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Prospective Longitudinal Study of Testosterone and Incident Depression in Older Men: The Health In Men Study

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Highlights

- Depression in older men has been associated with low circulating testosterone but data from prospective studies is limited and no previous studies have included data on testosterone's bioactive metabolites (dihydrotestosterone and estradiol)
- Longitudinal, prospective study of 3,179 older men followed up for nearly 10 years
- Low serum testosterone was associated with an 86% increased hazard of depression (HR 1.86, 95%CI 1.05-3.31) once relevant demographic, clinical and biochemical factors were taken into account
- Serum dihydrotestosterone, estradiol and calculated free testosterone were not associated with risk of depression
- Clinical trials of testosterone treatment in older men without pathological hypogonadism are needed to determine if testosterone has a role in treating and/or preventing depression in later life

ABSTRACT

Background: Depression in older men has been associated with low circulating testosterone concentration but data from prospective studies are limited.

Methods: We conducted a prospective longitudinal study in a community representative cohort of 3,179 older men free of clinically significant depressive symptoms at baseline. The main objective of this study was to determine if low serum testosterone, dihydrotestosterone and estradiol concentrations are associated with the development of depressive symptoms. Incident depression was assessed with the Patient Health Questionnaire and via an electronic health record database (The West Australian Data Linkage System). The main exposures of interest were serum testosterone, dihydrotestosterone and estradiol measured by liquid chromatography-mass spectrometry and calculated free testosterone in baseline blood samples (collected between 2001 to 2004).

Results: One hundred and thirty five men (4.2%) developed depression over a median follow up time of 9.4 years (range 8.4 to 10.9). Men with incident depression were older (median age 77.7 vs 76.1 years, $z=-3.82$, $p=0<0.001$) and were more likely to have cardiovascular disease (43.0% vs 32.6%, $\chi^2=6.32$, $p=0.012$) and diabetes (22.2% vs 13.2%, $\chi^2=8.95$, $p=0.003$). Low serum total testosterone (< 6.4 nmol/l) was associated with incident depression (HR 2.07, 95%CI 1.17-3.68) and this remained significant after adjustment for relevant potential confounding factors (HR 1.86, 95%CI 1.05-3.31). Low serum dihydrotestosterone, estradiol and calculated free testosterone were not associated with risk of depression.

Conclusions: Low serum total testosterone, but not calculated free testosterone, was associated with incident depression in this sample of older men.

Keywords: testosterone; depression; older men

1. INTRODUCTION

Depression affects about one in ten older adults and the symptoms are frequently chronic (Beekman et al., 2002; Pirkis et al., 2009). It is associated with increased number and duration of hospitalizations, reduced quality of life and increased medical morbidity, disability, suicide risk and mortality (Almeida et al., 2010; Blazer, 2002).

Testosterone (T), the main sex hormone in men, is essential for maintaining virilization and muscle mass and may also affect libido, mood regulation, bone health and cardiac disease (Borst and Mulligan, 2007; Hyde et al., 2012). It is produced and secreted by the testes under the control of the hypothalamic-pituitary-gonadal axis. Testosterone in the circulation is bound to sex hormone-binding globulin (SHBG) or albumin and can be converted to dihydrotestosterone (DHT) or estradiol (E_2). A small percentage of T is unbound or “free” (free T) (Yeap et al., 2012), and together with the albumin bound proportion, is considered to be “bioavailable” (Manni et al., 1985), although the free hormone hypothesis remains contentious (Mendel, 1989).

Among unselected older men there is a gradual and inconsistent decline in circulating T with increasing age (Mulligan et al., 1995) that may additionally be attributable to factors, such as obesity, cigarette smoking, diabetes, diet and possibly physical activity (Yeap et al., 2012). There is considerable phenomenological overlap between low circulating T and depression with symptoms of irritability, decreased libido and fatigue common to both. We have previously reported in this population that older men with depression have significantly lower total and calculated free T concentrations than their non-depressed counterparts (13.5 nmol/L vs 14.7 nmol/L, z=4.05, p<0.001) (Almeida et al., 2008). Men in the lowest quintile of calculated free T had increased odds of prevalent depression compared to men in the highest quintile (adjusted odds ratio [OR] 2.71, 95%CI 1.49-4.93) although this association was not significant with total T (adjusted OR 1.55, 95%CI 0.91-2.63). Testosterone was measured by immunoassay and free T was calculated using the Vermeulen formula (Vermeulen et al., 1999).

Several other epidemiological studies have investigated the association between low serum T and depressive symptoms, although findings have been inconsistent (Barrett-Connor et al., 1999; Kratzik et al., 2007; McIntyre et al., 2006; Seidman et al., 2002; Seidman and Walsh, 1999; Westley et al., 2015), and two large, cross-sectional studies have failed to find an association between low total T and depression (Berglund et al., 2011; Seidman et al., 2001). Data from the few prospective longitudinal studies available to date have been equally inconsistent (Joshi et al., 2010; Shores et al., 2005; Shores et al., 2004; T'Sjoen et al., 2005).

