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Title

Lower plasma testosterone or dihydrotestosterone, but not estradiol, are associated with symptoms of intermittent claudication in older men.

Short title

Sex steroids and claudication

Authors

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Disclosures

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Abstract

Objective

In men, testosterone (T) levels decline with age, and lower T predicts all-cause and cardiovascular mortality. However, the associations of T and its metabolites, dihydrotestosterone (DHT) and estradiol (E2), with symptomatic peripheral arterial disease remain unclear. We assessed associations of T, DHT and E2 with lower limb intermittent claudication in older men.

Design

Cross-sectional study.

Participants

Community-dwelling men aged 70-89 years resident in Perth, Western Australia.

Measurements

Intermittent claudication was ascertained by the Edinburgh Claudication Questionnaire. Early morning plasma T, DHT and E2 were assayed using liquid chromatography-tandem mass spectrometry.

Results

There were 268 men with intermittent claudication, and 2,435 without claudication or any leg pain. Men with non-specific leg pain (n=986) were excluded. After adjusting for age, smoking, BMI, waist:hip ratio, hypertension, dyslipidemia, diabetes, creatinine and prevalent cardiovascular disease (CVD), higher T was associated with reduced risk of having claudication (per 1 SD increase, odds ratio [OR]=0.80, 95% confidence interval [CI]=0.69-0.94, p=0.006; quartiles, Q4:Q1, OR=0.54, 95% CI=0.36-0.81). Higher DHT was associated with reduced risk of having claudication (per 1 SD increase, OR=0.86, 95% CI=0.73-1.00, p=0.048; Q4:Q1, OR=0.64, 95% CI=0.43-0.95). E2 was not associated with claudication (per 1 SD increase, OR=0.96, 95% CI=0.83-1.11, p=0.565; Q4:Q1, OR=0.88, 95% CI=0.60-1.29).

Conclusions

Lower T or DHT, but not E2, are associated with symptoms of intermittent claudication in older men. Reduced exposure to androgens may represent a causal factor or biomarker for symptomatic peripheral arterial disease. Further studies are needed to examine underlying mechanisms and evaluate therapeutic options in ageing men.

Introduction

Testosterone (T) is the primary androgen produced in men [1]. T is converted by 5α-reductase into dihydrotestosterone (DHT), a more potent ligand for the androgen receptor (AR), and by aromatase into estradiol (E2) a ligand for the estrogen receptor (ER)α or ERβ [1,2]. In men, T maintains virilisation and body composition, preserving lean mass and bone mineral density while reducing accumulation of fat [3]. Lower T levels have been associated with poorer health outcomes in ageing men, including higher all-cause and cardiovascular mortality [4,5]. Whether reduced T levels are causal factors or biomarkers for cardiovascular disease (CVD) in ageing men remains under debate as randomised clinical trials of testosterone have not been powered for the outcome of CVD events [2,6].

Lower T predicts incident stroke and transient ischaemic attack [7], but its association with other cardiovascular events such as myocardial infarction is inconsistent [8-10]. An equivocal association of lower DHT with ischaemic heart disease mortality was reported in one study [11]. E2 has been positively associated with the progression of carotid intima-media thickness and incident stroke [12,13], but negatively associated with mortality in different studies [14]. Therefore additional studies are needed to clarify the association of androgens encompassing both T and DHT, and estrogens with CVD-related outcomes.

Lower T levels have been associated with peripheral arterial disease (PAD) as assessed by comparing blood pressure in the leg and arm (Ankle Brachial Index, ABI) in some [15,16] but not all studies [17,18]. The ABI comprises the ratio of blood pressures measured over the posterior tibial artery at the ankle and the brachial artery in the arm, where an ABI<0.90 is used to define the presence of PAD [15]. However, it is unclear whether this association extends to the presence of claudication symptoms, or whether parallel or divergent associations are present for DHT and E2. We tested the hypothesis that T, DHT and E2 are differentially associated with intermittent claudication in older men.

Methods

Study population

The Health In Men Study (HIMS) is a population-based cohort study of community-dwelling older men who participated in a trial of screening for abdominal aortic aneurysm from Perth, Western Australia [19]. 12,203 men completed a questionnaire and attended for physical examination in Wave 1 (W1, 1996-1999). During Wave 2 (W2, 2001-2004), 4,248 men of these men then aged 70-89 years completed a second questionnaire, and attended for physical examination and venesection. The University of Western Australia Human Research Ethics Committee approved the study, and all men gave written informed consent.

Assessment of medical comorbidities

Men were considered to have hypertension if they reported this diagnosis at W1 or W2, or reported use of anti-hypertensive medication or had blood pressure ≥140/90 mmHg at W2. Dyslipidemia was defined as having fasting HDL <0.9 mmol/L, LDL ≥3.4 mmol/L, triglycerides ≥1.8 mmol/L or total cholesterol ≥5.5 mmol/L, or receiving lipid-lowering therapy at W2. Men diagnosed with diabetes, reporting use of glucose-lowering medication,

or with fasting or non-fasting glucose at W2 of ≥7 mmol/L or ≥11.1 mmol/L respectively, were considered to have diabetes [20]. Prevalent CVD was defined as self-reported history of angina, acute myocardial infarction, stroke or AAA by questionnaire responses in W1 and W2.

