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One year follow-up study of the association between chemical castration, sex hormones, beta-amyloid, memory and depression in men

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Summary The results of several recent studies suggest that estrogen and testosterone play an important role in the modulation of mood and cognitive function in women, and preliminary evidence indicates that these hormones may also modulate the levels of beta-amyloid ($A\beta$), a 4 Kilo Dalton peptide that is likely to be involved in the pathogenesis of Alzheimer's disease. However, the physiological and clinical effects of reversible castration remain unclear and no systematic data is currently available for men. We designed the present study to investigate the effects of reversible chemical castration on the mood and cognitive performance of men treated for prostate cancer, as well as its impact on the levels of plasma $A\beta$. Forty men with prostate cancer were clinically treated with androgen blockade therapy (flutamide and leuprolide) for 36 weeks and subsequently followed up for another 18 weeks after treatment was discontinued. All subjects received a comprehensive clinical, neuropsychological and biochemical evaluation that included the use of the Beck Depression (BDI) and Anxiety Inventories (BAI), several subtests of the Wechsler Memory and Intelligence Scales (Word Lists-WL, Verbal Paired Associates-VPA, Visual Reproduction-VR and Block Design-BD), and biochemical monitoring of changes in estrogen, testosterone and $A\beta$ levels. Chemical castration was associated with a rapid and marked decline in the levels of testosterone and estradiol, and significant increase in plasma $A\beta$ levels. Treatment was associated with increased BDI ($p = 0.004$) and BAI scores ($p < 0.001$), although such changes were of questionable clinical significance (i.e., few subjects had scores ≥ 13). CAMCOG ($p = 0.046$) and WL recall total scores ($p < 0.001$) improved significantly after androgen blockade treatment was discontinued, but visuospatial abilities, as assessed by BD, was not influenced by the introduction or discontinuation of treatment. There was a significant negative correlation between changes in $A\beta$ levels and subjects' WL total score

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change between weeks 36 and 54 ($r = -0.452$, $p = 0.012$). The results of this naturalistic study indicate that chemical castration is associated with a significant rise in the plasma levels of Aβ and, clinically, with increased depression and anxiety scores. The discontinuation of treatment is associated with better cognitive performance, most noticeably of verbal memory. The performance of subjects on the WL test was negatively correlated with plasma levels of Aβ, but the clinical significance of this finding remains to be determined.

1. Introduction

The results of several recent studies suggest that estrogen use is associated with significant clinical effects on mood and cognitive function. We have shown, in a randomised, double-blind, placebo-controlled trial, that estrogen replacement is an efficacious treatment of depression during the perimenopause (Soares et al., 2001), confirming and extending the results of previous observational surveys and small clinical trials (Zweifel and O'Brien, 1997; Schmidt et al., 2000). There is also tentative evidence that estrogen use after the menopause improves verbal memory and decreases the risk of Alzheimer's disease (AD) in later life (Tang et al., 1996; Kawas et al., 1997; Baldereschi et al., 1998; Waring et al., 1999; Hogervorst et al., 2002), although estrogen replacement is not indicated for the treatment of women with AD (Almeida and Barclay, 2001; Mulnard et al., 2000).

Testosterone is another sex hormone that seems to influence certain aspects of mood and cognition. Low testosterone levels are associated with docile conduct, whereas high levels correlate with impulsive and aggressive behavior (Dabs et al., 1996; Pope et al., 2000). A large survey of 5236 Vietnam veterans found a weak but significant inverse association between testosterone levels and the presence of depression as assessed by the Diagnostic Interview Schedule (Mazur, 1995). Schweiger et al. (1999) reported further evidence supporting the existence of a relationship between testosterone and depression—they found that their 15 male patients with major depression had significantly lower 24 hour testosterone secretion than 24 healthy controls. Hormone replacement has also been shown to decrease depressive scores among hypogonadal HIV-positive men randomised to treatment with either testosterone (up to 400 mg every other week) or placebo (Rabkin et al., 2000), and there is preliminary evidence suggesting that the response of hypogonadal men to antidepressant treatment may be significantly improved by the introduction of testosterone replacement (Pope et al., 2003).

A number of recent papers have also indicated the existence of an association between testosterone levels and cognitive function. One of the earliest clinical studies was reported by Janowsky et al. (1994): they randomised 56 older men to treatment with testosterone ($n = 27$) or placebo ($n = 29$). Testosterone use was associated with enhanced spatial abilities, as measured by the block design test, but had no obvious effect on delayed recall or visual reproduction abilities at the end of 3 months. Subsequent studies confirmed that testosterone supplementation improves certain aspects of cognitive function in both young and older adults (Postma et al., 2000; Wolf et al., 2000; Cherrier et al., 2001; Cherrier et al., 2002), but it is unclear whether the cognitive effects of testosterone only become apparent once normal to high plasma levels are achieved.

