CLINICAL STUDY

Elevated LH predicts ischaemic heart disease events in older men: the Health in Men Study

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Abstract

Context: Hypogonadism in men is associated with insulin resistance, elevations in pro-inflammatory cytokines and fibrinogen, and an atherogenic lipid profile. However, it is uncertain whether the age-related decline in testosterone is associated with ischaemic heart disease (IHD) events.

Objective: To determine whether testosterone and its associated hormones, sex hormone-binding globulin (SHBG) and LH, predict IHD events in older men.

Design: Prospective cohort study.

Methods: Between 2001 and 2004, 3637 community-dwelling men aged 70–88 years underwent a clinical assessment of cardiovascular risk factors and biochemical assessment of testosterone, SHBG and LH. Free testosterone was calculated using mass action equations. Participants were followed until December 2008 using electronic record linkage to capture IHD events (hospital admission or death).

Results: Mean follow-up was 5.1 years. During this period, 618 men (17.0%; 95% confidence interval (CI) 15.8, 18.3%) experienced an event, of which 160 were fatal. Men with higher baseline total or free testosterone levels experienced fewer IHD events (hazard ratio (HR)=0.89; 95% CI 0.82, 0.97 and HR=0.86; 95% CI 0.79, 0.94 for each one s.d. increase in total and free testosterone respectively). These associations were maintained after adjustment for age and waist:hip ratio but did not persist after adjustment for prevalent IHD or other cardiovascular risk factors. SHBG was not associated with IHD events. In contrast, higher LH levels were associated with reduced event-free survival in both univariate (HR=1.15; 95% CI 1.08, 1.22) and adjusted analyses (HR=1.08; 95% CI 1.01, 1.15).

Conclusions: Dysregulation of the hypothalamic–pituitary–gonadal axis may be a risk factor for IHD. Further studies of men with either elevated LH or low testosterone are warranted.

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Introduction

Ischaemic heart disease (IHD) is a leading cause of mortality and morbidity worldwide, accounting for 12% of deaths in 2004 (1). Currently ranked fourth, IHD is expected to become the second largest contributor to the global burden of disease by 2030 (1). Mortality rates for IHD vary across the globe, but a consistent male-to-female ratio of ~2:1 is observed (2). Behavioural differences between the sexes, such as higher rates of smoking in men, may partly account for this discrepancy but are insufficient to explain it. Some have therefore hypothesised a role for sex hormones in the aetiology of IHD (3).

Sex hormones appear to mediate several key sex differences in the cardiovascular system. Compared

with men, women have higher resting heart rates and a longer QT interval (4). The latter emerges at puberty and appears to be influenced by androgens; administration of testosterone shortens the QT interval in both sexes (5, 6). Similarly, sex differences in lipids and blood pressure develop at puberty. Both systolic and diastolic blood pressures are higher in men until ~60 years of age (7). Women have greater high-density lipoprotein (HDL) levels throughout the lifespan and less low-density lipoprotein (LDL) until menopause (8). The latter actions suggest that the male hormonal milieu could be detrimental to cardiovascular health, but favourable effects of androgens are also observed.

Testosterone levels correlate inversely with arterial stiffness, intima-media thickness and aortic diameter

(9, 10), predictors of cardiovascular events. Male hypogonadism is associated with insulin resistance, increased production of pro-inflammatory cytokines, elevated fibrinogen and an atherogenic lipid profile (11). Conversely, testosterone replacement increases antioxidant levels, and improves the cytokine profile, insulin sensitivity and abdominal obesity (12–14). These observations suggest a more complex role for testosterone and are consistent with the hypothesis that androgen deficiency plays a role in the pathogenesis of IHD.

