

Testosterone and gonadotropin levels in men with dementia

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Abstract

OBJECTIVES: Sex steroids such as testosterone and estradiol might protect the brain against Alzheimer's disease (AD). We previously found lower levels of testosterone in men with AD compared with controls. We wanted to assess levels of pituitary gonadotropins that regulate sex steroid levels, to determine whether primary or secondary hypogonadism was responsible for low levels of testosterone in cases.

METHOD: We included 45 men with AD (McKhann, 1987), 15 men with other types of dementia and 133 elderly controls from the Oxford Project to Investigate Memory and Ageing. Gonadotropins (follicle stimulating hormone or FSH and luteinizing hormone or LH), sex hormone binding globulin (SHBG, which determines the amount of free testosterone) and testosterone were measured using enzyme immunoassays.

RESULTS: We found no difference in average LH (8.7 ± 9 UI/L), FSH (13 ± 17 UI/L) or SHBG (44 ± 18 nmol/L) levels between AD cases and controls. Similar to our earlier findings, testosterone levels were significantly lower in men with AD (13 ± 6 nmol/L) compared with controls (17 ± 8 , O.R. = 0.92, 95% C.I. = 0.87 to 0.97, $p < 0.005$). Results were unchanged when controlled for age, SHBG and gonadotropin levels.

CONCLUSION: Although normal, the levels of gonadotropins were inappropriately low for the levels of testosterone. Our results support a preliminary conclusion that secondary hypogonadism occurs in men with AD. This could be a consequence of brain degeneration. This is contrary to an earlier study (Bowen, 1999) that found raised levels of gonadotropins in cases with AD, suggesting primary hypogonadism. Our cohort was younger than theirs and gonadotropin levels increase with age. We are enlarging our data set to investigate whether primary hypogonadism occurs in older cases with AD or whether secondary hypogonadism precedes cognitive dysfunction in men at risk for AD. If this is true, testosterone replacement therapy for hypogonadal men at risk for dementia may be indicated.

Abbreviations:

OPTIMA	Oxford Project To Investigate Memory and Ageing
FSH	Follicle Stimulating Hormone
LH	Luteinizing Hormone
ERT	Estrogen Replacement Therapy
TRT	Testosterone Replacement Therapy
TT	Total Testosterone
FT	Free Testosterone
FAI	Free Androgen Index
SHBG	Sex Hormone Binding Globuline
MMSE	Mini-Mental Status Examination
AD	Alzheimer's Disease
ODS	Other Dementia Syndromes
Con	Controls
C.V.	Coefficient of Variation
APOE ϵ 4	Apolipoprotein E ϵ 4
B.M.I.	Body Mass Index

Introduction

Sex steroid hormones such as testosterone and estradiol could protect against Alzheimer's disease (AD) [1]. Potential mechanisms include the promotion of non-amyloidogenic processing of the amyloid precursor protein [2], the modulation of nerve growth factor [1, 3–5], the prevention of hyperphosphorylation of tau [6] and the modulation of apolipoprotein E expression in the brain [7]. Though much testosterone is converted to estradiol in the brain, testosterone or 5 α -dihydrotestosterone also acts directly through androgen receptors on various brain functions. These may include neuroprotective actions against oxidative stress [8], apoptosis [9] and the toxicity of β -amyloid [10].

Several studies reported (non significantly) lower levels of testosterone in non-institutionalised men with AD [11, 12] and in institutionalised men with unspecified dementia [13, 14] compared with controls. These studies could not determine whether the lower levels of sex steroids in men with dementia were a cause or an effect of the disorder. Indirect evidence from the literature suggests that changes in the brain in AD could cause low TT. This evidence is based on measures of pituitary function. Two gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) produced by the pituitary, normally regulate sex steroid hormone production. While an earlier study suggested a decreased pituitary function with age in men with low testosterone [13], increased levels of LH and FSH with age in non demented men [14, 15] have been reported. Levels were further increased in men with AD compared with age-matched controls [14] and it was suggested that this in itself was a possible risk factor for cognitive decline and might contribute to an increase in plaques in the brain. In another cohort, LH (but not FSH) was also higher in men with AD than controls and cases with frontotemporal dementia, but this was no longer the case when the analyses were adjusted for age [16]. These authors did report that women with AD not using ERT had higher levels of LH and FSH compared to controls not using ERT, but not between the groups who were using ERT. It is thus unclear whether AD is associated with decreased or increased hypothalamo-pituitary function and whether this interacts with age.

