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# Neutral associations of testosterone, dihydrotestosterone and estradiol with fatal and non-fatal cardiovascular events, and mortality in men aged 17-97 years.

**Short Title:** Sex hormones and mortality

**Keywords:** Testosterone, dihydrotestosterone, estradiol, mortality, cardiovascular disease

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**Disclosures:**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**Abstract**

**Context:** Lower testosterone (T) is associated with poorer health outcomes in older men, however, the relationship between T, dihydrotestosterone (DHT) and estradiol (E2) with cardiovascular disease (CVD) in younger to middle-aged men remains unclear.

**Objectives:** We assessed associations between endogenous sex hormones with mortality (all-cause and CVD) and CVD events, in a cohort of men aged 17-97 years.

**Participants and methods:** Sex hormones were assayed using mass spectrometry in 2,143 men from the 1994/5 Busselton Health Survey. Outcomes to December 2010 were analysed.

**Results:** Of the 1,804 men included in the analysis, mean age was 50.3±16.8 years and 68.9% of men were aged <60. Mean follow-up period was 14.9 years. There were 319 deaths, 141 CVD deaths, and 399 CVD events. Compared to the full cohort, men who died had lower baseline T (12.0±4.4 vs 13.6±4.9 nmol/L), free T (181.9±52.9 vs 218.3±63.8 pmol/L) and DHT (1.65±0.64 vs 1.70±0.72 nmol/L), but higher E2 (64.0±32 vs 60.1±30.2 pmol/L). After adjustment for risk factors, T was not associated with mortality (adjusted HR=0.90, 95% CI 0.79-1.04; p=0.164 for every increase in 1 SD of T), CVD deaths (adjusted HR=1.04, 95% CI 0.84-1.29; p=0.708) or CVD events (adjusted HR=1.03, 95% CI 0.92-1.15, p=0.661). No associations were found for free T, DHT or E2. Results were similar for men older and younger than 60 years.

**Conclusions:** In predominantly middle-aged men, T, DHT and E2 do not influence mortality or CVD outcomes. This neutral association of hormones with CVD contrasts with prior studies of older men.

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## Introduction

Cardiovascular disease (CVD) is a major contributor to morbidity and mortality, particularly with increasing age [1]. Testosterone (T) levels decrease with age, comorbidities and obesity [2], and epidemiological studies have reported an inverse relationship between T levels and mortality risk [3]. However, existing randomized controlled trials (RCTs) of testosterone replacement therapy (TRT) have not been powered for cardiovascular end points. One RCT was terminated early due to adverse cardiovascular-related effects seen with T supplementation in older men with limited mobility [4]. However, higher initiation doses of testosterone gel was administered to men in that study compared to conventional practice [4]. Furthermore, no adverse signals were seen in other large RCTs of TRT [5, 6]. Meta-analyses generally do not show increased risk of CVD events [7]. Furthermore, observational studies suggest that TRT with subsequent normalisation of T levels is associated with favourable outcomes [8, 9].The use of TRT has increased in the past decade [10]. Recently, the Food and Drug Administration (FDA) has recommended that men be warned of potential adverse cardiovascular effects associated with TRT [11]. Given the prevalence of CVD, and the increase in use of TRT, the relationship between T and CVD risk requires further clarification.

Epidemiological studies assessing the relationship between endogenous T with mortality and CVD outcomes vary significantly according to population size and age range [3, 12]. Acknowledging heterogeneity in previous studies, overall results suggest an inverse relationship between T with CVD events and mortality [3, 13]. Liquid chromatography-tandem mass spectrometry (LC-MS) provides a more accurate measure of T compared to immunoassay [14], therefore some earlier studies may be limited by the use of immunoassay for measurement of sex hormones. Studies using LC-MS measured T have generally reported inverse associations between T and CVD events, however these studies have consisted of

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older populations [3, 13, 15-17]. Results from middle-aged populations using LC-MS measured T are conflicting [18, 19]. Earlier studies of middle-aged men using T measured by immunoassay have reported no association between T and acute myocardial infarction [20] and an inverse association of free and bioavailable T with CVD deaths at 9 but not 18 years [21], however these studies consisted of a smaller number of outcome events. Smith et al. reported no association of cortisol:T ratio with CVD events, but did not assess associations of T independent of cortisol [22]. Furthermore, T is converted by 5α-reductase into dihydrotestosterone (DHT), which is a more potent ligand for the androgen receptor, and by aromatase into estradiol (E2) [23]. Studies assessing the relationship between DHT and E2 with CVD are relatively limited. Our aim was to assess the relationship between T, DHT and E2 assayed using LC-MS with all-cause and CVD related mortality and with CVD events in community-dwelling men across a wide age span.