Testosterone is the major circulating androgen in males and is almost entirely bound to sex hormone-binding globulin (SHBG) and albumin. Less than 2% circulates unbound or 'free'. The free and albumin-bound portion is generally considered most active in target tissues. Testosterone levels reach a peak in early adulthood and then decline by 1–2% per year thereafter (15), although the clinical significance of this is uncertain. Epidemiological studies investigating whether testosterone levels are associated with IHD are conflicting. Some have reported that low testosterone predicts death from IHD (16), while others have found no association (17), or that higher testosterone levels are associated with increased IHD mortality (18). However, variability in sample size, duration of follow-up, age range and ability to capture endpoints with certainty limit the ability to draw conclusions from the data. Additionally, as far as we are aware, no prospective studies have explored the relationship between gonadotrophins and IHD in older men.

We therefore designed this study to explore associations between sex hormones and IHD in a large cohort of community-dwelling men aged 70–88 years at baseline, using electronic record linkage. We hypothesised that men with lower testosterone and higher LH levels would be more likely to experience an IHD event (either a hospital admission or death) during a follow-up period of up to 7 years.

Participants and methods

Study population

The Health in Men Study (HIMS) is a population-based study of men living in Perth, Western Australia, originally recruited for a trial of screening for abdominal aortic aneurysm (AAA) (19). Participants were randomly selected from the electoral roll (enrolment to vote being compulsory). Between 1996 and 1999 (wave 1, W1), 12 203 mostly Caucasian men aged 65 years and older attended a clinic and completed a questionnaire, providing a range of demographic and risk factor data. Approximately, 5 years later, 10 940 surviving men were invited to a follow-up study. Between 2001 and 2004 (wave 2, W2), 5585 men completed a second questionnaire, and 4263 of these attended a clinic. Early morning blood samples were obtained from 4249 clinic attendees. The human research ethics committee of the University of Western Australia approved the study.

Laboratory methods

Blood samples were collected at W2 between 0800 and 1030 h to minimise circadian variation. Serum was prepared immediately following phlebotomy and stored at -80°C until assayed. Biochemical assays were performed in the Biochemistry Department, PathWest, Royal Perth Hospital, Western Australia, as reported previously (20). Serum total testosterone, SHBG and LH were determined by chemiluminescent immunoassays on an Immulite 2000 analyser (Diagnostic Products Corp. Biomediq, Doncaster, Australia). Between-day imprecision was 11.2% at 7.2 nmol/l and 8.9% at 18 nmol/l for total testosterone; 6.7% at 5.2 nmol/l and 6.2% at 81 nmol/l for SHBG; and 6.4% at 2.3 IU/l and 5.8% at 19 IU/l for LH. The working ranges of the assays were 0.7–55 nmol/l for testosterone, 2–180 nmol/l for SHBG and 0.1–200 IU/l for LH. The normal ranges for these assays in men are 8–35 nmol/l for testosterone, 10–70 nmol/l for SHBG and 1–8 IU/l for LH. Free testosterone, the fraction not bound to SHBG or albumin, was estimated using mass action equations (21). The few men with undetectable results were assigned the lower limit of the working range. Serum glucose, LDL, HDL, total cholesterol and triglycerides were assayed using a Roche Hitachi 917 analyser (Roche Diagnostic GmbH).

Cohort for assessment of IHD events

Of the 4249 men providing sera at W2, testosterone and LH were successfully assayed in 4165 and SHBG in 4162. Of these, we excluded men with prostate cancer or who had undergone orchidectomy, and those receiving anti-androgens, GnRH analogues or testosterone therapy, leaving 3638 men (including three without SHBG). Of these 3638 men, one subsequently withdrew from the study, leaving 3637 participants for analysis.

Assessment of morbidity and mortality

We assessed morbidity and mortality via the Western Australian Data Linkage System (WADLS), which provides electronic linkage to the state's population health data collections (22). WADLS comprises records from the death registry, cancer registry and hospital morbidity data system (which includes all separations for public and private hospitals in the state dating from 1970). At the time of linkage, records up to 31 December 2008 were considered complete. As we could not be certain that hospital admissions were fully reported beyond this date, we truncated our analysis at this point.