We designed the present study with the aim of clarifying whether testosterone depletion and receptor blockade are associated with changes in mood and cognitive function in humans. We hypothesised that androgen deprivation would lead to a significant increase in depression scores and decline of performance on tests assessing visual memory and visuo-spatial abilities, but not verbal skills (there is preliminary evidence that androgens are directly and indirectly correlated with visuospatial and verbal abilities respectively—see van Goozen et al., 1995). We further hypothesized that the discontinuation of androgen deprivation would reverse the above-mentioned changes in mood and cognitive function. Finally, we hypothesised that hormone deprivation would increase plasma β-amyloid levels.

2. Subjects and methods

Men with prostate cancer who were prescribed intermittent androgen deprivation treatment with leuprolide 22.5 mg (SC) every three months and flutamide 250 mg (PO) tid were approached to

join the study. Leuprolide is a LHRH agonist that depletes men of testosterone and estradiol, whereas flutamide is used to block testosterone receptors. Participants commenced the hormonal therapy using flutamide 250 mg for one week before receiving leuprolide—this approach aimed to prevent the flare associated with the initial use of LHRH agonists. Androgen blockade was maintained for a total of 36 weeks, at which point subjects with Prostate Specific Antigen (PSA) < 4 ng/ml discontinued the therapy, but continued being followed-up for a total of 54 weeks or until their PSA > 4 ng/ml (whichever came first). Patients whose PSA levels remained higher than 4 ng/mL at the end of 36 weeks did not qualify for the 'off-treatment' period. We excluded from the study subjects who did not provide written informed consent, lived outside the Perth metropolitan area, were too ill to cope with the testing procedure/schedule, were from non-English speaking background, had a Cambridge Cognitive Examination for the Elderly (CAMCOG) score < 70 (to exclude subjects with dementia—Blessed et al., 1991), or presented with moderate to severe visual/auditory impairment or aphasia. Participants were recruited from the Department of Radiation Oncology at Sir Charles Gairdner Hospital in Perth, Western Australia. The study was approved by the Sir Charles Gairdner Hospital Ethics Committee.

2.1. Study design

This naturalistic study was specifically designed to investigate the psychological changes associated with chemical castration treatment over time. Eligible subjects were assessed one week prior to the introduction of treatment (pre-baseline) and again on the day they started flutamide (baseline). The pre-baseline assessment was introduced with the aim of familiarizing patients with the study's procedures and reducing practice effects associated with repetitive cognitive testing (i.e., baseline scores took the learning effect into account) (Benedict and Zgaljardic, 1998). Follow-up assessments took place 4, 12, 24 and 36 weeks after baseline. At 36 weeks, treatment was discontinued (the last injection of leuprolide was given at 24 weeks) and patients were re-assessed at 42, 48 and 54 weeks after baseline. Fig. 1 summarizes the follow-up assessments and progression of participants in the study.

2.2. Procedures and assessments

Subjects were asked to fast for 10 hours prior to each assessment. Blood was drawn every visit (except pre-baseline) between 8:30–10:00 am