Classification of lower limb pain

The presence of intermittent claudication was determined by responses to the Edinburgh Claudication Questionnaire (ECQ) completed at W2. The ECQ is an improved version of the WHO/Rose Questionnaire for use in epidemiological surveys and has 91% sensitivity and 99% specificity in comparison to the diagnosis of intermittent claudication made by a physician [21]. The questionnaire comprises seven items, asking whether pain or discomfort in the legs is present on walking, the distance before pain stops the walk, whether pain occurs with standing still or sitting, when walking uphill or hurrying, whether pain occurs with walking at ordinary pace on level ground, whether pain diminishes within 10 minutes of resting, and to mark the location of the pain on a diagram of both legs. A man was classified as positive in the ECQ if he had leg pain when walking (but not sitting or standing), which was relieved by standing still for 10 minutes or less, and which was not localised to a major joint. Men who reported leg pain who did not meet this definition were categorised as having atypical leg pain.

Laboratory assays

Blood samples were collected between 0800h and 1030h at W2. Plasma was prepared immediately following phlebotomy and stored at -80^{o}C until assayed. T, DHT and E2 were quantified within a single LC-MS run without derivatization using atmospheric pressure photo-ionisation in positive mode for androgens and negative mode with electrospray

ionisation for estrogens, from 200 µL samples as previously described [22,23]. The assay limits of detection were T 0.04 nmol/L, DHT 0.01 nmol/L and E2 3.4 pmol/L. Inter-assay coefficients of variation (CV, %) for high, medium and low reference concentrations were T: 3.9% at 29.8 nmol/L, 6.8% at 5.9 nmol/L, and 6.5% at 2.0 nmol/L; for DHT: 6.7% at 29.5 nmol/L, 9.1% at 5.7 nmol/L and 13.4% 1.9 nmol/L; and for E2 8.1% at 308 pmol/L and 8.6% at 103 pmol/L. Precision profiles (CV% plotted against mean) based on 49 consecutive runs displayed CV <6% for serum T levels (>0.4 nmol/L), <13% for serum DHT levels (>0.7 nmol/L) and <8% for serum E2 levels (>25 pmol/L). SHBG had been determined in an earlier series of assays by chemiluminescent immunoassay on an Immulite 2000 analyser (Diagnostic Products Corp.-Biomediq, Doncaster, Australia), with CV of 6.7% at 5.2 nmol/L and 6.2% at 81 nmol/L [24]. Free T was calculated from total T and SHBG using empirical formulae which in extensive validation studies correspond more closely with measured free T compared to calculations based on equilibrium binding equations [25].

Statistical analyses

The statistical package Stata version 11.1 (StataCorp, College Station, Texas, USA) was used to analyse the data. Baseline descriptive data were shown as mean and standard deviations (SD), or percentages (%). For continuous variables exhibiting a skewed (non-parametric) distribution, data were shown as median and interquartile range (25^{th} to 75^{th} percentiles). Comparisons of means were performed using two sample t tests with equal variances, which are robust for parametric and modestly skewed distributions with sufficiently large sample sizes [26]. Logistic regression was utilised to examine the associations of T, DHT, E2, calculated free T and SHBG with the presence of intermittent claudication. Hormonal data were modelled as continuous variables, and also in quartiles to explore whether non-linear or threshold effects were present. Adjustment for covariates was performed by incorporating

variables which might plausibly influence an association of sex hormones with this outcome. Analyses were initially adjusted for age, smoking, body mass index (BMI) and waist:hip ratio. Sequential adjustments were made for hypertension, dyslipidemia, diabetes and creatinine, and for prevalent CVD. A p value of <0.05 was considered significant.

Results

Characteristics of the study population

A flow chart of study participants is shown in Figure 1. There were 4,248 men who attended the HIMS Wave 2 assessment and provided a blood sample. Of these men 4,230 had total T, DHT and E2 assayed by LC-MS and 4,228 of these had ECQ results available. Exclusion of men on androgens (n=26), or anti-androgen therapy (n=77), with a history of orchidectomy (n=56) or prostate cancer (n=380) left 3,689 men. From these, men with non-specific leg pain (n=986) were also excluded, leaving a total of 2,703 men, of whom 2,435 had no leg pain and 268 men had intermittent claudication (9.9%).

FIGURE 1

Demographic, physical, medical and biochemical characteristics of the study cohort are shown in Table 1. Men with intermittent claudication were older, more likely to be current or past smokers, and had higher BMI and waist:hip ratios. Men with intermittent claudication were more likely to report hypertension, dyslipidemia, diabetes or prevalent CVD. Creatinine levels were higher in men with intermittent claudication compared to those without. Total and calculated free T, and DHT were lower in men with intermittent claudication compared to men with no leg pain. Mean total T in men with atypical leg pain was 12.5±4.9 nmol/L, intermediate between men who had no leg pain and those with intermittent claudication.

