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A J-shaped association between plasma testosterone and risk of ischemic arterial event in elderly men: The French 3C cohort study

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ABSTRACT

Objectives: Low plasma testosterone is associated with increased mortality in men. However, the relation between testosterone and cardiovascular disease is uncertain. We assessed the association of plasma sex hormones with the incidence of ischemic arterial disease (IAD) in elderly men.
Methods: We used data from the French Three-City prospective cohort study (3650 men aged >65 years). A case-cohort design was set up including a random sample of 495 men and 146 incident cases of first IAD event (112 coronary heart disease (CHD) and 34 strokes) after a 4-year follow-up. Plasma total and bioavailable testosterone, total estradiol and sex hormone-binding globulin (SHBG) were measured at baseline. Multivariate hazard ratios (HRs) and 95% confidence intervals for IAD were assessed using Cox model.
Results: After adjustment for cardiovascular risk factors, a J-shaped association between plasma total testosterone and IAD risk was found ($p < 0.01$). The HRs associated with the lowest and the highest total testosterone quintiles relative to the second quintile were 2.23 (95% CI: 1.02; 4.88) and 3.61 (95% CI: 1.55; 8.45) respectively. Additional analysis for CHD showed similar results (HR: 3.11, 95% CI: 1.27; 7.63 and HR: 4.75, 95% CI: 1.75; 12.92, respectively). Similar J-shaped association was observed between bioavailable testosterone and IAD risk ($p = 0.01$). No significant association of estradiol and SHBG with IAD was found.
Conclusion: High and low plasma testosterone levels are associated with an increased risk of IAD in elderly men. Optimal range of plasma testosterone may confer cardiovascular protection and these results may have clinical implications in the management of testosterone deficiency.

1. Introduction

Testosterone, the most important plasma androgen produced in men, progressively declines for the remainder of life about the age of thirty [1]. This decrease is thought to underlie many of symptoms and diseases of aging, and testosterone supplementation can provide beneficial metabolic effects in men who have signs and symptoms of hypogonadism [2,3]. Consistent data provided evidence for an association between low testosterone levels and increased risk of all-cause mortality in men [4]. However, the role of testosterone in the development of ischemic arterial diseases (IAD) remains uncertain.

Several studies have investigated the relation between endogenous testosterone and risk of death from cardiovascular diseases and they led to conflicting results. In the Health in Men Study, low testosterone levels were associated with increased of death from cardiovascular disease [5], whereas no significant association was found with androgen levels in the Rancho Bernado Study [6]. Moreover, higher levels of testosterone were associated with increased cardiovascular mortality in a prospective population-based cohort study [7]. For a long time large prospective studies failed to find significant associations between testosterone levels and risk of cardiovascular events in middle-aged men [8,9]. More recently, however, high serum testosterone levels were associated with a

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5-year reduced risk of cardiovascular events in a cohort study of elderly Swedish men [10]. This is consistent with the influence of testosterone levels on multiple risk factors for cardiovascular disease. Indeed, inverse associations were found between testosterone levels and obesity, diabetes, blood pressure and carotid atherosclerosis [11,12], whereas a positive association was reported between testosterone levels and HDL cholesterol [13].

Few studies focused on the relation between plasma testosterone levels and risk of cardiovascular events, especially in elderly men. In addition, since low and high testosterone levels have been associated with cardiovascular mortality, the relation between testosterone levels and risk of cardiovascular events may not be linear. Accordingly, we examined the association of plasma testosterone with the 4-year incidence of first IAD among men aged over 65 years from the French Three-City (3C) cohort study. Since sex hormone-binding globulin (SHBG) is an important determinant of total testosterone levels and testosterone may exert its effect by conversion to estradiol [1], plasma SHBG and estradiol were also taken into account.

2. Methods

2.1. Study population

The Three-City (3C) Study is a multicentric prospective cohort study aimed to evaluate the risk of coronary heart disease (CHD), stroke and dementia in older community-dwelling subjects. The Ethics Committee of the University of Hospital of Kremlin-Bicêtre approved the study protocol and an informed consent was obtained from each participant. The general methodology was described elsewhere [14]. Briefly, between 1999 and 2001, 9294 subjects (3650 men and 5644 women) aged over 65 years were selected from the electoral rolls of 3 French cities (Bordeaux, Dijon and Montpellier). At baseline trained psychologists or nurses gathered information on socio-demographic characteristics, education level, smoking status, consumption of alcohol, medical history and medication use during a face to face interview. In addition, blood pressure, height and weight were measured during a clinical examination.

