Cardiovascular Outcomes and All-cause Mortality Following Measurement of Endogenous Testosterone Levels

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Although reduced testosterone levels are common in aging populations, the clinical consequences remain to be further explored. We examined whether low total testosterone levels are associated with stroke (ischemic and hemorrhagic), myocardial infarction (MI), venous thromboembolism (VTE), and all-cause mortality in adult men. We conducted a cohort study in the Central Denmark Region (2000 to 2015). We included all men with a first-ever laboratory testosterone result and computed the 5-year risks of cardiovascular outcomes and all-cause mortality. Propensity score-weighted hazard ratios were computed, comparing persons with normal versus low testosterone levels. Individuals were censored at testosterone treatment during follow-up (3%). We identified 4,771 men with low testosterone levels and 13,467 with normal levels. Persons with low testosterone levels were older (median ages, 55 years vs 50 years) and had more co-morbidities than men with normal testosterone levels. Persons with low testosterone had higher 5-year risks of stroke (2.4% vs 1.5%), MI (1.5% vs 1.2%), VTE (1.4% vs 0.9%), and all-cause mortality (17.8% vs 6.8%) than persons with normal testosterone levels. After propensity score-weighting, the associations with cardiovascular outcomes reached unity. The 5-year hazard ratios were 1.14 (95% confidence intervals [CIs] 0.87 to 1.49) for stroke, 0.95 (95% CI 0.70 to 1.30) for MI, 1.10 (95% CI 0.78 to 1.55) for VTE, whereas it was 1.48 (95% CI 1.32 to 1.64) for all-cause mortality. In conclusion, low testosterone level was a strong predictor for cardiovascular outcomes and all-cause mortality in unadjusted models, however only the association between low testosterone and all-cause mortality persisted after adjustment for age and co-morbidity. **(Am J Cardiol 2019;123:1757−1764)**

Some recent studies suggest that testosterone therapy is associated with an increased risk of stroke, myocardial infarction (MI), and death.[1−5] As a consequence, testosterone has come under scrutiny by the US Food and Drug Administration.[6] Health Canada[7] also strongly warns about potential cardiovascular side effects in testosterone users, although the body of literature still lacks conclusive evidence. One explanation for the observed associations may be confounding by indication, as low levels of endogenous testosterone levels *per se* might be linked to stroke, MI, and death.[8−14] Additional analyses to clarify this association are therefore needed. In some countries, including Denmark, use of testosterone therapy has been limited, making it possible to examine the effect of endogenous testosterone levels on risk of cardiovascular outcomes. We therefore examined the risk of stroke, MI, venous thromboembolism (VTE), and all-cause mortality in men with normal versus low testosterone levels in a Danish cohort study.

Methods

This study was conducted in the Central Denmark Region from January 1, 2000 to November 1, 2015. This Region has a population of 1.3 million inhabitants (24% of the Danish population).[15] Denmark has a tax-supported health care system that guarantees unfettered access to medical care for all residents, as well as partial reimbursement of prescribed drugs.

The study population consisted of all male inhabitants with a first-ever testosterone measurement in the study period, where the person was 18 years or older at the time of the measurement. Data on testosterone measurements were obtained from the Clinical Laboratory Information System Research Database (LABKA), using Nomenclature for Properties and Units codes.[16] The database includes laboratory results from all hospitals, outpatient clinics, and general practices in the Central Denmark Region since 2000. Only men whose measurement had a nonmissing result were used and only individuals who lived in the

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Central Denmark Region at the time of the measurement were eligible for inclusion. Total testosterone laboratory methods included a variety of immunoassays and liquid chromatography-tandem mass spectrometry.