TABLE 1

Prevalence of intermittent claudication according to sex hormone levels

The prevalence of intermittent claudication in the 2,703 men according to deciles of T, DHT, E2 and calculated free T are shown in Figure 2. There was an inverse correlation of claudication risk with T, DHT and calculated free T (Figure 2A, B and C). There was no clear association of E2 in deciles with prevalence of claudication (data not shown). SHBG was inversely associated with prevalence of claudication (Figure 2D).

FIGURE 2

Associations of sex steroids with intermittent claudication in older men

Logistic regression models examining the association of sex hormones as continuous variables with the presence of intermittent claudication are shown in Table 2. After adjusting for age, smoking, BMI, waist:hip ratio, hypertension, dyslipidemia, diabetes, creatinine and prevalent CVD, higher T was associated with reduced risk of claudication (per 1 SD increase, odds ratio [OR]=0.80, 95% confidence interval [CI]=0.69-0.94) (Table 2A). Higher DHT was associated with reduced risk of claudication (per 1 SD increase, OR=0.86, 95% CI=0.73-1.00), but E2 was not (OR=0.96, 95% CI=0.83-1.11). The association of free T was similar to that of total T (per 1 SD increase in calculated free T, OR=0.81, 95% CI=0.70-0.94). Higher SHBG was associated with lower risk of claudication (OR=0.83, 95% CI=0.71-0.98) (Table 2A). When both total T and SHBG were included in the multivariable model the association of higher T with reduced risk of claudication persisted while the association of SHBG was

attenuated (Table 2B).

TABLE 2

Results for regression analyses with hormones modelled as quartiles are shown in Table 3. In the fully-adjusted model, men with T in the highest three quartiles had significantly decreased intermittent claudication risk compared with men in the lowest quartile (reference Q1: Q2 OR=0.60, 95% CI=0.42-0.87, Q3 OR=0.67, 95% CI=0.46-0.96, and Q4 OR=0.54, 95% CI=0.36-0.81). Men with DHT in the highest quartile had significantly reduced risk of intermittent claudication compared to men in the lowest quartile (Q4 vs Q1: OR=0.64, 95% CI=0.43-0.95). Quartiles of E2 were not associated with OR for claudication. Men with calculated free T in the highest two quartiles had reduced OR for intermittent claudication (reference Q1: Q3 OR=0.62, 95% CI=0.42-0.91, Q4 OR=0.65, 95% CI=0.43-0.98). Men with SHBG in the highest quartile of values had lower OR for intermittent claudication (reference Q1: Q4 OR=0.54, 95% CI=0.35-0.83).

TABLE 3

Intermittent claudication risk stratified according to lower T, DHT or E2

We performed regression analyses for risk of intermittent claudication according to T, DHT or E2 in the lowest quartile of values. Men with T or DHT in the lowest quartile (Q1) had increased OR for claudication (Q1 T: fully-adjusted OR=1.65, 95% CI=1.23-2.21, p<0.001, and Q1 DHT: OR=1.35, 95% CI=1.00-1.83, p=0.05). There was no association of E2 in the lowest quartile with claudication (Q1 E2: OR=1.15, 95% CI=0.85-1.56, p=0.36). For men with both T and DHT in Q1, the OR for claudication was similar to those with only T in Q1

(1.56 vs 1.65). For men with both T and E2 in Q1, the OR for claudication was similar to those with only T in Q1 (1.61 vs 1.65). Men with both T and SHBG in Q1 had OR for claudication similar to those with only T in Q1 (1.58 vs 1.65).

Discussion

In this study, higher levels of either T or DHT were associated with lower prevalence of intermittent claudication in older men, independently of conventional risk factors for vascular disease including prevalent CVD. A comparable association was seen with calculated free T, but not with E2. Adjustment for age and comorbidities had limited effect on the strength of associations of total T, DHT and calculated free T with intermittent claudication, consistent with the presence of robust and independent associations. Lower levels of androgens may predispose to intermittent claudication; alternatively low levels of androgens in men with intermittent claudication may be a result of the disease.

These findings contrast with the case-control study reported by Price et al in which levels of total and free T were not significantly different in 40 men with PAD defined using ABI and symptoms of intermittent claudication and 41 men without [17]. There was no association of E2 with PAD. In that study total T and E2 were measured by radioimmunoassay, and free T calculated as the ratio of total T to SHBG which is a particularly inaccurate estimate [27]. Our results also differ from the report by Maggio et al of 41 men with ABI <0.9 vs 378 men with ABI >0.9, where neither total T nor E2 measured by immunoassay were associated with the presence of PAD [18]. In that study SHBG was negatively associated with PAD [18]. This is consistent with our finding that higher SHBG was associated with lower risk of intermittent claudication, with the association present for men with SHBG in the highest quartile of values. Of note, we found that higher T was associated with lower risk of

intermittent claudication independently of SHBG. It is likely that the use of LC-MS and the larger sample size in our study enabled associations of T with symptomatic PAD to be defined with greater precision.