2.2. Follow-up and event ascertainment

After their recruitment, subjects have been followed every 2 years at home or at the study center for the detection of cardiovascular events and dementia. The 4-year follow-up was selected for the present analysis. IAD included CHD events (fatal or nonfatal) and ischemic stroke (fatal or nonfatal). Subjects who were hospitalized with stable or unstable angina pectoris, coronary dilatation, artery bypass, myocardial infarction, or subjects who died from CHD were classified as CHD cases. Nonfatal CHD events were documented by using hospital charts and practitioners' reports. CHD deaths were documented by reviewing hospital records, medical data obtained from family physicians or specialists, and proxy interviews (coded I210 to I219, I251 to I259, I461 and R960). All CHD events were adjudicated by an independent trained physicians committee. Stroke was defined as a rapid onset of neurological deficit lasting >24 h and confirmed by a lesion compatible with an acute stroke on computed tomography or magnetic resonance imaging of the brain. Imaging brain allowed drawing a distinction between ischemic or hemorrhagic stroke. We excluded hemorrhagic stroke for this analysis. Similarly, all stroke events were validated by an independent medical committee. The first ischemic arterial event has been chosen for subjects with both CHD and ischemic stroke during follow-up.

2.3. Case-cohort design

A case-cohort study was conducted from the 3C study to investigate the association of blood biomarkers with cardiovascular risk and dementia. This design has been detailed [15] and consists of a random sample from the total cohort as well as all incident first-time IAD cases. Advantages of the case-cohort versus nested case-control design have been described [16]. Briefly, 1254 subjects (759 women and 495 men) were randomly selected from the initial cohort after stratification by study center, sex and age. After exclusions including history of prostate cancer ($n = 17$), receiving hormonal therapy ($n = 5$), prevalent CHD ($n = 60$) or stroke ($n = 21$), 392 men were selected from the random subcohort. Data on incident ischemic arterial status were missing for 24 men. During the 4 years of follow-up, 345 men remained free of IAD and 23 incident IAD events occurred in this subcohort. Similar exclusion criteria were applied to the outside subcohort and 123 additional IAD cases were added for final comparisons. From the 146 incident cases of IAD, 112 were CHD events and 34 were ischemic strokes (Fig. 1).

2.4. Biochemical assessment of sex hormones

At baseline, blood samples were collected for 90% of the entire cohort in the morning after an overnight fast. EDTA plasmas were generated by 1 centrifugation ($3000 \times g$ at 4 °C) and aliquots were stored at −80 °C in 1-mL plastic tubes until use. A radioimmunoassay (RIA) method (Spectria® Orion Diagnostica, Espoo, Finland) was used to measure total testosterone (minimum detectable concentration (MDC) = 0.02 ng/mL) and estradiol (MDC = 2.0 pg/mL). The intra and inter-assay coefficients of variation (CVs) were 3.8% and 4.8% respectively for a total testosterone concentration of 3.2 ng/mL and 4.8% and 5.5% for a total testosterone concentration of 6.7 ng/mL. The intra and inter-assay CVs were 2.8% and 5.8% for a total estradiol concentration of 24 pg/mL. A solid-phase chemiluminescent immunometric assay (Immulite®, Siemens Health Diagnostic Products Llanberis, UK) was used to measure SHBG (MDC = 0.02 nmol/L). The intra and inter-assay CVs were 2.5% and 5.2% at 21 nmol/L. A bromocresol green colorimetric method was used to measure albumin with an MDC lower than 0.35 g/L. The intra and inter-assay CVs were 1.9% and 2.5% for an albumin concentration of 49.2 g/L. The bioavailable testosterone concentration was assessed by an indirect method after differential precipitations of testosterone bound to globulins with 50% ammonium sulfate and equilibration of the plasma sample with [^{3}H]-testosterone [17]. The intra and inter-assay CVs were 7.0% and 8.5% for a bioavailable testosterone concentration of 1.4 ng/mL.