to monitor PSA, oestradiol, testosterone and β-amyloid1–40 (Aβ) plasma level. Estradiol levels were estimated using IMMULITE 2000 (Immulite 2000 Estradiol L2KE2, Diagnostic Products Corp., Los Angeles, CA; Tello and Hernandez., 2000). Eestradiol is a solid phase, chemiluminescent competitive enzyme immunoassay. The solid phase, a polystyrene bead coated with a polyclonal rabbit anti-oestradiol antibody, is introduced into an Immulite test unit. Subjects' samples and alkaline-phosphatase-conjugated-E2 are simultaneously added to the test unit, and incubated for 60 minutes at 37 °C with intermittent agitation. During this time, E2 in the sample competes with the enzyme-labelled-E2 for a limited number of antibody binding sites on the bead. Unbound enzyme conjugate is removed by washing. Substrate is then added, and the test unit is incubated for a further 5 minutes as it is transported to the photomultiplier. The chemiluminescent substrate undergoes hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate, resulting in the sustained emission of light. The signal produced is inversely proportional to the concentration of E2 in the sample. Testosterone levels were determined using a one-step chemiluminometric competitive immunoassay. Sample, mouse monoclonal anti-testosterone antibody coated paramagnetic microparticles, testosterone acridinium-labelled conjugate and assay diluent were combined to create the reaction mixture. Testosterone present in the sample competes with the testosterone acridinium-labelled conjugate for binding with the anti-testosterone antibody coated paramagnetic microparticles, to form an antigen-antibody complex. Blocking reagent is included in the mixture, which binds to endogenous sex hormone binding globulin present in the sample, freeing all the testosterone for the competitive reaction above. After washing, trigger solutions, consisting of acidification with nitric acid (pH 2.1) and oxidation with hydrogen peroxide, followed by alkalinisation with sodium hydroxide and Triton X-100 are added to produce the light signal. An inverse relationship exists between the testosterone in the sample and the relative light units (RLUs) detected by the Architect (Abbott Architect-TES; Abbott Park, Illinois, USA; Ognibene et al., 2000). The analysis of plasma Aβ1–40 (Aβ) followed the procedures previously described by Mayeux and colleagues (1999)—plasma was stored at −70 °C immediately after collection. Aβ levels were determined using a combination of monoclonal antibody 6EI0 (specific to an epitope present on 1–16-amino acid residues of Aβ) and R162 (vs Ab1-
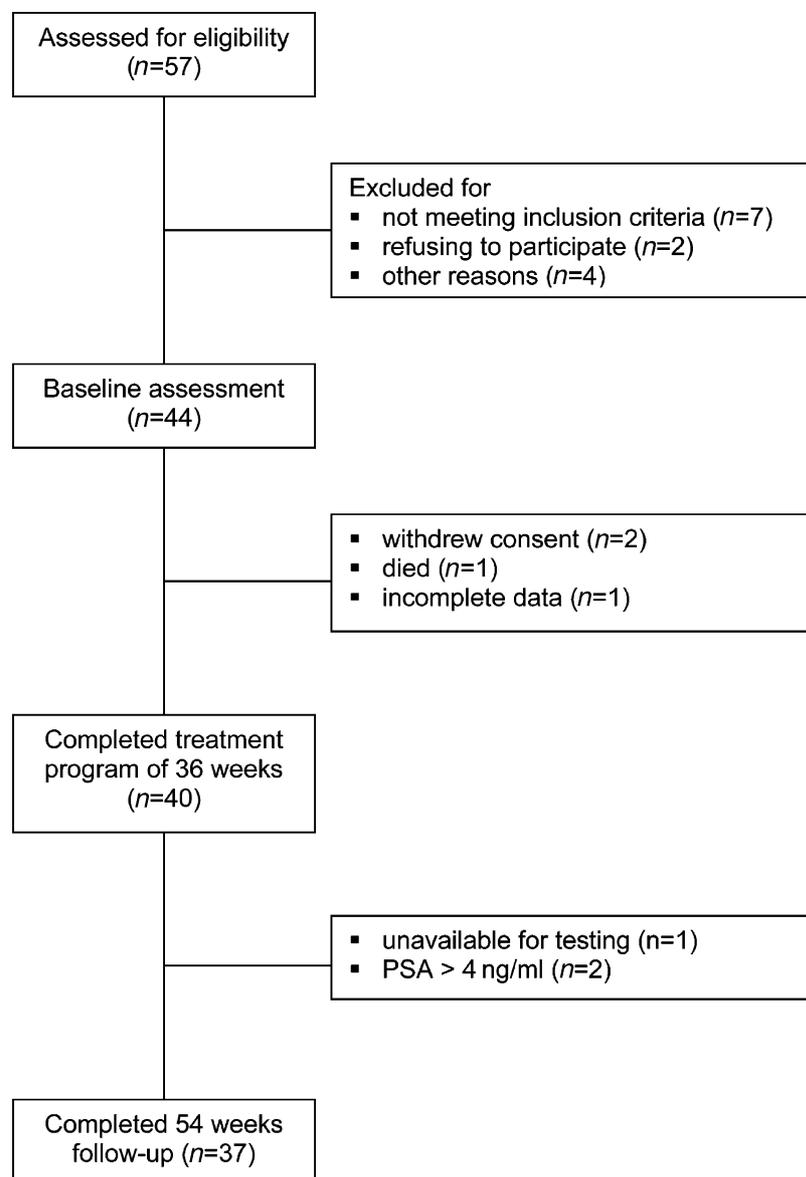
Fig. 1. Flow diagram of subjects' progress through the phases of the study.

40) antisera in a double-antibody sandwich enzyme-linked immunosorbent assay. The coefficient of variance for this assay in our laboratory was lower than 5%.

After breakfast, subjects underwent a comprehensive clinical and neuropsychological assessment that included the use of the following instruments:

- Beck Depression Inventory (BDI) (Beck and Steer, 1984). The BDI is a widely used self-rating scale that was designed to evaluate the severity of depression in clinical and research settings. It includes 21 questions with possible ratings ranging from 0 to 3. The scale is particularly useful in the assessment of negative thoughts associated with

depression. The BDI has high internal consistency (0.86 or greater) and is sensitive to change in the severity of depression (high scores are associated with increasing severity of depression).
- Beck Anxiety Inventory (BAI) (Beck et al., 1998). This self-rating scale includes 21 items describing common symptoms of anxiety that can be rated according to their intensity from 0 to 3. Internal consistency (alpha = 0.92) and test-retest reliability ratings ($r = 0.75$) are high, and so are different measures of validity.
- Cambridge Examination for Mental Disorders of the Elderly—Cognitive Battery Revised (CAMCO-G)(Roth et al., 1998). The CAMCOG is an instrument of general cognitive assessment divided into several subsections measuring various

aspects of cognitive functioning: orientation, language, memory, attention and concentration, praxis, perception, calculation, and executive functions. The total score can range from zero to 105, and is highly correlated with the MMSE total score (which can also be computed from the CAMCOG). Reported test-retest reliability scores are greater than 0.8.

