Lower Testosterone Levels Predict Incident Stroke and Transient Ischemic Attack in Older Men

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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: This was a prospective observational study with median follow-up of 3.5 yr.

Setting: Community-dwelling, stroke-free older men were studied.

Participants: A total of 3443 men at least 70 yr of age participated in the study.

Main Outcome Measures: Baseline serum total testosterone, SHBG, and 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/liter and <222 pmol/liter) 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 comorbidity, lower total testosterone predicted increased incidence of stroke or TIA (hazard ratio = 1.99; 95% confidence interval, 1.33–2.99). Lower free testosterone was also associated (hazard ratio = 1.69; 95% confidence interval, 1.15–2.48), whereas 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 that raise circulating testosterone levels might prevent cerebrovascular disease in men. **(*J Clin Endocrinol Metab* 94: 2353–2359, 2009)**

Most circulating testosterone is bound to 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 because lower testosterone concentrations have been associated with increased incidence of metabolic syndrome and type 2 diabetes in middle-aged and older men

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2008-2416 Received November 5, 2008. Accepted April 1, 2009.
First Published Online April 7, 2009

Abbreviations: CI, Confidence interval; HDL, high-density lipoprotein; HR, hazard ratio; TIA, transient ischemic attack.

(3–6). Additionally, lower testosterone levels are associated with carotid intima-media thickness, lower extremity peripheral arterial disease, and aortic atherosclerosis (7–10). However, despite the relationship between lower testosterone levels and conditions associated with either the 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 nonfatal cardiovascular events (17).

In cross-sectional and longitudinal observational studies, reverse causation needs to be considered because 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 randomized 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).

Subjects and 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, a total of 4263 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 4165 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 3638 men available for analysis (24). Of these men, a further 195 were excluded because they had a prior diagnosis of stroke or TIA, leaving 3443 men to be included in the longitudinal analysis. Height (in centimeters), weight (in kilograms), girth at hips and waist (in centimeters), 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-yr 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 tumors, metastatic tumors, 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, TX).

Identification of men with incident stroke or TIA

We followed participants using the WADLS between baseline assessment and December 31, 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 hemorrhage; I61, intracerebral hemorrhage; I63, cerebral infarction; I64, stroke, not specified as hemorrhage or infarction; and G45, transient cerebral ischemic attacks and related syndromes.

Laboratory assays

Blood samples were collected between 0800 and 1030 h. Serum was prepared immediately after phlebotomy and stored at −80 C until assayed. Biochemical and hormone assays were performed in the Biochemistry Department, PathWest, Royal Perth Hospital, Western Australia. Serum total testosterone, SHBG, and 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/liter and 8.9% at 18 nmol/liter; for SHBG, 6.7% at 5.2 nmol/liter and 6.2% at 81 nmol/liter; and for LH, 6.4% at 2.3 IU/liter and 5.8% at 19 IU/liter. The working range of the testosterone assay was 0.7–55 nmol/liter; the sensitivities of the SHBG and LH assays were 2 nmol/liter and 0.1 IU/liter, respectively. The established reference intervals for these assays are total testosterone, 8–35 nmol/liter; SHBG, 10–70 nmol/liter; and LH, 1–8 IU/liter. Fasting serum glucose, total and high-density lipoprotein (HDL) cholesterol, and triglycerides were assayed using a Roche Hitachi 917 analyzer (Roche Diagnostic GmbH, Mannheim, Germany). Between-day imprecision for glucose was 2.9% at 4.8 mmol/liter and 2.2% at 15.2 mmol/liter; for cholesterol, 2.3% at 3.2 mmol/liter and 2.1% at 6.7 mmol/liter; for HDL, 2.4% at 0.8 mmol/liter and 2.5% at 1.7 mmol/liter; and for triglycerides, 4.8% at 0.9 mmol/liter and 2.4% at 2.0 mmol/liter. Free testosterone was calculated using mass action equations as described by Vermeulen *et al.* (28).

Definition of hypertension and dyslipidemia

Hypertension was defined as a recorded blood pressure of at least 140/90 mm Hg or having a diagnosis of hypertension or receiving treatment for high blood pressure. Dyslipidemia was defined as having HDL below 0.9 mmol/liter, low-density lipoprotein of at least 3.4 mmol/liter, triglycerides of at least 1.8 mmol/liter, or total cholesterol of at least 5.5 mmol/liter, or receiving lipid-lowering therapy.