Our results extend the report by Tivesten et al [15] which assessed ABI rather than symptoms of PAD. In that report from the Osteoporotic Fractures in Men Study (MrOS) higher T or lower E2 were associated with ABI in 2,784 men aged (mean±SD) 75.4±3.2 years [15]. After adjustment for age, site of study, smoking, BMI, diabetes and hypertension, T in the lowest quartile was associated with increased risk of ABI<0.90, as was free T. In the fully adjusted model free E2 but not total E2 in the highest quartile was associated with increased risk of ABI<0.90 [15]. Our study supports this finding by demonstrating a similar association of T with symptoms of lower limb intermittent claudication, and provides parallel data for DHT.

Our results contrast with the report by Haring et al of 1,422 men aged 61.0±9.5 years from the Framingham Heart Study using a composite definition of PAD which included ABI<0.90, symptoms of calf pain on exertion relieved with rest or a history of lower limb revascularisation [16]. In that cohort 4.5% had an ABI<0.90, 3.6% reported intermittent claudication and 6.3% met the composite definition of PAD. After adjustment for age, waist circumference, smoking, lipids, diabetes, hypertension and CVD, neither total nor free T were associated with PAD, although free T in the lowest quartile was associated with lower ABI [16]. E2 was not associated with either ABI or PAD. Since the Framingham cohort is younger compared with HIMS, it is possible that the associations of lower androgens with PAD are only evident in older men.

Strengths of our study include the large sample of community-dwelling older men, careful phenotyping of the cohort with assessment of comorbidities, and the assessment of T, DHT and E2 using an established LC-MS methodology. In the statistical analyses, we were able to adjust for conventional risk factors such as age and smoking, and specifically for the presence of hypertension, dyslipidemia, diabetes and prevalent CVD. We acknowledge several limitations of the study. The assessment of intermittent claudication was made using questionnaire responses, rather than dedicated clinical review and did not include routine imaging. The hormone results are based on blood sampling at a single timepoint, albeit collected early in the morning to minimise any confounding from circadian variation. We did not have serial blood samples, nor did we re-assess intermittent claudication longitudinally, therefore the analysis is cross-sectional which precludes our ability to infer causality. It is possible that lower androgen levels are a consequence of ill-health [2], representing a biomarker rather than a causal factor.

We adjusted for multiple variables including conventional cardiovascular risk factors and prevalent CVD, but it is possible that these may not have captured all relevant covariates. The association of lower hormone levels with intermittent claudication may be due to an underlying common factor not accounted for in the analysis. We excluded a large number of men with atypical leg pain. Most of these men were likely to have had musculoskeletal pain rather than genuinely non-specific pain. Some may have had both claudication and another cause of pain. Although this sub-group represents some loss of sensitivity of the ECQ, their inclusion with the claudication group would reduce specificity markedly. Intermittent claudication is induced by walking therefore it is possible that some men who were sedentary or purposefully limited ambulation to distances that did not induce claudication might have been misclassified. Assessment of activity or ambulation was not adjusted for in the analysis.

Men returned for assessment in 2001-2004 after a previous assessment in 1996-1999. Thus a "healthy survivor" effect may be present. Men in HIMS are predominantly Caucasian in ethnicity therefore we cannot extrapolate these findings to other ethnic groups, nor can we draw any conclusions as to any associations in women.

Of note, we found no association of E2 with intermittent claudication in our cohort. This finding contrasts with reports that higher circulating E2 predicted progression of carotid intima-media thickness in a study of 313 middle-aged men [12] and CVD events in the Framingham cohort of 2,084 middle-aged men [8]. E2 correlated inversely with ABI in the 2,784 older men from MrOS [15]. These analyses in which E2 was measured by immunoassay rather than mass spectrometry suggest an adverse association of E2 level with outcomes of vascular disease. However, in a separate analysis of 3,014 men with mean age of 75 years from MrOS Sweden, T and E2 were assayed by gas chromatography-mass spectrometry and men with both low T and low E2 had the highest risk of death [14]. Therefore, the exact contribution of E2 to CVD risk in older men remains to be clarified. Our study in a large cohort of older men with E2 measured using LC-MS provides no evidence that E2 improves stratification of intermittent claudication risk above that obtained by measurement of T and DHT. It is possible that higher E2 functions as a risk predictor in middle-aged men and that its association with clinical syndromes of CVD might be attenuated or even reversed with age. Alternatively, tissue concentrations of E2 derived from local action of aromatase may be more powerful determinants of pro- or anti-atherosclerotic actions and these may not be exactly mirrored by circulating hormone levels [28].