Some of these prospective studies have relied on the use of health records and relatively brief follow up (Shores et al., 2005; Shores et al., 2004), while others have failed to account for potentially confounding variables (Joshi et al., 2010; Shores et al., 2005; Shores et al., 2004; T'Sjoen et al., 2005). None of these studies have considered T's circulating potent and bioactive metabolites, DHT and E_2.

We designed this large, prospective cohort study to investigate if low circulating T and its metabolites are associated with the development of depressive symptoms in men free of depression at study entry. We hypothesized that men with low total and calculated free T, DHT and E_2 would be more likely to develop clinically significant depressive symptoms over time.

2. METHODS

2.1 Study design and setting

This is a prospective longitudinal analysis of older men aged 71-88 years who participated in the Health in Men Study (HIMS) conducted in Perth, Western Australia (Jamrozik et al., 2000). Baseline data for these analyses were collected between 2001 and 2004. The men were subsequently assessed for incident depression via self-reported questionnaires in 2008 and 2011/2012. We additionally followed these men via health records obtained from the Western Australian Data Linkage System (WADLS) until December 31, 2012. The WADLS records data on all acute hospital admissions, hospital movements and psychiatric outpatient contacts of all Western Australian residents (Holman et al., 2008).

2.2 Participants

The design and the recruitment of participants for HIMS have been described elsewhere (Jamrozik et al., 2000; Norman et al., 2009). In brief, 12,203 men aged 65 years and older were randomly recruited from the electoral roll (voting is compulsory in Western Australia) between 1996 and 1998 to take part in an abdominal aneurysm screening trial (wave 1). Between 2001 and 2004 (baseline for this study – wave 2), 5,438 men returned for reassessment that included a detailed questionnaire, the short version of the Geriatric Depression Scale (GDS-15) (Yesavage et al., 1982), and in 4,261 men, a physical examination and blood sample. Testosterone assay was not available in 32 men and another 26 men were excluded because they were taking testosterone supplements. A further 125 men were excluded due to history of orchidectomy (n=56) or the use of anti-androgen medication (n=69).

In order to restrict the analyses to incident depression, we excluded all men with prevalent depression, including those with self-reported past history of depression (n=183) and all participants who scored 4 or higher on the GDS-15 during the baseline assessment (n=687). We chose a relatively low cut-off score on this scale to ensure high sensitivity for depression and to minimize the risk of including potentially prevalent cases in the analyses (Marc et al., 2008). Finally, we excluded another 29 men who had a diagnosis of depression recorded in WADLS prior to the baseline assessment but had denied past or current depression. A total of 3,179 men fulfilled the study's entry criteria (Figure 1).

Figure 1.

2.3 Ethics

The Human Research Ethics Committee of the University of Western Australia approved this study protocol and all men offered written informed consent to participate. The research was conducted in accordance with the Declaration of Helsinki recommendations for the conduct of clinical research.

2.4 Outcome of interest: incident depression

Participants completed the Patient Health Questionnaire (PHQ-9) (Kroenke et al., 2001) during the 2008 and 2011/2012 assessments. The PHQ-9 is a self-rated depression-screening tool that rates each of the nine DSM-IV criteria for major depression on a scale of 0-3. Participants scoring 10 or greater were considered to show evidence of clinically significant symptoms of depression (incident depression). This cut-point is associated with 88% sensitivity and specificity for the diagnosis of a major depressive episode according to DSM-IV criteria (Kroenke et al., 2001; Phelan et al., 2010). Additional diagnoses of incident depression were sought from the WADLS utilizing the International Classification of Diseases (ICD) ninth (codes 296.2, 296.3, 296.82, 296.90, 298.0 and 311) and tenth revisions (codes F32, F33, F34.1 and F38.10) during the follow up period. WADLS brings together all death records, acute hospital admissions, hospital movements, cancer registry, as well as psychiatric outpatient contacts for all residents of Western Australia since 1980 (Holman et al., 2008).