2.5. Statistical analysis

Baseline characteristics of men are presented as frequencies for categorical variables, as arithmetic means and standard deviation for continuous variables and, as geometric mean and interquartile range (Q1–Q3) for log-transformed continuous variables. Body mass index (BMI) was calculated as weight (kg) divided by the height squared (m^2). Smoking status was defined in three categories (never, past and current). Hypertension was characterized according to the World Health Organization criteria by a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg, and/or by using a current blood-pressure-lowering therapy. Hypercholesterolemia was defined as a total cholesterol ≥6.2 mmol/L (2.4 g/L) and/or a cholesterol-lowering therapy and diabetes as a fasting blood glucose of 7 mmol/L and/or a treatment for diabetes. Baseline characteristics of men with and without incident ischemic arterial disease were compared by chi-square test and 2-tailed Student's *t*-test. Hazard ratios (HRs) for cardiovascular events and 95% confidence intervals (95% CI) were computed with

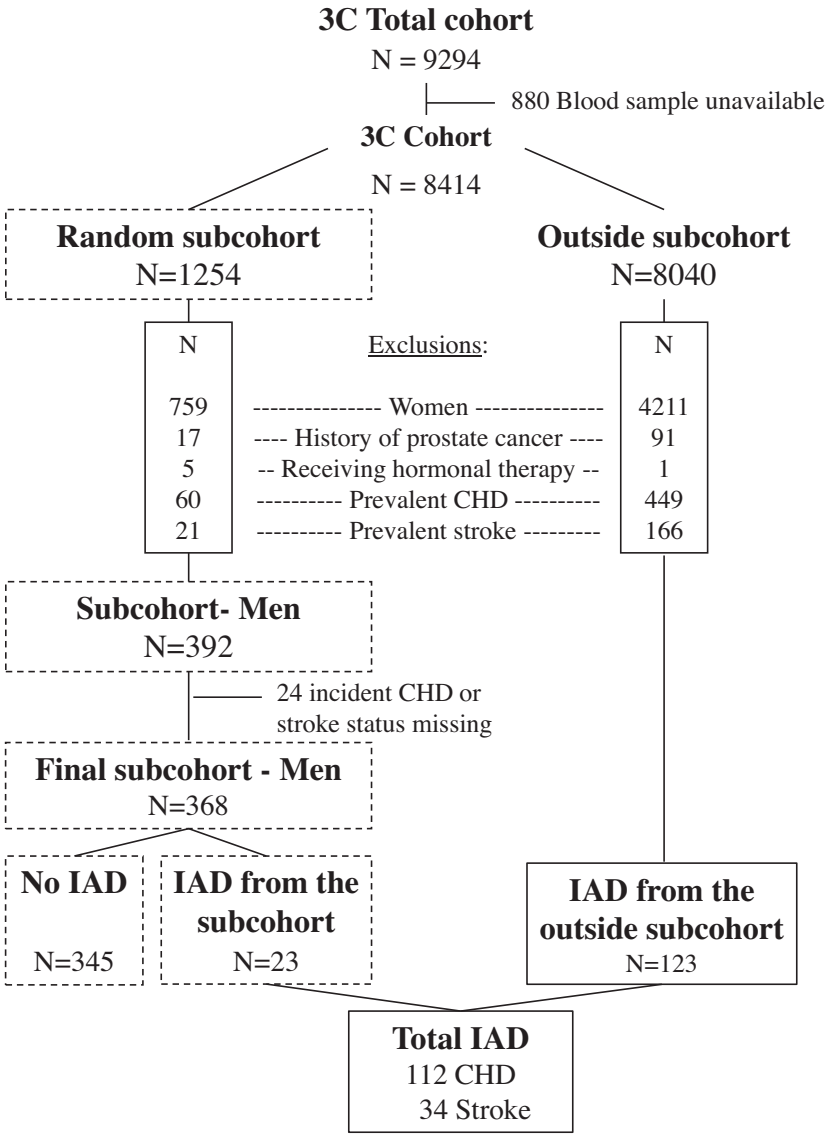

Fig. 1. Flowchart of the 3C case-cohort study for the investigation of first ischemic arterial event in relation to sex hormone levels in men.