Androgens exert potential anti-atherogenic effects directly on the vasculature, or indirectly via favourable associations with cardiovascular risk factors including insulin sensitivity and

circulating lipid profiles [2]. T may also improve perfusion via vasodilatory actions or neovascularisation effects [29]. However, adequately powered randomised clinical trials of T therapy to detect differences in hard clinical outcomes such as incident CVD events are lacking [2,6]. A Cochrane Review identified four randomised controlled trials of sex hormones in patients with lower limb atherosclerosis, one of which was subsequently excluded due to methodological concerns [30]. The remaining three trials involved 109 subjects with intermittent claudication or critical leg ischaemia, and showed that short term T therapy produced no significant improvement in outcomes such as walking distance, muscle blood flow or symptom scores [30]. The authors concluded that these findings could reflect limited data rather than the lack of a real effect. Given our findings that lower T and DHT levels are independently associated with claudication in older men, further studies are warranted to determine whether a causal link exists and to explore the potential for improving clinical outcomes. A recent observational study of elderly US war veterans reported testosterone treatment was associated with better survival [31]. However, bias in the non-randomised design allowing for preferential treatment of healthier men with testosterone limits the interpretation of those findings [32]. More definitive, randomised controlled trials of T interventions in older men could consider incorporating symptoms of intermittent claudication into the baseline assessment and as an outcome measure. Given the unexpected finding of cardiovascular adverse events in the study by Basaria et al of T intervention in older men with limited mobility, careful selection of participants and monitoring for cardiovascular events would be appropriate [33].

In conclusion, lower T or DHT levels, but not E2, were associated with intermittent claudication in older men. Reduced circulating androgens may be a causal factor or a biomarker for this symptomatic manifestation of PAD, and further research is needed to

explore potential underlying mechanisms and options for improved risk stratification and intervention.

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Figure legends

Figure 1

Participant flow chart showing how the cohort of 2,703 men was derived.

Figure 2

Percentages of the 2,703 community-dwelling men aged 70-89 years reporting lower limb intermittent claudication pain according to deciles of (A) testosterone, (B) dihydrotestoterone, (C) calculated free testosterone and (D) sex hormone-binding globulin.

Table 1

Characteristics of 2,703 community-dwelling men aged 70-89 years, stratified according to whether no leg pain or intermittent claudication was present. Data are shown as number (%) or mean ± SD.

Variable	No claudication N=2,435	Claudication N=268	P value
Age 71-74 yrs	981 (40.3)	73 (27.2)	
75-79 yrs	1,022 (42.0)	117 (43.7)	0.006
80-84 yrs	368 (15.1)	57 (21.3)	<0.001
85-89 yrs	64 (2.6)	21 (7.8)	<0.001
Completed high school	1,215 (49.9)	117 (43.7)	0.052
Never smoker	870 (35.7)	52 (19.4)	
Past smoker	1,442 (59.2)	191 (71.3)	<0.001
Current smoker	122 (5.0)	25 (9.3)	<0.001
BMI ≥25 kg/m^2	1,502 (61.7)	187 (69.8)	0.01
WHR ≥0.90	2,010 (82.6)	249 (92.9)	<0.001
Hypertension	1,838 (75.5)	229 (86.7)	<0.001
Dyslipidemia	1,711(70.3)	224 (83.6)	<0.001
Diabetes	338 (13.9)	59 (22.0)	<0.001
CVD	665 (28.6)	146 (56.6)	<0.001
Creatinine (µmol/L)	91.3 ± 29.3	105.6 ± 37.4	<0.001
Total Testosterone (nmol/L)	13.5 ± 4.8	11.7 ± 4.6	<0.001
Calculated free T (pmol/L)	189.9 ± 53.5	169.3 ± 54.1	<0.001
Dihydrotestosterone (nmol/L)	1.5 ± 0.7	1.3 ± 0.7	<0.001
Estradiol (pmol/L)	73.8 ± 29.3	71.1 ± 28.0	0.165

Table 2

A: Associations of T, DHT, E2, calculated free T and SHBG as continuous variables with presence of claudication in 2,703 community-dwelling men aged 70-89 years. B: Multivariable model incorporating both T and SHBG. OR=odds ratio per 1 SD increase in hormone values, CI=confidence interval.