2.5 Baseline hormone measurements

The main exposures of interest were serum total and calculated free T, DHT and E_2. Blood was collected at baseline between 08h00 and 10h30. Plasma was separated from blood cells immediately and stored at -80 °C until assayed. Total T, DHT and E_2 were quantified within a single liquid chromatography-tandem mass spectrometry (LC-MS) run without derivatization using atmospheric pressure photoionization in positive mode for androgens and negative mode for E_2 as previously described (Yeap et al., 2012). The coefficient of variation for total T was < 6% for T > 0.4nmol/L. Free T was calculated using an empirical formula that requires assay of SHBG (Sartorius et al., 2009). SHBG was determined by chemiluminescent immunoassay on an Immulite 2000 analyzer (Diagnostic Products Corp., Biomediq, Doncaster, Australia). Reference ranges and determinants of total and free T, DHT and E_2 have already been established in this population (Yeap et al., 2012). Low total serum T was defined as < 6.4 nmol/L, low calculated free T as < 103.6 pmol/L, low DHT as < 0.49 nmol/l and low E_2 as < 27.6 pmol/L

2.6 Other variables of interest at baseline

We recorded the age of participants (in years) at the time of the baseline assessment and this was also categorized into quartiles. Education was dichotomized at the level of high school completion and participants were grouped as smokers (current or past) or never smokers. We considered participants to be risky drinkers if they consumed more than two standard drinks a day (http://www.nhmrc.gov.au/publications/synopses/ds10syn.htm). Men were considered to be physically active if they reported at least 150 minutes or more of vigorous (e.g. fast walking, jogging or swimming) or non-vigorous (e.g. slow walking, Tai Chi, yoga) activity per week.

Cognition was assessed with the Mini-Mental Status Examination (MMSE), with participants scoring less than 24 presumed to have evidence of cognitive impairment (Folstein et al., 1975). Body mass index (kg/m^2) and blood pressure (within 2 mm Hg) were measured according to standard procedures and cut-offs defined according to World Health Organization (WHO) definitions (Whitworth, 2003; WHO, 2000). Participants were deemed to have cardiovascular disease (CVD) if they reported a past or current history of angina, myocardial infarction or stroke.

Fasting serum glucose, creatinine, and cholesterol were analyzed with a Roche Hitachi 917 analyzer and thyroid stimulating hormone (TSH) was measured using a Roche Elecsys 2010 analyzer. Diabetes was classified if they had a prior diagnosis of diabetes, were on antidiabetic medication or had fasting blood glucose of ≥ 7.0 mmol/L at baseline. Estimated glomerular filtration rate (eGFR) was calculated using the Cockcroft-Gault equation: [(140-age) x weight (kg)]/(creatinine x 0.8136). Total plasma homocysteine (tHcy) concentration was assessed as previously described (Araki and Sako, 1987).

2.7 Statistical analyses

Data were analyzed with Stata version 12.1 (StataCorp, College Station, Texas). The distribution of data was investigated with histograms and the skewness/kurtosis test. Means and standard deviations (SD) were used to summarize normally distributed variables, median and interquartile range (IQR) for skewed data and frequencies and percentages for categorical variables. We used Student t tests to compare differences between normally distributed continuous variables and the Mann-Whitney test (z statistic) for skewed data. Pearson's chi squared tests were used for categorical data.

We used Cox proportional-hazards regression models to calculate hazard ratios (HR) for incident depression. Participants were censored at the time of depression diagnosis or death (all participants were assumed to remain in the study due to the use of data linkage follow up). We first performed a univariate analysis with depression as the outcome variable and variables that were significantly associated with depression were included in the multivariate analyses. We investigated the association of T, DHT and E_2 and incident depression as dichotomous variables using the cut-offs described above. Three statistical models were used: a crude (unadjusted) model, one adjusted for age alone and a fully adjusted (age, cardiovascular disease and diabetes) model. Associations between T and age were explored with simple linear regression models. We additionally performed a survival analysis using the Kaplan-Meier estimator with log-rank test and Poisson regression was used to calculate the incident rate ratio (IRR) of depression according to T status.

3. RESULTS

3.1 Population characteristics at baseline

The analyses included 3,179 men. Table 1 shows the population characteristics according to total T status. Men with low total T were marginally older (median age 77.4, interquartile range [IQR] 74.1-80.0 vs 76.1 years, IQR 74.1-78.9 z=-2.24, p=0.025), were more likely to have diabetes (22.8% vs 13.0%, χ^2=13.72, p<0.001) and had higher BMI (median 28.3, IQR 26.1-30.8 vs 26.1, IQR 24.1-28.4, z=-7.82, p<0.001). Men with more prominent depressive symptoms at baseline were less likely to have had a blood test and serum T measurement:

mean GDS-15 2.94 (standard deviation [SD] 2.83) for men who did not have a serum T measurement vs 2.15 (SD 2.26) for men who did (t=10.16, p<0.001).

Table 1.

3.2 Follow-up

The men were followed up for 9.4 years (range 8.4 to 10.9). Of the original cohort, 48.7% (n=1,548) and 34.3% (n=1,090) were available for re-assessment in 2008 and 2011/2012 respectively and 1,037 died (median time from baseline to death was 6.2 years, range 1 month to 11 years) – figure 1.