## Methods

*Study population*

The Busselton Health Study (BHS) population is the coastal region of Busselton in Western Australia with a predominantly Anglo-Celtic population. The BHS includes a series of cross-sectional surveys conducted between 1966-1987. Surviving participants of these surveys were invited to participate in a follow-up survey in 1994/95. 2,143 men aged 17-97 years participated in the follow-up survey and provided blood samples for analysis. The 1994/95 survey was approved by the Human Research Ethics Committee of the University of Western Australia and all participating men provided written consent. The Human Research Ethics Committee of the Department of Health of Western Australia gave permission to access the hospital admission and death records for these survey participants from 1 January 1980 to 31 December 2010 using record linkage to the Register of Deaths and the Western Australian

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Hospital Morbidity Data System, which records all hospital admissions to public and private hospitals in Western Australia [24].

*Assessment of medical comorbidities*

Methods used in the Busselton Health Survey have previously been described [25]. All men completed a comprehensive health and lifestyle questionnaire and underwent a physical assessment at baseline. Alcohol consumption was labelled 'light' if consumption was ≤140g/week and 'heavy' if consumption was >140g/week. Body mass index (BMI) was defined as weight (kg) divided by height (m) squared. Further assessment of medical comorbidities was performed using their history of hospital admissions. *International Classification of Diseases*, 9th revision (ICD-9) codes were used for events up to 1987, ICD-9-CM codes for events up to 30 June 1999, and ICD-10-AM codes for subsequent events. History of CVD at baseline was defined as having any hospital admission for CVD (ICD-9 390-459) during the 15 years before the survey (i.e. 1980-1994). History of non-skin cancer was based on any non-skin cancer registrations (ICD-10 C00-42, C45-97) before the survey. Diabetes was based on self-reported doctor-diagnosed diabetes or use of glucose-lowering treatment at the survey, or a history of hospital admissions with a diagnosis of diabetes (ICD-9 250). Hypertension was defined based on self-reported use of antihypertensive medications at the survey or a history of hospital admissions with hypertension (ICD-9 401-405). History of chronic obstructive pulmonary disease (COPD) was based on a history of hospital admissions with this diagnosis (ICD-9 490-496) or percent predicted FEV1 < 60% as measured at the survey.

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*Biochemical assessment*

Early morning blood samples were collected after an overnight fast and serum was stored at -70°C until time of analysis. Serum T, DHT and E2 were quantified within a single LC-MS run without derivatization using atmospheric pressure photo-ionization for positive mode for androgens and negative mode for oestrogens, from 200µl samples as previously described [26]. Between-run imprecision for T was 8.6% at a concentration of 5.3 nmol/l and 7.9% at 26.9 nmol/l. For DHT, it was 11.3% at a concentration of 1.3 nmol/l and 9.1% at 5.3 nmol/l, and for E2, it was 14.5% at a concentration of 73 pmol/l and 9.9% at 279 pmol/l. Luteinising hormone (LH) was assayed using a two-step noncompetitive chemiluminometric immunoassay (Abbott Architect, Abbott Diagnostics, North Ryde, NSW, Australia) with between-run imprecision of 5.6% at 4.8 IU/l. Sex hormone binding globulin (SHBG) was assayed using a solid-phase, two-site enzyme immunometric assay with chemiluminescent substrate (Immulite 2000xPi; Siemens Healthcare, Bayswater, Vic., Australia) with between-run imprecision of 3.4% at 39.4nmol/l. Free T was calculated using an empirical formulae, which provides closer concordance with measured free T compared with calculations based on equilibrium binding equations [27]. Fasting serum cholesterol, high-density lipoprotein (HDL) and triglycerides (TG) were determined by standard enzymatic methods on a Hitachi 747 analyser (Roche Diagnostics, Castle Hill, NSW, Australia). Glucose was measured using a hexokinase assay. C-reactive protein (CRP) was measured using a particle-enhanced immunoturbidimetric assay on a Modular analyser (Roche Diagnostics, Mannheim, Germany). Interassay precision was 3.9% at 1.6 mg/l and 1.6% at 5 mg/l.