A:

Variable	Range	Univariate OR (95% CI) p-value	Model 1 OR (95% CI) p-value	Model 2 OR (95% CI) p-value	Model 3 OR (95% CI) p-value
T (nmol/L)	0.31-46.50	0.66 (0.57-0.76) <0.001	0.73 (0.63-0.84) <0.001	0.78 (0.67-0.90) 0.001	0.80 (0.69-0.94) 0.006
DHT (nmol/L)	0.12-7.20	0.73 (0.63-0.84) <0.001	0.81 (0.69-0.93) 0.004	0.84 (0.72-0.98) 0.024	0.86 (0.73-1.00) 0.048
E2 (pmol/L)	2.3-237.9	0.91 (0.80-1.04) 0.165	0.95 (0.83-1.08) 0.411	0.97 (0.84-1.11) 0.620	0.96 (0.83-1.11) 0.565
Free T (pmol/L)	5.2-699.0	0.66 (0.58-0.76) <0.001	0.75 (0.65-0.86) <0.001	0.78 (0.67-0.90) 0.001	0.81 (0.70-0.94) 0.005
SHBG (nmol/L)	5.6-181.0	0.77 (0.66-0.89) <0.001	0.75 (0.64-0.88) <0.001	0.83 (0.71-0.96) 0.016	0.83 (0.71-0.98) 0.025

B:

Variable	Range	Univariate OR (95% CI) p-value	Model 1 OR (95% CI) p-value	Model 2 OR (95% CI) p-value	Model 3 OR (95% CI) p-value
T (nmol/L)	0.31-46.50	0.64 (0.54-0.77) <0.001	0.76 (0.63-0.91) 0.004	0.78 (0.65-0.94) 0.011	0.82 (0.68-0.99) 0.041
SHBG (nmol/L)	5.6-181.0	1.01 (0.85-1.19) 0.942	0.89 (0.74-1.08) 0.236	0.95 (0.79-1.14) 0.602	0.94 (0.78-1.13) 0.484

Model 1: adjusted for age, smoking, body mass index and waist:hip ratio

Model 2: adjusted for variables in model 1, and for hypertension, dyslipidemia, diabetes and creatinine

Model 3: adjusted for variables in model 2, and for prevalent cardiovascular disease

Table 3

Associations of T, DHT, E2 and calculated free T and SHBG with claudication examined by logistic regression in 2,703 community-dwelling men aged 70-89 years. Hormones are analysed as quartiles, with the lowest quartile (Q1) as the reference group. OR=odds ratio, CI=confidence interval.

		Range	No Claudication N (%)	Claudication N (%)	Univariate OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
T (nmol/L)	Q1	0.31-9.82	527 (21.6)	100 (37.3)				
	Q2	9.82-12.53	607 (24.9)	63 (23.5)	0.55 (0.39-0.77)	0.59 (0.42-0.84)	0.63 (0.44-0.89)	0.60 (0.42-0.87)
	Q3	12.56-15.72	629 (25.8)	60 (22.4)	0.50 (0.36-0.71)	0.57 (0.41-0.81)	0.64 (0.45-0.92)	0.67 (0.46-0.96)
	Q4	15.75-46.50	672 (27.6)	45 (16.8)	0.35 (0.24-0.51)	0.45 (0.30-0.66)	0.51 (0.34-0.76)	0.54 (0.36-0.81)
DHT (nmol/L)	Q1	0.12-0.92	547 (22.7)	86 (33.0)				
	Q2	0.93-1.34	585 (24.3)	64 (24.5)	0.70 (0.49-0.98)	0.73 (0.52-1.04)	0.73 (0.51-1.05)	0.71 (0.49-1.04)
	Q3	1.34-1.83	614 (25.5)	64 (24.5)	0.66 (0.47-0.93)	0.79 (0.55-1.12)	0.82 (0.58-1.18)	0.86 (0.59-1.24)
	Q4	1.83-7.20	664 (27.6)	47 (18.0)	0.45 (0.31-0.65)	0.56 (0.38-0.83)	0.62 (0.42-0.91)	0.64 (0.43-0.95)
E2 (pmol/L)	Q1	3.4-53.6	611 (25.2)	75 (28.2)				
	Q2	54.0-70.1	588 (24.3)	68 (25.6)	0.94 (0.67-1.33)	0.93 (0.66-1.33)	0.98 (0.69-1.41)	0.92 (0.63-1.33)
	Q3	70.2-89.9	608 (25.1)	64 (24.1)	0.86 (0.60-1.22)	0.88 (0.62-1.26)	0.90 (0.63-1.30)	0.81 (0.56-1.19)
	Q4	90.0-237.9	616 (25.4)	59 (22.2)	0.78 (0.54-1.12)	0.86 (0.60-1.25)	0.91 (0.62-1.32)	0.88 (0.60-1.29)
Free T (pmol/L)	Q1	5.2-150.4	515 (21.6)	91 (34.6)				
	Q2	150.4-182.6	579 (24.2)	74 (28.1)	0.72 (0.52-1.01)	0.83 (0.59-1.16)	0.86 (0.61-1.21)	0.79 (0.55-1.13)

