$]; and E_2 [$F(1,11) = 14.8$, $P < .01$]. There were no significant differences among the 3 treated groups (patch and 50- and 100-mg T) for T, DHT, or E_2 at baseline. See the Table for hormone values before and during treatment.

For the DHT gel study, repeated measures MANOVA with time (baseline and days 30 and 90) as the repeated factor, and T, DHT, and E_2 as the dependent measure, was conducted for the treatment and placebo groups. An overall interaction effect for time and condition was observed [$F(4,28) = 2.91$, $P < .05$]. For serum T levels, a significant group by time interaction was evident [$F(2,14) = 6.6$, $P < .01$] with T decreasing in the treatment group and the placebo group remaining stable between day 30 and baseline [$F(1,7) = 9.6$, $P < .05$] and day 90 and baseline [$F(1,7) = 6.9$, $P < .05$]. Post hoc comparisons revealed T levels were significantly decreased from baseline levels in the treatment group at days 30 ($P < .01$) and 90 ($P < .05$). The treatment group had significantly lower T levels at day 30 of treatment [$F(1,7) = 46.3$, $P < .01$] and day 90 [$F(1,7) = 7.4$, $P < .01$] compared with the placebo group. Similarly, DHT evidenced a significant group by time interaction [$F(2,14) = 4.9$, $P < .05$] with DHT increasing and the placebo group remaining stable between day 30 and baseline [$F(1,7) = 6.6$, $P < .05$] and day 90 and baseline [$F(1,7) = 6.1$, $P < .05$]. Post hoc comparisons revealed DHT levels were significantly raised from baseline levels in the treatment group at days 30 ($P < .01$) and 90 ($P < .01$). E_2 levels did not appreciably change.

Neuropsychological Measures

For the T gel study, repeated measures MANOVA with group as the independent factor (treatment vs placebo) and time (baseline and days 90 and 180) as the repeated factor, spatial memory (route test) and verbal memory (proactive interference and story recall) and divided attention (Stroop test) as dependent measures revealed a significant omnibus interaction effect [$F(5,27) = 3.29$, $P < .05$]. Significant improvement across days occurred for story recall [$F(2,30) = 3.52$, $P < .05$] in the T-treated group (combined T gel and T patch). Planned comparisons between on-treatment (days 90 and 180) and baseline for each test revealed significant improvement in immediate story recall at day 180 compared with baseline in the treated group [$F(1, 15) = 4.59$, $P < .05$; Figure 1] and delayed story recall at day 180 compared with baseline [$F(1,15) = 8.6$, $P < .01$; Figure 1]. Pairwise comparisons revealed a significant increase in story recall in the treated group for immediate story recall ($P < .05$) and delayed story recall ($P < .01$). There were no significant differences between groups at baseline or at days 90 or 180. Although the treatment group demonstrated an improvement in performance on the route test, a measure of spatial memory, this change did not reach statistical significance ($P < .09$). No significant changes were noted on the Stroop test, a measure of divided attention, or the

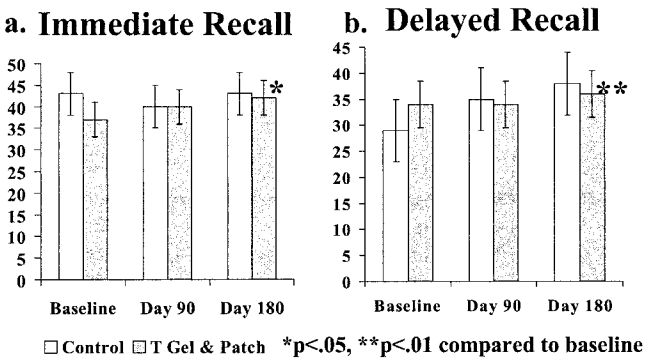

Figure 1. Mean bits of information recalled on the story recall test for immediate and delayed recall for the testosterone (T) gel study at days 90 and 180 of treatment for T-treated and testing control group. Dark bars represent the T-treated hypogonadal men (T gel and T patch together) and white bars represent the eugonadal testing-only control group. Standard error bars represent standard error of measurement. Asterisks located between bars indicate a significant difference between on-treatment time points and baseline.

proactive interference test, a measure of verbal working memory.

