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Title:

Lower testosterone levels predict incident stroke and transient ischemic attack in older men.

Short title:

Low testosterone and stroke or TIA.

Precis:

Lower testosterone levels are associated with increased incidence of stroke and transient ischemic attack in older men.

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Abstract

Context

Lower circulating testosterone concentrations are associated with metabolic syndrome, Type 2 diabetes, carotid intima-media thickness and aortic and lower limb arterial disease in men. However, it is unclear whether lower testosterone levels predict major cardiovascular events.

Objective

We examined whether lower serum testosterone was an independently significant risk factor for symptomatic cerebrovascular events in older men.

Design

Prospective observational study with median follow-up of 3.5 years.

Setting

Community-dwelling, stroke-free older men.

Participants

3,443 men aged ≥70 years.

Main outcome measures

Baseline serum total testosterone, sex hormone-binding globulin (SHBG) and luteinising hormone (LH) were assayed. Free testosterone was calculated using mass action equations. Incident stroke or transient ischemic attack (TIA) was recorded.

Results

A first stroke or TIA occurred in 119 men (3.5%). Total and free testosterone concentrations in the lowest quartiles (<11.7 nmol/L and <222 pmol/L) were associated with reduced event-free survival (p=0.014 and p=0.01 respectively). After adjustment including age, waist-hip ratio, waist circumference, smoking, hypertension, dyslipidemia and medical co-morbidity, lower total testosterone predicted increased incidence of stroke or TIA (hazard ratio=1.99,

95% CI 1.33-2.99). Lower free testosterone was also associated (HR=1.69, 95% CI 1.15-2.48), whilst SHBG and LH were not independently associated with incident stroke or TIA.

Conclusions

In older men, lower total testosterone levels predict increased incidence of stroke or TIA after adjusting for conventional risk factors for cardiovascular disease. Men with low-normal testosterone levels had increased risk. Further studies are warranted to determine whether interventions which raise circulating testosterone levels might prevent cerebrovascular disease in men.

Introduction

Most circulating testosterone is bound to sex hormone-binding globulin (SHBG) or albumin, with a small fraction of unbound or free testosterone. Among men, both total and free testosterone levels decline with increasing age, and the decline is steeper for free compared with total testosterone (1,2). This characteristic hormonal change of male aging is of interest, as lower testosterone concentrations have been associated with increased incidence of metabolic syndrome and Type 2 diabetes in middle-aged and older men (3-6). Additionally, lower testosterone levels are associated with carotid intima-media thickness (CIMT), lower extremity peripheral arterial disease and aortic atherosclerosis (7-10). However, despite the relationship between lower testosterone levels and conditions associated with either increased risk or presence of atherosclerosis, it is unclear whether lower testosterone levels independently predict morbidity and mortality from cardiovascular disease. In studies of middle-aged and older men, low total or free testosterone concentrations were associated with higher overall mortality and with mortality from cardiovascular, cancer and respiratory causes (11-13). However, other studies have reported negative or conflicting findings (14-

16). Furthermore, it is not clear whether lower testosterone levels are associated with non-fatal cardiovascular events (17).

In cross-sectional and longitudinal observational studies reverse causation needs to be considered, as systemic illness can result in lower testosterone levels (18). Therefore it is possible that lower testosterone levels might be a marker for, rather than a cause of subsequent poorer health outcomes in older men, which could account for its association with overall mortality rather than morbidity and mortality due to cardiovascular disease. Existing randomised trials of testosterone therapy in men have not been designed or powered to detect treatment-related differences in cardiovascular outcomes (19-22). Thus additional data addressing the key question of whether lower testosterone concentrations are an independently significant risk factor for vascular events in each of the cerebral, coronary and peripheral arterial circulations, would inform planning of intervention trials exploring cardiovascular outcomes. We sought to test the hypothesis that in community-dwelling older men, lower testosterone levels are independently associated with higher incidence of stroke and transient ischemic attack (TIA).

