

Original Research

Time-varying testosterone level and risk of myocardial infarction and stroke among hypogonadal men: a longitudinal study in Germany

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Abstract

Background and objective: Findings on the association between testosterone and cardiovascular risk in men have been inconsistent. Previous studies investigated this association based on testosterone level measured at one single point in time (e.g., testosterone level at the baseline visit), which might not be relevant for the future cardiovascular events that occurred many years later, given the dynamic changes of testosterone level over time. Additionally, as compared to patients without prior cardiovascular history, those with prior cardiovascular events are more likely to have pathological changes in coronary or peripheral vasculature, and therefore more susceptible to cardiovascular events when exposed to risk factors. However, whether time-varying testosterone level affects differently between patients with prior cardiovascular events and those without is unknown. The objective of this study was to investigate the association between time-varying testosterone level and risks of myocardial infarction and stroke, and compared the associations stratified by patient's prior cardiovascular event history. **Material and methods:** We used data of 376 hypogonadal men from a registry study in Germany with up to 11 years' follow-up and applied Cox proportional hazards regression models to investigate the proposed association. **Results:** No association was found between baseline testosterone level and risks of myocardial infarction and stroke. When taking into account the changes of testosterone level over time and including testosterone as a time-varying covariate in the model, the adjusted hazard ratio (HR) for myocardial infarction associated with 1 nmol/L increase in testosterone level was 0.78 (95% CI: 0.64, 0.97); no association between time-varying testosterone level and stroke was found. The associations between testosterone level and cardiovascular risk were not different among patients with prior cardiovascular event history and those without. **Conclusion:** One baseline measurement of testosterone level may be insufficient to predict one's future risk of cardiovascular events; regular monitoring of testosterone levels may be useful to assess the short-term cardiovascular risk in men.

Keywords: Testosterone; Time-varying; Cardiovascular risk; Cohort; Longitudinal

1. Introduction

Cardiovascular disease remains a major cause of premature death and chronic disability worldwide. Compared to women, men have a higher risk of acute coronary syndromes, ST-elevation myocardial infarction before age 60, and on average develop coronary heart disease, the most common type of cardiovascular disease, 7–10 years earlier than women do [1].

Cardiovascular disease is closely related to atherosclerosis, a pathological process when plaque made up of fat, cholesterol, and calcium forms in the walls of the arteries [2]. Rupture of atherosclerotic plaques may lead to thrombotic events, such as myocardial infarction and stroke, which can cause sudden death or significant morbidity [3]. There has been a focus on the role of testosterone deficiency in atherosclerosis among men, which was thought to contribute to the higher risk of cardiovascular disease in male gender [4–8].

Testosterone is responsible for the principal male sex differentiation and maintenance of libido, and closely associated with lipid and glucose metabolism that have been linked to cardiovascular health [9,10]. There is a dynamic change of testosterone level in men over the life course. Typically, testosterone levels increase during puberty and adolescence, reaching their peak in young to middle adulthood and then gradually decreasing when males enter their later adulthood. A condition of suppressed circulating testosterone levels and physical and psychological symptoms in aging men is called late-onset hypogonadism (LOH) [11,12]. Animal models have consistently demonstrated an anti-atherogenic action of testosterone in males, whereas testosterone deficiency promotes the early stages of atherogenesis [13,14]. Studies have also shown that testosterone deficiency was associated with higher carotid intima media thickness [15–17], which is a surrogate marker of atherosclerosis that is closely related to ab-



normal glucose metabolism and lipid profile as well as a strong predictor of future clinical ischemic cardiac and cerebrovascular events [18–20].

Although testosterone deficiency has been associated with atherosclerosis and predictors of cardiovascular disease, inconsistent findings were reported regarding the role of testosterone in cardiovascular risk [21]. In addition to the different demographic makeup of the study populations, various statistical methods applied, and insufficient length of follow-up and sample sizes, another possible explanation is that prior studies were using testosterone level measured at one single point in time as the exposure, such as baseline testosterone level, which might not be relevant for the cardiovascular event that occurred years later, given the dynamic changes of serum testosterone level over time and the possible long time period in between baseline measurement and disease occurrence later on [21–23]. It might make more sense to use testosterone levels close to the time when cardiovascular events occurred as the exposure when investigating the association between testosterone levels and the cardiovascular risk. Additionally, participants of these studies were normally restricted to those who had the same prior cardiovascular event status (either free of prior cardiovascular events or had prior cardiovascular events) [24]; whether testosterone level affects differently among patients with and without prior cardiovascular events is unknown. As compared to patients without prior cardiovascular history, those with prior cardiovascular events are more likely to have comorbidities and pathological changes (e.g., atherogenesis) in coronary or peripheral vasculature, and therefore are hypothesized to be more susceptible to cardiovascular events when exposed to risk factors.