3.3 Incident depression during follow-up

Incident depression developed in 135 (4.2%) men (41 established during the 2008 assessment, 22 during 2011/2012 and 72 from WADLS). Table 2 shows that the men with depression were older (median age 77.7, IQR 75.0-80.0 vs 76.1 years, IQR 74.1-78.9 z=-3.82, p=0<0.001) and were more likely to have cardiovascular disease (43.0% vs 32.6%, χ^2=6.32, p=0.012) and diabetes (22.2% vs 13.2%, χ^2=8.95, p=0.003). The concentration of SHBG was similar between the two groups: incident depression 41.4 nmol/L, SD 16.7, not depressed 42.3 nmol/L, SD 16.5; t=0.64, p=0.523.

Table 2.

3.4 Incident depression and testosterone

Low serum total T was associated with incident depression (HR 2.07, 95%CI 1.17-3.68 – Table 2) and this remained statistically significant after adjustment for age, cardiovascular disease and diabetes (HR 1.86, 95%CI 1.05-3.31 – Table 3). Low serum DHT was not associated with depression hazard (adjusted HR 0.66, 95%CI 0.29-1.51 – Table 3). A J-shaped relationship with incident depression was noticed for DHT but not for T (supplementary figures A1 and A2 in the appendix). Serum E2 and calculated free T were not associated with depression risk.

Table 3.

Men with normal serum T concentrations had improved depression-free survival (Log-rank test p=0.011) although the effect only became apparent after approximately 5 years (Figure 2). The adjusted incidence rate ratio of depression associated with low serum T was 1.56 (95%CI 0.88 to 2.77; adjusted for age, diabetes and cardiovascular disease).

Figure 2.

3.5 Effect of age

Serum T decreased slightly for every year of age (β= -0.03, t=-2.72, p=0.007). The crude proportion of men with depression and low serum T (< 6.4 nmol/l) increased with age relative to those with higher serum T (≥ 6.4 nmol/l), apart from the oldest age group (Figure 3) but the interaction of age and T concentration was non-significant (z=-0.23, p=0.856), suggesting that the effect of low serum T on depression risk was independent of age.

Figure 3.

3.6 Sensitivity analysis excluding potentially undiagnosed cases

Three men were diagnosed with depression within a year of their baseline assessment. We re-ran the analyses after excluding these men as potentially undiagnosed prevalent cases of depression. The adjusted depression hazard associated with low serum T was similar to the result for the whole sample (HR 1.88, 95%CI 1.06 to 3.43) and the other analyses were essentially unchanged.

4. DISCUSSION

In this sample of older men, we found that the risk of developing depression over a period of 9.4 years was higher in those with low baseline total serum T and that increased risk remained significant once the analyses were adjusted for relevant factors, notably age, lifestyle factors and medical comorbidities. The risk was not increased with calculated free T or circulating concentrations of T's bioactive metabolites DHT and E_2.

This study has a number of strengths and weakness. We studied a large, community-derived cohort of older men and had available data on a range of factors that may confound the relationship between T and depression. To the best of our knowledge, this is the largest prospective study in this area to date and we were able to include measurements of serum T and its bioactive metabolites DHT and E_2 by LC-MS in the analyses. We excluded men with prevalent depression and used well-validated instruments and administrative health data to assess for depression. We additionally excluded men who may have had surgical or chemical-induced T deficiency. The cohort was followed up for nearly 10 years.

While we were able to account for a range of potential confounders in our analyses, there is always the possibility of residual confounding e.g. other medical comorbidities other than those included in this study. We excluded men with depression at baseline from the longitudinal analysis to minimize confounding from reverse causality. Nevertheless, we cannot dismiss the possibility that a common underlying factor could be responsible for both low baseline T and increased risk of depression during follow-up. We also acknowledge that the diagnosis of depression relied on cut-off scores on self-rating depression scales and data linkage rather than structured interviews. Furthermore, the approach to assess depression during follow up relied on data linkage and scores on the PHQ-9, whereas the baseline assessment was based on the GDS-15. This could have led to ascertainment bias and may explain why the incidence of depression in our sample was low (4.2%). The most likely consequence of such bias would have been loss of power due to the misclassification of some cases as non-cases. Alternatively, one could interpret these results as an indication of healthy participant bias and survivorship bias, which would lead to a decreasing number of older men with mental disorders being available for follow up (Almeida et al., 2014).