Methods

Study population

The origins and characteristics of the Health In Men Study (HIMS) have been described in depth elsewhere (23). Briefly, between October 2001 and August 2004, 4,263 community-dwelling men participated in the study by completing a health questionnaire and providing an early morning blood sample for analysis of biochemistry and hormone levels. Available sera were assayed to provide hormone data for 4,165 men. After exclusion of men receiving hormonal therapy, men receiving any form of testosterone supplementation and those with

prostate cancer, there were hormone results for 3,638 men available for analysis (24). Of these men, a further 195 were excluded because they had a prior diagnosis of stroke or TIA, leaving 3,443 men to be included in the longitudinal analysis. Height (in centimetres), weight (in kilograms), girth at hips and waist (in centimetres) and blood pressure were measured using standard procedures. Physical activity and alcohol use had been ascertained by questionnaire previously (23). The Human Research Ethics Committee of the University of Western Australia approved the study protocol, and all participants provided written informed consent.

Assessment of medical comorbidity

We used the Charlson weighted index (25) to determine the presence of significant medical comorbidity in our sample. This takes into account 17 common medical conditions that predict 1-year mortality: myocardial infarction, congestive heart failure, peripheral arterial disease, cerebrovascular disease, dementia, chronic pulmonary disease, connective tissue disease, ulcer disease, liver disease, diabetes (including diabetes with end organ damage), hemiplegia, renal disease, leukemia, lymphoma, other tumours, metastatic tumours and AIDS. Medical diagnoses are weighted for severity and summed to provide a weighted index of medical comorbidity. For this purpose administrative medical information was obtained from the Western Australian Data Linkage System (WADLS) (26). Briefly, WADLS links together records from the Mental Health Information System, cancer register, death register and hospital morbidity data (which includes codes for multiple medical diagnoses for all admissions to private and public hospitals). Data were collected from 1990 to the time of blood sampling, providing a measure of recent comorbidity. Coding algorithms to define medical comorbidities followed the procedures described by Quan et al (27) and were calculated using Stagg's Charlson index Stata routine (StataCorp, College Station, Texas).

Identification of men with incident stroke or TIA

We followed up participants using the WADLS between baseline assessment and 31 December 2006. Men who were admitted to hospital with a first-ever recorded diagnosis of stroke or TIA during follow-up were identified according to the following ICD-10 diagnostic codes: H34.1 central retinal artery occlusion, I60 subarachnoid haemorrhage, I61 intracerebral haemorrhage, I63 cerebral infarction, I64 stroke, not specified as haemorrhage or infarction and G45 transient cerebral ischemic attacks and related syndromes.

Laboratory assays

Blood samples were collected between 0800 and 1030 hours. Serum was prepared immediately following phlebotomy and stored at -80^{o}C until assayed. Biochemical and hormone assays were performed in the Biochemistry Department, PathWest, Royal Perth Hospital, Western Australia. Serum total testosterone, sex hormone binding globulin (SHBG) and luteinizing hormone (LH) were determined by chemiluminescent immunoassays on an Immulite 2000 analyzer (Diagnostic Products Corp. Biomediq, Doncaster, Australia). Between-day imprecision (coefficient of variation) for testosterone was 11.2% at 7.2 nmol/L and 8.9% at 18 nmol/L, for SHBG: 6.7% at 5.2 nmol/L and 6.2% at 81 nmol/L, and for LH: 6.4% at 2.3 IU/L and 5.8% at 19 IU/L. The working range of the testosterone assay was 0.7 – 55 nmol/L; the sensitivities of the SHBG and LH assays were 2 nmol/L and 0.1 IU/L, respectively. The established reference intervals for these assays are total testosterone 8 – 35 nmol/L, SHBG 10 – 70 nmol/L and LH 1 – 8 IU/L. Fasting serum glucose, total and high density lipoprotein (HDL) cholesterol, and triglycerides were assayed using a Roche Hitachi 917 analyser (Roche Diagnostic GmbH, Mannheim, Germany). Between-day imprecision for glucose was 2.9% at 4.8 mmol/L and 2.2% at 15.2 mmol/L, for

cholesterol: 2.3% at 3.2 mmol/L and 2.1% at 6.7 mmol/L, for HDL: 2.4% at 0.8 mmol/L and 2.5% at 1.7 mmol/L, and for triglycerides: 4.8% at 0.9 mmol/L and 2.4% at 2.0 mmol/L. Free testosterone was calculated using mass action equations as described by Vermeulen at al (28).