In this study, we used data from 376 hypogonadal men with up to 11 years' follow-up in a registry-based study in Germany that contain serum testosterone levels measured at each visit and corresponding cardiovascular event status. We treated testosterone level as a time-varying variable and fitted data using Cox proportional hazards regression models to investigate the effect of time-varying testosterone level on cardiovascular risk at each visit, which took into account the dynamic changes of testosterone level over time. For comparison, we also evaluated the association between baseline testosterone level and cardiovascular risk. All the analyses were conducted stratified by patients' prior cardiovascular event status.

2. Methods

2.1 Study population

We used de-identified data from a registry study in Germany. Seven hundred and seventy-six hypogonadal men were recruited from one urology center in Bremerhaven, Germany from 2004 to 2016. Hypogonadism diagnosis was confirmed if they had total testosterone level ≤ 12.1 nmol/L and symptoms such as decreased libido, erectile dysfunction, depression, and fatigue, as assessed by

the Aging Males' Symptoms scale (AMS). The threshold of 12.1 nmol/L was selected based on clinical experience and confirmed by Bhasin *et al.* [25]. Ethical guidelines formulated by the German Ärztekammer (German Medical Association) for observational studies in patients receiving standard treatment were followed. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of German Medical Association. Eligible participants without contraindications were given option of testosterone therapy at study entry. Four hundred of them decided to receive testosterone therapy, whereas three hundred and seventy-six opted against testosterone therapy for various reasons including the concern about the testosterone therapy safety issues. Our current study only used data from the participants who did not take treatment to avoid the influence of exogenous testosterone administration.

2.2 Testosterone measurement and cardiovascular outcome ascertainment

Participants were followed semi-annually for updates in serum testosterone levels. Cardiovascular events (i.e., myocardial infarction and stroke) occurring during follow-up were recorded. Cardiovascular events were partly reported in the form of "physician letters" from the hospital or the cardiologist/neurologist/family physician, and partly by patients themselves or relatives. The latter usually occurred when already scheduled patient visits had to be postponed due to an event. Detailed description of lab measurements/methods and patients' medication use have been published elsewhere [26].

2.3 Statistical analysis

In the descriptive analysis, characteristics among patients who experienced cardiovascular events and those who did not during the study period were presented.

Cox proportional hazards regression models were fitted to the data to investigate the association of time-varying testosterone as well as baseline testosterone levels and the risk of cardiovascular events. When using time-varying testosterone level as the exposure, we modeled the association between testosterone measured at each visit (level changes over time/across visits) and its corresponding risk of cardiovascular events; when using baseline testosterone level as the exposure, we modeled the association between testosterone level measured at baseline (a constant number over time) and the risk of cardiovascular events that occurred during the study period. Covariates that are closely related to the risk of cardiovascular risk including age at study entry, baseline smoking/drinking status, baseline comorbidities (type 2 diabetes, hypertension, dyslipidemia), body mass index (BMI) and family history of coronary heart disease were included in the model. Cardiovascular risk

Table 1. Characteristics of participants, by endpoint outcome.