Testosterone levels were categorized according to age-specific reference values for normal and low levels (Supplementary Table 1). If a man's first-ever measurement had a result classified as high, the patient was excluded from the study. Patients with prostate cancer before the test, identified from the Danish National Patient Registry (DNPR), also were excluded, because most have low testosterone levels due to antiandrogen therapy, and we were unable to identify a sufficient number of comparators with normal testosterone levels for these patients. The DNPR is an ongoing population-based registry. It has collected data on admission and discharge dates as well as diagnoses from all nonpsychiatric hospitals since 1977 and on emergency room and outpatient clinic visits since 1995. Each hospital discharge or outpatient visit is recorded in the DNPR with one primary diagnosis and one or more secondary diagnoses coded according to the *International Classification of Diseases, Eighth Revision* between 1977 and 1993 and *Tenth Revision* thereafter. We also excluded individuals treated with exogenous testosterone and antiandrogen therapy within 90 days before the first testosterone test results. The index date was the date of the first-ever testosterone result.

Outcomes included 1-year and 5-year risk of first-time stroke, MI, and VTE identified in the DNPR, based on primary and secondary diagnoses from inpatient and outpatient hospital contacts.[17] Emergency room diagnoses were not considered due to the assumed low positive predictive value of diagnoses in this setting since they are initial working diagnoses.[17] Secondarily, we also examined all-cause mortality. These data were obtained from the Danish Civil Registration System, which provides daily updates on vital statistics, including dates of emigration and death.[18]

Data on the most recent albumin level, follicle-stimulating hormone status, and luteinizing hormone status (all categorized into missing, low, normal, and high) within the previous year or at the index date were retrieved from the LABKA database. Using all inpatient and outpatient clinic diagnoses, data on several co-morbidities and potential causes of low testosterone within 10 years before the index date were retrieved from the DNPR.[17]

We also retrieved information on various filled prescriptions within 90 days before the index date from the Aarhus University Prescription Database, using the Anatomical Therapeutic Chemical Classification System.[19,20] The Aarhus University Prescription Database contains complete information on all prescriptions redeemed in the Central Denmark Region since 1998. All Nomenclature for Properties and Units, Anatomical Therapeutic Chemical, and *International Classification of Diseases* codes are provided in Supplementary Table 2.

For each outcome-specific analysis, persons were followed from the date of their first-ever testosterone laboratory test until the date of the outcome, death (unless the outcome of interest was all-cause mortality), date of emigration, 1 or 5 years of follow-up depending on the analysis, or 31 December 2015, whichever occurred first. For each outcome, patients with a previous event were excluded from the analysis (e.g., when VTE was the outcome, patients with previous VTE were excluded). In addition, individuals were censored at testosterone treatment during follow-up. We described individuals with low and normal testosterone levels according to the covariates listed above and presented these data only for individuals included in the all-cause mortality analysis. In addition, the number of individuals initiating testosterone treatment at hospitals or through redemption of a prescription during follow-up was tabulated. We calculated incidence rates of the outcomes per 100 person-years and calculated 1-year and 5-year risks of the outcomes, comparing men with low versus normal testosterone levels. We also plotted cumulative risks, accounting for death as a competing risk.

Each patient's propensity score were estimated with generalized boosted models using the covariates shown in Table 1 and then transformed the propensity score into inverse probability of treatment weights (IPTW),[21] which permits estimation of an average treatment effect in the treated population.[22] We computed hazard ratios (HRs) with 95% confidence intervals (CIs) using Cox regression analysis before and after propensity score weighting, to compare individuals with low testosterone levels to those with normal levels. To assess the balance of covariates after propensity score weighting we estimated the standardized difference for all covariates included in the propensity score.[22] Furthermore, we estimated and compared the empirical cumulative distribution function for each of the continuous covariates.[22] Because age may modify the effect of testosterone level, we stratified the analyses by age groups.

We examined proportionality of hazards assumption using log(-log) plots, and the assumption was found to be appropriate. All analyses were conducted using SAS version 9.4 (SAS Institute, Cary, North Carolina). The study was approved by the Danish Data Protection Agency (record number: 2013-41-1924). According to Danish law, use of registry data does not require informed consent from patients.