In addition, it is unclear whether alterations in gonadotropin and/or TT levels are specific to AD or relate to dementia in general. This could be tested by investigating whether these alterations also occur in men with other types of dementia or neurological degenerative disease. In the current study we further investigated whether serum gonadotropin (LH and FSH) levels were elevated or decreased in a well-studied cohort of men with AD and with other types of dementia as compared with controls without cognitive impairment, while controlling for age. The present study also investigated testosterone and SHBG levels from a later visit than the ones reported before and also included more participants to confirm our original finding [17].

Method

Subjects

The Oxford Project To Investigate Memory and Ageing (OPTIMA) was started in 1988 and has since recruited over 600 subjects. For the present study, serum samples of n = 193 participants were available. The cases (possible, probable and definite AD and other types of dementia) and controls were followed up yearly with a complete medical examination, which also included history taking, brain scanning, blood sampling and neuropsychological testing [18]. Diagnoses of dementia were made according to the National Institute of Neurological and Communicative Disorders and Stroke / the Alzheimer's Disease and Related Disorders Association' (NINCDS/ADRDA [19]) criteria in life and the 'Consortium to Establish a Registry for Alzheimer's Disease' (CERAD) histopathological criteria for post mortem confirmation of 29 of the AD cases [20]. Our clinical diagnoses (of the living cases) were found to have good validity (with respect to post-mortem histopathological diagnosis) and interrater-reliability [21].

Biochemistry

All assays were performed using non-fasting serum samples that had been stored at -70° C. Serum was collected between 10 and 12 a.m. We analyzed FSH and LH using enzyme immunoassays (Bayer® Immuno 1, Bayer Corporation, 511 Benedict Avenue, Tarrytown, N.Y. 10591-5097 U.S.A., Technicon 1 Immunoassay). For FSH, the total coefficient of variation (C.V.) ranged between 2.8–3.2% and the within-run C.V. was between 1.6 and 2.4%. The sensitivity of this assay was set at 0.1 IU/L. Correlation with the Abbott Imx immunoassay was 0.99 in the range of 1 to 133 IU/L. For LH, the total C.V. ranged between 2.8–4.5% and the within-run C.V. was between 0.15 and 1.13%. The sensitivity of this assay was set at 0.3 IU/L. Correlation with the DPC radio-immunoassay was 0.98 in the range of 1 to 59 IU/L. Total testosterone was analyzed using a competitive enzyme immunoassay (Bayer® [22]). The total C.V. ranged between 3.0–12.9% and the within-run C.V. was between 2.4 and 12.9%. The sensitivity of this assay was set at 0.25 nmol/L. Correlation with the DPC C-A-C was 0.98 in the range of 0.05 to 15 ng/mL. SHBG levels were investigated using an immuno-enzymo-metric

assay (Immulite® 2000 SHBG). The total C.V. ranged between 4.2–6.6% and the within-run C.V. was between 2.3 and 6.6%. The sensitivity was 0.02 nmol/L. Correlation with the DPC Immulite assay was 0.95 in the range of 10 to 170 nmol/L. We calculated the free androgen index (FAI), an indicator of free testosterone unbound to SHBG, as TT/SHBG.

Statistical analyses

Because LH and FSH data were skewed, we used their log value for analyses. TT and SHBG had a normal distribution. We used univariate general linear models with simple contrasts (using the controls as a reference group) to examine whether there were differences in gonadotropins and confounds between AD cases (possible, probable and definite, n = 45), cases with other types of dementia (n = 15) and controls (n = 133), co-varied for age. If age had a significant main effect, we also looked at the interaction between age and group. Separate logistic regression analyses were done for AD and controls to assess the relative contribution of gonadotropins, age, testosterone and SHBG as potential risk factors. Analyses were done in SPSS 10.0 [23] and the level of significance was set at 0.05 (two-tailed).