Q3	182.7-216.3	637 (26.7)	51 (19.4)	0.45 (0.32-0.65)	0.54 (0.37-0.78)	0.59 (0.40-0.86)	0.62 (0.42-0.91)
Q4	216.4-699.0	657 (27.5)	47 (17.9)	0.40 (0.28-0.59)	0.55 (0.37-0.81)	0.61 (0.41-0.92)	0.65 (0.43-0.98)
SHBG (nmol/L) Q1	5.6-31.5	569 (23.8)	87 (33.1)				
Q2	31.6-39.6	579 (24.2)	67 (25.5)	0.76 (0.54-1.06)	0.71 (0.50-1.00)	0.83 (0.58-1.19)	0.89 (0.62-1.30)
Q3	39.7-50.4	608 (25.5)	66 (25.1)	0.71 (0.51-1.00)	0.65 (0.46-0.93)	0.77 (0.54-1.10)	0.82 (0.56-1.19)
Q4	50.5-181.0	632 (26.5)	43 (16.3)	0.44 (0.30-0.65)	0.42 (0.28-0.63)	0.53 (0.35-0.79)	0.54 (0.35-0.83)

Model 1: adjusted for age, smoking, body mass index and waist:hip ratio

Model 2: adjusted for variables in model 1, and for hypertension, dyslipidemia, diabetes and creatinine

Model 3: adjusted for variables in model 2, and for prevalent cardiovascular disease

Figure 1