IHD morbidity

Hospital admissions after W2 were captured from WADLS morbidity data. Admissions in which the ICD-10 coded

primary diagnosis was I20 (angina pectoris), I21 (acute myocardial infarction), I22 (subsequent myocardial infarction), I23 (certain current complications following acute myocardial infarction), I24 (other acute IHDs) or I25 (chronic IHD), or in which a coronary revascularisation procedure was performed, were considered IHD events. Revascularisation procedures were identified with ICD-10 Australian Modification (ICD-10-AM) codes from blocks 669–681.

IHD mortality

Primary cause of death was ascertained from WADLS mortality data. This dataset contains both the original death certificate and ICD codes generated from these data and other sources by the Australian Bureau of Statistics (ABS). For the final year of deaths (2008), ABS coding was not yet available. These deaths were coded by Z H and reviewed by B B Y and P E N. Deaths in which IHD appeared in the text of part 1 of the death certificate (e.g. acute myocardial infarction, atherosclerotic heart disease and chronic IHD) were considered IHD deaths. For previous years, records containing ICD-10 codes in the range I21–I25 were flagged as cases.

Assessment of prevalent IHD

Men with pre-existing IHD were identified from self-reported questionnaire data at W1 and W2 and from WADLS hospital records flagged with the ICD-10/ICD-10-AM codes given previously, or with ICD-8, ICD-9 or ICD-9-CM diagnosis codes 410–414, or with Code of Surgical Operations (COSO) procedure code 304, International Classification of Procedures in Medicine (ICPM) codes 5–360 to 5–363, or ICD-9-CM procedure code 36.x.

Other items of interest

Height (in centimetres), weight (in kilograms) and blood pressure were measured at W1 and W2. Questionnaire data at W1 and W2 and biochemical assessment data at W2 were used to identify men with dyslipidaemia or diabetes. Questionnaire and clinical data at W1 and W2 were used to identify men with hypertension. Men were asked about tobacco use at both time points. As illness may reduce testosterone levels, we performed adjustments for medical comorbidity with Charlson's weighted comorbidity index (23). Hospital morbidity records from 1990 to W2 were used to build this measure.

Statistical analysis

The statistical package Stata, version 11.1, was used to analyse the data (StataCorp, 2010). Pearson's χ^2 test was used to assess associations in categorical variables between groups. For continuous variables, *t*-tests or Mann–Whitney *U* tests were used to assess normal and

non-normal data respectively. To explore whether the risk of experiencing an IHD event differed by hormone level, we calculated age-standardised rates for each quintile of the hormones via the direct method, using the age distribution of the HIMS cohort at the time of blood sampling. Cox proportional hazard models were used to test associations between hormone levels and IHD events (hospital admission and/or death). Hormonal parameters were entered into the models as Z-scores, placing them on a common metric-free scale. Hazard ratios (HR) reflect the effect of a one s.d. increase in hormone level. Adjustments were made for age, waist:hip ratio, hypertension, dyslipidaemia, Charlson's index, smoking, diabetes and prevalent IHD. We adjusted for waist:hip ratio rather than other measures of obesity, as it may be the best predictor of IHD mortality (24). Assessment of the Schoenfeld residuals confirmed the proportional hazards assumption. All tests were two sided, and *P* values <0.05 were considered statistically significant.

Results

Mean duration of follow-up was 5.1 years (range 0.1–7.2 years). During this period, 618 men (17.0%) experienced an IHD event (hospital admission or death), of which 160 were fatal. There were 605 deaths from all causes; the proportion due to IHD was therefore 26.4% (95% confidence interval (CI) 23.0, 30.2%).

Characteristics of men experiencing an IHD event

Baseline demographic, biochemical and clinical characteristics of men who did and did not experience an event are shown in Table 1. Men experiencing an event were older, had lower total and free testosterone levels and higher LH levels, and were more likely to have established risk factors for IHD such as dyslipidaemia and hypertension. A larger proportion of these men had experienced a previous non-fatal IHD event.