- Word Lists (WL) (WMS-III; Wechsler, 1997). WL I measures immediate and delayed memory for verbal material. Subjects are presented with a list of 12 semantically unrelated words, and then asked to recall as many words as possible. This procedure is repeated for three additional trials for a total of four learning trials (range 0 to 48). Then, the examiner reads a new word list and asks the subject to recall the words on this list and, subsequently, on the first list. In WL II, the examinee is asked to recall the first list or words (delayed recall). Then the examiner reads a list of 24 words and asks the examinee to identify each word as either one he or she was asked to remember of a new word (recognition) (range 0 to 24). For the purposes of this study, we computed and analyzed the 'recall total score' (sum of trials 1 to 4), 'recognition total score' and 'percent retention' [(delayed recall/word list recall for trial 4) $\times$ 100].
- Verbal Paired Associates (VPA) (WMS-III; Wechsler, 1997). The VPA uses the same test procedures described for WL and produces measures of immediate and delayed cued recall for semantically unrelated pairs of words; i.e., during the recall phase of the test, the examiner reads aloud one word of the pair and the examinee is asked to recall the word that was associated with the former. As previously described for Word Lists, the performance of subjects on this test was summarised by the recall total score (range 0 to 32), recognition total score (range 0 to 24) and percent retention.
- Visual Reproduction (VR)(WMS-III; Wechsler, 1997). This is a test of visual memory that also involves constructional abilities. Subjects are asked to design, from memory, a complex figure immediately after presentation or after a delay of 25–35 minutes. Performance on this test can be evaluated by the recall total score (sum of scores for designs A-E) (range 0 to 104) and percent retention [(delayed recall total score/recall total score) $\times$ 100].
- Block Design (BD)(WAIS-III; Wechsler, 1997). This is a constructional test in which the subject is presented with four or nine colored blocks. The aim is to use the blocks to construct replicas of 10 designs printed in a booklet. This is a

sensitive test of visuospatial organisation, with the total possible score ranging from 0 to 68 points.

2.3. Data analysis

Data were analyzed using the statistical package 'Stata 7.0' (StataCorp, 2002). The data was initially explored and summarized with descriptive statistics. Multivariate analysis of variance for repeated measures was used to investigate the progression of scores over time, the number of degrees of freedom being equivalent to the number of repeated measures minus 1. The strength of the association between variables was assessed with the Pearson correlation coefficient (r) or Spearman's rho in the case of ranked data.

3. Results

Fifty-seven patients were approached to join the study—7 did not meet the inclusion criteria, 2 failed to provide informed consent, and 4 were not available for testing. The follow-up data for one other subject had to be discarded because of missing baseline information, two withdrew consent, and one man died during the active treatment phase. Hence, 40 men with prostate cancer commenced treatment and were available for follow-up during the active treatment phase of 36 weeks (Fig. 1). Subjects' age ranged from 44 to 83 years (mean = 72.4, SD = 7.5). Thirty-two men were married. Their formal education ranged from 12 to 20 years (mean = 14.9, SD = 1.4) and all came from English-speaking background. Their PSA level at baseline ranged from 5.9 to 556 ng/mL (mean = 51.6, SD = 106.7).

Fig. 2 illustrates changes in testosterone and oestradiol levels during the follow-up period associated with chemical castration (weeks 0 to 36) and after the discontinuation of treatment (weeks 36 to 54) (3 subjects excluded for weeks 42, 48 and 54 because of illness relapse or non-availability for assessment). Analysis of variance for repeated measures showed that testosterone level varied significantly between baseline and week 36 (effect of time—$F = 215.8$ (df = 4), $p < 0.001$), as well as from week 36 to week 54 (effect of time—$F = 30.6$ (df = 3), $p < 0.001$). A similar pattern was observed for plasma estradiol ($F = 83.7$ (df = 4), $p < 0.001$ and $F = 22.5$ (df = 3), $p < 0.001$, respectively). Data on plasma Aβ was available at all points for 30/37 of the men who completed the 54 weeks of follow-up. Nine of the thirty-seven cases could not be utilized because of tech-
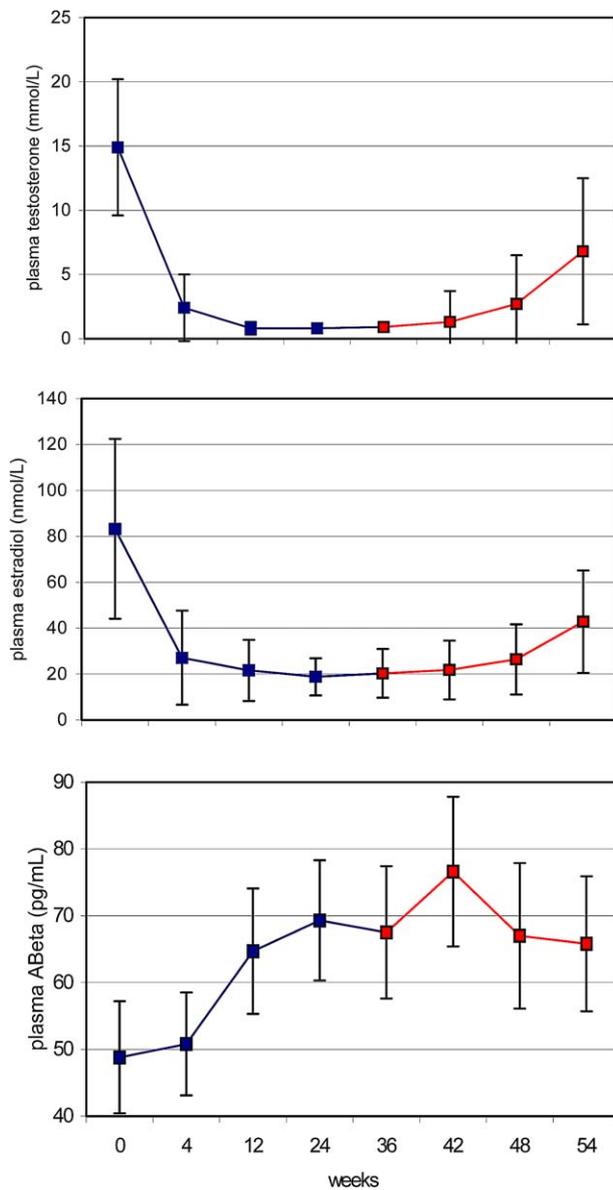
Fig. 2. Plasma testosterone (top), estradiol (middle) and β-amyloid1–40 (Abeta) (bottom) levels of men receiving hormonal deprivation therapy. The blue lines indicate mean plasma levels during the period of chemical castration (baseline to week 36), red lines indicate the mean plasma levels during the 'off treatment' period (weeks 36 to 54). The vertical bars represent the standard deviation.