Results

We identified 13,467 individuals with a normal testosterone level and 4,771 individuals with a low level (Table 1 and Figure 1). The cohort of persons with low testosterone was older than the cohort of persons with a normal level (median age, 55 years vs 50 years, respectively). Persons with low testosterone had a substantial higher prevalence of co-morbidities than persons with normal testosterone levels. The prevalence of hypogonadism, hypopituitarism, Klinefelter's syndrome, Down's syndrome, testicular torsion, varicocele, cryptorchidism, and orchitis were all below 1% in the study population as a whole. After propensity score weighting, the standardized difference of all covariates were less than 0.1. Furthermore, the empirical cumulative distribution functions of continuous variables was almost identical after weighting. Both indicate that covariate balance was achieved after weighting. During the first 5 years of follow-up, 485 individuals (~3% of the study population) initiated testosterone treatment.

Persons with low testosterone had a higher 1-year risk of stroke (1% vs 0.5%), MI (0.7% vs 0.4%), VTE (0.7% vs 0.3%), and all-cause mortality (9% vs 2%) than persons

Table 1
Characteristics of individuals with normal and low testosterone levels, Denmark, 2000 to 2015

	Unweighted cohorts		Propensity score weighted cohorts	
Variable	Low testosterone	Normal testosterone	Low testosterone	Normal testosterone
Number of men	4,771	13,467	4,771.0	4,470.7
Median age (25th−75th percentiles)	55.4 (38.3−69.2)	50.4 (33.8−63.4)	55.4 (38.3−69.2)	54.8 (37.9−68.5)
Albumin level				
Low	794 (17%)	734 (5.5%)	794.0 (17%)	652.1 (15%)
Normal	2,072 (43%)	5,772 (43%)	2,072.0 (43%)	1,985.2 (44%)
High	371 (7.8%)	1,031 (7.7%)	371.0 (7.8%)	336.4 (7.5%)
Missing	1,534 (32%)	5,930 (44%)	1,534.0 (32%)	1,496.9 (34%)
Co-morbidities				
Myocardial infarction	216 (4.5%)	301 (2.2%)	216.0 (4.5%)	172.4 (3.9%)
Congestive heart failure	292 (6.1%)	295 (2.2%)	292.0 (6.1%)	233.5 (5.2%)
Peripheral vascular disease	270 (5.7%)	354 (2.6%)	270.0 (5.7%)	214.9 (4.8%)
Cerebrovascular disease	400 (8.4%)	602 (4.5%)	400.0 (8.4%)	338.6 (7.6%)
Dementia	48 (1.0%)	45 (0.3%)	48.0 (1.0%)	29.3 (0.7%)
Chronic pulmonary disease	502 (11%)	780 (5.8%)	502.0 (11%)	436.4 (9.8%)