IHD events by quintiles of hormones

Age-standardised event rates by quintiles of hormones are given in Table 2. Rates were slightly lower across increasing quintiles of total and free testosterone, and higher across increasing quintiles of LH, but this reached statistical significance only for LH.

Hormonal associations with IHD events

As illustrated in Fig. 1, total testosterone and LH levels appeared to predict IHD events in a graded manner, with lower quintiles of total testosterone and higher quintiles of LH associated with reduced event-free

Table 1 Baseline demographic, biochemical and clinical characteristics of men who did and did not experience an IHD event (fatal and non-fatal). The data is expressed either as mean $\pm$ s.d. or as *n* (%).

Variable	No IHD event (<i>n</i> =3019)	IHD event (<i>n</i> =618)	<i>P</i> value
Age (years)	76.8 $\pm$ 3.5	77.9 $\pm$ 3.8	<0.001
WHR	0.97 $\pm$ 0.1	0.98 $\pm$ 0.1	<0.001
T testosterone (nmol/l)	15.5 $\pm$ 5.7	14.9 $\pm$ 5.3	0.013
F testosterone (pmol/l)	280 $\pm$ 99	267 $\pm$ 83	0.002
SHBG (nmol/l)	42.4 $\pm$ 16.4	42.7 $\pm$ 18.0	0.599
LH (IU/l)	5.6 $\pm$ 4.9	6.5 $\pm$ 6.7	0.004
Hypertension	2286 (75.7)	492 (79.6)	0.038
Dyslipidaemia	2241 (74.2)	505 (81.7)	<0.001
Diabetes mellitus	450 (14.9)	123 (19.9)	0.002
Smoking status			0.171
Never smoked	1026 (34.0)	190 (30.7)	
Ex-smoker	1823 (60.4)	398 (64.4)	
Current smoker	170 (5.6)	30 (4.9)	
Charlson's index			<0.001
0	1895 (62.8)	264 (42.7)	
1–2	790 (26.2)	229 (37.1)	
3–4	230 (7.6)	87 (14.1)	
$\geq$ 5	104 (3.4)	38 (6.2)	
Prevalent IHD	840 (27.8)	364 (58.9)	<0.001

IHD, ischaemic heart disease; WHR, waist-hip ratio; T, Total; F, Free.

survival. Compared with the highest quintile, free testosterone levels in the lowest quintile also appeared to be associated with reduced survival. No association was apparent for SHBG.

These associations were tested with Cox proportional hazard models (Table 3). In univariate analyses, higher levels of total testosterone (HR=0.89; 95% CI 0.82, 0.97) and free testosterone (HR=0.86; 95% CI 0.79, 0.94) were associated with fewer IHD events. These associations were maintained after adjustment for age and waist:hip ratio but did not persist after adjustment for prevalent IHD or other risk factors. In both univariate (HR=1.15; 95% CI 1.08, 1.22) and full multivariate analyses (HR=1.08; 95% CI 1.01, 1.15), higher LH levels were associated with reduced event-free survival. Performing additional adjustment for total or free testosterone did not substantially alter the association between LH and IHD (data not shown). SHBG was not associated with IHD events.

Hormonal associations with IHD mortality

In univariate analyses, higher free testosterone levels were associated with reduced mortality from IHD (HR=0.76; 95% CI 0.63, 0.92). This association was maintained after adjustment for age and waist:hip ratio, but not after adjustment for prevalent IHD or other risk factors. A univariate association was also observed between higher SHBG levels and IHD mortality (HR=1.17, 95% CI 1.02, 1.34) but did not persist after adjustment for age or other covariates. Higher LH levels were associated with mortality from IHD in both

univariate (HR=1.26; 95% CI 1.16, 1.38) and full multivariate (HR=1.14; 95% CI 1.04, 1.25) models. Performing additional adjustment for total or free testosterone did not substantially alter the association between LH and IHD mortality (data not shown).