Characteristics	Had CV events (n = 79)		Did not have CV events (n = 297)		<i>p</i> -value ^a
	Mean ± SD N (%)	Range	Mean ± SD N (%)	Range	
Age at study entry (years)	63.49 ± 4.75	49–73	64.06 ± 4.66	45–74	0.3456
Baseline testosterone (nmol/L)	9.49 ± 1.22	5.89–12.13	9.75 ± 1.12	5.89–11.79	0.0941
BMI (kg/m ²)	31.86 ± 4.77	23.20–46.02	29.66 ± 3.94	22.15–46.98	0.0003
Smoker	42 (53.16)	-	96 (32.32)	-	0.001
Alcohol user	50 (63.29)	-	135 (45.45)	-	0.005
Hypertension	68 (86.08)	-	216 (72.73)	-	0.014
Diabetes	46 (58.23)	-	107 (36.03)	-	<0.001
Dyslipidemia	55 (69.62)	-	179 (60.27)	-	0.128
Family history of coronary heart disease	37 (46.84)	-	62 (20.88)	-	<0.001
Prior CV event	34 (43.04)	-	73 (24.58)	-	0.001
Follow-up (years)	7.82 ± 1.51	4–10	7.35 ± 1.79	2–11	0.0181

^a *p*-value for *t*-test if the variable is continuous; *p*-value for Chi-Square test if the variable is categorical.

CV, cardiovascular; SD, standard deviation; BMI, body mass index.

was presented in a hazard ratio (HR) and its 95% confidence interval (CI).

Analyses were stratified by prior cardiovascular event status; those with prior cardiovascular events and those without prior cardiovascular events. By the time of study, we had information available on whether patients had prior myocardial infarction, stroke and diagnosis of coronary artery disease. “With prior cardiovascular events” indicates the patient had one of the three prior conditions. The significance of the interaction term between time-varying testosterone level or baseline testosterone level and prior cardiovascular event status was also tested to examine whether the effects of testosterone level on cardiovascular events were different in the two strata. Outcome variables were myocardial infarction, stroke, or any cardiovascular events (myocardial infarction or stroke).

All analyses were performed with Stata/MP 14.0 (StataCorp LLC, College Station, TX, USA).

3. Results

As shown in Table 1, the characteristics were in general different in participants who experienced cardiovascular events and who did not during the study period. As compared to the participants who did not experience cardiovascular events, those who had cardiovascular events during the study period had higher BMI, were more likely to be a smoker or an alcohol user, have hypertension, diabetes, family history of coronary heart disease, and prior cardiovascular events, and had longer follow-up (*p*-value < 0.05). There was no difference in age at study entry, baseline testosterone level, and the proportions of patients with dyslipidemia in the two groups (*p*-value > 0.05).

Table 2 presents the results of Cox proportional hazards regression of cardiovascular events on baseline testosterone levels, stratified by patients’ prior cardiovascular event status. Among patients without prior cardiovascu-

lar events, the adjusted HR for any cardiovascular event, myocardial infarction, and stroke associated with 1 nmol/L increase in baseline testosterone level was 0.76 (95% CI: 0.61, 0.96), 0.69 (95% CI: 0.53, 0.89), and 0.78 (95% CI: 0.56, 1.09), respectively. Among patients with prior cardiovascular events, the adjusted HRs for any cardiovascular event, myocardial infarction, and stroke associated with 1 nmol/L increase in baseline testosterone level were 1.06 (95% CI: 0.83, 1.36), 1.15 (95% CI: 0.79, 1.66), and 1.02 (95% CI: 0.75, 1.39), respectively.

By including testosterone as a time-varying covariate, as shown in Table 3, among patients without prior cardiovascular events, the adjusted HRs for any cardiovascular event, myocardial infarction, and stroke associated with 1 nmol/L increase in time-varying testosterone level were 0.75 (95% CI: 0.60, 0.94), 0.64 (95% CI: 0.49, 0.84), and 0.79 (95% CI: 0.57, 1.10), respectively. Among patients with prior cardiovascular events, the adjusted HRs for any cardiovascular event, myocardial infarction, and stroke associated with 1 nmol/L increase in time-varying testosterone level were 0.93 (95% CI: 0.74, 1.17), 1.02 (95% CI: 0.73, 1.43), and 0.96 (95% CI: 0.70, 1.33), respectively.

Though stratum-specific HRs for cardiovascular events seemed different among participants with prior cardiovascular events and those without, the interaction terms between time-varying testosterone level or baseline testosterone level and patients’ prior cardiovascular event status were not different from 0 (*p*-value > 0.05; results not shown), indicating the effects of testosterone level (either time-varying or baseline) on cardiovascular risk were not different in the two populations based on formal statistical tests. Thus, collapsed tables describing the associations between time-varying testosterone level or baseline testosterone and cardiovascular events, regardless of participants’ prior cardiovascular event status were presented.