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*Ascertainment of incident deaths and cardiovascular events*

Follow-up for hospital admissions and deaths were available until the end of 2010, amounting to a total of 16 years of follow-up. Outcome events were ascertained from hospital admissions and death records. Three outcomes were analysed, which were time to death from any cause, time to death from CVD and time to first fatal or non-fatal CVD event. Non-fatal CVD events were defined as a hospital admission with a principal diagnosis of coronary heart disease (ICD-9 410-414; ICD-10 I20-25), stroke (ICD-9 430-437; ICD-10 I60-68, G45), congestive heart failure (ICD-9 428; ICD-10 I50) or peripheral arterial disease (ICD-9 440-448; ICD-10 I70-79). Deaths from CVD were ascertained based on deaths with underlying cause of death coded as diseases of the circulatory system (ICD-9 390-459; ICD-10 I00-99, G45).

*Statistical analysis*

SAS version 9.4 was used to analyse the data. Baseline data was assessed and expressed as mean and standard deviation (SD) for continuous data, and a percentage for categorical data. Cox proportional hazards regression analysis was used to examine associations of endogenous sex hormones, SHBG and LH with risk of all-cause and CVD mortality, and incidence of CVD events. Sex hormones were analysed based on effect estimates for 1 SD increase in hormone level, and also based on quartiles. Adjustments were made for factors which might plausibly affect these associations. Analyses were adjusted for age (model A1), then additional adjustments were made for smoking, vigorous exercise, alcohol and BMI (model A2). Further adjustments were then made for diabetes, history of CVD, COPD, non-skin cancer history, systolic blood pressure (SBP), hypertension and lipid lowering therapy (model A3). The fully adjusted model also included cholesterol, HDL, log TG, log CRP and log creatinine (model A4). Analyses were performed for the full cohort, for 1,501 men

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without any history of CVD at baseline, and for 570 men with comorbidities (CVD, diabetes, or BMI >30) at baseline. Further analyses were performed including an interaction term between age and T, and restricting follow-up to the first 8 years. A p-value of <.05, or a 95% confidence interval (CI) that did not cross 1.0 was considered significant.

## Results

*Baseline characteristics*

After excluding men who were taking androgens and anti-androgens, men who had a history of orchidectomy or prostate cancer and restricting to men without missing key hormone or other variables, 1,804 men were included in the analysis. The mean follow-up period from baseline assessment to the time of death or end of follow-up was 14.9 years. Baseline demographic, physical and biochemical data are shown for the entire cohort, stratified according to survival status and absence of CVD at baseline (table 1). The mean (SD) of T level in the full cohort was 13.6±4.9 nmol/L and 183 (10%) men had T levels <8 nmol/L. During the period of the study, there were 319 men who died, 141 of whom died of CVD, and 399 men who experienced a fatal or nonfatal CVD event. 1,501 men were free of CVD at baseline. There were 191 deaths, 71 CVD deaths and 234 CVD events in the CVD-free subgroup. In men who had T levels of <8.0 nmol/L, there were 54 deaths, 23 CVD deaths and 60 CVD events.

Younger to middle-aged men represented a greater portion of the cohort, with 68.9% of men aged <60 years. Compared to the entire cohort, participants who died were older, had a higher BMI, were more likely to be ex-smokers, less active and had more comorbidities. They also had higher log CRP and log creatinine levels. T (12.0±4.4 vs 13.6±4.9 nmol/l), free T (181.9±52.9 vs 218.3±63.8 pmol/l) and DHT levels (1.65±0.64 vs 1.70±0.72 nmol/l) were

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lower in those who died compared to the entire cohort. E2, SHBG and LH levels were higher in those who died compared to the entire cohort.