Definition of hypertension and dyslipidemia

Hypertension was defined as a recorded blood pressure ≥140/90 or having a diagnosis of hypertension or receiving treatment for high blood pressure. Dyslipidemia was defined as having 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.

Statistical analysis

Data were analysed using the statistical software packages Stata (version 10, StataCorp, College Station, Texas) and SPSS (version 15, SPSS Inc, Chicago, USA). Group comparisons were performed using either Student's *t* test or the Mann Whitney test in the case of non-parametrically distributed variables. Kaplan Meier plots were constructed to show stroke and TIA-free survival according to quartiles of baseline total testosterone, free testosterone, SHBG and LH, and comparisons made using the log-rank test. Cox regression (proportional hazards model) was performed to analyse the association between baseline hormone values and time to first stroke/TIA, including adjustment for potential confounders and for known cardiovascular risk factors. Total and free testosterone, SHBG and LH met the criteria for use of the proportional hazards model. All *p* values were two-tailed with a level of <0.05 considered significant.

Results

Incident stroke and TIA occurring during follow-up

Median (interquartile range) duration of follow-up was 3.5 (2.8-4.2) years. Five men had a fatal first stroke and 114 men had a non-fatal first stroke or TIA, representing 3.5% of the cohort. The frequency of specific diagnoses is shown in Table 1. The majority of events were cerebral infarction, stroke not specified as haemorrhage or infarction, and TIA.

TABLE 1

Characteristics of men who experienced a first stroke or TIA

Baseline characteristics of the 3,443 men grouped according to whether or not a stroke or TIA occurred during follow-up are shown in Table 2. Men who experienced a first stroke or TIA during follow-up were older than men who did not. While baseline total and free testosterone levels were lower in men who experienced a first stroke or TIA, only free testosterone levels were significantly different (260±84 vs 280±97 pmol/L, p=0.03).

TABLE 2

Stroke and TIA-free survival according to baseline hormone levels

Men were stratified according to quartiles of baseline total testosterone, calculated free testosterone, SHBG and LH. There was not a dose-response gradient across quartiles of total or free testosterone (Figure 1). The occurrence of stroke or TIA was evaluated in men with total testosterone, free testosterone and SHBG in the lowest quartile of values compared with the three remaining quartiles. Men with lower total testosterone or lower free testosterone had significantly lower stroke and TIA-free survival with p=0.014 and p=0.01, respectively (supplementary Figure S1A, B). Lower SHBG did not discriminate for stroke and TIA-free

survival (Figure 1C). As an elevated LH is indicative of primary gonadal failure, men with LH in the highest quartile of values were compared with the lowest three quartiles. There was no difference in event-free survival in men with higher LH (Figure 1D).

FIGURE 1

Multivariate analysis of hormonal predictors for incident stroke/TIA

To evaluate independent associations between hormone values and incident stroke or TIA, age, waist:hip ratio, waist circumference, medical comorbidity (Charlson index), smoking status, presence or absence of diabetes, exercise history, past alcohol intake, use of aspirin or clopidogrel and the presence of hypertension and dyslipidemia were accommodated in the multivariate model (Table 3). A total testosterone level in the lowest quartile (<11.7 nmol/L) predicted incident stroke or TIA with a hazard ratio (HR) of 1.99. Men with free testosterone in the lowest quartile (<222 pmol/L) were also at increased risk of stroke or TIA, with a HR of 1.69. Neither lower SHBG nor higher LH predicted incident stroke or TIA. There was negligible change in these results when adjustment was made for BMI in place of waist circumference, or when blood pressure and lipid values were considered as continuous variables (data not shown).