For the DHT gel study, an examination of age revealed a large discrepancy between the mean age of the treatment group (77) and the placebo group (68) despite random assignment; therefore age was used as a covariate in the analysis. A repeated measures MANCOVA with group as the independent factor (treatment vs placebo) and time (baseline and days 30 and 90) as the repeated factor, spatial memory (route test and Spatial Array Learning Test [SALT]) and verbal memory (proactive interference and story recall) and divided attention (Stroop test) as dependent measures and with age as a covariate revealed a significant omnibus effect of time [$F(10,16) = 3.03$, $P < .05$] and a significant time by age interaction effect [$F(10,16) = 2.77$, $P < .05$]. Planned comparisons between on-treatment (days 30 and 90) and baseline for each test revealed a significant interaction effect for spatial memory (route test) at day 30 compared with baseline [$F(1, 6) = 6.33$, $P < .05$; Figure 2], and spatial memory (SALT) at day 30 compared with baseline [$F(1,6) = 9.4$, $P < .05$; Figure 2]. Pairwise comparisons revealed that the significant interaction effect for the route test was due to a significant increase in the treatment group at day 30 compared with baseline ($P < .05$). The significant interaction effect for the SALT was due to a greater increase in the treatment group at days 30 and 90. Post hoc comparisons revealed no other significant differences between means. No significant changes were noted on the proactive interference test and paragraph recall measures of verbal memory or the Stroop test, a measure of divided attention.

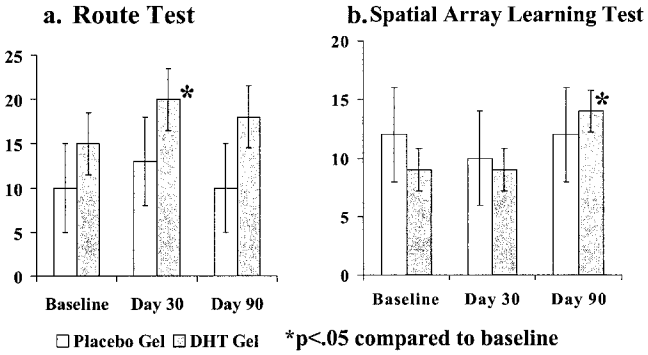

Figure 2. Mean number of points correctly recalled on the route test and spatial array learning test in the DHT and placebo gel groups at days 30 and 90 of treatment. Dark bars represent the DHT gel–treated hypogonadal men and white bars represent the hypogonadal men treated with placebo gel. Standard error bars represent standard error of measurement. Asterisks located between bars indicate a significant difference between on-treatment time points and baseline.

Discussion

Our results indicate that hypogonadal men demonstrate significant improvements in verbal memory in response to T supplementation with significantly increased serum T, DHT, and E_2 levels, and older hypogonadal men demonstrate improved spatial memory in response to DHT treatment, with significantly increased serum DHT levels. Hypogonadal men receiving T gel demonstrated a beneficial increase in spatial memory; however, this did not reach statistical significance. We did not observe significant changes on all neuropsychological measures or on measures of language or attention. These results are consistent with previous findings of improved verbal ability in hypogonadal men receiving T replacement (Alexander et al, 1998) and for improved spatial and verbal memory in older eugonadal men receiving T treatment (Janowsky et al, 1994; Alexander et al, 1998; Janowsky et al, 2000; Cherrier et al, 2001a).

Results of improved verbal and visual memory with no significant changes in language or attention suggests that androgens may have selective effects on brain structures that underlie memory functions. Androgen effects on memory may be mediated through several mechanisms. First, T alters neurotransmitter levels in numerous brain regions involved in memory and spatial processing, including the hippocampus and basal forebrain and includes catecholamine, (α aminobutyric acid), GABA and serotonin receptors, and N-methyl-D-aspartate–mediated depolarization (Pouliot et al, 1996; Sagrillo and Selmanoff, 1997; Sumner and Fink, 1998; Adler et al, 1999; Kritzer et al, 1999; Kritzer, 2000). T has excitatory effects on both baseline and kindled hippocampal slice excitability and restores choline acetyltransferase (ChAT) immunoreactive neuron loss from gonadectomy in the anterior cingulate, posterior parietal region, and hippocampus (Nakamura et al, 2002; Smith et al, 2002). Second, the