As shown in Table 4, after adjustment, no association was found between baseline testosterone level and the

Table 2. Cox proportional hazards regression of cardiovascular events on baseline testosterone levels, by prior cardiovascular event status.

	Without prior CV events (N = 269)			With prior CV events (N = 107)		
	HR (95% CI)			HR (95% CI)		
	Any CV event	MI	Stroke	Any CV event	MI	Stroke
Baseline testosterone (nmol/L)	0.76 (0.61, 0.96)	0.69 (0.53, 0.89)	0.78 (0.56, 1.09)	1.06 (0.83, 1.36)	1.15 (0.79, 1.66)	1.02 (0.75, 1.39)
Age at study entry (years)	1.02 (0.95, 1.09)	0.99 (0.91, 1.07)	1.04 (0.94, 1.14)	0.99 (0.91, 1.06)	0.95 (0.87, 1.03)	0.97 (0.89, 1.07)
BMI (kg/m ²)	1.15 (1.03, 1.28)	1.17 (1.03, 1.33)	1.06 (0.92, 1.22)	0.99 (0.92, 1.06)	0.92 (0.83, 1.03)	1.04 (0.95, 1.15)
Smoker	1.74 (0.92, 3.32)	1.42 (0.64, 3.15)	2.05 (0.82, 5.12)	1.62 (0.78, 3.37)	1.16 (0.41, 3.24)	1.84 (0.73, 4.66)
Alcohol user	1.11 (0.60, 2.04)	0.94 (0.44, 2.01)	1.07 (0.44, 2.61)	1.71 (0.79, 3.71)	2.12 (0.67, 6.66)	1.06 (0.43, 2.60)
Type 2 diabetes	1.18 (0.59, 2.36)	1.12 (0.48, 2.60)	1.95 (0.72, 5.28)	2.04 (0.98, 4.22)	4.02 (1.27, 12.72)	0.87 (0.34, 2.23)
Hypertension	1.56 (0.39, 3.25)	1.12 (0.48, 2.61)	1.84 (0.59, 5.68)	1.67 (0.21, 13.29)	-	1.52 (0.18, 13.12)
Dyslipidemia	1.05 (0.39, 2.81)	1.16 (0.33, 4.07)	1.52 (0.36, 6.44)	5.18 (1.11, 24.20)	2.24 (0.41, 12.40)	5.76 (0.69, 47.97)
Family history of coronary heart disease	2.01 (0.96, 4.20)	1.63 (0.66, 4.00)	2.35 (0.82, 6.68)	2.98 (1.39, 6.37)	3.85 (1.27, 11.64)	3.10 (1.21, 7.95)

CV, cardiovascular; HR, hazard ratio; CI, confidence interval; MI, myocardial infarction; BMI, body mass index.

Table 3. Cox proportional hazards regression of cardiovascular events on time-varying testosterone levels, by prior cardiovascular event status.