Results

Characteristics of the cohort

The characteristics of the cohort are shown in Table 1. There was no difference in age between AD and controls, but cases with other types of dementia (ODS) were 7 years younger on average. ODS cases had lower FSH, TT and FAI levels than controls. There was also a trend for lower LH and for higher SHBG in this group compared with controls. For AD cases, there were no differences in the biochemistry variables, except for TT and FAI levels, which were both lower. The GLM analyses also showed that there were main effects of age for LH [F(1,89) = 7.00, p = 0.009], FSH [F(1,189) = 20.61, p = 0.0001] and SHBG [F(1,186) = 28.44, p = 0.0001], which all increased with age and FAI, which decreased with age [F(1,186) = 36.34, p = 0.0001]. TT showed no main effect of age (p = 0.63). We repeated analyses including the age x group interaction. This interaction only showed a trend for LH [F(2,107) = 2.83, p = 0.06] but none of the other GLMs had a significant age x group interaction. To investigate this interaction, we performed separate non parametric Spearman's rank correlation analyses per group which showed that there was no correlation between age and LH (p = 0.23) or FSH (p = 0.51) in the ODS group, but for both AD cases (LH: p = 0.09, FSH: rho = 0.30, p < 0.05) and controls (LH: rho = 0.29, p < 0.001, FSH: rho = 0.31, p < 0.0001), older men had higher levels than younger men.

Table 1. Cohort characteristics (mean values and SD), p-values are based on adjusted* GLM

	Controls (n=133)	AD (n=45)	ODS (n=15)	Group F	p-value	Con-ODS p-value	Con-AD p-value
<i>Demographics</i>							
Age	74.7 (9.0)	72.5 (9.3)	65.2 (12.2)	7.26	0.001	0.0001	0.18
MMSE	28.1 (2.3)	18.9 (7.3)	22.6 (5.4)	78.60	0.0001	0.0001	0.0001
<i>Biochemistry</i>							
LH (IU/L)	8.6 (8.4)	8.1 (10.0)	5.3 (7.0)	1.96	0.15	0.06	0.14
FSH (IU/L)	12.8 (14.9)	12.0 (16.1)	5.6 (4.7)	2.73	0.07	0.03	0.23
TT (nmol/L)	17.2 (7.7)	13.3 (5.7)	13.3 (5.8)	6.31	0.002	0.04	0.001
SHBG (nmol/L)	43.2 (18.3)	43.9 (17.2)	45.5 (17.4)	1.91	0.15	0.06	0.44
FAI (TT/SHBG)	0.43 (0.19)	0.33 (0.15)	0.34 (0.19)	11.35	0.0001	0.05	0.001

* For age

Table 2. Logistic regression predicting Alzheimer's disease

	O.R.	unadjusted 95% C.I.	p-value	adjusted O.R.	95% C.I.	p-value
TT (nmol/L)	0.92	0.87–0.97	0.003	0.90	0.84–0.95	0.0001
LogFSH				0.66	0.35–1.24	0.20
LogLH				1.22	0.62–2.40	0.57
Age (years)				0.97	0.93–1.01	0.10
SHBG (nmol/L)				1.03	1.00–1.05	0.03
FAI (TT/SHBG)	0.04	0.004–0.34	0.003	0.009	0.001–0.11	0.0001
LogFSH				0.61	0.32–1.16	0.13
LogLH				1.36	0.69–2.68	0.37
Age (years)				0.96	0.92–1.00	0.04

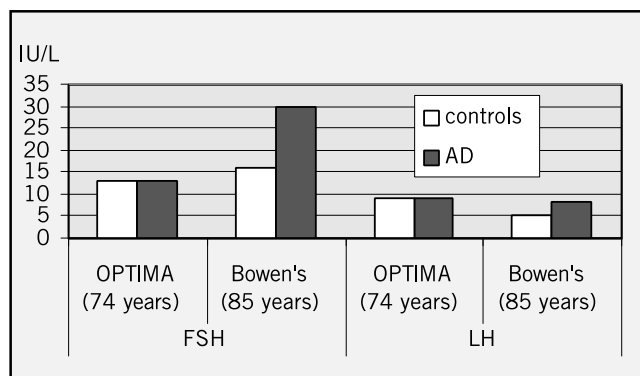


Figure 1. Comparison of mean gonadotropin levels from an older (Bowen et al., 2000) & younger (OPTIMA, 2002) cohort (average age given).