nical problems associated with the collection and storage of some of the blood samples, which resulted in missing data for some of the time-points. Fig. 2 shows the changes in plasma Aβ over time. Aβ levels increased significantly between baseline and week 36 ($F = 2.7$, df = 4, $p = 0.036$), but there was no obvious change between week 36 and 54 ($F = 1.0$, df = 3, $p = 0.385$).

Table 1 summarizes the results of mood and cognitive assessments from baseline to week 54.

The complete follow-up data set was not available for 8 subjects for the following reasons: one subject had to recommence treatment between week 48 and 54 due to relapse of illness, three subjects were unavailable for one assessment, two were too unwell to reliably complete all the tasks, one subject did not discontinue treatment after week 36, and one subject refused to complete all the assessment battery on several visits. Analysis of variance for repeated measures showed that the total CAMCOG score improved significantly between week 36 and 54 ($p = 0.046$), although such a change lacked any obvious clinical meaning. Similarly, we observed that the Word List total recall score, as well as the Verbal Paired Associates total recall score, improved significantly once androgen deprivation therapy was discontinued ($p < 0.001$ and $p = 0.002$, respectively) (Table 1). Yet again, the improved cognitive performance could not be explained by changes in the BDI ($r = 0.123$, $p = 0.454$; $r = 0.137$, $p = 0.406$ respectively) and BAI scores ($r = 0.104$, $p = 0.529$; $r = -0.241$, $p = 0.139$ respectively).

Contrary to our prediction, subjects' performance on the Visual Reproduction and Block Design tests was not influenced by the introduction or discontinuation of hormonal treatment, although percent retention of material on the Visual Reproduction test seems to have increased progressively throughout the trial (Table 1).

There was no obvious association between changes in testosterone levels and the performance of subjects on the CAMCOG ($r = -0.07$, $p = 0.665$), WL total score ($r = -0.05$, $p = 0.773$), and VPA total score ($r = -0.124$, $p = 0.472$). A similar association pattern was found for estradiol levels ($r = -0.06$, $p = 0.713$; $r = 0.172$, $p = 0.315$; $r = -0.135$, $p = 0.432$ respectively).

Aβ levels were available for 30 study participants at week 36 and 54. There was a significant negative correlation between changes in Aβ levels and subjects' WL total score change between weeks 36 and 54 ($r = -0.452$, $p = 0.012$; $p = 0.036$ after Bonferroni correction for multiple comparisons). However, this association was not noticeable for VPA ($r = 0.09$, $p = 0.641$) or CAMCOG total score ($r = -0.02$, $p = 0.934$). Likewise, the association between change in Aβ level and VR percent retention between week 36 and 54 was not significant ($r = 0.125$, $p = 0.509$). The correlation between change in Aβ and estradiol ($r = -0.03$, $p = 0.858$) and testosterone levels ($r = 0.19$, $p = 0.319$) between weeks 36 and 54 was not significant.

Changes of cognitive scores for the CAMCOG, WL, VPA and VR tests between weeks 36 and 54

Table 1 Depression, anxiety and cognitive scores associated with the 'on' (italicized area) and 'off' phase of androgen deprivation therapy for the treatment of prostate cancer (information reported only for subjects who completed all relevant assessments)