*Association between T with deaths and CVD outcomes*

There was an inverse association of T with overall mortality (HR=0.81, 95% CI 0.71-0.93; p=0.002, for every 1 SD increase in T) and CVD events (HR=0.88, 95% CI 0.79-0.98; p=0.024) in the age-adjusted model (table 2). However, this association was not significant in the fully adjusted model (HR=0.90, 95% CI 0.79-1.04; p=0.164, and HR=1.03, 95% CI 0.92-1.15; p=0.661 respectively). There was no association of T with CVD deaths (HR=1.04, 95% CI 0.84-1.29; p=0.708) in the fully adjusted model. In the CVD-free cohort, T did not influence risk of death (HR=0.88, 95% CI 0.74-1.05; p=0.151), CVD deaths (HR=0.96, 95% CI 0.72-1.28, p=0.777) or CVD events (HR=1.01, 95% CI 0.87-1.17, p=0.887) (table 2). Similar results were seen when follow-up was restricted to the first 8 years, and in men with comorbidities at baseline (supplementary tables 1&2). In the fully-adjusted model, T in the highest quartile was associated with an increased risk of CVD events compared to the rest of the men (p=0.039), however this difference was not significant in the CVD-free sub-cohort (p=0.524) or across T quartiles (p=0.138) (supplementary table 3). A relationship of highest quartile T versus the rest of the quartiles was also not observed for all-cause or CVD deaths in the total cohort, and the CVD-free sub-cohort (supplementary table 3). After adjustments, T <8nmol/L was not associated with all-cause or CVD deaths, or CVD events (supplementary table 4). The associations of T with these outcomes were similar in men <60 years and men ≥60 years (supplementary table 5).

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*Association between free T with deaths and CVD outcomes*

There was an inverse age-adjusted association between free T and all-cause mortality (HR=0.79, 95% CI 0.69-0.91; p=0.001) (table 3). However this was also not significant after adjusting for other risk factors (HR=0.88, 95% CI 0.76-1.03; p=0.111) (table 3). When free T was analysed in quartiles, there was no association with all-cause mortality, CVD mortality or CVD events (data not shown). There were similar findings in the CVD-free subgroup (table 3).

*Associations between other hormone variables with deaths and CVD outcomes*

DHT was associated with a lower incidence of death in the age-adjusted model, but not in the fully-adjusted model (table 4). Both DHT and E2 were not associated with all-cause or CVD mortality, or CVD events when analysed as continuous levels, quartiles or when the highest quartile was compared to the rest of the quartiles (tables 4&5, supplementary tables 6&7). When analysed as a continuous variable, SHBG was inversely associated with CVD events in the age-adjusted model (HR=0.85, 95% CI 0.76-0.94; p=0.003), however this was not significant after adjustment for other risk factors (HR= 1.03, 95% CI 0.92-1.15; p=0.623) (data not shown). No associations were seen when SHBG was analysed in quartiles (data not shown). LH was not associated with all-cause or CVD mortality, or CVD events (data not shown).

**Discussion**

In this cohort of community-dwelling men aged 17-97 years, there were apparent age-adjusted associations between higher T, free T and DHT with lower all-cause mortality, and higher T and SHBG with fewer CVD events, and higher SHBG with fewer CVD events. These associations were not significant in the fully adjusted models. No associations were

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seen for E2 or LH with mortality or CVD. The effect of T was similar in men older and younger than 60 years.

Our results are consistent with the findings from the Atherosclerosis Risk in Communities (ARIC) study in which no association was seen between T with CVD events or mortality, or markers of preclinical atherosclerosis in a cohort of 1,558 men aged 45-64 years [18]. By contrast, the European Male Aging Study (EMAS) which included 2,599 men aged 40-79 years, found that men with T <8nmol/L had increased risk of mortality [19]. We observed a trend towards increased CVD events in men with the highest quartile of T compared to the rest of the cohort. This is supportive of previously observed 'U- or J-shaped' associations between T and adverse health outcomes [28, 29], and of observational studies of TRT demonstrating a stroke signal in men with high achieved serum T levels [9]. However, this isolated finding should be interpreted with caution as no significant differences in associations were seen when T was assessed in quartiles or as a continuous variable, and these associations were not present for all-cause or CVD deaths, or in men free of CVD at baseline.

Our results indicate that associations of low T with mortality and CVD events generally seen in older men are not present in this cohort spanning a wide age-range. A previous meta-analysis by Araujo et al. showed an inverse association between T and all-cause and CVD mortality but noted considerable heterogeneity between studies [3]. When these studies were stratified by age, an inverse association was seen for studies which included men >60 years, but not in studies of men ≤60 years [3]. In our cohort, we found no interaction effect between age and T on mortality or CVD events, however our population may not be sufficiently large to test this effect. Araujo et al. also found that follow-up period contributed to heterogeneity in relative risk (RR), with greater RR seen in subjects followed for <9.6 years [3]. This may

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reflect suppression of T by pre-existent subclinical disease in shorter-term studies. In our cohort, limiting follow-up to 8 years did not change our results. There was a low prevalence of baseline comorbidities in this predominantly middle-aged cohort, therefore serial measurements of T would be required to clarify the relationship between T and adverse health over time.