a weighted Cox proportional hazards regression model adapted for the case-cohort design [18]. We used the weighting method proposed by Barlow [19]. As age is strongly related to arterial disease, age was used as the timescale for all analyses as recommended [20]. Two models are presented: the first one was adjusted for age (timescale) and center, and the second one was adjusted for age (timescale), center and traditional cardiovascular risk factors, including BMI, smoking status, diabetes, hypertension and hypercholesterolemia. The proportional hazards assumption was tested graphically using log-minus-log plots. Likelihood-ratio tests were used to assess the deviation from linearity of the relation between sex hormones levels and IAD. Nonlinear (J-shaped or U-shaped) associations between sex hormones and IAD were also evaluated including linear and quadratic terms for sex hormones in the models. Sex hormones levels were examined as quintiles based on distribution in men from the final subcohort. We have chosen the second quintile as a reference group for J-shaped associations. No significant interactions between hormones levels and IAD risk factors were detected. We performed statistical analyses using standard procedures from the Statistical Analysis System (SAS 9.2 Institute Inc., Cary, NC, USA).

3. Results

Baseline characteristics of men with and without incident ischemic arterial disease are showed in Table 1. Cases were significantly older than noncase subjects (73.4 and 74.7 years respectively, $p = 0.01$). BMI was similar in both groups. The proportion of smokers was significantly different among cases and noncase subjects. Cases of IAD tended to be more hypertensive, hypercholesterolemic, and were more likely to have diabetes (20.8% and 10.7% respectively, $p < 0.01$) than noncases. Similar sex hormone mean levels were found among men with and without ischemic arterial disease.

Table 2 shows the baseline characteristics of the whole sample and by quintiles of plasma total testosterone levels. Body mass index decreased with increasing levels of total testosterone (p for trend <0.01). Similarly, men with the lowest testosterone levels were more likely to have diabetes (borderline significant $p = 0.06$). Levels of plasma estradiol and SHBG were positively associated with total testosterone levels (p for trend < 0.01 for both).

HRs for IAD, CHD, and ischemic stroke by quintiles of plasma testosterone are showed in Table 3. A significant J-shaped

Table 1
Baseline characteristics of men with and without incident ischemic arterial event during a 4-year follow-up from the 3C case-cohort study.

	Noncases (n = 345)	Ischemic arterial event cases (n = 146)	p
Age, years	73.4 (5.4)	74.7 (5.2)	*0.01*
BMI[a], kg/m^2	26.1 (3.2)	26.1 (3.1)	*0.95*
Study center			
Bordeaux	73 (21.2)	33 (22.6)	
Dijon	173 (50.1)	76 (52.1)	
Montpellier	99 (28.7)	37 (25.3)	*0.92*
Education level[b]			
Less then grade school	87 (25.3)	30 (20.6)	
Grade school or high school	99 (28.8)	44 (30.1)	
High school validated or university	158 (45.9)	72 (49.3)	*0.44*
Smoking			
Never	111 (32.2)	63 (43.2)	
Former	206 (59.7)	70 (47.9)	
Current	28 (8.1)	13 (8.9)	*0.04*
Daily alcohol consumption[c]			
None	9 (2.6)	9 (6.2)	
Former	7 (2.0)	6 (4.1)	
≤2 drinks	217 (63.3)	89 (61.4)	
>2 drinks	110 (32.1)	41 (28.3)	*0.07*
Medical history			
Hypertension	267 (77.4)	124 (84.9)	*0.22*
Diabetes[d]	37 (10.7)	30 (20.8)	*<0.01*
Hypercholesterolemia	141 (40.9)	69 (47.3)	*0.13*
Hormone levels			
Total testosterone, ng/mL	5.6 (1.9)	5.7 (2.1)	*0.17*
Bioavailable testosterone, ng/mL	3.2 (1.0)	3.3 (1.1)	*0.18*
Total estradiol[e], pg/mL	18.3 (14.4; 24.3)	18.3 (15.5; 23.8)	*0.78*
SHBG, nmol/L	24.6 (18.3; 32.8)	26.0 (19.2; 35.7)	*0.24*

Data are expressed as mean (SD) unless otherwise stated as n (%) or geometric mean (interquartile range).
[a] 2 Missed values.
[b] 1 Missed value.
[c] 3 Missed values.
[d] 2 Missed values.
[e] 1 Missed value.