	Pre-baseline	Baseline N = 40	Week 4 N = 40	Week 12 N = 40	Week 24 N = 40	Week 36 N = 40	p* 0–36	Week 36 N = 35	Week 42 N = 35	Week 48 N = 35	Week 54 N = 35	p* 36–54
BDI—mean (SD)	–	5.4 (4.8)	6.2 (4.5)	6.9 (4.4)	7.5 (5.1)	7.1 (4.8)	0.004	6.7 (3.9)	6.0 (4.6)	6.0 (3.0)	5.9 (4.4)	0.149
BAI—mean (SD)	–	4.7 (6.1)	5.0 (5.5)	8.5 (7.1)	8.9 (7.1)	8.5 (6.0)	<0.001	8.3 (5.7)	7.4 (5.2)	7.4 (4.4)	7.0 (5.7)	0.238
CAMCOG total score (SD)	–	95.2 (6.4)	95.8 (6.4)	96.0 (7.7)	96.1 (7.0)	95.2 (7.6)	0.404	95.6 (7.2)	96.5 (6.7)	96.1 (6.9)	96.9 (6.6)	0.046
		N = 38	N = 38	N = 38	N = 38	N = 38		N = 31	N = 31	N = 31	N = 31	
WL—recall total score (SD)	25.7 (7.5)	29.8 (7.3)	30.3 (8.1)	29.7 (8.1)	30.4 (8.1)	30.5 (8.0)	0.753	30.7 (8.5)	32.3 (8.5)	32.7 (9.4)	35.1 (8.8)	<0.001[B]
WL—recognition score (SD)	22.0 (2.0)	22.9 (2.4)	22.2 (1.5)	22.4 (2.1)	22.5 (1.8)	22.3 (2.4)	0.649	22.4 (2.4)	22.7 (2.2)	22.4 (2.3)	22.8 (2.2)	0.288
WL—percent retention (SD)	51.1 (33.2)	62.1 (28.2)	58.1 (28.9)	58.2 (29.2)	59.9 (31.4)	62.9 (32.1)	0.784	62.6 (32.4)	62.5 (33.0)	58.5 (35.6)	60.4 (37.1)	0.842
VPA—recall total score (SD)	11.3 (8.0)	13.9 (9.6)	15.2 (9.1)	15.0 (8.7)	16.2 (9.0)	17.2 (9.3)	0.003[B]	16.3 (9.9)	17.4 (10.2)	18.0 (9.9)	18.8 (9.3)	0.008[B]
VPA—recognition score (SD)	23.0 (2.5)	23.7 (0.8)	23.9 (0.2)	23.7 (0.5)	23.8 (0.5)	23.9 (0.4)	0.055	23.8 (0.4)	23.9 (0.2)	23.8 (0.4)	23.8 (0.5)	0.397
VPA—percent retention (SD)	67.0 (43.1)	81.4 (43.5)	86.8 (39.7)	95.0 (55.2)	106.6 (57.7)	102.1 (51.4)	0.090	101.3 (57.8)	92.2 (41.6)	97.1 (41.0)	111.1 (58.7)	0.406
VR—recall total score (SD)	62.2 (19.4)	74.1 (16.4)	74.7 (17.6)	74.6 (17.8)	74.0 (18.2)	75.6 (14.9)	0.872	74.4 (15.8)	75.1 (17.9)	76.0 (16.9)	76.8 (17.0)	0.419
VR—percent retention (SD)	67.0 (43.1)	76.8 (25.8)	74.2 (25.3)	82.3 (20.5)	85.4 (24.2)	84.5 (23.7)	0.009[B]	86.0 (23.4)	84.5 (22.3)	89.6 (22.5)	91.6 (20.0)	0.099[B]
BD—total score (SD)	30.7 (10.4)	34.2 (10.2)	35.4 (11.3)	34.7 (11.2)	34.3 (11.8)	35.4 (11.8)	0.517	34.5 (12.0)	34.9 (13.4)	35.2 (13.3)	35.7 (12.7)	0.538

*p-value refers to the probability, in an analysis of variance for repeated measures, that the observed scores changed by chance over the specified period of time. B = p-value after Bonferroni correction for multiple comparisons. BDI = Beck Depression Inventory, BAI = Beck Anxiety Inventory, CAMCOG = Cambridge Cognitive Examination for Mental Disorders of the Elderly, WL = Word List Test of the Wechsler Memory Scale III, VPA = Verbal Paired Associates Test of the Wechsler Memory Scale III, VR = Visual Reproduction Test of the Wechsler Memory Scale III, BD = Block Design Test of the Wechsler Adult Intelligence Scale III.

were not significantly associated with changes in the BDI ($r = -0.01$, $p = 0.940$; $r = 0.12$, $p = 0.454$; $r = 0.14$, $p = 0.406$ and $r = 0.23$, $p = 0.151$ respectively) or BAI scores ($r = 0.06$, $p = 0.702$; $r = 0.10$, $p = 0.529$; $r = -0.24$, $p = 0.139$ and $r = 0.15$, $p = 0.376$ respectively).

