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# Long-term measures of free testosterone predict regional cerebral blood flow patterns in elderly men

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**Abstract**

We previously reported that high circulating free testosterone (T) was associated with better performance on tests of memory, executive function, and spatial ability, and with a reduced risk for Alzheimer's disease. In this study, we report that free T levels, measured on multiple occasions over 14 years, predict regional cerebral blood flow (rCBF) measured by PET in 40 older men. Voxel-based regression, indicated that higher Free T was associated with increased rCBF in the hippocampus bilaterally (extending to the parahippocampal gyrus on the right), anterior cingulate gyrus, and right inferior frontal cortex. Total T concentrations were positively correlated with rCBF in the left putamen, bilateral thalamus, and left inferior frontal cortex and negatively correlated with amygdala rCBF bilaterally. These findings suggest that endogenous T influences brain physiology in regions critical for memory and attention and provide one mechanism through which T may affect cognitive function.
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*Keywords:* Testosterone; Steroid hormones PET; Regional cerebral blood flow; Aging; Neuroimaging

## 1. Introduction

In men, total testosterone (T) levels decline by approximately 50% from ages 30–80 [19] and as many as 68% of men over age 70 can be classified as hypogonadal based on their endogenous free T concentrations [14]. This progressive loss of T with age in men has significant physiological consequences including decreased muscle mass, reduced bone density and sexual dysfunction [12]. Among men with suspected andropause, memory loss was the third most commonly reported symptom after sexual dysfunction and general weakness [37]. Despite the high morbidity associated with hypogonadism, there have been few studies specifically investigating the cognitive and neural effects of T supplementation in elderly men.

Gonadal steroids exert powerful behavioral and neural effects in non-human species [2-4,11]. Of particular interest to researchers investigating hormonal contributions to human abilities is the observation that T facilitates rodent spatial memory [32,40], a behavior which is dependent on the integrity of the hippocampus [28].

Although the effects of T on human brain and cognitive function are not clear, both associational [1,24] and randomized intervention trials [6,15] suggest that T may play an important role in the maintenance of cognitive function. In a series of studies [23–25], we have investigated the cognitive and neurological consequences of androgen depletion in elderly men. In one study, we followed 574 men for a mean duration of 19 years. We collected multiple serum samples for determination of total T, sex hormone binding globulin (SHBG), and a free T index, and prospectively evaluated presence or absence of a diagnosis of Alzheimer's disease

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(AD). High free T levels were associated with a significantly reduced risk of AD. In a second study, we investigated age-associated decreases in endogenous T concentrations and declines in neuropsychological performance among 407 non-demented older men. Higher free T was associated with higher scores on visual and verbal memory and visuospatial functioning and with a reduced rate of decline in visual memory. These results suggest that higher free T levels in older men may protect against cognitive decline and the development of AD.

Despite the findings from animal and human studies that T may influence cognition and the brain, there are few studies that assess the effects of T on human brain physiology. In this study, we examined associations between T levels and PET measurements of regional cerebral blood flow (rCBF) in 40 non-demented men aged 59–85. Regression analyses were performed separately for total and free T levels, based on multiple T measures over a mean 13.9 (8.4) year follow-up. We report that higher free T predicts increased rCBF in the hippocampal–parahippocampal complex, anterior cingulate and right inferior frontal region.