TABLE 3

Stratification of testosterone concentration

To determine whether the higher HR for incident stroke or TIA in men with total testosterone in the lowest quartile of values was accounted for solely by increased risk in men with unequivocally low levels of total testosterone a further multivariate analysis was

conducted. Men with total testosterone <8 nmol/L had HR=1.39 (95% CI 0.64-3.02) for incident stroke or TIA (7 men out of 172). By contrast, men with low to normal total testosterone levels (≥8 and <11.7 nmol/L) had HR=2.08 (95% CI 1.35-3.20) for incident stroke or TIA (32 men out of 664) in the adjusted model, compared with men with total testosterone ≥11.7 nmol/L.

Discussion

The key finding in our study was that lower total testosterone levels at baseline predicted a higher incidence of stroke or TIA in community-dwelling older men after adjustment for potential confounders and known risk factors for cardiovascular disease. There was a comparable association between lower free testosterone levels and increased risk of stroke or TIA. Men with total testosterone <8 nmol/L did not show a statistically significant increased risk of stroke/TIA, possibly due to the smaller number of men and lower number or events seen in this group. However, men with total testosterone <11.7 nmol/L as a whole had a higher incidence of stroke or TIA. Thus this increased risk was not confined to men with unequivocally low testosterone levels but involved men with values in the lowest quartile. The absence of a dose-response gradient across all four quartiles of total or free testosterone is not in keeping with the causal criteria described by Hill, as one would anticipate that progressively lower concentrations of total or free testosterone would be associated with increasing incidence of cerebrovascular events. Our results suggest that if total or free testosterone were causally related to strokes or TIAs, then the relationship between the two would not be subject to gradient but to threshold effect (i.e., a minimum concentration of testosterone would need to be achieved before a substantial increase in the risk of strokes/TIAs became apparent). The existence of such a threshold effect is a potential limitation of the Hill criteria, as acknowledged by others (29), and highlights the difficulties

of inferring causality from observational data. These results support the hypothesis that reduced circulating androgens are independently associated with higher incidence of clinically significant cardiovascular disease-related events during male aging, and the concept that preserving circulating testosterone in older men might reduce their burden of ill-health.

Previous studies have reported associations between lower testosterone and surrogate markers of cardiovascular disease, including presence or incidence of metabolic syndrome and diabetes, higher CIMT, reduced ankle-brachial index and presence of calcified aortic atheroma (3-10). In longitudinal studies, lower total testosterone levels predicted increased overall mortality in men aged 40 years and above (11-13). However, the increase in mortality was related not only to cardiovascular disease, but also to cancer (12) and respiratory conditions (13). In other studies of men aged 61-87, 45-59 and 40-70 years, lower total testosterone was not associated with increased mortality (14-16). Instead, higher free testosterone was associated with mortality from ischemic heart disease (16). One recent study found that in men 65 years and older, only those with testosterone, insulin-like growth factor 1 and dehydroepiandrosterone sulfate in the lowest quartiles of values had higher mortality, while lower testosterone levels alone did not predict mortality in the fully adjusted analysis (30). Therefore it was unclear whether the relationship between lower testosterone levels and conditions associated with cardiovascular disease translates into an excess of clinically significant cardiovascular events which would warrant preventive intervention. In a prospective study of 2,084 middle-aged men without cardiovascular disease at baseline, testosterone levels did not predict incidence of cardiovascular disease from coronary, cerebrovascular or peripheral vascular disease or heart failure (17). However, that study was smaller and involved a cohort of middle-aged men, in contrast to our findings in a larger

study of older men. Our findings also contrast with the report by Abbott et al of 2,197 men aged 71-93 years from the Honolulu-Asia Aging Study (31). In that study, stroke risk varied negligibly from lowest to highest quintiles of total testosterone, from 10.4/1000 person-years for testosterone ≤12 nmol/L to 9.0/1000 for testosterone >21.5 nmol/L. Possible explanations for this discrepancy may involve the smaller size of the study, or the differences in ethnicity between the two populations as Abbott et al studied a cohort of men with Japanese ancestry whilst the Health In Men Study cohort is predominantly Caucasian (23). Thus our data support lower total testosterone concentration as a marker of increased cardiovascular risk in older men, and for the first time confirm its role as an independent predictor of clinically significant events in the cerebral vascular territory. Further studies are required to examine the hypothesis that interventions which raise testosterone levels in older men might protect against the incidence of stroke and TIA.