BDI scores increased significantly during the initial 36 weeks of active treatment ($F = 4.1$, df $= 4$, $p = 0.004$) and declined somewhat thereafter ($F = 1.8$, df $= 3$, $p = 0.149$) (Table 1). Three participants had BDI scores greater than 12 at baseline, which is suggestive of clinically significant depression. This number increased to 7/40 at week 24 and declined to 4/40 at the end of follow-up. A similar pattern was observed for BAI scores: they rose from a mean level of 4.3 at baseline to 8.3 at week 36 ($F = 8.5$, df $= 4$, $p < 0.001$), but subsequently failed to return to baseline ($F = 1.4$, df $= 3$, $p = 0.238$) (Table 1). Only 2/40 subjects had BAI scores greater than 12, suggestive of clinically significant anxiety, but this rate went up to 10/40 at week 24 and did not change substantially until the end of follow-up. The Pearson correlation between change in BDI and BAI scores from baseline to week 36 was 0.45 ($p = 0.003$). The correlation between change in BDI and BAI scores from week 36 to week 54 was 0.30 ($p = 0.068$). There was no association between changes in BDI or BAI scores between baseline and week 36 with changes in Aβ levels ($p \geq 0.1$).

4. Discussion

The results of the present study indicate that hormonal suppression and testosterone receptor blockade are associated with a significant rise in Aβ plasma level. It may be argued that such an association lacks statistical support, as we were unable to demonstrate a significant inverse correlation between changes in amyloid and hormone levels over time. However, it is worth noting that there was a clear temporal relationship between hormonal castration and the rise in Aβ levels (Fig. 2), and the lack of statistical association between these variables can be explained by marked floor effect of hormone levels associated with treatment. In other words, there was not sufficient change in testosterone and estradiol levels over time to produce a statistical correlation with Aβ. Clinically, these changes were related to an increase in depression and anxiety scores. In addition, we found that discontinuation of treatment was linked to an improvement on a general measure of cognitive function (CAMCOG), as well as on recall total scores for the Words List and the Verbal Paired Associates tests. The percentage retention for the Visual Reproduction test also

increased after treatment discontinuation, but treatment use or withdrawal had no obvious effect on the scores of the Block Design test. Taken together, these results suggest that marked decline in estradiol and testosterone levels are associated with significant changes in mood and memory, but not visuospatial abilities. We should also consider the possibility that the cognitive and mood changes observed in the present study might be due to the direct effect of leuprolide. However, this seems an unlikely explanation for our findings, as previous studies have clearly shown that the mental effects of leuprolide treatment are completely reversed by estrogen supplementation (Berman et al., 1997; Sherwin and Tulandi, 1996).

An obvious and expected effect of chemical castration is the rapid decline in the plasma levels of estradiol and testosterone. We observed a marked fall in the levels of sex hormones during the initial four weeks of therapy, which was only partly reversed during the follow-up period of four months after treatment was discontinued (Fig. 2). The cognitive effects of such dramatic hormonal changes were inconsistent, with the scores for some of the tests improving, but remaining the same for others (Table 1). Two opposing forces may have influenced the cognitive performance of our subjects throughout the study: decline in hormonal levels and repetitive testing. We had hypothesized that chemical castration would be associated with a significant deterioration of visuospatial abilities (as measured by the Block Design test) and visual memory (as measured by the Visual Reproduction test), and that treatment cessation would reverse such changes. Our results indicate that neither the introduction nor the cessation of treatment influenced visuospatial scores, suggesting that hormone levels and learning had no obvious impact on performance after baseline. Other studies have reported that testosterone supplementation improves the total score (Janowsky et al., 1994) as well as the mean time required to complete the Block Design task (Cherrier et al., 2001), but such effects were equivocal and only became apparent once supra-physiological levels of testosterone were achieved in those experiments. Moreover, we are unable to dismiss the possibility that Block Design scores would have improved once testosterone levels had returned to baseline (most subjects remained hypogonadal at the end of the follow-up). In contrast, the performance of participants on the Visual Reproduction Test suggests that the study procedures were not associated with total immediate recall scores, but had a significant impact on delayed recall (as measured by the

percentage retention). Learning is likely to have played an important role in this regard, particularly during the 'on treatment' period. The correlation between sex hormone levels and subjects' cognitive performance was not obvious, although this may be partly explained by marked floor effect of hormonal levels (i.e., levels could not get any lower after 4 weeks and recovery was protracted and incomplete after treatment was discontinued). We also acknowledge that the performance of our men in some of the cognitive subscores investigated (e.g., recognition for Words) may have been subject to ceiling effect and, for this reason, no obvious change could be observed at follow-up.

A similar rationale could be used to explain the steady improvement in subjects' performance on the Verbal Paired Associates task, but this would not be sufficient to account for the results observed for the Word List test. There is no evidence that subjects' total scores were being influenced by learning during the 'on treatment' phase or, if they were, they were being counterbalanced by deteriorating performance due to other factors (i.e., the learning effect associated with repetitive testing in our study may have been of the same magnitude as the negative cognitive consequences associated with lack of sex hormones). The significant increase in recall total scores during the 'off phase' suggests that one or more treatment factors were contributing to improve performance. For example, the changes in cognitive function observed after 36 weeks may have become apparent partly because the learning effect became dissipated and enabled the cognitive effects of sex hormones to come to the forefront. Changes in the WL total score were not significantly correlated with changes in hormone levels, or BDI and BAI scores. Interestingly, however, we found that such cognitive changes were significantly associated with changes in the plasma levels of $A\beta$.