Lower DHT has been associated with poorer outcomes in older men. The HIMS cohort showed an inverse association of DHT with all-cause mortality and stroke incidence, but not incidence of myocardial infarction [15, 28]. Similarly, the CHS showed an inverse association between DHT and all-cause mortality and incident stroke, but a curvilinear association with CVD [30]. Studies of DHT in middle-aged men are lacking. DHT was not studied in either the ARIC or the EMAS populations [18, 19]. One study including middle-aged men using DHT measured by immunoassay showed no relationship between DHT and ischaemic heart disease mortality [31]. Our results suggest that DHT does not influence mortality and CVD outcomes in predominantly middle-aged men.

Of note, in age-adjusted analyses, we saw inverse associations of T, free T and DHT with all-cause mortality, and of T with CVD events. However, none of these associations remained significant as soon as adjustment for comorbidities and cardiovascular risk factors were performed. In particular, adjustment for lipids, CRP and creatinine rather than other conventional CVD risk factors, nullified the association of T and free T with mortality. Adjustment for these confounders may have accounted for differences in our findings compared to that of Pye *et al* (21). Thus apparent associations of higher androgens with better health and reduced CVD risk [26, 32] may reflect confounding from the presence of dyslipidaemia, inflammation or renal dysfunction. Restricting the analysis to men with

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baseline comorbidities showed similar associations, however, there was less power to detect significant associations given a smaller number of men (n=570) in this population.

E2 originates from peripheral conversion of T through enzymatic aromatization but the relationship of E2 with CVD remains unclear. One study reported an inverse association between E2 as measured by immunoassay and CVD mortality [21] and another study showed an inverse association with all-cause mortality in elderly men when E2 was measured by gass chromatography-mass spectrometry [33]. By contrast, two studies using E2 measured by immunoassay reported increased risk of death [34] or stroke [35]. However, consistent with our findings, most studies have shown a neutral association between E2 with CVD and all-cause mortality [28, 36], and CVD events [15, 16, 29, 36, 37]. Neither ARIC nor EMAS assayed E2 [18, 19]. Apart from the HIMS and MrOs cohort, most of these studies utilised immunoassay to measure E2 concentrations. Therefore our findings reinforce the concept that estrogens, even when measured accurately using LC-MS, are not a robust predictor of mortality and CVD in men.

Experimental studies have shown that SHBG-bound testosterone may be biologically active through megalin, an endocytic receptor, which acts as a pathway for cellular uptake of SHBG-bound testosterone [38]. Epidemiological studies assessing the association between SHBG with all-cause and CVD mortality have shown conflicting results. In older men, a neutral association was seen in one study [33] while an inverse association was observed for CVD mortality in another [39]. In middle-aged men, Menke et al. demonstrated an increased risk of CVD mortality with low SHBG levels at 18 years but not at 9 years of follow-up [21], while Araujo et al did not find a significant association between SHBG and all-cause or ischaemic heart disease mortality after adjustment for confounders [31]. Our findings do not support the concept that SHBG independently modulates cardiovascular risk. In older men, a

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higher LH level has been associated with increased CVD mortality [17]. In our present study of predominantly middle-aged men, LH was not associated with mortality or CVD.

We acknowledge several limitations in our study. This is an observational study and we therefore cannot infer causality. Men in the BHS were community-dwelling survivors from previous surveys, therefore a 'healthy-survivor' effect may be present and our results may be more applicable to healthier men. Outcomes were measured based on single measures of endogenous sex hormones at baseline, we did not have serial hormone measures. However, blood samples were collected in the early morning to minimize the effect of circadian variation on hormone levels. Lastly, the BHS consists of a population from a predominantly Anglo-Celtic background, and these results cannot be extrapolated to men of other backgrounds.

Strengths of our study include a large study population spanning a wide range of ages with a long period of follow-up whereby a large number of outcome events occurred, increasing our statistical power to define associations. Sex hormones were measured using LC-MS, which provides a more accurate measure compared to immunoassays. Outcome events were ascertained through the WADLS, which captures mortality data and hospital admissions for the entire state of Western Australia, and systematic adjustment for a range of risk factors was performed.

*Conclusion*

In conclusion, T exhibits neutral associations with all-cause or CVD mortality, or incident CVD events in a cohort of predominantly middle-aged men. Further studies are required to clarify the age-dependent effects of T, and to examine the relationship between T and disease over time.