Another potential source of error could be ascribed to the fact that biochemical and clinical data were only available during the baseline assessment, so that subsequent variations in serum T, DHT and E_2 and clinical factors (e.g. changes in cardiovascular risk) could not be examined. Taken together, these potential sources of error would most likely have biased the results of the study towards the null hypothesis, thereby suggesting that the observed associations between low T and incident depression could be even stronger. Our finding of an association between incident depression and total rather than calculated free T is slightly at odds with our previous cross-sectional study in this population (Almeida et al., 2008) and it is difficult to be certain if this association is clinically important. There was however a trend towards a higher risk of depression in those with low calculated free T (adjusted HR 1.58, 95%CI 0.85-2.94) and the two studies utilized different methods to calculate this. Lastly, the proportion of men with incident depression and low total T in our study was low (13/135, 9.6%).

The mechanism by which T may play a role in mood regulation is largely unknown. It cannot be excluded from an observational study that depression itself and/or its sequelae (e.g. reduced sexual activity) (Hsu et al., 2015a) or consequences (e.g. cognitive decline or physical activity) (Hsu et al., 2014; Hsu et al., 2015b) may cause a reduction in circulating T although the prospective design and exclusion of prevalent cases of depression from the analyses would have helped minimize this possibility. There may be interactions between T and the hypothalamic-pituitary-adrenal axis and between T and brain neurotransmitters associated with modulating mood (Pompili et al., 2012; Rubinow et al., 2005). Testosterone appears to modulate brain monoamine oxidase levels with evidence of increased serotonin levels in the cerebrospinal fluid of healthy volunteers administered T supplements (Daly et al., 2001). Testosterone is aromatized to E_2 and this has also been linked to mood regulation in men (Almeida et al., 2004). Lastly, T appears to have a role in γ-aminobutyric acid (GABA) transmission that may be implicated in mood regulation (Henderson et al., 2006).

The current study should also be evaluated in the context of previous longitudinal research in this area. Shores and colleagues (Shores et al., 2004) examined computerized clinical records of 278 older male veterans. None of the men had a prior diagnosis of depression and were classified as “hypogonadal” if their total T was ≤ 6.94 nmol/L or free T ≤ 30 pmol/L. The men were followed up for 2 years via their medical records. Depressive illness was diagnosed in 21.7% of hypogonadal men versus 7.1% of those with normal T (χ^2=6.00, p=0.010). A Kaplan-Meier survival analysis showed a significant difference between hypogonadal and eugonadal men in time to diagnosed depression (log-rank test χ^2=6.9, p=0.008). The same authors (Shores et al., 2005) subsequently studied a larger sample of 748 men from the same population with low serum T defined at a higher cutpoint (≤ 7.95 nmol/L). Analyses were controlled for age and some medical comorbidities. Men with low serum T again had a greater 2-year incidence of depression (18.5% vs 10.4%, df=1, p=0.006) and a shorter time to onset of depression (log-rank χ^2=8.1, df=1, p=0.004). Analyses did not however take into account the effects of various lifestyle factors, BMI, cognition and the follow up period was relatively short. The exclusive use of clinical records is also a concern given the inherent problems of case ascertainment and the need to exclude prevalent cases and may in part explain the higher incidence of depression than in our study (4.2%)

T’Sjoen and colleagues (T'Sjoen et al., 2005) enrolled 236 community-dwelling men age 70 and above and assessed them on two occasions roughly 4 years apart. Clinically relevant depression (GDS-15 score of ≥ 11) was not related to circulating T either cross-sectionally or in the longitudinal analysis. In contrast, findings from the Longitudinal Aging Study

Amsterdam reported a positive association between low serum T, measured by an immunoassay, and depression where men with calculated free T concentrations below 0.22 nmol/L were at increased risk of depressive symptoms (HR 1.99, 95%CI 1.17-3.37) (Joshi et al., 2010). The authors took into account a range of potential confounding factors but the study population was relatively small (n=518) and they were unable to exclude men with testosterone deprivation (i.e. previous orchidectomy or those on anti-androgen therapy) or individuals taking testosterone supplements.

A number of clinical trials of T treatment have been conducted to date. A systematic review and meta-analysis (Zarrouf et al., 2009) identified seven trials of participants with DSM diagnosed depression and evaluation of treatment response through the Hamilton Depression Rating Scale. The meta-analysis showed a positive effect for T therapy on HAM-D response when compared to placebo ($z=4.04$, $p<0.001$). This effect was also evident in hypogonadal men ($z=3.84$, $p<0.001$) but the small study size and relatively strict inclusion criteria limits the external validity of this study. A more recent meta-analysis by Amanatkar and colleagues (Amanatkar et al., 2014) of 16 trials (n=944) reported a positive effect of T treatment on mood ($z=4.59$, $p<0.001$. The effect was most marked in hypogonadal men (effect size 4.19, $p<0.001$).