For the ODS cases, low LH (but not FSH) was correlated with low FAI ($\rho = 0.71$, $p < 0.005$), but this was not apparent for AD cases ($p = 0.33$) or controls ($p = 0.17$). Stepwise backward linear regression analyses were used to assess which factors were associated with low TT levels per group. For the ODS cases, LH and FSH (to a lesser extent) determined TT levels ($\beta = 0.84$, $p = 0.01$, 95% C.I. = 1.60 to 9.40 resp. $\beta = -0.79$, $p = 0.01$, 95% C.I. = -8.14 to -1.17). However, for controls, a younger age ($\beta = -0.16$, $p = 0.06$, 95% C.I. = -0.28 to -0.004) and high SHBG levels ($\beta = 0.49$, $p = 0.0001$, 95% C.I. = 0.14 to 0.28) and for AD only high SHBG ($\beta = 0.37$, $p = 0.01$, 95% C.I. = 0.03 to 0.22) levels were associated with low TT. Logistic regression analyses revealed that low TT and high SHBG levels were significant independent risk factors for AD. Similar results were achieved for low FAI (Table 2).

Discussion

We replicated our earlier findings and those of others of low testosterone in men with dementia [13, 14, 17]. However, we did not find that LH and FSH levels were different between AD cases and controls. These results are similar to those of a larger cohort [16] but deviate from another which showed elevated LH and FSH levels in cases with dementia [14].

We attempted to explain these differences. Comparing the cohorts, we found that LH levels differed between controls of the two cohorts (ours and Bowen's) but not between the AD cases. Firstly, blood sampling differences may have accounted for the differences in levels. When blood samples are left at room temperature for a longer period of time, conversion of LH can be expected to take place. The control samples from Bowen's cohort were received from general practices where this could be more likely to occur. FSH is a more robust measure, has a longer half life and shows less circadian fluctuations than LH and could therefore be expected to be less sensitive to this type of confound. For FSH, the largest difference was between the cases of the cohorts (see also Short *et al.*, 2001). Secondly,

Bowen's cases were long-term care facility residents and our cases were community dwelling, which could be hypothesized to confound. Abbasi *et al* (1993) also included institutionalized cases but found the opposite: that they were more likely to have lower levels of LH than controls. Unfortunately, they did not measure LH in all participants and a direct comparison between cases and controls was not made. Interestingly, their cases were on average 10 years younger (65 years of age) than ours. This brings us to a third possible explanation. FSH and LH levels have been found to increase with age [14, 15]. Similarly, our group with other types of dementia, who were 10 years younger on average than the AD cases and controls, had much lower LH and FSH values than both groups. The cohort that showed higher gonadotropin levels in cases [14] was on average 11 years older (85 years of age) than ours. The mean values of gonadotropins for each cohort are displayed in Figure 1. Particularly FSH levels were much higher in their cases. The study that found no difference between their male cases with dementia and controls [16] was, in terms of age (79 years) between the two studies. Their median value for LH (5.5 averaged over both groups) was similar to ours (5.9) but their median averaged FSH level was slightly higher (8.6) compared with ours (6.6). Interestingly, again the median level of FSH for their cases was almost twice as high as our cases. This could suggest that in older AD cases (> 79 years of age), levels of gonadotropin levels (particularly for FSH) are higher than those of controls. We are currently enlarging our data set in order to test whether there is an interaction between age and group for gonadotropin levels. This interaction between age x group in cases could possibly also explain why Short *et al.* found higher LH and FSH in women with AD not using ERT compared to controls not using ERT, but not between the groups who were using ERT as women using ERT, in general, are younger compared to those who are not [24]. In addition, any inference regarding gonadotropins in estrogen users is difficult because estrogen suppresses gonadotropin levels.