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Table 1. Baseline characteristics of cohort (n = 1,804). Table shows mean (SD) or percent.

| Variable (units) | Died | | Cohort | |
|---|---|---|---|---|
| | Yes (n = 319) | No (n = 1,485) | All (n = 1,804) | CVD-free (n = 1,501) |
| Age (years) | 70.4 (11.0) | 46.0 (14.6) | 50.3 (16.8) | 47.5 (16.1) |
| Smoking | | | | |
| Never | 27.3 | 46.1 | 42.8 | 44.6 |
| Ex | 58.9 | 37.1 | 41.0 | 37.4 |
| Current | 13.8 | 16.8 | 16.2 | 18.0 |
| Exercise vigorous - yes | 30.4 | 65.6 | 59.4 | 64.2 |
| Alcohol | | | | |
| Non | 3.1 | 4.4 | 4.2 | 4.5 |
| Ex | 17.6 | 4.6 | 6.9 | 6.1 |
| Light | 45.8 | 48.6 | 48.1 | 47.8 |
| Heavy | 33.5 | 42.4 | 40.9 | 41.5 |
| BMI ($kg/m^2$) | 27.0 (3.7) | 26.3 (3.4) | 26.5 (3.4) | 26.3 (3.4) |

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| | | | | |
|---|---|---|---|---|
| Diabetes (%) | 16.9 | 3.8 | 6.2 | 4.6 |
| CVD history (%) | 40.1 | 11.8 | 16.8 | - |
| COPD history (%) | 8.8 | 1.4 | 2.7 | 1.5 |
| Non-skin cancer history (%) | 6.6 | 1.2 | 2.2 | 1.3 |
| SBP (mmHg) | 137.5 (19.6) | 124.5 (13.9) | 126.8 (15.8) | 125.2 (14.4) |
| Hypertension (%) | 46.7 | 10.5 | 16.9 | 9.5 |
| Cholesterol (mmol/l) | 5.64 (0.95) | 5.49 (1.05) | 5.52 (1.04) | 5.48 (1.05) |
| HDL (mmol/l) | 1.18 (0.35) | 1.21 (0.29) | 1.21 (0.30) | 1.21 (0.30) |
| log Triglycerides (mmol/l) | 0.35 (0.52) | 0.16 (0.56) | 0.20 (0.55) | 0.17 (0.55) |
| Lipids medication (%) | 7.2 | 2.2 | 3.0 | 1.4 |
| log CRP | 0.82 (1.23) | 0.13 (1.18) | 0.25 (1.22) | 0.17 (1.20) |
| log Creatinine | 4.65 (0.22) | 4.56 (0.12) | 4.58 (0.15) | 4.56 (0.12) |
| Testosterone (nmol/l) | 12.0 (4.4) | 14.0 (4.9) | 13.6 (4.9) | 13.8 (4.9) |
| Free testosterone (pmol/l) | 181.9 (52.9) | 226.1 (63.2) | 218.3 (63.8) | 223.0 (63.9) |
| Dihydrotestosterone (nmol/l) | 1.65 (0.64) | 1.71 (0.73) | 1.70 (0.72) | 1.70 (0.72) |
| Estradiol (pmol/l) | 64.0 (32.0) | 59.2 (29.8) | 60.1 (30.2) | 59.9 (29.8) |
| SHBG (nmol/l) | 34.7 (14.3) | 27.8 (11.8) | 29.1 (12.5) | 28.4 (12.3) |
| LH (IU/l) | 5.74 (5.67) | 3.61 (2.14) | 3.99 (3.18) | 3.82 (2.92) |

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Table 2. Hazard ratios and 95% CI for T in relation to deaths, CVD deaths and CVD events for the full cohort and CVD-free sub-cohort after progressive adjustment for risk factors. Hazard ratio is for a standard deviation change of T of 4.90 nmol/l.