In conclusion, we found a positive association between low serum T and incident depression in a community-derived sample of older men although this effect was not apparent with serum DHT and E_2 or calculated free T. This is consistent with other epidemiological studies that have reported an association between lower circulating T and depressive symptoms in older men. Clinical trial evidence supporting the use of T treatment for depression has not produced compelling results other than in men with genuine T deficiency. Larger trials of older men without pathological hypogonadism at risk of depression could help establish whether T treatment has any pharmacological role in treating and/or preventing depression in later life although these trials will have to be mindful of safety concerns (Xu et al., 2013).

Conflict of interest

The authors declare no conflicts of interest.

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Contributors

Andrew Ford and Osvaldo Almeida designed the study and wrote the article. Osvaldo Almeida, Paul Chubb, Jonathan Golledge, Graeme Hankey, Bu Yeap, David Handelsman and Leon Flicker acquired the data that Andrew Ford analyzed. All authors contributed to data interpretation, reviewed the article and approved it for publication.

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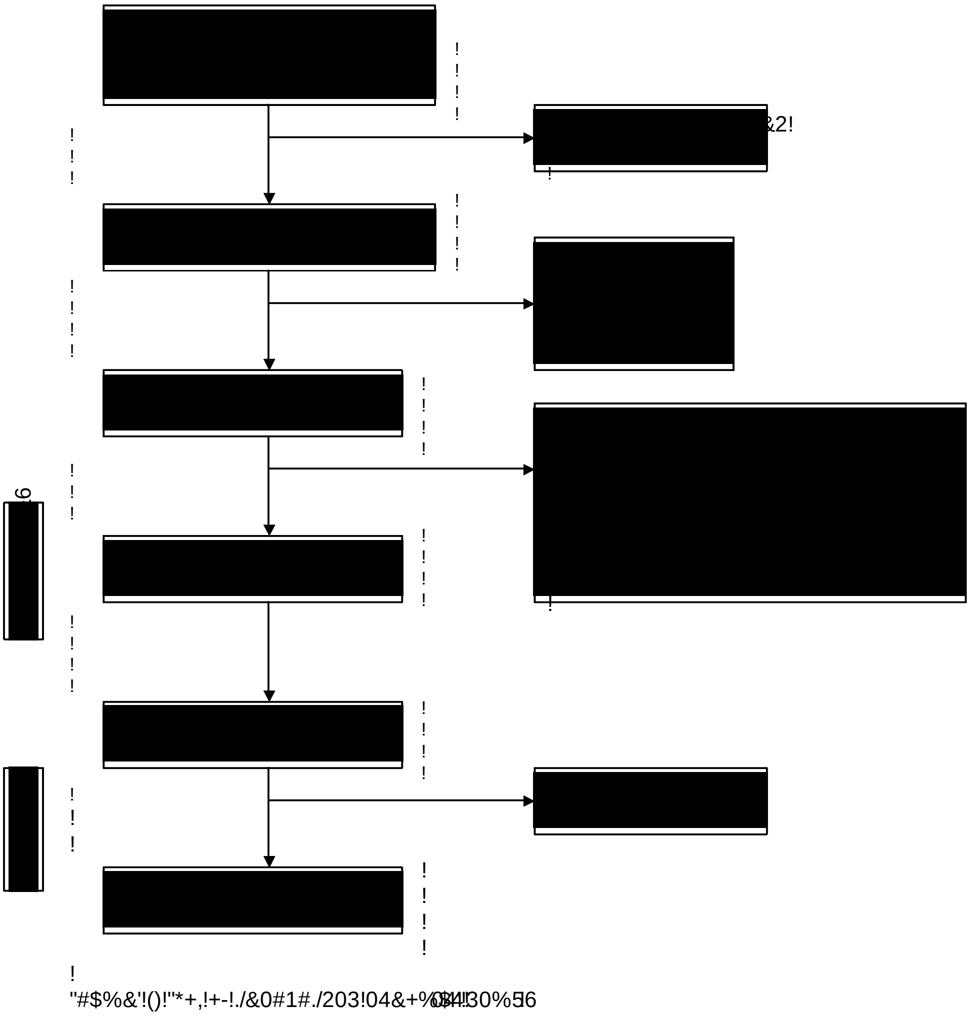

Figure 1. Flow of participants through the study

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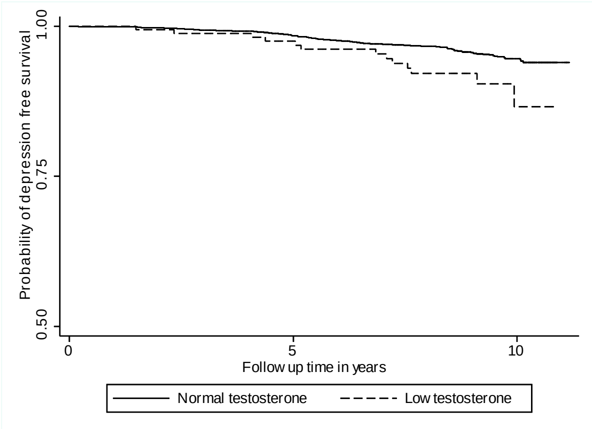