Assessment of reverse causality

To explore the possibility of reverse causality, we performed a sensitivity analysis in which we examined the predictors of IHD events in men with (*n*=1204) and without (*n*=2433) prevalent IHD. As expected with reduced power, the adjusted association between LH and IHD events was attenuated but remained essentially unchanged in men with (HR=1.08; 95% CI 0.99, 1.17) and without (HR=1.08; 95% CI 0.95, 1.22) prevalent IHD. Although we attempted to control for medical comorbidity, the possibility remains that illness at the time of blood sampling may have affected sex hormone levels. We therefore repeated our analyses after excluding 134 men who died within 2 years of blood collection. After these exclusions, LH levels continued to predict IHD mortality (HR=1.18; 95% CI 1.06, 1.31) and events (HR=1.09; 95% CI 1.02, 1.17).

IHD mortality and events by gonadal status

The association observed between IHD events and LH, but not total or free testosterone, could still indicate a

Table 2 Ischaemic heart disease events (fatal and non-fatal) by quintiles of hormones. Age-standardised rate is per 1000 person-years.

Hormone quintile	Events/ men at risk (<i>n</i> / <i>n</i>)	Follow-up person- years	Age- standardised rate	95% CI
Testosterone				
1 (lowest)	130/715	3295	40.7	33.7, 47.7
2	131/727	3406	38.7	32.0, 45.4
3	127/737	3569	34.4	28.4, 40.5
4	122/723	3462	34.8	28.6, 41.0
5 (highest)	108/735	3547	30.5	24.5, 36.4
Free testosterone				
1 (lowest)	142/721	3312	43.2	35.8, 50.5
2	118/718	3432	33.7	27.5, 39.8
3	125/732	3562	33.8	27.8, 39.8
4	121/730	3455	35.7	29.2, 42.1
5 (highest)	112/733	3500	32.7	26.5, 39.0
SHBG				
1 (lowest)	123/723	3336	39.3	31.9, 46.6
2	122/729	3436	35.8	29.4, 42.1
3	132/727	3501	38.5	31.8, 45.1
4	125/726	3470	35.6	29.1, 42.0
5 (highest)	116/729	3520	29.1	23.7, 34.6
LH				
1 (lowest)	104/724	3462	30.0	24.2, 35.9
2	116/729	3478	35.7	29.1, 42.3
3	131/728	3499	36.2	30.0, 42.5
4	118/727	3465	33.8	27.6, 40.1
5 (highest)	149/729	3374	43.6	36.1, 51.1

CI, confidence interval.

Figure 1 Kaplan–Meier survival curves showing associations between ischaemic heart disease events (fatal and non-fatal) and quintiles of total testosterone (A), free testosterone (B), SHBG (C) and LH (D).

relationship between testosterone and IHD, as LH levels might better reflect androgen status in older men. To investigate this hypothesis, we stratified men by categories of hypogonadism using previously published Australian criteria (25) and examined IHD events in each group (Table 4).

After 5 years, the proportion of men experiencing an event did not differ between those with normal hormone levels and those classified as having hypogonadotrophic

hypogonadism or Leydig cell impairment. However, the proportion of men with hypergonadotrophic hypogonadism who experienced a new event was double that of the normal group (35.0 vs 15.8%). There was also no difference in IHD deaths between normal men and men with hypogonadotrophic hypogonadism. However, mortality due to IHD in those with Leydig cell impairment (8.6%) or hypergonadotrophic hypogonadism (11.5%) was at least twice that of normal men (3.4%) and men with hypogonadotrophic hypogonadism (3.9%).

Discussion

In this prospective study of community-dwelling men aged 70 years and older, higher LH levels were associated with greater likelihood of experiencing an IHD event. This association was maintained after adjustment for potential confounders and established cardiovascular risk factors. To our knowledge, this is the first time that LH levels have been identified as a marker of IHD risk in older men.