association was found between total testosterone and the risk of IAD (p for J-shaped 0.04). After adjustment for traditional cardiovascular risk factors, this association persisted (p for J-shaped < 0.01). The adjusted-HR associated with the lowest and the highest total testosterone quintiles relative to the second quintile were 2.23 (95% CI: 1.02; 4.88) and 3.61 (95% CI: 1.55; 8.45) respectively. Similar associations between total testosterone and the risk of CHD were observed in both model (adjusted-HR: 3.11 95% CI (1.27; 7.63) and adjusted-HR: 4.75 95% CI (1.75; 12.92), p for J-shaped < 0.01). No significant association was found for stroke. Likewise, a J-shaped association was also observed between plasma bioavailable testosterone and IAD risk (p for J-shaped = 0.01). After adjustment for cardiovascular risk factors, the J-shaped relation was only significant for ischemic stroke, whereas a borderline

Table 2
Baseline characteristics of the entire sample and by quintile group of plasma testosterone.

	Total testosterone quintile						
	Whole sample (n = 491)	<3.94 ng/mL (n = 101)	3.94–4.88 ng/mL (n = 89)	4.88–5.88 ng/mL (n = 97)	5.88–6.89 ng/mL (n = 106)	>6.89 ng/mL (n = 106)	p for trend
Age, years	73.7 (5.4)	73.7 (5.4)	74.2 (5.5)	73.9 (5.4)	74.2 (5.5)	73.6 (5.1)	*0.57*
BMI[a], kg/m^2	26.1 (3.2)	27.1 (3.9)	26.3 (2.8)	27.1 (3.9)	26.0 (3.9)	24.9 (2.8)	*<0.01*
Current Smoking	41 (8.4)	5 (4.9)	7 (7.9)	6 (6.2)	12 (12.4)	11 (10.4)	*0.08*
Education level ≥ high school or university[b]	230 (46.9)	43 (43.0)	46 (51.7)	48 (49.5)	48 (49.5)	50 (47.2)	*0.94*
Daily alcohol consumption >2 drinks[c]	151 (30.7)	33 (33.0)	29 (32.9)	31 (32.0)	26 (26.5)	32 (30.5)	*0.45*
Medical history							
Hypertension	391 (79.6)	85 (84.2)	68 (76.4)	79 (81.4)	76 (77.5)	83 (78.3)	*0.39*
Diabetes[d]	67 (13.7)	16 (15.8)	16 (18.2)	14 (14.4)	12 (12.4)	9 (8.5)	*0.06*
Hypercholesterolemia	210 (42.8)	41 (40.6)	39 (43.8)	43 (44.3)	36 (36.7)	51 (48.1)	*0.57*
Hormone levels							
Total testosterone, ng/mL	5.6 (1.9)	3.1 (0.7)	4.4 (0.3)	4.4 (0.3)	4.4 (0.3)	8.3 (1.2)	–
Bioavailable testosterone, ng/mL	3.2 (1.1)	1.9 (0.5)	2.6 (0.3)	3.2 (0.4)	3.6 (0.5)	4.5 (0.9)	*<0.01*
SHBG, nmol/L	25.0 (18.9; 33.6)	14.4 (11.3; 19.1)	17.4 (13.6; 22.4)	17.3 (14.2; 22.7)	19.7 (16.6; 24.9)	23.9 (19.7; 29.2)	*<0.01*
Total estradiol[e], pg/mL	18.3 (14.7; 24.2)	21.2 (17.0; 26.0)	23.0 (16.7; 30.3)	24.2 (18.2; 31.8)	27.0 (21.1; 35.3)	30.1 (22.8; 38.4)	*<0.01*

Data are expressed as mean (SD) unless otherwise stated as n (%) or geometric mean (interquartile range), respectively.
[a] 2 Missed values.
[b] 1 Missed value.
[c] 3 Missed values.
[d] 2 Missed values.
[e] 1 Missed value.

Table 3
HRs for first ischemic arterial disease, CHD, and stroke events across quintiles of plasma testosterone.