Connective tissue disease	193 (4.0%)	459 (3.4%)	193.0 (4.0%)	178.8 (4.0%)
Ulcer disease	171 (3.6%)	236 (1.8%)	171.0 (3.6%)	127.9 (2.9%)
Mild liver disease	103 (2.2%)	184 (1.4%)	103.0 (2.2%)	94.5 (2.1%)
Diabetes without end-organ damage	522 (11%)	803 (6.0%)	522.0 (11%)	456.0 (10%)
Hemiplegia	31 (0.6%)	40 (0.3%)	31.0 (0.6%)	20.1 (0.4%)
Moderate to severe renal disease	219 (4.6%)	214 (1.6%)	219.0 (4.6%)	162.4 (3.6%)
Diabetes with end-organ damage	295 (6.2%)	430 (3.2%)	295.0 (6.2%)	247.7 (5.5%)
Moderate to severe liver disease	36 (0.8%)	49 (0.4%)	36.0 (0.8%)	30.4 (0.7%)
AIDS	9 (0.2%)	41 (0.3%)	9.0 (0.2%)	10.2 (0.2%)
Hypogonadism	15 (0.3%)	11 (0.1%)	15.0 (0.3%)	10.2 (0.2%)
Hypopituitarism	37 (0.8%)	35 (0.3%)	37.0 (0.8%)	23.8 (0.5%)
Klinefelter's syndrome	8 (0.2%)	11 (0.1%)	8.0 (0.2%)	6.3 (0.1%)
Down's syndrome	7 (0.1%)	3 (0.0%)	7.0 (0.1%)	4.3 (0.1%)
Testicular torsion	5 (0.1%)	21 (0.2%)	5.0 (0.1%)	4.8 (0.1%)
Varicocele	8 (0.2%)	30 (0.2%)	8.0 (0.2%)	8.6 (0.2%)
Cryptorchidism	25 (0.5%)	53 (0.4%)	25.0 (0.5%)	22.6 (0.5%)
Orchitis	54 (1.1%)	134 (1.0%)	54.0 (1.1%)	45.1 (1.0%)
Chronic kidney disease	222 (4.7%)	244 (1.8%)	222.0 (4.7%)	183.9 (4.1%)
Myxedema	46 (1.0%)	72 (0.5%)	46.0 (1.0%)	32.7 (0.7%)
Obesity*	240 (5.0%)	255 (1.9%)	240.0 (5.0%)	196.7 (4.4%)
Alcoholism	331 (6.9%)	718 (5.3%)	331.0 (6.9%)	297.1 (6.6%)
Hypertension	785 (17%)	1,234 (9.2%)	785.0 (17%)	693.4 (16%)
Any cancer (except prostate cancer)	586 (12%)	1,195 (8.9%)	586.0 (12%)	532.6 (12%)
Illicit drug abuse	30 (0.6%)	38 (0.3%)	30.0 (0.6%)	17.6 (0.4%)
Comedications				
ACE/ARB	966 (20%)	1,785 (13%)	966.0 (20%)	863.4 (19%)
Beta-blockers	595 (13%)	959 (7.1%)	595.0 (13%)	525.2 (12%)
Statins	881 (19%)	1,405 (10%)	881.0 (19%)	779.2 (17%)
Low-dose aspirin	711 (15%)	1,121 (8.3%)	711.0 (15%)	612.2 (14%)
Clopidogrel	102 (2.1%)	157 (1.2%)	102.0 (2.1%)	98.0 (2.2%)
Vitamin K antagonists	186 (3.9%)	344 (2.6%)	186.0 (3.9%)	177.5 (4.0%)
Diuretics	818 (17%)	941 (7.0%)	818.0 (17%)	684.8 (15%)
NSAID	679 (14%)	1,396 (10%)	679.0 (14%)	589.3 (13%)
Opiods	785 (17%)	1,132 (8.4%)	785.0 (17%)	666.9 (15%)
Antidepressants	688 (14%)	1,153 (8.6%)	688.0 (14%)	583.1 (13.0%)
Antipsychotics	199 (4.2%)	308 (2.3%)	199.0 (4.2%)	163.9 (3.7%)
Erectile dysfunction drugs	29 (0.6%)	151 (1.1%)	29.0 (0.6%)	30.2 (0.7%)