Statistical analysis

Data were analyzed using the statistical software packages Stata version 10 (StataCorp) and SPSS version 15 (SPSS Inc., Chicago, IL). Group comparisons were performed using either Student's *t* test or the Mann-Whitney test in the case of nonparametrically distributed variables. Kaplan Meier plots were constructed to show stroke and TIA-free survival according to quartiles of baseline total testosterone, free testoster-

TABLE 1. Frequency of specific nonfatal events

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

Five men experienced a fatal first stroke, and 114 men experienced a nonfatal first stroke or TIA during follow-up.

one, SHBG, and LH, and comparisons made using the log-rank test. Cox regression (proportional hazards model) was performed to analyze 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) yr. Five men had a fatal first stroke, and 114 men had a nonfatal 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 hemorrhage or infarction, and TIA.

Characteristics of men who experienced a first stroke or TIA

Baseline characteristics of the 3443 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. Although 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/liter; $P = 0.03$).

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 (Fig. 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 (Supplemental Fig. 1, A and B, published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Lower SHBG did not discriminate for stroke and TIA-free survival (Supplemental Fig. 1C). Because 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 (Supplemental Fig. 1D).

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/liter) predicted incident stroke or TIA with a hazard ratio (HR) of 1.99. Men with free testosterone in the lowest quartile (<222 pmol/liter) 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).

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

TABLE 2. Baseline characteristics of men who experienced a first stroke or TIA during follow-up compared with men who did not

	+ Stroke/TIA (n = 119)	No stroke/TIA (n = 3324)	*P* value
Age (yr)	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[a]	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/liter)	5.3 (5.0–5.9)	5.4 (5.0–5.9)	0.18
Total testosterone (nmol/liter)	14.5 ± 5.1	15.5 ± 5.6	0.07
Free testosterone (pmol/liter)	260 ± 84	280 ± 97	0.03
SHBG (nmol/liter)	42.8 ± 14.3	42.3 ± 16.8	0.75
LH (IU/liter)	4.6 (3.4–7.1)	4.3 (3.0–6.5)	0.08

Data are shown as median (interquartile range), percentage of men in each category, or mean ± SD.

[a] Percentages do not add up to 100 because one man did not answer the question on smoking status.

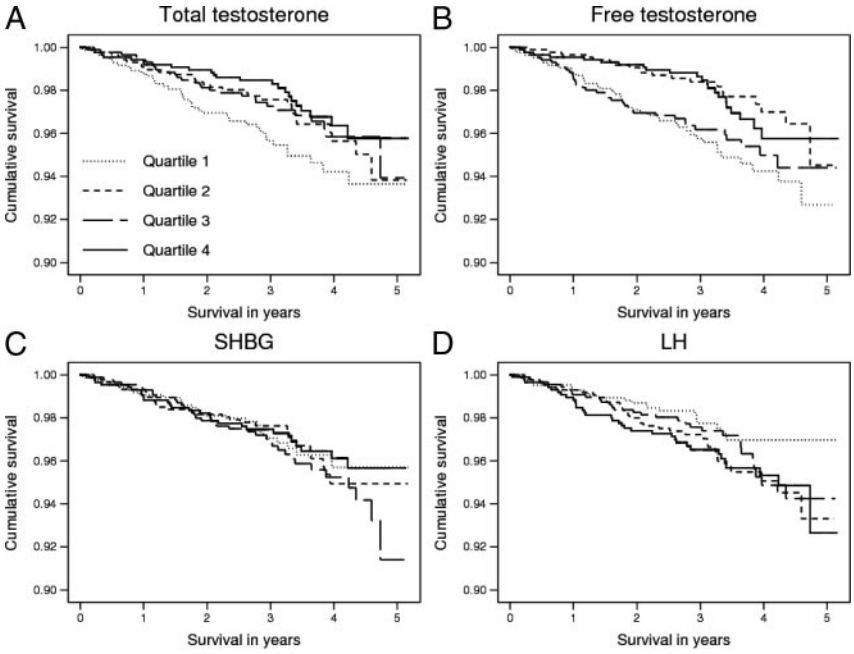

FIG. 1. Kaplan Meier plots showing associations between baseline hormone levels (shown in quartiles) with stroke and TIA-free survival in community-dwelling older men. A, Total testosterone; B, free testosterone; C, SHBG; D, LH.