	Ischemic arterial disease			CHD			Ischemic stroke		
	No. of events	Model 1 HR (95%CI)	Model 2 HR (95%CI)	No. of events	Model 1 HR (95%CI)	Model 2 HR (95%CI)	No. of events	Model 1 HR (95%CI)	Model 2 HR (95%CI)
Total testosterone, ng/mL									
Q1 <3.94	31	1.78 (0.89; 3.56)	2.23 (1.02; 4.88)	27	2.29 (1.04; 5.03)	3.11 (1.27; 7.63)	4	0.76 (0.20; 2.88)	0.77 (0.17; 3.44)
Q2 [3.94; 4.88]	19	1.00 (referent)	1.00 (referent)	12	1.00 (referent)	1.00 (referent)	7	1.00 (referent)	1.00 (referent)
Q3 [4.88; 5.88]	28	1.72 (0.83; 3.54)	1.97 (0.86; 4.50)	21	1.95 (0.84; 4.55)	2.36 (0.89; 6.25)	7	1.36 (0.40; 4.64)	1.52 (0.38; 6.14)
Q4 [5.88; 6.89]	29	1.88 (0.92; 3.84)	2.16 (0.96; 4.86)	19	1.86 (0.80; 4.31)	2.29 (0.88; 6.01)	10	2.01 (0.63; 6.45)	2.10 (0.59; 7.45)
Q5 ≥ 6.89	39	2.74 (1.36; 5.52)	3.61 (1.55; 8.45)	33	3.40 (1.52; 7.60)	4.75 (1.75; 12.92)	6	1.48 (0.39; 5.53)	1.84 (0.41; 8.21)
p for J-shaped		*0.04*	*<0.01*		*0.03*	*<0.01*		*0.65*	*0.41*
Bioavailable testosterone, ng/mL									
Q1 <2.35	28	1.27 (0.66; 2.45)	1.52 (0.73; 3.16)	23	1.21 (0.60; 2.43)	1.49 (0.68; 3.24)	5	1.49 (0.34; 6.65)	1.55 (0.27; 9.08)
Q2 [2.35; 2.87]	25	1.00 (referent)	1.00 (referent)	21	1.00 (referent)	1.00 (referent)	4	1.00 (referent)	1.00 (referent)
Q3 [2.87; 3.34]	23	1.07 (0.54; 2.11)	1.15 (0.54; 2.48)	17	0.92 (0.45; 1.91)	0.99 (0.44; 2.26)	6	1.74 (0.39; 7.82)	1.77 (0.32; 9.88)
Q4 [3.34; 4.02]	38	1.75 (0.94; 3.29)	2.08 (1.02; 4.25)	26	1.44 (0.72; 2.87)	1.74 (0.80; 3.81)	12	3.53 (0.97; 12.85)	4.05 (0.84; 19.50)
Q5 ≥ 4.02	32	1.77 (0.93; 3.37)	1.99 (0.90; 4.39)	25	1.53 (0.76; 3.06)	1.67 (0.71; 3.93)	7	3.28 (0.79; 13.53)	4.15 (0.71; 24.33)
p for J-shaped		*0.05*	*0.01*		*0.15*	*0.07*		*0.10*	*0.03*

Model 1: adjusted for study center.
Model 2: adjusted for study center, body mass index, diabetes, hypertension, hypercholesterolemia and smoking status.

significant association was observed for CHD. No significant association between the estradiol/total testosterone ratio and the risk of IAD was found. No significant association of plasma total estradiol and SHBG with IAD was detected. Adjusting testosterone-IAD event association for SHBG or estradiol and vice versa made no substantial change to the results (Data not shown).

4. Discussion

In this French prospective population study of older men, a J-shaped association of total and bioavailable testosterone with the risk of first IAD was found after adjustment for traditional cardiovascular risk factors. To the best our knowledge, this is the first result showing that both low and high levels of testosterone are associated with an increased risk of cardiovascular disease. In contrast, no significant association of estradiol and SHBG levels with IAD risk was detected.

Previous cohort studies reported conflicting data on the association between testosterone levels and the risk of cardiovascular events. Early findings failed to detect an association between testosterone levels and incident cardiovascular disease [6,8,9,21]. However, a recent study including 2416 men aged 69–81 years showed that serum testosterone levels were inversely associated with the risk of cardiovascular events [10]. Low testosterone levels were also related with an increased risk of incident stroke or transient ischemic attack in the HIMS study including 3443 men aged over 70 years [22]. Moreover, a meta-analysis [4] and a recent report among 3637 community-dwelling men aged 70–88 years [5] showed a relationship between low testosterone levels and increased all-cause and cardiovascular mortality. Interestingly, further meta-analysis recently showed that low testosterone levels predicted risk for cardiovascular disease in elderly men but not in healthy middle aged men [23]. Thus, the ability to draw conclusions from the observational data is limited by differences in population age range, but also by differences in hormone assay, duration of follow-up and definition of clinical outcomes. However, our results confirm and extend the previous findings showing that low testosterone levels is an independent predictor of IAD risk in elderly men.