Our results show low levels of TT combined with low to normal LH and FSH in cases with dementia suggesting secondary (central) hypogonadism. This could result from damage to the hypothalamo-pituitary axis. This in turn could be due to vascular disease, infections, trauma or tumors, but it is also seen after acute illness or during chronic systemic disease. While none of the participants had acute disease, all of the above factors are also risk factors associated with cognitive deficiency and dementia. It is thus unclear whether lower TT is a co-morbid effect of dementia (caused by other risk factors) or results from secondary hypogonadism which could be secondary to general brain degeneration. The SHBG results could shed more light on this. For both controls and AD, low SHBG levels were associated with low TT but high SHBG was an independent risk factor for AD even though average levels were not different between cases and controls. How can these seemingly contradictory results be explained?

Firstly, SHBG will increase in cachexia or general wasting syndrome, which occurs in the later phases of dementia and which is also known to deregulate the hormonal milieu. However, the cases in our study were in the mild to moderate phases and nobody had yet progressed to the later phases of the disease. We also earlier did not find differences in body mass index (B.M.I.) between cases and controls [17] and post hoc analyses of this cohort also revealed no significant differences in their B.M.I. Secondly, low SHBG was associated with low TT levels in both cases and controls. Modest reductions in SHBG levels may be encountered in individuals with hypothyroidism, Cushing's syndrome, hyperprolactinemia and in those receiving growth hormone treatments or glucocorticoids. Co-morbidity could thus potentially decrease SHBG levels (and subsequently increase free levels of testosterone) and be a confound. However, patients with AD are often remarkably healthy, apart from their cognitive impairments in the earlier phases of the disease. In addition, both low TT and low FAI, as an indicator of free testosterone, had independent (of SHBG) associations with AD. Although levels were not different, surprisingly, *high* SHBG was an independent risk factor for AD. Elevated levels of SHBG may be observed during administration of drugs, such as phenytoin, that promote hepatic enzyme induction, in hepatic cirrhosis and in hyperthyroidism. While subclinical hyperthyroidism was more prevalent in cases than controls [17], our earlier analyses showed that TSH levels, as a measure of thyroid function, (but also alcohol abuse which would affect the liver function), were related to AD independent of TT. Lastly, SHBG is known to simply increase when TT decreases, which could also explain its independent association with AD.

There is a possibility that secondary hypogonadism actually precedes the dementia and is a risk factor for it. As discussed in the introduction, there is a strong case for the biological protective mechanisms of testosterone from animal studies and hence for the potential of testosterone in the treatment of cognitive impairment [2, 3]. However, evidence from human studies that testosterone can preserve cognitive function in older men by testosterone replacement therapy (TRT) is still inconclusive [25–32]. We have hypothesized that there is a window of time – and possibly an optimum dose range – in which TRT would be most effective for cognitive maintenance. Preliminary data-analyses of a cohort of healthy non-demented men between 47 and 72 years of age from the Maastricht Aging Study showed level dependent effects of endogenous testosterone on verbal memory, but this was not found in non-demented men over 72 using data of the Foresight Challenge cohort [25]. We also found that men who had not (yet) developed AD, but had the apolipoprotein E ϵ 4 genotype (*APOE* ϵ 4), a risk factor for AD, had lower levels of testosterone, independent of sex hormone binding globulin (SHBG) and age [11]. These results are open to various interpretations. One is that *APOE* ϵ 4, among other factors, lowers testosterone in male controls and that low testosterone, whether or not

as a consequence of *APOE* ϵ 4 status, contributes to the onset of AD. Another interpretation is that *APOE* ϵ 4 lowers testosterone in controls and that AD results in a lowering of testosterone levels for other reasons. However, combined, these findings could implicate that middle-aged men who are genetically at risk for AD could benefit from dose-dependent TRT. We are currently investigating longitudinal data of our cohort to establish whether secondary hypogonadism (with low TT as a by-product) precedes cognitive dysfunction in controls and cases with mild cognitive impairment and dementia.

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