| | Full cohort | | Sub-cohort | |
|---|---|---|---|---|
| | HR (95% CI) | p-value | HR (95% CI) | p-value |
| Deaths | | | | |
| A1 | 0.81 (0.71 , 0.93) | 0.002 | 0.85 (0.72, 1.00) | 0.048 |
| A2 | 0.83 (0.73 , 0.95) | 0.008 | 0.87 (0.73 , 1.03) | 0.097 |
| A3 | 0.87 (0.76 , 1.00) | 0.045 | 0.86 (0.73 , 1.02) | 0.093 |
| A4 | 0.90 (0.79 , 1.04) | 0.164 | 0.88 (0.74 , 1.05) | 0.151 |
| CVD deaths | | | | |
| A1 | 0.85 (0.70 , 1.03) | 0.098 | 0.92 (0.71 , 1.20) | 0.533 |
| A2 | 0.87 (0.71 , 1.07) | 0.194 | 0.92 (0.70 , 1.22) | 0.581 |
| A3 | 0.94 (0.77 , 1.15) | 0.546 | 0.96 (0.72 , 1.27) | 0.758 |
| A4 | 1.04 (0.84 , 1.29) | 0.708 | 0.96 (0.72 , 1.28) | 0.777 |
| CVD events | | | | |
| A1 | 0.88 (0.79 , 0.98) | 0.024 | 0.95 (0.82 , 1.09) | 0.428 |
| A2 | 0.93 (0.83 , 1.04) | 0.193 | 0.97 (0.84 , 1.12) | 0.693 |
| A3 | 0.97 (0.87 , 1.08) | 0.585 | 0.99 (0.86 , 1.13) | 0.852 |
| A4 | 1.03 (0.92 , 1.15) | 0.661 | 1.01 (0.87 , 1.17) | 0.887 |

A1 – age
A2 – A1, smoking, vigorous exercise, alcohol, BMI
A3 – A2, diabetes, CVD history, COPD history, non-skin cancer history, SBP, hypertension, lipids medication
A4 – A3, cholesterol, HDL, (log) triglycerides, (log) CRP, (log) creatinine

Table 3. Hazard ratios and 95% CI for Free T in relation to deaths, CVD deaths and CVD events for the full cohort and CVD-free sub-cohort after progressive adjustment for risk factors. Hazard ratio is for a standard deviation change of free T of 63.79 pmol/l.

| | Full cohort | | Sub-cohort | |
|---|---|---|---|---|
| | HR (95% CI) | p-value | HR (95% CI) | p-value |
| Deaths | | | | |
| A1 | 0.79 (0.69 , 0.91) | 0.001 | 0.84 (0.71 , 1.09) | 0.061 |
| A2 | 0.82 (0.71 , 0.95) | 0.007 | 0.87 (0.72 , 1.04) | 0.130 |
| A3 | 0.86 (0.74 , 0.99) | 0.038 | 0.86 (0.72 , 1.04) | 0.123 |
| A4 | 0.88 (0.76 , 1.03) | 0.111 | 0.87 (0.72 , 1.05) | 0.157 |
| CVD deaths | | | | |
| A1 | 0.85 (0.69 , 1.06) | 0.149 | 0.97 (0.72 , 1.30) | 0.823 |
| A2 | 0.88 (0.71 , 1.10) | 0.274 | 0.96 (0.71 , 1.31) | 0.806 |
| A3 | 0.94 (0.75 , 1.18) | 0.618 | 1.00 (0.73 , 1.37) | 0.977 |
| A4 | 1.04 (0.82 , 1.31) | 0.763 | 0.99 (0.72 , 1.37) | 0.959 |
| CVD events | | | | |

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| | | | | |
|---|---|---|---|---|
| A1 | 0.89 (0.79 , 1.00) | 0.058 | 0.95 (0.81 , 1.10) | 0.482 |
| A2 | 0.94 (0.83 , 1.06) | 0.325 | 0.97 (0.83 , 1.14) | 0.718 |
| A3 | 0.98 (0.87 , 1.11) | 0.774 | 0.98 (0.84 , 1.15) | 0.832 |
| A4 | 1.02 (0.90 , 1.15) | 0.767 | 0.98 (0.84 , 1.16) | 0.854 |

A1 – age
A2 – A1, smoking, vigorous exercise, alcohol, BMI
A3 – A2, diabetes, CVD history, COPD history, non-skin cancer history, SBP, hypertension, lipids medication
A4 – A3, cholesterol, HDL, (log) triglycerides, (log) CRP, (log) creatinine

Table 4. Hazard ratios and 95% CI for DHT in relation to deaths, CVD deaths and CVD events for the full cohort and CVD-free sub-cohort after progressive adjustment for risk factors. Hazard ratio is for a standard deviation change for DHT of 0.72 nmol/l.