ACE/ARB, angiotensin-converting enzyme/angiotensin II receptor blockers; AIDS, acquired immune deficiency syndrome; NSAID, nonsteroidal anti-inflammatory drugs.

* Defined as hospital-based diagnoses of obesity. Data are counts (%), unless otherwise stated. The characteristics were tabulated for the individuals where all-cause mortality was the outcome of interest.

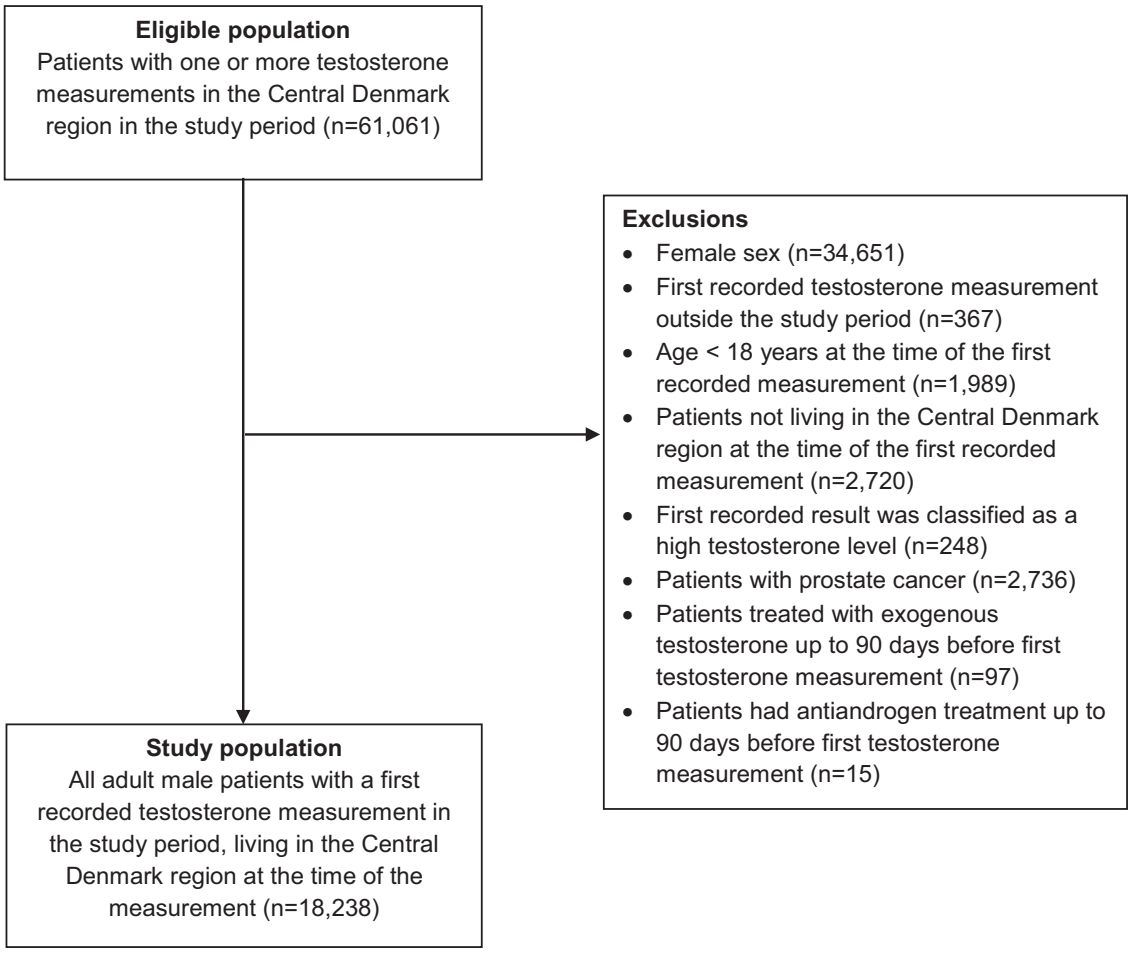

Figure 1. Flow chart of the study population.

with normal testosterone levels (Table 2 and Figure 2). Correspondingly, the 1-year unadjusted HRs was increased for stroke, MI, VTE, and all-cause mortality. Using 5 years of follow-up period, the unadjusted HRs were attenuated but remained elevated for all outcomes.

After accounting for measured confounders using IPTW, the cumulative incidence of stroke, MI, and VTE were comparable for persons with low and normal testosterone, whereas the cumulative incidence of all-cause mortality remained higher for persons with low testosterone levels (Figure 2). After applying IPTW, the 1-year HRs were 1.33 (95% CI 0.84 to 2.09) for stroke, 1.47 (95% CI 0.91 to 2.38) for MI, 1.10 (95% CI 0.65 to 1.85) for VTE, and 2.08 (95% CI 1.72 to 2.52) for all-cause mortality. The 5-year HRs for cardiovascular outcomes reached unity. For all-cause mortality, the association was attenuated but persisted (HR 1.48, 95% CI 1.32 to 1.64).

In analyses stratified by age, the associations were broadly consistent across all age groups with few exceptions (Supplementary Tables 3-6).

Discusssion

In this cohort study, in unadjusted models, a low testosterone level was a strong predictor for increased risk of stroke, MI, VTE, and all-cause mortality, especially in the first year, but also during 5 years. However, the increased risks of cardiovascular outcomes were largely explained by increased age and co-morbidity levels in persons with a low testosterone level. Thus, the associations were greatly attenuated after accounting for differences in these variables.