hippocampus that underlies declarative memory and spatial abilities contains both androgen (Roof and Havens, 1992; Fink et al, 1999; Poletti and Martini, 1999; Beyenburg et al, 2000; Hammond et al, 2001) and estrogen receptors (Luine, 1994; Gundlah et al, 2000; Osterlund et al, 2000a,b; Shughrue and Merchenthaler, 2000; Neele et al, 2001; Savaskan et al, 2001). Testosterone may act directly through the androgen receptor and/or via aromatization to E_2 acting on estrogen receptors. Both types of estrogen receptors ERα and ERβ have been found in the rat hippocampus, and recent in vivo estrogen-binding studies suggest that estrogen is likely involved in cognition as well as neuroprotection in the hippocampus and basal forebrain (Shughrue and Merchenthaler, 2000; Shughrue et al, 2000). Behavioral effects of ERα knockout mice also suggest that estrogen has effects on learning and memory (Rissman et al, 1999). Estrogen-treated vs estrogen-deprived rats demonstrate increased choline uptake and higher levels of choline acetyltransferase in the hippocampus and frontal cortex, and these increases are associated with better performance on a behavioral memory task (Gibbs, 1996; Farr et al, 2000; Gibbs, 2000a,b). Selective effects of androgens on memory functions may be secondary to direct effects of androgens acting on the androgen receptor as well as indirect effects from aromatization to E_2 interacting with estrogen receptors localized in the hippocampus (Naftolin et al, 1975).

Men with IHH have been found to have impairments in spatial abilities (Buchsbaum and Henkin, 1980; Hier and Crowley, 1982) and memory for both verbal and visual information and spatial attention (Cappa et al, 1988; Kertzman et al, 1990). In the present study, hypogonadal men treated with T gel or T patch improved significantly on verbal memory (Figure 1). Our results appear to be most consistent with the findings of Alexander et al (1998) who examined 33 hypogonadal men receiving testosterone replacement therapy and found that hypogonadal men were impaired in their verbal fluency compared with eugonadal men at baseline, which significantly improved with T treatment.

Positive effects on verbal memory observed in the T-treated but not DHT-treated participants may be due to the rise in E_2 levels from aromatization of T in the T-treated group. Although hypogonadal men in both studies received androgen supplementation, only the treated group in the T gel study demonstrated a significant increase in verbal memory. Significant improvements in verbal recall have been found in healthy older women and patients with Alzheimer disease receiving estrogen replacement treatment (Sherwin, 1988; Phillips and Sherwin, 1992; Kampen and Sherwin, 1994; Sherwin and Tulandi, 1996; Resnick et al, 1998; Hogervorst et al, 1999; Shaywitz et al, 1999; Duff and Hampson, 2000; Asthana et al, 2001; Neele et al, 2001). In men, exogenous increases in estrogen have been shown to improve verbal memory for a paired associate learning task, a task that generally favors women (Miles et al, 1998). These findings, along with studies examining sex differences in cognitive abilities, suggest that estrogen may have selective benefits on verbal abilities and verbal memory (Slabbekoorn et al, 1999). In the T gel study, improved verbal memory was noted for both immediate and delayed recall on the story recall test. Improvement was also found on the route test, a measure of spatial memory; however, this did not achieve statistical significance ($P < .09$). The lack of a significant change in spatial memory in the T-treated group is not consistent with previous studies demonstrating a beneficial effect of T on spatial abilities and may be secondary to sample size and/or power limitations.

Our findings indicate that older, hypogonadal men treated with DHT gel demonstrate a significant increase in spatial memory. Beneficial changes in cognition using a nonaromatizable androgen such as DHT have not been previously reported. Because DHT cannot be aromatized into E_2, the improvement in spatial memory is likely a direct androgen effect, rather than an indirect effect from an increase in E_2. A significant decrease in T levels was also observed, suggesting that DHT may have effects on cognition independent of T. Although DHT is the most potent natural androgen and a metabolite of T, it has not been widely used therapeutically. A few studies have indicated beneficial therapeutic use in hypogonadal and older, androgen-deficient men (Chemana et al, 1982; de Lignieres, 1993; Wang et al, 1998; Ly et al, 2001). The formation of DHT in the body is achieved by the enzyme 5-reductase (5-R). There are 2 isoforms of 5-R (5-R type 1 and 5-R type 2; Russell and Wilson, 1994). Both forms reduce androgens; however, the affinity for the 5-R type 1 isoform is much lower than the 5-R type 2. The enzyme isoform 5-R type 1 appears to be widely distributed in tissue, whereas 5-R type 2 is specific to androgen-dependent tissues (eg, prostate, testes) and brain (Poletti and Martini, 1999). The enzyme isoform 5-R type 2 can be found in the hypothalamus and hippocampus (Melcangi et al, 1998; Poletti and Martini, 1999) and has been detected in the human hippocampus and medial temporal regions (Saatman et al, 1997; Stoffel-Wagner et al, 1998, 2000). DHT binds to the androgen receptor (AR) more potently than T, and lower concentrations are needed to activate the transcription of androgen-dependent genes (Grino et al, 1990; Lu et al, 1999). Further examination of DHT action in the brain independent of T may help elucidate the mechanism of action of androgens on cognition. These findings of improved spatial memory in response to DHT administration may also help explain mixed findings of previous studies using T administration (Sih et al, 1997; Wolf et al, 2000).