## 2. Materials and methods

### 2.1. Study population

Subjects were male volunteers participating in the Baltimore Longitudinal Study of Aging (BLSA), a study performed by the National Institute on Aging (NIA) [33]. Participants are generally healthy volunteers who returned every 2 years to the NIA clinical research program for comprehensive medical, physiological and neuropsychological evaluations. Androgen data were available from a large pool of BLSA participants whose blood samples were assayed for androgen as part of a study of prostate health and disease. A subset of these men were also participants in the BLSA Neuroimaging Study [31] and returned each year for structural MRI measures and PET studies of brain activity, using $^{15}$O-labeled water to measure cerebral blood flow, an index tightly coupled to brain metabolism. None of the volunteers in the present study met NINCDS-ADRDA criteria for probable or possible Alzheimer's disease [22], and participants were free of severe cardiovascular and other medical diseases at enrollment. The present analyses were based on men who had completed three PET scans: baseline, 2-year, and 4-year follow-ups. The final sample was comprised of 40 men (after excluding 2 additional men with major depression) and included 38 Caucasians and 2 African-Americans. All but two of the men were right-handed. The mean age (S.D.) at baseline T assessment was 57.2 (11.7) years and the duration of follow-up averaged 13.9 (8.4) years between baseline T measures and initial PET study, with the mean age at initial PET study of 71.1 (7.2) years. The mean difference between the last T measure and the first PET scan was 0.17 (1.12) years. Mean level of education was 16.5 (3.0) years, and mean scores on the mini-mental state exam were 28.6 (1.7) and 28.7 (1.5) at initial and 4-year follow-up PET scans, respectively. This protocol was approved by the local Institutional Review Board, and all subjects provided written informed consent to participate at each visit.

### 2.2. Hormone determinations

Blood samples were collected prospectively at each visit since 1963 and stored at −70° C. As part of the BLSA prostate study, 3621 samples from the serum bank were analyzed to examine the longitudinal changes in serum T in 901 men [14]. For each subject, samples selected for assay were those from the visits closest to 10, 15, and 20 years prior to the most recent visit and then as many as were available within 10 years from the most recent visit. Blood samples were drawn between 6 a.m. and 8 a.m. after an overnight fast. All serum total testosterone (TT) and SHBG measurements were performed at Covance Laboratories, McLean, VA. The free testosterone index (FTI) was calculated by dividing serum T by SHBG. The FTI, calculated from radioimmunoassay of total T and SHBG, has been shown to be well correlated with measures of free T by dialysis (AFTC) and bioavailable T by ammonium sulfate precipitation (BT) and is simpler to obtain [26]. Both Vermeulen et al. [39] and Morley et al. [26] have recently demonstrated that calculation of an index from T and SHBG, as determined by immunoassay, provides a rapid, simple, and reliable index of bioavailable T, highly correlated with AFTC or BT except in pregnancy where SHBG concentrations are very high.

Details of the hormonal assay have been published previously [14]. Testosterone levels were determined in duplicate using $^{125}$I, double antibody RIA kits obtained from Diagnostic Systems Laboratories (Webster, TX). Minimum detectible T levels averaged 0.42 nmol/L, with intra- and interassay coefficients of variance, respectively, of 4.8% and 5.7% at concentrations of 7.74 and 7.29 nmol/L, and 3.3% and 6.4% at concentrations of 44.7 and 42.9 nmol/L. SHBG concentrations were measured using RIA kits purchased from Radim (Liege, Belgium) which employ $^{125}$I labeled SHBG and PEG-complexed second antibody. The sensitivity of the SHBG assay was approximately 10 nmol/L. The CV at 5 nmol/L was 22% and at 25 nmol/L 5%, with intra- and interassay coefficients of variance, respectively, of 3.4% and 10.8% at concentrations of 22 and 19 nmol/L, and 1.8 % and 7.7% at concentrations of 117 and 118 nmol/L.

Preliminary analysis of data from the samples stored between 1961 and 1995 revealed a significant increase in T level with length of storage, independent of subject age. On investigation, we were able to demonstrate that the increase was due to a date-related assay artifact. This increase in T with length of storage was linear, with a slow constant rate over time. A mixed effects model was utilized to adjust T for the date effect with all values adjusted to 1995, the year when the samples were analyzed [14]. Because this adjustment was linear and constant, it was unlikely to substantially

alter the relative rank ordering of individuals within this sample.

The mean (S.D.) number of testosterone assessments in the study was 5.09 (1.85) over an average span of 14.0 (8.65) years. Mean total T level was 13.82 (2.68) nmol/L with an annualized rate of decline of 0.16 (0.17) nmol/L per year. Mean FTI was 0.19 (0.08) with an annualized rate of decline of 0.005 (0.003) nmol/nmol per year. In the present analysis, we used the mean FTI and TT values as indicators of individual differences in long-term T levels.