| | Full cohort | | Sub-cohort | |
|---|---|---|---|---|
| | HR (95% CI) | p-value | HR (95% CI) | p-value |
| Deaths | | | | |
| A1 | 0.88 (0.77 , 0.99) | 0.037 | 0.91 (0.77 , 1.07) | 0.241 |
| A2 | 0.89 (0.79 , 1.01) | 0.078 | 0.92 (0.78 , 1.09) | 0.358 |
| A3 | 0.92 (0.81 , 1.05) | 0.206 | 0.93 (0.79 , 1.11) | 0.433 |
| A4 | 0.95 (0.83 , 1.08) | 0.403 | 0.94 (0.79 , 1.11) | 0.471 |
| CVD deaths | | | | |
| A1 | 0.87 (0.72 , 1.05) | 0.151 | 0.98 (0.74 , 1.28) | 0.860 |
| A2 | 0.89 (0.74 , 1.09) | 0.266 | 1.01 (0.76 , 1.35) | 0.917 |
| A3 | 0.90 (0.73 , 1.11) | 0.321 | 1.02 (0.77 , 1.37) | 0.870 |
| A4 | 0.98 (0.79 , 1.20) | 0.824 | 1.02 (0.76 , 1.38) | 0.870 |
| CVD events | | | | |
| A1 | 0.95 (0.86 , 1.06) | 0.390 | 1.03 (0.90 , 1.18) | 0.662 |
| A2 | 1.00 (0.90 , 1.11) | 0.971 | 1.06 (0.92 , 1.22) | 0.394 |
| A3 | 0.99 (0.89 , 1.11) | 0.912 | 1.08 (0.94 , 1.24) | 0.284 |
| A4 | 1.02 (0.92 , 1.14) | 0.667 | 1.09 (0.95 , 1.26) | 0.212 |

A1 – age
A2 – A1, smoking, vigorous exercise, alcohol, BMI
A3 – A2, diabetes, CVD history, COPD history, non-skin cancer history, SBP, hypertension, lipids medication
A4 – A3, cholesterol, HDL, (log) triglycerides, (log) CRP, (log) creatinine

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Table 5. Hazard ratios and 95% CI for E2 in relation to deaths, CVD deaths and CVD events for the full cohort and CVD-free sub-cohort after progressive adjustment for risk factors. Hazard ratio is for a standard deviation change for E2 of 30.25 pmol/l.

| | Full cohort[1] | | Sub-cohort[2] | |
|---|---|---|---|---|
| | HR (95% CI) | p-value | HR (95% CI) | p-value |
| Deaths | | | | |
| A1 | 0.99 (0.89 , 1.10) | 0.851 | 0.94 (0.81 , 1.09) | 0.403 |
| A2 | 0.98 (0.88 , 1.09) | 0.712 | 0.91 (0.78 , 1.06) | 0.247 |
| A3 | 1.00 (0.89 , 1.12) | 0.964 | 0.88 (0.75 , 1.03) | 0.120 |
| A4 | 1.00 (0.89 , 1.12) | 0.995 | 0.88 (0.75 , 1.04) | 0.136 |
| CVD deaths | | | | |
| A1 | 1.10 (0.94 , 1.29) | 0.232 | 1.03 (0.81 , 1.31) | 0.789 |
| A2 | 1.08 (0.92 , 1.27) | 0.334 | 1.02 (0.80 , 1.30) | 0.861 |
| A3 | 1.10 (0.94 , 1.30) | 0.219 | 1.01 (0.79 , 1.30) | 0.907 |
| A4 | 1.14 (0.97 , 1.35) | 0.119 | 1.03 (0.80 , 1.33) | 0.827 |
| CVD events | | | | |
| A1 | 0.98 (0.89 , 1.08) | 0.673 | 0.95 (0.83 , 1.09) | 0.500 |
| A2 | 0.97 (0.87 , 1.07) | 0.501 | 0.94 (0.82 , 1.08) | 0.380 |
| A3 | 0.97 (0.88 , 1.07) | 0.543 | 0.91 (0.79 , 1.04) | 0.173 |
| A4 | 1.00 (0.91 , 1.10) | 0.971 | 0.93 (0.81 , 1.07) | 0.304 |

[1] n = 1,700 [2] n = 1,405

A1 – age

A2 – A1, smoking, vigorous exercise, alcohol, BMI

A3 – A2, diabetes, CVD history, COPD history, non-skin cancer history, SBP, hypertension, lipids medication

A4 – A3, cholesterol, HDL, (log) triglycerides, (log) CRP, (log) creatinine

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