The association between low testosterone levels and cardiovascular events or mortality could be mediated through known effects of testosterone on cardiovascular risk factors [24]. In agreement with several studies [25], a strong inverse relation between obesity and plasma testosterone levels was observed in our study. Obesity activates an inflammatory process, especially in adipose tissue where increased circulating levels of cytokines contribute to proinflammatory [2] and prothrombotic state [26]. In addition, diabetes, a major contributory factor to the development of cardiovascular disease in men, has also been associated with increased prevalence of low testosterone levels. However, in our study, adjustment for traditional cardiovascular risk factors including BMI and diabetes made little change to the results. Other mechanisms including unfavorable changes in haemostasis [27] could therefore be involved.

In our study, high testosterone levels were also associated with an elevated risk of IAD. This finding is consistent with the MMAS study showing an association between high free testosterone levels and increased risk of ischemic heart disease mortality [7]. Higher endogenous testosterone levels were also related to an increased risk of CHD among elderly men in the MrOS study [28]. Besides, a recent placebo-controlled clinical trial of elderly men reported increased rates of adverse cardiovascular events in the group treated by a testosterone gel [29]. Although other trials found no similar adverse effect, we can hypothesize that high testosterone levels may have deleterious effects on cardiovascular

disease through the development of hypertension and congestive heart failure, especially in older men [30].

The question of whether changes in endogenous testosterone levels play a role in the development of IAD remains largely unsolved. Based on observational studies, the existence of an independent association between levels of endogenous testosterone and IAD risk does not necessarily imply that this relationship be causal. Although altered androgens levels could contribute to atherothrombosis through several potential mechanisms, low testosterone could be merely a marker of poor general health and/or aging without any causal role in cardiovascular disease. This hypothesis is supported by evidence that both acute and chronic illnesses reduce testosterone production [1]. Thus, a reverse causation could explain in part our results. In our study, data analysis was done among men who were free of cardiovascular disease at baseline, thus arguing against a substantial role of prevalent IAD. Furthermore, exclusion of the first few months of follow-up data did not affect materially our results. However, a reverse causation due to subclinical disease cannot be ruled out.

A major strength of our study relates to the prospective population-based and multicenter cohort design with a high participation rate during the 4 years of follow-up. Another strength is the adequate validation of incident cases of CHD and stroke by two independent committees of experts using hospital charts and practitioners' reports. Third, information on comorbidities and medications including drugs altering testosterone levels was systematically collected by direct interview.

A limitation of our study is the small number of incident cases, which may yield a lack of statistical power, especially for subgroup analyses and for testing potential interactions of sex hormones with cardiovascular risk factors on IAD. A second limitation pertains to the specific-method variability in sex hormones levels measurements performed by RIAs compared with the state-of-the-art mass spectrometry methodology. However, our method comparison data showed that RIA underestimated the mass spectrometry-based methodology values but provided accurate results in term of relative ranking by hormone level [31], which is the pre-requisite for adequate risk estimation in epidemiology. Thirdly, our results were based on a single measurement of biomarkers and the potential within-subject variability of sex hormones might have resulted in disease lack of statistical power. However, such a limitation lead to underestimate, not cause, associations between sex hormone and IAD risk. In addition, all plasma samples were collected in the morning thus reducing changes in sex hormones due to diurnal variation [32]. Finally, the 3C study population consisted of older volunteers with a better global health than the general population and our results may not apply to the whole men population.

In conclusion, high and low plasma testosterone levels are associated with an increased risk of arterial ischemic event in elderly men. Our data suggest that optimal range of plasma testosterone may confer protection against cardiovascular events. Testosterone may be useful for improving cardiovascular health among men with age-associated low levels of testosterone. However, more data are needed to assess the safety of testosterone supplementation, especially in older men with high prevalence of cardiovascular risk factors.

Contributors

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Competing interests

The authors declare no conflict of interest.

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Ethical approval

The Ethics Committee of the University of Hospital of Kremlin-Bicêtre approved the study protocol and an informed consent was obtained from each participant.

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