### 2.3. PET acquisition

PET scans were acquired on a GE 4096+ PET scanner, which provides 15 slices of 6.5 mm resolution FWHM. CBF was measured using [$^{15}$O] water and the bolus technique with 2.8 GBq per scan injected intravenously. Images were acquired for 60 s after total brain counts reached a threshold of 11,000 counts/s, and rCBF was measured in nCi/cm$^3$. Measured attenuation correction was obtained using transmission scans. At each visit, subjects received three PET scans under three different conditions: rest with eyes open, verbal delayed recognition task, figural delayed recognition task, with an interval of 13 min between successive injections. Order of conditions was counter-balanced across subjects. PET scans obtained during the resting condition at baseline and 2-year and 4-year follow-ups were used in the present analysis.

### 2.4. Image processing

For each year separately, the three PET images for different conditions were realigned using SPM99, and the mean of the three images was co-registered to the respective structural MRI scan. Next, each MRI was transformed to the Talairach stereotaxic space, using the STAR algorithm for elastic transformation [7], and the MRI transformation parameters were applied to the co-registered PET images. A uniform transformation was then applied to all images to account for differences between our implementation and the SPM99/Montreal Neurological Institute (MNI) definition of the Talairach coordinate space. Finally, images were smoothed using a 12 mm cubic filter. Normalization of the PET images to the global brain activity (expressed in nCi/cm$^3$ and scaled to a mean global value of 50) was performed using proportional scaling to the gray matter mean.

### 2.5. Statistical analysis

The primary statistical analysis was performed using SPM99 and the mean normalized PET image across resting conditions acquired at baseline, 2-year and 4-year follow-up. We used voxel-based multiple regression analysis with mean T values (FTI and total T in separate analyses) and mean age at the time of T assessment as independent predictors. Mean images, rather than individual images for each year, were used in the primary analysis to increase reliability of PET assessments and for comparability with the use of the mean T and age values over multiple years as predictors. Significant associations are reported at $p < 0.01$ statistical threshold, with a spatial extent threshold of 100 voxels. Secondary correlational analyses were performed using individual images for each year, separately, and mean T and age values as predictors as an internal reliability check to confirm findings based on mean images. Using the $x$, $y$, $z$ coordinates corresponding to local maxima for each region significant in the primary analyses, a small volume search was conducted for each region for each year. A spherical volume with radius of 10 mm and a significance threshold of $p < 0.01$ without spatial extent correction was specified. Regional localization for all analyses was determined using the Talairach coordinates [36] and overlays on the SPM99 T1-weighted MRI provided by the MNI. These definitions were verified using standard anatomic atlases.

## 3. Results

Results of the SPM multiple regression analyses showing significant associations between rCBF and mean T levels are presented for free T and TT in Table 1. Positive associations between the mean FTI and regional brain activity were observed in the hippocampus bilaterally, extending to the parahippocampal gyrus on the right, the right middle and inferior temporal cortex, the anterior cingulate and the cerebellum bilaterally (Fig. 1). There were no regions showing negative associations between the FTI and rCBF. For TT, a different pattern of associations was observed. Positive associations between mean TT and regional activity were seen in left subcortical structures, including the putamen, globus pallidus, and thalamus, and in left inferior frontal cortex (Fig. 2). Negative associations between TT and rCBF were observed in the amygdala and uncal region bilaterally. Secondary analyses for each year separately confirmed the associations between the FTI and rCBF across all years, with the following exceptions: year 1 right hippocampus/parahippocampal gyrus, year 3 right inferior temporal region, year 1 and year 5 right inferior frontal. Secondary analyses using the individual PET scan images for each year also confirmed the positive associations between Total T and rCBF in the thalamus and negative associations between Total T and rCBF in the uncus/amygdala region.