Strengths of this study include the large sample size, the longitudinal nature of the analysis, the focus on older men and the fact that men were community-dwelling and not selected on the basis of an existing medical condition. Furthermore, as severe illness can result in reduced testosterone levels (18), we adjusted for medical comorbidity in the analysis and excluded men with prior stroke or TIA in order to address the potential issue of reverse causation. We used data linkage encompassing mortality and hospital morbidity databases to track the occurrence of stroke or TIA in this cohort (26). Therefore all the non-fatal events analysed were of sufficient severity to necessitate hospital admission.

Limitations of our study include the use of a single blood sample and our lack of opportunity to gather multiple samples for serial hormone assays. In the study by Abbott et al (31), men in the top quintile of serum estradiol experienced a two-fold excess risk of stroke compared

with men in the lower four quintiles. Thus Abbott et al (31) found that higher estradiol levels were associated with increased risk of stroke while testosterone levels were not, calling into question the potential value of hormone supplementation in this setting. In that study estradiol and testosterone were assayed by quantitative competitive immunoassay using an Immulite 2000 analyser. The Immulite 2000 platform was also used in our study, and it provides an acceptable measurement of serum testosterone in men (32,33). However, as we did not measure serum estradiol, we are unable to clarify this issue further. It should also be remembered that different testosterone assays may give varying results, and calculation of free testosterone may not provide an exact estimate of circulating free testosterone measured by equilibrium dialysis (34,35). However, these methods have been used extensively in large studies where measurement of total testosterone by mass spectrometry and free testosterone by equilibrium dialysis might be impractical. Finally, only a minority of the men experienced a first stroke or TIA during the period of the study. Therefore, there is a possibility that the results could change as additional events occur during extended follow-up of this cohort into the future.

In our study, both total and free testosterone independently predicted incident stroke or TIA. There was also no association between lower SHBG and incident stroke or TIA. Also, we did not find any relationship between higher LH and incident stroke or TIA, despite low testosterone levels operating as a feedback mechanism to stimulate LH production in normal physiology (18). This could be accounted for by the fact that many older men with low testosterone levels would not exhibit elevated LH, as aging is associated with both reduced testicular responses to LH and incomplete hypothalamo-pituitary compensation for the fall in total and free testosterone levels (36).

Putative mechanisms by which lower testosterone levels could contribute to an increased burden of cardiovascular disease range from the loss of beneficial effects of testosterone on endothelial function and vasodilation to epidemiological correlations between testosterone and more favourable lipid profiles (18,37,38). In a castrated rat model, testosterone treatment enhanced recovery from stroke, supporting in a different context a potential beneficial effect of testosterone on neurological function (39). Lower testosterone is also associated with higher body mass index and fat mass, which are recognised cardiovascular risk factors, and testosterone replacement therapy in hypogonadal men reduces circulating inflammatory cytokines (40). Overviews of randomised controlled trials have not shown a benefit of testosterone supplementation in cardiovascular risk reduction, but these studies were not designed or powered to detect this outcome (19-22). At present, testosterone therapy should only be offered to men who meet accepted diagnostic criteria for the diagnosis of androgen deficiency, including symptoms suggestive of hypogonadism and confirmed low testosterone levels measured in early morning blood samples (41,42). The exact threshold for defining a "low" testosterone level is subject of debate. A threshold of <10.4 nmol/L has been proposed below which a diagnosis of androgen deficiency could be considered (41). A recent consensus statement suggests that men with total testosterone levels >12 nmol/L generally would not require testosterone supplementation, whereas men with levels consistently <8 nmol/L should be considered for testosterone treatment (42). Therefore we tested the *a priori* hypothesis that men in with total testosterone in the lowest quartile of values (<11.7 nmol/L) would have increased risk of stroke/TIA. We subsequently stratified these men into those with levels <8 nmol/L and those with levels ≥8 and <11.7 nmol/L to determine whether the increased risk of stroke/TIA was confined to men with unequivocally low total testosterone levels, or spread amongst the men in the lowest quartile. Importantly, our findings indicate that men with total testosterone levels that are not unequivocally low

(≥8 nmol/L and <11.7 nmol/L) have an increased incidence of stroke or TIA. Therefore, planning of prospective studies to clarify the issue of whether interventions which raise testosterone levels could prevent cardiovascular events in aging men should give particular consideration to men with low to normal testosterone levels.