Relatively few prospective studies have explored the relationship between endogenous sex hormones and IHD in elderly men. Most have focused on cardiovascular disease generally and have comprised varying sample sizes and age ranges. Khaw *et al.* (16) reported an association between low total testosterone levels and all-cause, cardiovascular and IHD mortality in a nested case–control analysis comprising 2314 men aged

Table 3 Cox proportional hazard models exploring hormone levels and associations with subsequent IHD events (fatal and non-fatal) and mortality.

Variable	Outcome					
	IHD event			IHD mortality		
	HR	95% CI	P value	HR	95% CI	P value
Total testosterone						
Model 1	0.89	0.82, 0.97	0.007	0.86	0.73, 1.02	0.082
Model 2	0.92	0.84, 0.99	0.042	0.87	0.74, 1.04	0.121
Model 3	0.94	0.87, 1.02	0.145	0.91	0.77, 1.08	0.273
Model 4	0.96	0.88, 1.04	0.321	0.93	0.78, 1.10	0.374
Free testosterone						
Model 1	0.86	0.79, 0.94	0.001	0.76	0.63, 0.92	0.004
Model 2	0.90	0.83, 0.99	0.022	0.82	0.69, 0.99	0.035
Model 3	0.93	0.85, 1.01	0.086	0.86	0.72, 1.03	0.098
Model 4	0.94	0.87, 1.02	0.167	0.88	0.74, 1.05	0.164
SHBG						
Model 1	1.01	0.93, 1.09	0.831	1.17	1.02, 1.34	0.025
Model 2	1.00	0.92, 1.09	0.986	1.12	0.96, 1.29	0.139
Model 3	1.00	0.92, 1.09	0.984	1.12	0.97, 1.28	0.117
Model 4	1.01	0.93, 1.10	0.740	1.12	0.97, 1.29	0.109
LH						
Model 1	1.15	1.08, 1.22	<0.001	1.26	1.16, 1.38	<0.001
Model 2	1.11	1.04, 1.19	0.001	1.21	1.10, 1.34	<0.001
Model 3	1.09	1.02, 1.16	0.012	1.17	1.07, 1.29	0.001
Model 4	1.08	1.01, 1.15	0.022	1.14	1.04, 1.25	0.006

Model 1, univariate; Model 2, adjusted for age and waist:hip ratio; Model 3, model 2+ prevalent IHD; Model 4, model 3+ hypertension, dyslipidaemia, smoking status, diabetes and Charlson's weighted co-morbidity index. Hazard ratios indicate the effect of a one s.d. increase in hormone level. CI, confidence interval; IHD, ischaemic heart disease; HR, hazard ratio.

Table 4 Five-year risk of ischaemic heart disease events (fatal and non-fatal) and mortality by categories of hormone status. Table does not include the data of 97 men with total testosterone > 15 nmol/l and LH > 12 IU/l.

Category	IHD event			IHD mortality		
	%	95% CI	P value	%	95% CI	P value
Normal (<i>n</i> =3225) Testosterone ≥ 8 nmol/l LH ≤ 12 IU/l	15.8	14.5, 17.2	–	3.4	2.8, 4.1	–
Hypogonadotrophic hypogonadism (<i>n</i> =133) Testosterone < 8 nmol/l LH ≤ 12 IU/l	21.0	14.9, 29.1	0.069	3.9	1.6, 9.0	0.599
Leydig cell impairment (<i>n</i> =126) Testosterone 8–15 nmol/l LH > 12 IU/l	19.6	13.4, 28.0	0.113	8.6	4.7, 15.3	<0.001
Hypergonadotrophic hypogonadism (<i>n</i> =56) Testosterone < 8 nmol/l LH > 12 IU/l	35.0	23.6, 49.9	0.001	11.5	5.3, 23.8	0.010

P values are for log-rank test between men with normal hormone status and categories of hypogonadism.

42–78 years. Compared with men with total testosterone levels in the highest quartile, men in the lowest quartile were almost twice as likely to have died from IHD. Low testosterone levels were also associated with cardiovascular and all-cause mortality in the Rancho Bernardo Study (26). In this sample of 794 men aged 50–91 years, a graded increase in risk across decreasing quartiles of both total and bioavailable (non-SHBG bound) testosterone was observed. We have also previously reported associations between low free testosterone and incident stroke and transient ischaemic attack, and prevalent AAA in this cohort (10, 27). Higher LH levels were also associated with AAA in the latter analysis.