## 4. Discussion

The results of the present study demonstrate that endogenous total and free T measured over a mean of 14 years are significant predictors of patterns of neural activity as determined by PET rCBF, a measure tightly coupled with regional cerebral metabolism under typical conditions. Higher free T was associated with increased rCBF in the hippocampus bilaterally, right parahippocampal gyrus, the anterior cingu-

Table 1
Significant associations between endogenous free and total testosterone levels and regional CBF

| | Brodman area (BA) | *x* | *y* | *z* | *t*-Value |
|---|---|---|---|---|---|
| Free T—positive associations | | | | | |
| R parahippocampal gyrus | 36 | 26 | −34 | −14 | 4.05 |
| L parahippocampal gyrus/HIP | 36/28 | −32 | −20 | −20 | 3.17 |
| R middle temporal gyrus | 21 | 60 | −34 | −10 | 3.39 |
| R inferior temporal gyrus | 20 | 54 | −24 | −28 | 2.81 |
| R cerebellum | | 18 | −70 | −24 | 3.13 |
| L cerebellum | | −24 | −68 | −28 | 3.27 |
| R cingulate | 24/32 | 2 | 40 | 2 | 3.36 |
| L medial frontal gyrus | 10 | −4 | 54 | 6 | 2.86 |
| R inferior frontal gyrus | 47 | 38 | 38 | −12 | 2.86 |
| Total T—positive associations | | | | | |
| L putamen | | −28 | 10 | −8 | 3.62 |
| R thalamus | | 22 | −16 | 2 | 3.48 |
| L thalamus | | −18 | −16 | 4 | 3.13 |
| L inferior frontal gyrus | 45 | −36 | 28 | 4 | 3.18 |
| Total T—negative associations | | | | | |
| R uncus/amygdala | 28 | 22 | 2 | −24 | 4.27 |
| L uncus/amygdala | 28 | −14 | 6 | −24 | 3.56 |

*x*, *y*, *z* are the coordinates of the local maxima in MNI space.