There is currently substantial evidence indicating that $A\beta$ is a key-component of the pathophysiological pathway that leads to the development of AD. Several studies have shown that high concentrations of $A\beta$ are neurotoxic and that the presence of both estradiol and testosterone reduce its production and attenuates its toxicity in vitro and in vivo (Gouras et al., 2000; Petanceska et al., 2000; Pike, 2001). We had previously shown, in a case-series of 6 subjects, that chemical castration was associated with increased plasma $A\beta$ levels (Gandy et al., 2001), and that this form of treatment might be associated with rapid cognitive decline in patients with AD (Almeida et al., 2001). Furthermore, a recently published randomized, placebo-controlled clinical trial of estradiol ther-

apy for women with Alzheimer's disease showed that active treatment was associated with a significant reduction of plasma $A\beta40$ at the end of 8 weeks, although this effect was only apparent for women who had never used hormone replacement therapy before (Baker et al., 2003). These results are consistent with our own findings and suggest that estradiol and testosterone are directly or indirectly involved in the metabolic pathway of $A\beta$ in humans.

In a longitudinal study, higher plasma levels of $A\beta42$ at entry predicted subsequent development of AD after multivariate analysis allowing for age, education, APOE genotype and ethnic group (Mayeux et al., 1999). In addition, Mehta et al. (2001) found that patients with AD have higher plasma levels of $A\beta$ than controls, but were unable to show an association between $A\beta$ plasma level and cognitive performance as measured by the MMSE. Our results confirm that there is no association between changes in $A\beta$ plasma level and performance on the CAMCOG (which is a measure of general cognitive function like the MMSE), but indicate the presence of a significant inverse correlation between changes in the concentration of plasma $A\beta$ and verbal memory scores. Preliminary evidence from animal studies shows that the infusion of $A\beta$ into the cerebral ventricle decreases the activity of soluble protein kinase C in the hippocampus and produces memory deficits (Olariu et al., 2002). At present, however, there is no direct evidence that the concentration of $A\beta$ in the plasma is associated with the concentration of $A\beta$ in the brain. Furthermore, the lack of a clear association between improved memory performance after the discontinuation of androgen blockade therapy and plasma $A\beta$ would suggest that, in humans, plasma levels of $A\beta$ do not directly drive cognitive performance. The long-term clinical implications of such a change of plasma $A\beta$ are less clear and will require further research (for example, would the menopause lead to a sustained increase in plasma $A\beta$ and risk of AD?).

The results of the Women's Health Initiative Study (WHIS), however, have shown that the risks associated with hormone replacement therapy (estrogen plus progestin) outweigh its potential benefits (Rossouw et al., 2002). In addition, the cognitive performance of postmenopausal women aged 65 years or older treated with HRT does not improve when compared to women treated with placebo for 4.2 years. In fact, significant cognitive decline (deterioration of 8–10 points on the Modified Mini-Mental State Examination score) was more likely to occur in women treated with estro-

gen plus progestin than placebo (Rapp et al., 2003). The results of the arm of the WHIS that used estrogen replacement only (as opposed to estrogen plus progestin) are not as yet available and we are not aware of any large trials designed to investigate the cognitive effects of testosterone replacement in humans. As a consequence, it remains unclear whether estrogen and testosterone replacement treatment will ever be considered a practical preventative strategy to reduce the burden of cognitive impairment in later life.

We have also found that BDI scores increased significantly during the 'on treatment' period and tended to decline after the chemical castration was discontinued. However, the number of people with clinically significant depressive symptoms did not change significantly throughout the study. A similar pattern was observed for anxiety scores. A large survey of 5236 Vietnam veterans found a weak but significant association between testosterone levels and depression, as assessed by the Diagnostic Interview Schedule (DIS)(Mazur, 1995). There is also preliminary evidence that testosterone secretion is lower in depressed than non-depressed men (Schweiger et al., 1999) and that testosterone supplementation increases response to antidepressant therapy in patients with treatment-resistant depression (Pope et al., 2003; Seidman and Rabkin, 1998). Animal studies indicate that estrogen increases the uptake and synthesis of serotonin (5-HT), upregulates 5-HT1 and downregulates 5-HT2 receptors. The hormone also increases the turnover of noradrenaline, decreases noradrenaline uptake from the synaptic cleft, inhibits monoamino oxidase activity and enhances beta-adrenoreceptor binding. Taken together, these findings suggest that, like most antidepressant medications, estrogen acts as a serotonergic and noradrenergic agonist (see Almeida & Barclay, 2001 for review). Clinical evidence for the mood-regulating effect of estrogen is relatively robust for women (Schmidt et al., 2000; Soares et al., 2001), but is less well established for men. Our study may have lacked the necessary power to detect clinically significant changes in mood associated with chemical castration, but its results are consistent with the hypothesis that sex hormones modulate certain aspects of mood.

In summary, the results of this naturalistic study indicate that chemical castration is associated with a significant rise in the plasma levels of $A\beta$ and, clinically, with increased depression and anxiety scores. The discontinuation of treatment is associated with changes in cognitive performance, most noticeably of verbal memory, but the clinical implications of such preliminary findings remain to be determined.

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