In summary, lower total testosterone levels predict higher incidence of stroke or TIA in community-dwelling older men after adjustment for potential confounders and conventional risk factors for cardiovascular disease. The higher risk of these cerebrovascular events is not limited to men with unequivocally low total testosterone levels, but is present in men with low to normal testosterone concentrations. Further studies are needed to determine whether interventions which raise testosterone levels could prevent cerebrovascular disease in aging men.

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Tables

Table 1

Five men experienced a fatal first stroke and 114 men a non-fatal first stroke or TIA during follow-up. The frequency of specific non-fatal events is shown.

ICD code	Diagnosis	n
H34.1	Central retinal artery occlusion	2
I60	Subarachnoid haemorrhage	3
I61	Intracerebral haemorrhage	8
I63	Cerebral infarction	53
I64	Stroke, not specified as haemorrhage or infarction	21
G45	Transient cerebral ischemic attacks and related syndromes	27

Table 2

Baseline characteristics of men who experienced a first stroke or TIA during follow-up (n=119) compared with men who did not (n=3324). Data are shown as median (interquartile range), percentages of men in each category or mean ±SD.

	+ stroke/TIA n=119	no stroke/TIA n=3324	*p* value
Age (years)	78.4 (76.0-81.2)	76.1 (74.0-79.0)	<0.001
Waist:hip ratio	0.97 ± 0.06	0.97 ± 0.07	0.98
Charlson index (%)	0: 56 1-2: 34 3-4: 6 ≥5: 4	0: 63 1-2: 27 3-4: 7 ≥5: 3	0.41
Smoker (%)	No: 33 Ex: 61 Yes: 5*	No: 34 Ex: 61 Yes: 5	0.96
Hypertension (%)	71	76	0.23
Dyslipidemia (%)	75	76	0.75
Diabetes (%)	12	15	0.32
Glucose (mmol/L)	5.3 (5.0-5.9)	5.4 (5.0-5.9)	0.18
Total testosterone (nmol/L)	14.5 ± 5.1	15.5 ± 5.6	0.07
Free testosterone (pmol/L)	260 ± 84	280 ± 97	0.03
SHBG (nmol/L)	42.8 ± 14.3	42.3 ± 16.8	0.75
LH (IU/L)	4.6 (3.4-7.1)	4.3 (3.0-6.5)	0.08

* percentages do not add up to 100 as one man did not answer the question on smoking status.

Table 3

Cox regression analyses of baseline hormone concentrations as predictors of incident stroke or TIA in community-dwelling older men.

	Hazard Ratio*	95% CI	*p* value
Lower total testosterone (<11.7 nmol/L)	1.99	1.33-2.99	0.001
Lower free testosterone (<222 pmol/L)	1.69	1.15-2.48	0.008
Lower SHBG (<31.4 nmol/L)	1.23	0.77-1.95	0.382
Higher LH (≥6.55 IU/L)	1.09	0.73-1.63	0.677