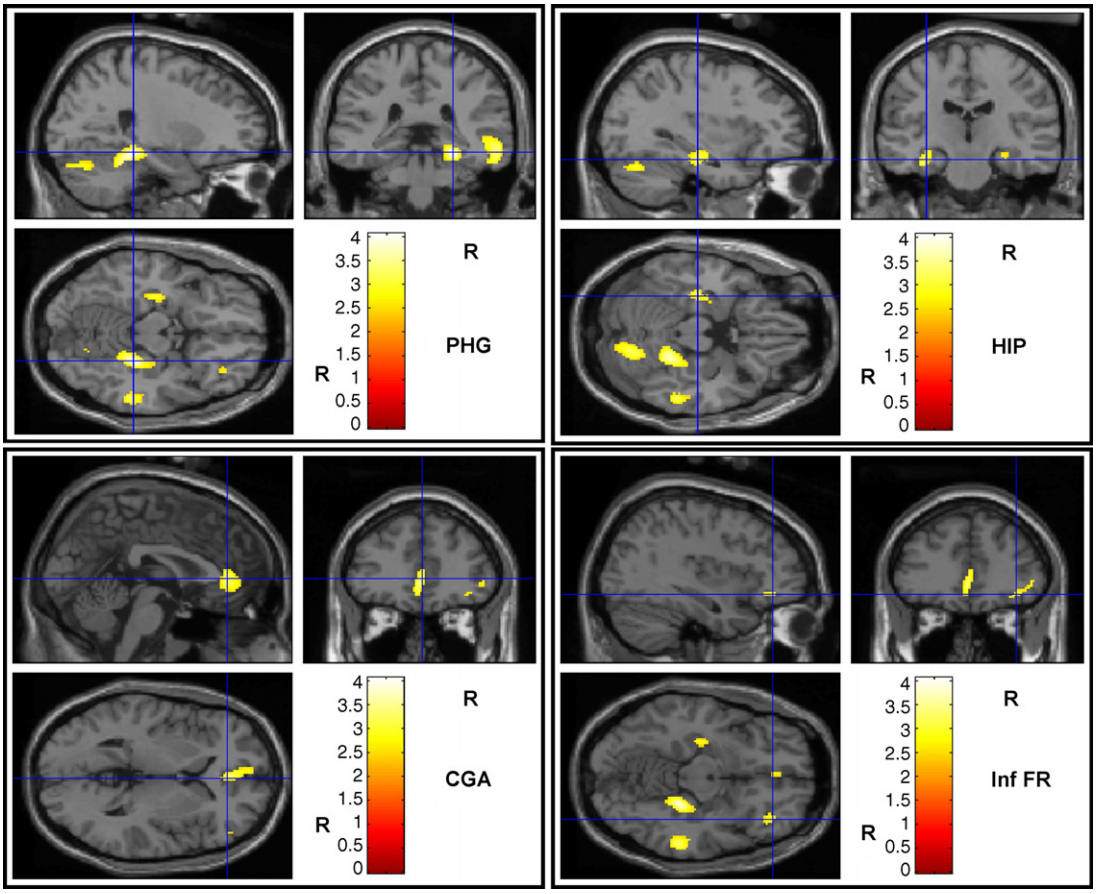


Fig. 1. Positive associations between Free T and regional CBF. Cross-hairs reflect the localization of the local maxima in MNI space (*x*, *y*, *z* coordinates). Abbreviations: PHG, parahippocampal gyrus; HIP, hippocampus; CGA, anterior cingulate; Inf FR, inferior frontal.

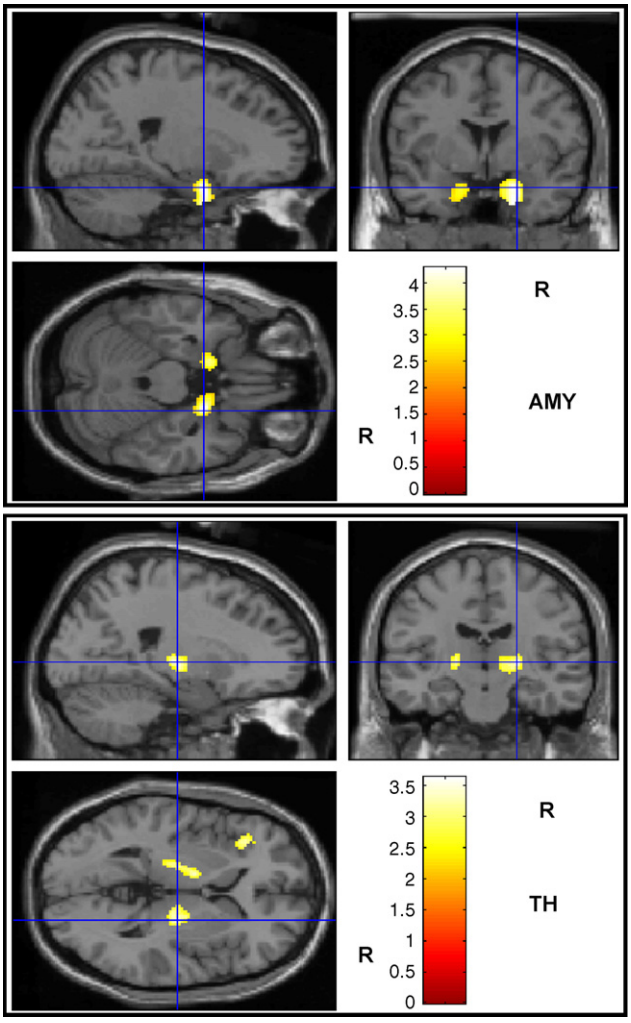


Fig. 2. Significant negative and positive associations between Total T and regional CBF in amygdala (AMY) and thalamus (TH), respectively. Crosshairs reflect the localization of the local maxima in MNI space (*x*, *y*, *z* coordinates).

late gyrus and right inferior frontal region. There were no brain regions showing negative correlations between rCBF and free T. Total T was associated with increased rCBF in the thalamus bilaterally and reduced activity in the amygdala and uncal regions. These findings suggest one way in which circulating total and/or free T concentration may modulate cognitive and neural function in older men.