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Figure 2. Kaplan-Meier estimate of survival free of depression according to testosterone status. Men with normal testosterone concentrations had improved survival (Log-rank test p=0.011) although the effect only became apparent after approximately 5 years. The adjusted incidence rate ratio of depression associated with low testosterone was 1.56 (95%CI 0.88 to 2.77; adjusted for age, diabetes and cardiovascular disease)

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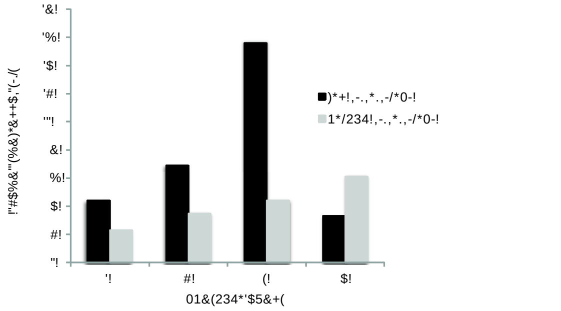

Figure 3. Incident depression in men with low versus normal testosterone by age quartile. The proportion of men with depression and low total testosterone increased with age although not in the oldest group. The proportion of men in age quartile 3 with low testosterone (<6.4nmol/L) compared to those with normal testosterone (≥6.4nmol/l) was significantly higher (χ^2=11.06, p=0.001). The age ranges of the relevant quartiles were as follows: quartile 1(71 to 74 years), quartile 2 (74 to 76 years), quartile 3 (76 to 79 years) and quartile 4 (79 to 89 years).

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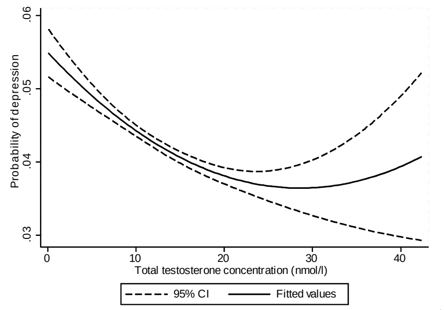

Figure A1. Two-way quadratic prediction of the probability of incident depression according to plasma total testosterone concentration (adjusted for age, cardiovascular disease and diabetes).

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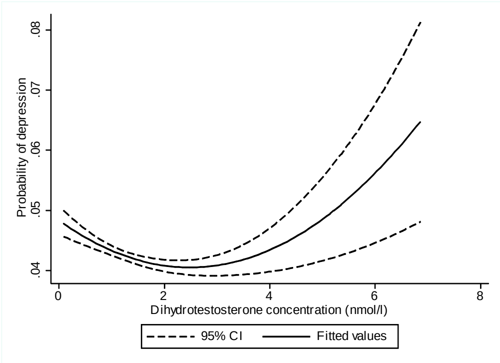

Figure A2. Two-way quadratic prediction of the probability of incident depression according to plasma dihydrotestosterone concentration (adjusted for age, cardiovascular disease and diabetes).

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Table 1. Characteristics of the study sample according to testosterone status

	Total testosterone < 6.4 nmol/l n=180	Total testosterone ≥ 6.4 nmol/l n=2,999	Statistic	P value
Demographics				
Age, median (IQR) years	77.4 (74.1-80.0)	76.1 (74.1-78.9)	z=-2.24	0.025
Education, completed high school or better, n (%)	90 (50.0)	1,484 (49.5)	χ^2=0.02	0.896
Lifestyle				
Ever smoked, n (%)	124 (69.3)	1,912 (63.8)	χ^2=2.20	0.138
Alcohol ≥ 14 units/wk[a]	53 (34.4)	768 (31.0)	χ^2=0.77	0.381
Physically active, n (%)	113 (62.8)	2,071 (69.1)	χ^2=3.11	0.078
Clinical				
MMSE, median (IQR)	26.0 (25-28)	27.0 (25-28)	z=1.54	0.125
BMI, median (IQR)	28.3 (26.1-30.8)	26.1 (24.1-28.4)	z=-7.82	<0.001
Systolic BP mm Hg, mean (SD)	149.1 (19.8)	148.0 (20.1)	t=-0.70	0.482
Diastolic BP mm Hg, mean (SD)	74.5 (10.8)	73.3 (10.4)	t=0.95	0.340
Cardiovascular disease, n (%)	70 (38.9)	979 (32.7)	χ^2=2.98	0.084
Diabetes, n (%)	41 (22.8)	391 (13.0)	χ^2=13.72	<0.001
Biochemistry				
eGFR , mean (SD)	75.7 (21.9)	78.0 (18.2)	t=1.65	0.100
TSH, median (IQR) mU/l	2.0 (1.4-3.1)	2.0 (1.4-2.8)	z=-1.12	0.264
Chloesterol, mean (SD) mmol/l	4.8 (1.1)	4.9 (0.9)	t=1.93	0.053
Homocysteine, median (IQR) µmol/l	12.1 (10.0-15.9)	12.3 (10.2-14.8)	z=-0.10	0.922