Previous studies have examined the association between endogenous testosterone level, mortality, and cardiovascular outcomes.[8,11,14] However, many were limited by low numbers of events,[13,23,24] reported only surrogate end points for cardiovascular outcomes (e.g., degree of aortic atherosclerosis),[23–25] and did not assess individual cardiovascular outcomes or included data on VTE.[8,11] Our analysis thus complements the literature by providing data on the association between low testosterone levels and several cardiovascular outcomes, accounting for several potential confounders, within a uniformly organized health care system, with complete individual-level linkage of data in various registries.

A previous meta-analysis of 19 studies examined the association between endogenous testosterone and atherosclerosis, stroke, MI, ischemic heart disease, death from coronary artery disease, and all-cause mortality.[11] In total, 18 studies had data on total testosterone level, with follow-up ranging between 3 and 15 years. A weak protective

Table 2
Risk of stroke, myocardial infarction, venous thromboembolism, and all-cause mortality in men with normal and low testosterone levels

Outcome by testosterone level	0–1 year of follow-up				0–5 years of follow-up			
	No. at risk/No. of events	Incidence rate* (95% CI)	Unadjusted hazard ratio (95% CI)	Hazard ratio after IPTW weighting (95% CI)	No. at risk/No. of events	Incidence rate* (95% CI)	Unadjusted hazard ratio (95% CI)	Hazard ratio after IPTW weighting (95% CI)
Stroke								
Normal	13,034/65	0.55 (0.43; 0.70)	1.00 (ref)	1.00 (ref)	13,034/199	0.47 (0.41;0.54)	1.00 (ref)	1.00 (ref)
Low	4,469/45	1.17 (0.87; 1.57)	2.13 (1.46; 3.11)	1.33 (0.84;2.09)	4,469/105	0.79 (0.65;0.95)	1.67 (1.32;2.12)	1.14 (0.87; 1.49)
Myocardial infarction								
Normal	13,026/50	0.42 (0.32; 0.55)	1.00 (ref)	1.00 (ref)	13,026/154	0.36 (0.31; 0.42)	1.00 (ref)	1.00 (ref)
Low	4,460/29	0.75 (0.52; 1.08)	1.77 (1.12; 2.80)	1.47 (0.91; 2.38)	4,460/65	0.49 (0.38; 0.62)	1.33 (1.00; 1.78)	0.95 (0.70; 1.30)
Venous thromboembolism								
Normal	13,266/42	0.35 (0.26; 0.47)	1.00 (ref)	1.00 (ref)	13,266/113	0.26 (0.22; 0.31)	1.00 (ref)	1.00 (ref)
Low	4,656/33	0.82 (0.59; 1.16)	2.37 (1.50; 3.73)	1.10 (0.65; 1.85)	4,656/65	0.47 (0.37; 0.60)	1.78 (1.31; 2.41)	1.10 (0.78; 1.55)
All-cause mortality								
Normal	13,467/263	2.14 (1.89; 2.41)	1.00 (ref)	1.00 (ref)	13,467/919	2.08 (1.95; 2.22)	1.00 (ref)	1.00 (ref)
Low	4,771/421	10.25 (9.31; 11.27)	4.75 (4.07; 5.54)	2.08 (1.72; 2.52)	4,771/848	5.95 (5.56; 6.36)	2.83 (2.58; 3.11)	1.48 (1.32; 1.64)

CI, confidence interval; IPTW, inverse probability of treatment weighting.
* Per 100 person-years. For nonfatal outcomes, individuals with previous events were excluded, for example, when stroke was the outcome, individuals with previous stroke were excluded to assess first-time events.