These findings are internally consistent with our previous research on larger samples drawn from the BLSA. In these earlier studies, we found higher free T was associated with reduced risk for AD and with enhanced verbal and visual memory, sustained attention and cognitive flexibility, and increased spatial function. Moreover, higher free T was associated with a reduced *rate of decline* in visual memory, a measure that may be an early predictor of a subsequent diagnosis of AD [17,42]. Selective correlations between free T and performance on tests of memory and attention/set-shifting parallel our observations of associations between Free T and rCBF in the hippocampus and anterior cingulate gyrus, which respectively subserve some aspects of these cognitive functions [5]. Moreover, our finding that higher free T was associated with increased hippocampal function may have important clinical implications. The hippocampus subserves both verbal and visual memory and shows pathology early in the course of Alzheimer's disease. Our results suggest the possibility that higher free T may enhance hippocampal blood flow and neural activity, exerting beneficial effects on memory and cognitive function and serving as a factor that may protect against the development of AD.

Associations between total T and rCBF did not show the same pattern of results, with positive associations most pronounced for some subcortical structures and negative associations with the amygdala. It is important to emphasize that in our previous studies, which were based on the same population of subjects, only free T was associated with cognitive function and risk for Alzheimer's disease with total T showing no significant associations. The differences in results for free and total T most likely reflect the difficulties in relating circulating total T concentrations to CNS function, as only the free or loosely bound portion (reflected in the free T index) is bioavailable.

Numerous investigations support the biological plausibility of a protective effect of T on cognitive function. Regions of the rat brain thought to subserve aspects of spatial learning, including the hippocampus, are targets of gonadal steroids [16,32]. In adult animals, androgen receptors have been found in high concentration in hippocampal CA1 pyramidal cells [18]. Androgen treatment may prevent NMDA excitotoxicity in hippocampal CA1 neurons [29] and may facilitate recovery after injury by promoting fiber outgrowth and sprouting in hippocampal neurons [27]. Moreover, T administration increases nerve growth factor (NGF) levels in the hippocampus, septum and neocortex and induces an up-regulation of NGF receptors in the forebrain [38]. In rodents, strain-specific age-related deficits in long-term memory have been associated with decreased serum testosterone concentrations [8], and androgen treatment modulates memory deficits in mice expressing human Apolipoprotein E epsilon 4 (ApoE4), a genetic risk factor for AD[30]. T decreases β amyloid secretion from rat cortical neurons [10] and reduces β amyloid-induced neurotoxicity in cultured hippocampal neurons [9]. Moreover, T was found to be protective of human primary neurons in culture, and this neuroprotection was independent of estrogen action [13]. Taken together, these findings suggest that T may exert important neurotrophic and neuroprotective effects and play an important role in β amyloid biochemistry.

Observational studies and some small randomized clinical intervention trials suggested that hormone therapy in women may exert beneficial effects on specific cognitive functions [21] and may reduce the incidence and delay the onset of Alzheimer's disease [20,41]. Recent findings from the Women's Health Initiative Memory Study (WHIMS) have illustrated the complexities in interpreting these earlier findings. Data from the large multi-center randomized

trials of WHIMS indicated an increased risk for dementia among older postmenopausal women assigned to combination estrogen plus progestin therapy compared to placebo [35] and in the combined sample comparing active treatment (with or without progestin) to placebo [34]. Findings from the Women's Health Initiative suggest that much caution is warranted in interpreting the present observational data, but also highlight the relative dearth of comparable studies of the effects of T in men.

Although our data suggest the possibility that free T may enhance hippocampal and cingulate blood flow in elderly men, a causal impact of T on brain physiology cannot be confirmed from correlational data. An alternative interpretation of our data that cannot be excluded is that activity in brain regions such as the hippocampus or amygdala may exert downstream effects on the hypothalamic-pituitary axis and indirectly affect circulating T levels. Nor can we eliminate the possibility that high Total and free T levels may serve as a marker for rather than a cause of enhanced cognitive and neural function. In addition, we cannot determine from these data whether the association between T and rCBF is mediated via androgen or estrogen pathways as T is aromatized to estradiol in the CNS.

Results of the current study, taken together with those of recent epidemiological and intervention studies suggest that the maintenance of high free T levels in men may contribute to the maintenance of normal cognitive and neural function. Intervention studies are warranted to evaluate the safety of T administration in men and to confirm whether T can enhance cognitive and brain function and possibly delay the onset of AD.

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