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 below 8 nmol/liter had HR = 1.39 [95% confidence interval (CI), 0.64–3.02] for incident stroke or TIA (seven of 172 men). By contrast, men with low to normal total testosterone levels (≥8 and <11.7 nmol/liter) had HR = 2.08 (95% CI, 1.35–3.20) for incident stroke or TIA (32 of 664 men) in the adjusted model, compared with men with total testosterone of at least 11.7 nmol/liter.

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 less than 8 nmol/liter 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 less than 11.7 nmol/liter 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 it 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, because 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 rather 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 carotid intima-media thickness, 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 yr 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 yr, 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 yr and older, only those with testosterone, IGF-I, and dehydroepiandrosterone sulfate in the lowest quartiles of values had higher mortality, whereas 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 that would warrant preventive intervention. In a prospective study of 2084 middle-

TABLE 3. Cox regression analyses of baseline hormone concentrations as predictors of incident stroke or TIA in community-dwelling older men

	HR[a]	95% CI	*P* value
Lower total testosterone (<11.7 nmol/liter)	1.99	1.33–2.99	0.001
Lower free testosterone (<222 pmol/liter)	1.69	1.15–2.48	0.008
Lower SHBG (<31.4 nmol/liter)	1.23	0.77–1.95	0.382
Higher LH (≥6.55 IU/liter)	1.09	0.73–1.63	0.677

[a] 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.

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.* (31) of 2197 men aged 71–93 yr from the Honolulu-Asia Aging Study. In that study, stroke risk varied negligibly from lowest to highest quintiles of total testosterone, from 10.4/1000 person-years for testosterone no greater than 12 nmol/liter to 9.0/1000 for testosterone above 21.5 nmol/liter. Possible explanations for this discrepancy may involve the smaller size of the study or the differences in ethnicity between the two populations because Abbott *et al.* (31) studied a cohort of men with Japanese ancestry, whereas 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, because 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 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 nonfatal events analyzed 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 2-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, whereas 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 analyzer. The Immulite 2000 platform was also used in our study, and it provides an acceptable measurement of serum testosterone in men (32, 33). However, because 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 lower total and free testosterone independently predicted incident stroke or TIA. There was 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 because 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 favorable 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 recognized cardiovascular risk factors, and testosterone replacement therapy in hypogonadal men reduces circulating inflammatory cytokines (40). Overviews of randomized 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 less than 10.4 nmol/liter 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 above 12 nmol/liter generally would not require testosterone supplementation, whereas men with levels consistently less than 8 nmol/liter should be considered for testosterone treatment (42). Therefore, we tested the *a priori* hypothesis that men with total testosterone in the lowest quartile of values ($<$11.7 nmol/liter) would have increased risk of stroke/TIA. We subsequently stratified these men into those with levels below 8 nmol/liter and those with levels of at least 8 and less than 11.7 nmol/liter to determine whether the increased risk of stroke/TIA was confined to men with unequivocally low total testosterone levels or spread among the men in the lowest quartile. Importantly, our findings indicate that men with total testosterone levels that are not unequivocally low ($\geq$8 nmol/liter and $<$11.7 nmol/liter) have an increased incidence of stroke or TIA. Therefore, planning of prospective studies to clarify the issue of whether interventions that 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 that raise testosterone levels could prevent cerebrovascular disease in aging men.

Acknowledgments

The authors thank Tricia Knox and the staff of the Department of Biochemistry, PathWest, Royal Perth Hospital, Western Australia, for their assistance in performing the hormone assays; Peter Feddema from DPC-Biomediq, Australia, for his assistance with sourcing hormone assay kits and reagents; and Dr. Kieran McCaul, Senior Research Fellow, Centre for Health and Ageing, University of Western Australia, for his advice regarding the statistical analyses. We thank the staff and management of Shenton Park Hospital for providing space in which to conduct follow-up clinics. We especially thank all the men and staff who participated in the Western Australian Abdominal Aortic Aneurysm Program and the Health In Men Study.

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Hormone assays were funded by a Clinical Investigator Award (to B.B.Y.) from the Sylvia and Charles Viertel Charitable Foundation, New South Wales, Australia. The Health In Men Study was funded by the National Health and Medical Research Council of Australia Project Grants 279408, 379600, 403963, and 513823.

Disclosure Summary: The authors have no conflicts of interest to declare.

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