For hypogonadal men in the T gel study, beneficial

changes in verbal memory were observed at both on-treatment time points (days 90 and 180) compared with baseline but achieved statistical significance at day 180 of treatment. For the older hypogonadal men receiving DHT gel, beneficial changes in spatial memory were evident at days 30 and 90 of treatment compared with baseline and achieved statistical significance at day 90 of treatment. Beneficial cognitive effects of T treatment in hypogonadal men has been observed after 6 weeks of treatment (Alexander et al, 1998) and from 3 to 8 weeks of treatment in eugonadal older men (Janowsky et al, 1994; Alexander et al, 1998; Janowsky et al, 2000; Cherrier et al, 2001a). Improvement in verbal memory observed after 6 months of treatment or spatial memory after 3 months of treatment has not been previously reported and suggests that beneficial effects of androgens on cognition may occur over a longer time period than previously reported. A similar time period has been observed with regard to declines in verbal memory from lack of E_2 in women who have undergone saliphoorphorectomy (Sherwin, 1988). These results suggest that beneficial effects of hormones may be evident several months after onset of treatment.

Previous studies demonstrating beneficial effects of androgens on cognition have utilized patch or injection methods of hormone delivery. Injection methods can result in a significant rapid rise in T levels within 24 to 48 hours following injection followed by a significant decline to baseline or below baseline within 5 to 7 days depending on the dose (see Matsumoto et al, 1994). Similarly, patch delivery methods also result in a rapid rise in T levels within several hours of application followed by a significant drop back to baseline or below baseline within 24 hours. Percutaneous T and DHT gel with daily application results in a reliable steady-state dosage level between daily application (see Swerdloff et al, 2000, for 24 T gel pharmacokinetics, and Wang et al, 1998, for DHT gel pharmacokinetics). Thus observed improvements in cognition in the present study are not likely secondary to temporary or ''peak'' effects of serum hormone levels. Hypogonadal men in the T gel study evidenced significant increases in T, DHT, and E_2 levels. Typically, DHT levels do not significantly increase in response to T supplementation from injection methods. The observed increase in DHT levels in the T gel study may be secondary to relatively high amounts of 5α-reductase found in skin. However, high levels of DHT may also be due to methodological issues of the DHT assay, such as incomplete oxidation in the sample assay. Although we cannot completely rule out the latter issue, our findings of significantly increased DHT levels are consistent with another study demonstrating increased DHT levels in response to T gel (Wang et al, 1998).

In summary, our results indicate that mildly hypogonadal men demonstrate significant improvements in verbal memory in response to T supplementation, and older hypogonadal men demonstrate improved spatial memory in response to DHT supplementation. Hypogonadal men receiving T gel also demonstrated a beneficial increase in spatial memory; however, this did not reach statistical significance. Findings of improved spatial memory from DHT supplementation and improved verbal memory from T supplementation in hypogonadal men have not been previously reported. Despite these novel findings, our results must be interpreted with caution. The sample sizes in these studies are small, and therefore our findings will need to be replicated with a larger sample. Further, a prospective study designed to directly compare T vs DHT effects on cognition will provide additional information regarding the mechanism of androgen effects and will allow us to make conclusions regarding the relative contribution of T, DHT, and E_2 in cognitive processing. Despite these cautions, our results are consistent with significant improvements from T supplementation in the area of verbal ability for hypogonadal men and spatial memory in older eugonadal men. Although change in cognition is not typically a primary treatment consideration for hypogonadal men, our results demonstrate that beneficial changes in cognition may also occur with androgen replacement therapy and that beneficial changes in cognition can occur using a nonaromatizable androgen.

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