	Without prior CV events (N = 269)			With prior CV events (N = 107)		
	HR (95% CI)			HR (95% CI)		
	Any CV event	MI	Stroke	Any CV event	MI	Stroke
Time-varying testosterone (nmol/L)	0.75 (0.60, 0.94)	0.64 (0.49, 0.84)	0.79 (0.57, 1.10)	0.93 (0.74, 1.17)	1.02 (0.73, 1.43)	0.96 (0.70, 1.33)
Age at study entry (years)	1.02 (0.95, 1.08)	0.99 (0.91, 1.06)	1.03 (0.94, 1.14)	0.98 (0.91, 1.06)	0.95 (0.87, 1.03)	0.98 (0.89, 1.07)
BMI (kg/m ²)	1.13 (1.02, 1.25)	1.15 (1.01, 1.30)	1.04 (0.90, 1.19)	0.99 (0.92, 1.06)	0.93 (0.84, 1.04)	1.04 (0.94, 1.15)
Smoker	1.78 (0.94, 3.37)	1.44 (0.66, 3.17)	2.05 (0.83, 5.10)	1.60 (0.77, 3.30)	1.17 (0.42, 3.28)	1.81 (0.72, 4.56)
Alcohol user	1.12 (0.61, 2.06)	0.94 (0.44, 2.01)	1.11 (0.45, 2.74)	1.81 (0.84, 3.90)	2.25 (0.72, 7.00)	1.09 (0.45, 2.65)
Type 2 diabetes	1.13 (0.57, 2.25)	1.07 (0.46, 2.48)	1.80 (0.67, 4.80)	1.94 (0.92, 4.08)	4.00 (1.26, 12.75)	0.86 (0.34, 2.22)
Hypertension	1.63 (0.78, 3.41)	1.19 (0.50, 2.79)	1.91 (0.62, 5.91)	1.69 (0.21, 13.49)	-	1.51 (0.18, 12.95)
Dyslipidemia	0.96 (0.36, 2.58)	0.99 (0.28, 3.48)	1.40 (0.33, 5.94)	4.81 (1.04, 22.29)	2.04 (0.38, 11.05)	5.65 (0.68, 46.71)
Family history of coronary heart disease	1.90 (0.91, 3.95)	1.45 (0.60, 3.52)	2.27 (0.80, 6.46)	2.83 (1.33, 6.03)	3.54 (1.19, 10.52)	3.08 (1.20, 7.89)

CV, cardiovascular; HR, hazard ratio; CI, confidence interval; MI, myocardial infarction; BMI, body mass index.

risk of any cardiovascular event, myocardial infarction, or stroke. As shown in Table 5, by including testosterone as a time-varying covariate, the adjusted HR for any cardiovascular event associated with 1 nmol/L increase in testosterone level was 0.85 (95% CI: 0.72, 0.99); the adjusted HR for myocardial infarction associated with 1 nmol/L increase in testosterone level was 0.78 (95% CI: 0.64, 0.97); no association between time-varying testosterone level and stroke was found. This result matched our *post hoc* analysis results using stratified proportional hazards models (results not shown).

4. Discussion

We conducted a cohort study and followed 376 hypogonadal men for up to 11 years to investigate the association of time-varying testosterone level and baseline testosterone level with the risk of cardiovascular events, stratified by patients' prior cardiovascular event status. We did not find any difference in the effects of testosterone level (either baseline or time-varying) on the risk of cardiovascular events among patients with prior cardiovascular events and

those without, based on formal statistical tests. Regardless of patients' prior cardiovascular event status, a lower time-varying testosterone level was associated with a higher risk of myocardial infarction, but not of stroke; no association was found between baseline testosterone level and the risk of cardiovascular events.

Srinath *et al.* [22] investigated the association between baseline endogenous plasma testosterone level and incident clinical cardiovascular events using data of 1558 male participants from the Atherosclerosis Risk in Communities (ARIC) study. Although low baseline testosterone level was associated with cardiovascular risk factors such as higher BMI and greater waist circumference at baseline, no association was found between baseline testosterone level and cardiovascular event incidence, which was consistent with our findings. This is possibly because the baseline testosterone level might be irrelevant for the cardiovascular events that occurred many years later. In a recent study, Gagliano-Jucá *et al.* [21] reviewed large prospective cohort studies that assessed cardiovascular outcomes and used reliable assays for the measurement of testosterone levels; inconsistent findings were reported. Yeap *et al.* [27], Ohls-

Table 4. Cox proportional hazards regression of cardiovascular events on baseline testosterone levels.

	Total (N = 376)		
	HR (95% CI)		
	Any CV event	MI	Stroke
Baseline testosterone (nmol/L)	0.91 (0.77, 1.07)	0.85 (0.69, 1.05)	0.90 (0.72, 1.13)
Age at study entry (years)	1.00 (0.95, 1.05)	0.97 (0.92, 1.03)	1.01 (0.94, 1.08)
BMI (kg/m ²)	1.05 (0.98, 1.11)	1.02 (0.95, 1.11)	1.06 (0.98, 1.14)
Smoker	1.78 (1.12, 2.84)	1.43 (0.78, 2.62)	1.84 (0.99, 3.44)
Alcohol user	1.42 (0.90, 2.23)	1.36 (0.76, 2.43)	1.10 (0.60, 2.03)
Type 2 diabetes	1.59 (0.98, 2.57)	1.98 (1.05, 3.75)	1.28 (0.66, 2.51)
Hypertension	1.77 (0.90, 3.49)	1.35 (0.60, 3.06)	1.88 (0.70, 5.05)
Dyslipidemia	2.00 (0.92, 4.36)	1.74 (0.66, 4.56)	2.33 (0.75, 7.24)
Family history of coronary heart disease	2.34 (1.42, 3.85)	2.08 (1.09, 3.99)	2.60 (1.34, 5.05)
Prior CV event	1.59 (0.96, 2.64)	1.28 (0.66, 2.51)	2.04 (1.06, 3.94)