effect of a one-standard-deviation increase in total testosterone (overall risk ratio = 0.89, 95% CI 0.83 to 0.96) was reported, with a stronger association in men above age 70.[11] Another meta-analysis of 12 studies found that low endogenous testosterone was associated with increased risk of all-cause mortality (overall relative risk = 1.35, 95% CI 1.13 to 1.62), and cardiovascular mortality (overall relative risk = 1.25, 95% CI 0.97 to 1.60).[8] Consistent with these findings, a recent meta-analysis also found that low testosterone was a predictor for cardiovascular morbidity and mortality, in both unadjusted and fully adjusted models.[14]

Our analyses suggested that the increased risk of cardiovascular outcomes associated with low testosterone were driven mainly by age and co-morbidity, both of which themselves can contribute to reduced testosterone levels. As the CIs of the effect estimates for 1-year cardiovascular outcomes after applying IPTW were relatively wide, we cannot exclude entirely an association between testosterone level and some cardiovascular outcomes. However, this does not necessarily imply a causal link, as our findings could be susceptible to residual confounding (e.g., we lacked data on disease severity such as cancer stage and/or unmeasured confounding (e.g., physical activity, smoking, and alcohol abuse).

The strength of present study lies in its population-based design. As well, earlier studies found high positive predictive values of diagnoses in the DNPR of MI (~97%), ischemic stroke (~97%), and VTE (~88%), and somewhat lower positive predictive values for hemorrhagic stroke (~65% to 75%).[17,26] Our study also has some limitations. First, we had no valid information on what time of day the sample was drawn, which is known to affect the level of testosterone.[27] Nonetheless, timing of testosterone blood sampling may be independent of subsequent testosterone level, suggesting nondifferential misclassification, which could have biased the results against the null. Data were almost entirely missing on free testosterone levels (99%) in the LABKA registry, as all analyses for this laboratory test were performed in another Danish region during 2000 to 2009, and thus the test results were not available in the LABKA registry. It is also likely that free testosterone levels as well as luteinizing hormone and follicle-stimulating hormone are rarely measured in the primary health care sector as part of the initial diagnostic work-up. Therefore, these results were not available at baseline, but may have been present at a later stage, for example after referral to a specialist outpatient hospital clinic. Testosterone is a non-routine laboratory test, and will only be performed in those with a suspected reason to draw it. Laboratory tests were not standardized, which we were unable to account for in the analyses. However, a study found overall good correlations several immunoassays and the liquid chromatography-tandem mass spectrometry method.[28] Thus this issue is likely to be of minor importance.

Our study may have implications for clinical practice and future research. First, the prevalence of conditions related to hypogonadism for example, testicular torsion, were low, suggesting that almost all of the hypogonadism observed in routine clinical care is age-and co-morbidity-related. Second, persons with low testosterone levels have a higher absolute risk of dying and a higher absolute risk of