Studies focussing on markers of atherosclerosis, rather than on cardiovascular events, have also reported associations between low testosterone levels and development or worsening of the disease. Hougaku *et al.* (28) found that total testosterone levels were inversely associated with carotid stiffness in longitudinal analyses of 206 men aged 33–95 years. Hak *et al.* (29) measured calcification of the abdominal aorta in 504 men aged 55–89 years. Men with total or bioavailable testosterone levels in the highest, compared with the lowest tertile, were less likely to have severe atherosclerosis at baseline and were less likely to develop or show progression of the condition after a follow-up period of 6.5 years.

However, in the Tromsø study, comprising 1568 men with a mean age of 59 years, neither total nor free testosterone was associated with cardiovascular or IHD deaths, although lower free testosterone levels were associated with all-cause mortality (17). A smaller Finnish study of 187 men aged 71–72 years found that low total testosterone was associated with all-cause but not cardiovascular mortality (30). Price *et al.* (31) found that neither oestradiol nor total or free testosterone levels were significantly associated with peripheral arterial disease in a nested case–control study of 81 elderly men. The Framingham Heart Study of 2084

middle-aged men was also unable to demonstrate an association between testosterone levels and cardiovascular events but observed a protective effect for oestradiol (32). An apparent adverse effect of higher testosterone levels has also been reported. In a subset of the Massachusetts Male Aging Study comprising 1686 men aged 40–70 years, there was a graded increase in the risk of IHD mortality across increasing quintiles of free testosterone (18).

Interventional data are also conflicting. Intravenous testosterone increased the time to exercise-induced myocardial ischaemia in 14 men with coronary artery disease (33) and increased flow-mediated reactivity of the brachial artery in a small crossover study of 22 men with IHD (34). In another crossover study of 22 men with low testosterone randomised to oral testosterone undecanoate or placebo for 8 weeks, testosterone improved measures of arterial stiffness, though did not improve angina symptoms (35). In contrast, a recent randomised placebo-controlled trial of transdermal testosterone in 209 older men with low testosterone and mobility limitations was terminated early due to safety concerns (36). Adverse cardiovascular events were experienced by 23 men in the treatment group, compared with five in the placebo group. However, the majority of participants were hypertensive, which may have been exacerbated by the high testosterone dose used. Another recent study of 50 middle-aged hypogonadal men randomised to intramuscular testosterone undecanoate or placebo for 24 months did not report any cardiovascular safety concerns (37).

We propose three hypotheses to explain the association between higher LH levels and greater IHD events: i) androgen deficiency is detrimental to the cardiovascular system, but LH better reflects prevailing testosterone levels; and/or ii) LH has an independent effect on the cardiovascular system, with elevated levels being harmful; or iii) elevated LH is not directly involved in the pathogenesis of IHD but is rather an epiphenomenon of another disease process. The first hypothesis is

supported by evidence of testosterone's immune-modulating properties. In both *in vitro* and *in vivo* studies, testosterone reduces inflammation and production of pro-inflammatory cytokines, which are considered to play a role in the development of atherosclerosis (12, 38). The normal endothelium is permeable to LDL, which accumulates in the arterial wall, provoking an inflammatory response and the eventual formation of an atherosclerotic plaque. Continued production of inflammatory cytokines compromises the stability of the plaque, leading to erosion or rupture, and an acute coronary syndrome (9). Testosterone also has anti-thrombotic actions and an inverse relationship with fibrinogen levels (38). A role in lipid metabolism is also hypothesised. Androgen deficiency is associated with an atherogenic lipid profile (11), while testosterone therapy generally lowers total cholesterol and LDL, although decreases in HDL are also reported (39). Animal and human studies also suggest direct vasoactive properties. Dose-dependent vasodilatory effects are observed in the presence of androgen receptor (AR) antagonists and in testicular feminised mice (40, 41). Alternatively, some of the favourable effects attributed to testosterone may be at least partly due to oestradiol, which is also thought to have beneficial actions. Aromatase deficiency in males is associated with a range of metabolic disturbances and increased risk of cardiovascular disease, despite adequate testosterone levels (42). Nonetheless, while testosterone supplementation has been shown to reduce atherosclerosis in both orchidectomised wild-type and AR knock-out mice fed a high-fat diet, efficacy was superior in mice with a functional AR, supporting an independent role for testosterone (43).