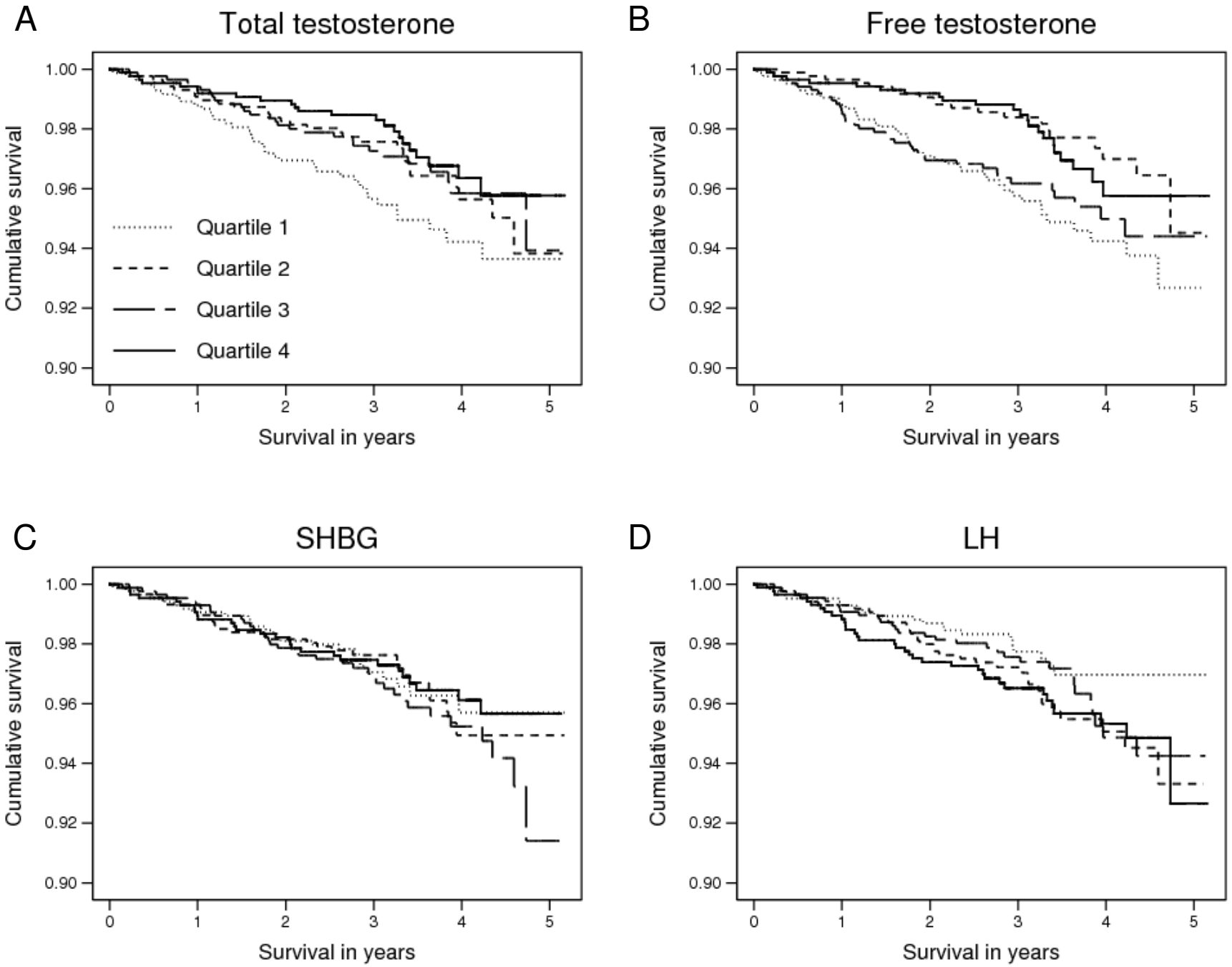
* adjusted for adjusted for age, waist:hip ratio, waist circumference, medical comorbidity (Charlson index), smoking status, presence or absence of diabetes, exercise history, past alcohol intake, use of aspirin or clopidogrel and the presence of hypertension and dyslipidemia.

Figure Legends

Figure 1

Kaplan Meier plots showing associations between baseline hormone levels (shown in quartiles) with stroke and TIA-free survival in community-dwelling older men. A: Total testosterone. B: Free testosterone. C: SHBG. D: LH.

Figure 1


A
Total testosterone
B
Free testosterone
C
SHBG
D
LH
Cumulative survival
1.00
0.98
0.96
0.94
0.92
0.90
0
1
2
3
4
5
Survival in years
Quartile 1
Quartile 2
Quartile 3
Quartile 4