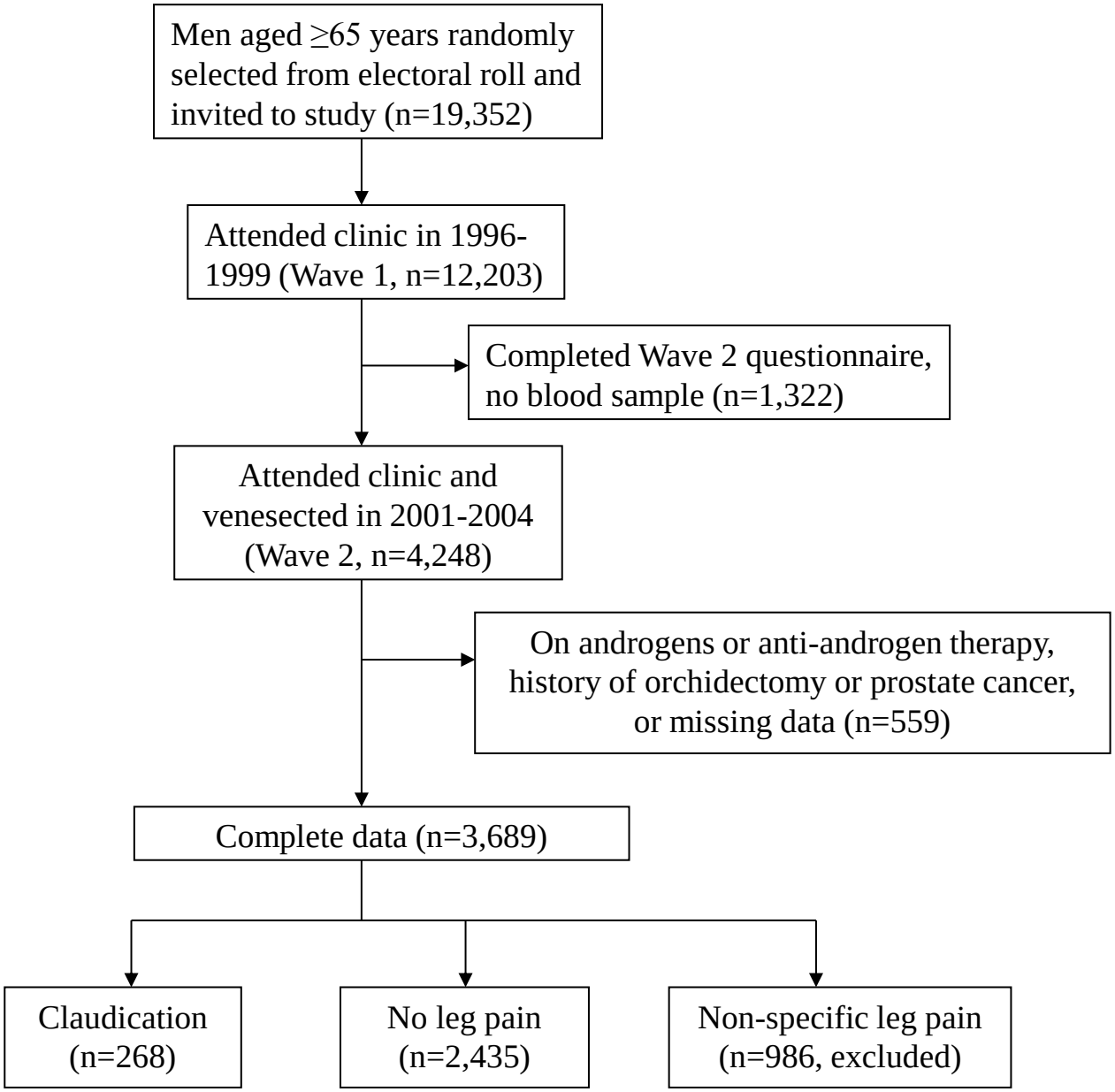
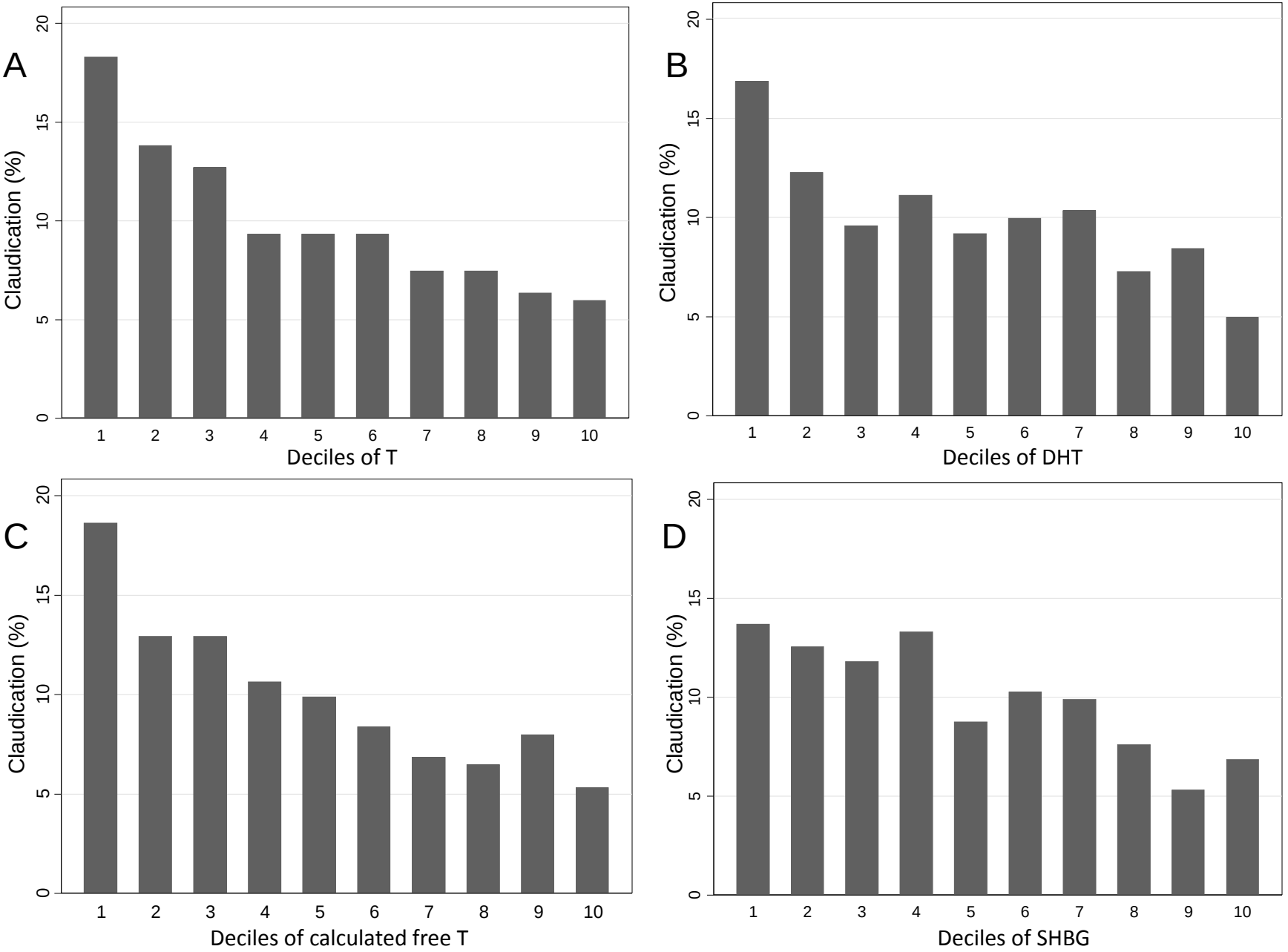

Men aged ≥65 years randomly selected from electoral roll and invited to study (n=19,352)
Attended clinic in 1996-1999 (Wave 1, n=12,203)
Completed Wave 2 questionnaire, no blood sample (n=1,322)
Attended clinic and venesected in 2001-2004 (Wave 2, n=4,248)
On androgens or anti-androgen therapy, history of orchidectomy or prostate cancer, or missing data (n=559)
Complete data (n=3,689)
Claudication (n=268)
No leg pain (n=2,435)
Non-specific leg pain (n=986, excluded)

Figure 2


A
Claudication (%)
0
5
10
15
20
1
2
3
4
5
6
7
8
9
10
Deciles of T
B
Claudication (%)
0
5
10
15
20
1
2
3
4
5
6
7
8
9
10
Deciles of DHT
C
Claudication (%)
0
5
10
15
20
1
2
3
4
5
6
7
8
9
10
Deciles of calculated free T
D
Claudication (%)
0
5
10
15
20
1
2
3
4
5
6
7
8
9
10
Deciles of SHBG