[a] n=2,628

Abbreviations: IQR – interquartile range, MMSE – mini-mental state examination, BMI – body mass index, BP – blood pressure, eGFR – estimated glomerular filtration rate, TSH – thyroid stimulating hormone

Table 2. Univariate association with incident depression

Exposure	Incident depression N=135	No depression N=3,044	Hazard Ratio	95% CI
Demographics				
Age quartile 1, n (%)	19 (14.1)	777 (25.5)	Ref	
Age quartile2, n (%)	29 (21.5)	766 (25.2)	1.44	0.81-2.58
Age quartile3, n (%)	40 (29.6)	755 (24.8)	2.01	1.16-3.47
Age quartile 4, n (%)	47 (34.8)	746 (24.5)	2.57	1.50-4.39
Education, completed high school or better, n (%)	75 (55.6)	1,499 (49.3)	1.25	0.89-1.76
Lifestyle				
Ever smoked, n (%)	77 (57.0)	1,959 (64.4)	0.77	0.54-1.08
Alcohol ≥ 14 units/wk, n(%)[a]	35 (33.0)	786 (31.2)	1.11	0.74-1.66
Physically active, n (%)	92 (68.2)	2,092 (68.7)	0.94	0.66-1.36
Clinical				
MMSE <24, n (%)[b]	22 (17.6)	353 (12.5)	1.58	0.99-2.50
BMI <25, n (%)	43 (31.9)	1,061 (34.9)	Ref	
BMI 25-29.9, n (%)	71 (52.6)	1,561 (51.4)	1.11	0.76-1.61
BMI ≥ 30, n (%)	21 (15.6)	418 (13.8)	1.23	0.76-2.07
Hypertension, n (%)	99 (73.3)	2,260 (74.2)	0.91	0.62-1.34
Cardiovascular disease, n (%)	58 (43.0)	991 (32.6)	1.66	1.18-2.34
Dyslipidemia, n (%)	91 (67.4)	1,981 (65.1)	1.03	0.72-1.48
Diabetes, n (%)	30 (22.2)	402 (13.2)	1.90	1.27-2.85
Biochemistry				
Total testosterone < 6.4 nmol/l, n (%)	13 (9.6)	167 (5.5)	2.07	1.17-3.68
Free testosterone < 103.6 pmol/l, n (%)[c]	11 (8.3)	167 (5.6)	1.77	0.95-3.28

Dihydrotestosterone < 0.49 nmol/l, n (%)[d]	6 (4.5)	180 (6.0)	0.78	0.35-1.78
Estradiol < 27.6 pmol/l, n (%)	8 (6.0)	105 (3.5)	1.79	0.88-3.67
eGFR ≤ 60 ml/min, n (%)	22 (16.3)	447 (14.7)	1.33	0.84-2.10
TSH ≥ 4 mIU/l, n (%)[e]	16 (12.2)	332 (11.2)	1.17	0.69-1.97
Total plasma homocysteine ≥ 15mmol/l, n (%)	40 (29.6)	728 (23.9)	1.42	0.98-2.06

[a] n=2,628, [b] n=2,941, [c] n=3,120, [d] n=3,123 [e] n=3,090

Abbreviations: 95%CI – 95% confidence interval, MMSE – mini-mental state examination, BMI – body mass index, eGFR – estimated glomerular filtration rate, TSH – thyroid stimulating hormone

Table 3. Hazard ratios of incident depression according to sex hormone status

	Crude HR (95%CI)	Age adjusted HR (95%CI)	Fully* adjusted HR (95%CI)
Low total testosterone (< 6.4 nmol/l)	2.07 (1.17-3.68)	2.00 (1.12-3.54)	1.86 (1.05-3.31)
Free testosterone < 103.6 pmol/l,	1.77 (0.95-3.28)	1.64 (0.88-3.05)	1.58 (0.85-2.94)
Dihydrotestosterone < 0.49 nmol/l	0.78 (0.35-1.78)	0.77 (0.34-1.75)	0.66 (0.29-1.51)
Estradiol < 27.6 pmol/l	1.79 (0.88-3.66)	1.68 (0.82-3.43)	1.73 (0.84-3.53)

* Adjusted for age, cardiovascular disease and diabetes
Abbreviations: HR – hazard ratio, 95%CI – 95% confidence interval