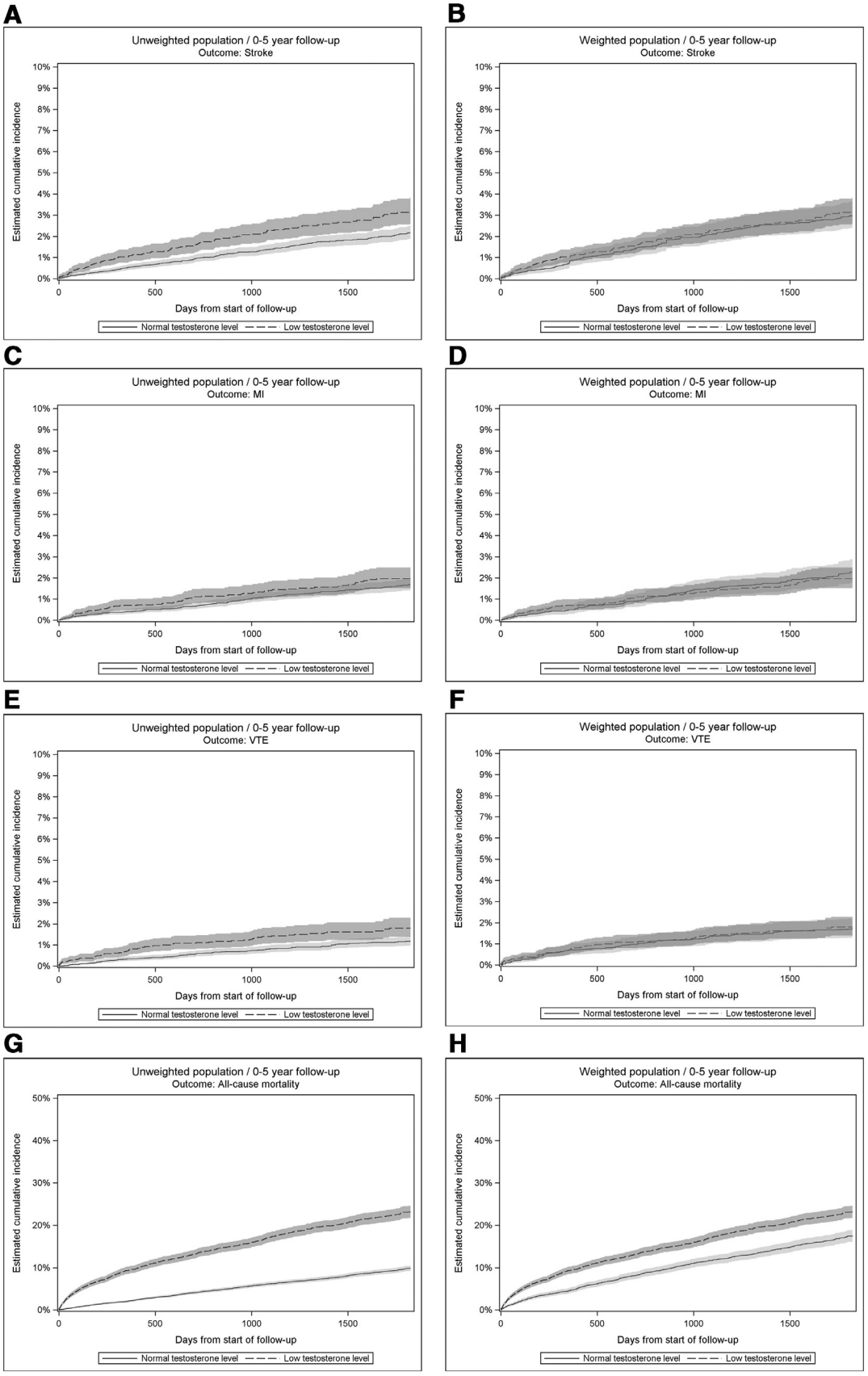

Figure 2. Cumulative incidence curves for stroke (*A-B*), myocardial infarction (MI) (*C-D*); venous thromboembolism (VTE) (*E-F*), and all-cause mortality (*G-H*) in men with low and normal testosterone levels, graphically illustrating risks in unweighted and weighted cohorts. The gray shaded areas represent 95% confidence intervals.

cardiovascular outcomes than individuals with normal testosterone levels. This suggests that the endogenous testosterone level is a potential marker of poor health, although our results do not suggest that low testosterone is an independent risk factor. Third, our study highlights the importance of taking into account confounding by low testosterone level in future pharmacoepidemiological studies on the safety and benefit of testosterone therapy.

In this cohort study, men with low total testosterone levels experienced more stroke, MI, VTE and had higher all-cause mortality than men with testosterone levels in the normal range. However, the associations between low testosterone levels and cardiovascular outcomes were mainly attributable to higher age and level of co-morbidity.

Author Contribution

All authors conceived the idea and designed the study. T. B.R performed the statistical analyses. All authors interpreted the data and reviewed the literature. K.A drafted the first manuscript. All authors critically reviewed the manuscript and approved the final version for submission. C.F.C has the overall responsibility of the accuracy of the data and the manuscript.

Ethics Approval

As this study did not involve patient contacts or any interventions, it was not necessary to obtain permission from the Danish Scientific Ethical Committee.

Disclosures

JBL is an employee of RTI International, an independent, non-profit research organization that performs contract work on behalf of governmental agencies and pharmaceutical companies. The remaining co-authors have no conflicts of interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.amjcard.2019.02.042.

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