However, when we stratified by categories of hypogonadism, we observed a difference in IHD events, with men with hypergonadotrophic hypogonadism more likely to experience an event than those with hypogonadotrophic hypogonadism. This could suggest a more complicated relationship, with LH directly involved in the pathogenesis of IHD, perhaps mediated via the LH receptor (LHR). Although originally thought to be present only in gonadal cells, the LHR has since been identified in extragonadal sites (44), including smooth muscle and vascular tissue (45, 46). Berndt *et al.* (45) reported that the expression of LHR was associated with endothelial cell proliferation in the mouse aorta. LH might therefore theoretically exert a direct effect on the heart or associated vascular structures. In particular, the increased IHD mortality in men with low normal testosterone and elevated LH suggests that it is not the absolute level of testosterone that determines risk but rather the degree of hypothalamic–pituitary–gonadal axis disruption. Leydig cell impairment (compensated hypogonadism) has been postulated as a distinct syndrome (25, 47), but this is the first time that an important clinical consequence has been observed. Therefore, our results suggest that assessment of

androgen deficiency in men should include risk stratification based on both testosterone and LH levels.

Alternatively, it is possible that our observations reflect underlying disease, with illness directly reducing testosterone production at the testicular level or indirectly via suppression of gonadotrophins. However, the latter is unlikely given that we found an association between IHD and hypergonadotrophic hypogonadism. Furthermore, we assessed and performed adjustments for medical comorbidity. Nonetheless, the possibility of residual confounding cannot be entirely eliminated, highlighting the difficulty of establishing causality in observational analyses.

Strengths of our study include the large, population-based sample, relatively narrow age range compared with previous studies, and use of electronic record linkage to capture endpoints. The latter is a key strength of this study, facilitating complete ascertainment of hospital admissions and mortality, and may explain why we found an association while other studies have not. Additionally, we limited our endpoints to a small and precisely defined range, compared with other studies that have included a disparate class of cardiovascular disorders (48). Limitations of our study include collection of a single blood sample and use of the Vermeulen method to calculate free testosterone. However, this method is widely used in other studies and is considered to provide a reasonable estimate of actual levels. We did not have the resources to collect additional samples or to assay other hormones such as oestradiol. We also cannot exclude the possibility that some men may have been misdiagnosed or their admissions or death certificates coded imprecisely. For example, the ICD contains a number of ill-defined cardiovascular disease codes, which are used frequently in some countries. However, coding of mortality is considered precise in Australia (49), and the similarity of our findings with regard to mortality and both fatal and non-fatal events is reassuring. Additionally, validation studies of the WADLS have established very high consistency between clinical coding and medical records (50).

In summary, this study suggests that higher LH levels are associated with increased IHD events in men. The possibility of an independent role for LH in the pathogenesis of IHD requires further investigation. Although not statistically significant, there appeared to be a graded relationship between decreasing testosterone levels and increasing risk, suggesting that androgen deficiency could be involved in the aetiology of IHD. A graded rather than threshold effect suggests that risk may not be confined to men with the very lowest levels. However, given conflicting data from other observational studies and safety concerns raised by a recent trial, it would be premature to suggest testosterone therapy for the prevention of IHD. Further studies are warranted, with particular emphasis on men with elevated LH in addition to those with low testosterone levels.

Declaration of interest

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

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