HR, hazard ratio; CI, confidence interval; CV, cardiovascular; MI, myocardial infarction; BMI, body mass index.

Table 5. Cox proportional hazards regression of cardiovascular events on time-varying testosterone levels.

	Total (N = 376)		
	HR (95% CI)		
	Any CV event	MI	Stroke
Time-varying testosterone (nmol/L)	0.85 (0.72, 0.99)	0.78 (0.64, 0.97)	0.90 (0.72, 1.13)
Age at study entry (years)	1.00 (0.95, 1.05)	0.97 (0.92, 1.03)	1.01 (0.94, 1.08)
BMI (kg/m ²)	1.04 (0.98, 1.10)	1.01 (0.94, 1.09)	1.05 (0.97, 1.13)
Smoker	1.76 (1.10, 2.80)	1.40 (0.76, 2.58)	1.83 (0.98, 3.43)
Alcohol user	1.42 (0.91, 2.24)	1.34 (0.75, 2.39)	1.10 (0.60, 2.03)
Type 2 diabetes	1.52 (0.94, 2.46)	1.89 (1.00, 3.58)	1.22 (0.63, 2.37)
Hypertension	1.82 (0.93, 3.56)	1.39 (0.62, 3.13)	1.94 (0.73, 5.19)
Dyslipidemia	1.96 (0.90, 4.28)	1.64 (0.62, 4.33)	2.35 (0.76, 7.31)
Family history of coronary heart disease	2.29 (1.39, 3.77)	1.99 (1.04, 3.82)	2.59 (1.34, 5.03)
Prior CV event	1.58 (0.95, 2.61)	1.26 (0.64, 2.46)	2.07 (1.07, 4.00)

HR, hazard ratio; CI, confidence interval; CV, cardiovascular; MI, myocardial infarction; BMI, body mass index.

son *et al.* [28], Soisson *et al.* [16], and Magnani *et al.* [29] found a negative association between testosterone level and the risk of adverse cardiovascular outcomes including transient ischemic attack, myocardial infarction, unstable angina, and atrial fibrillation, whereas Arnlov *et al.* [30], Abbott *et al.* [31], Vikan *et al.* [15], Haring *et al.* [10], and Shores *et al.* [32] did not find any association between testosterone and the cardiovascular risk. In addition to the different study population characteristics and the small number of events, which might contribute to the discrepancies in their study findings, Gagliano-Jucá *et al.* [21] also highlighted some limitations of these studies, including one single testosterone measurement, which was suboptimal given the variation in serum testosterone levels over time.

In our study, besides baseline testosterone level, we also assessed the association between time-varying testosterone level and the risk of cardiovascular events. We found lower time-varying testosterone level was associated with a higher risk of myocardial infarction. A large population-

based retrospective matched-cohort study of men aged 66 years or older with a median follow-up of 5 years conducted in Canada assessed the effect of testosterone therapy exposure duration on all-cause mortality and a composite cardiovascular outcome (consisting of myocardial infarction, cerebrovascular accident, and venous thromboembolic event). Unlike our analysis, where continuous time-varying exposure (testosterone level) was added to the model to evaluate the effect of testosterone level at each visit and the risk of cardiovascular events, the authors used the time-varying testosterone therapy exposure and assessed the cumulative effect of testosterone therapy on mortality. They found that the mortality was progressively lower with increasing exposure to testosterone, with a decreased risk for patients with the highest tertile of exposure (HR = 0.60, 95% CI: 0.45, 0.80). Both their study and ours considered the dynamic changes of exposure and applied advanced statistical techniques to address that, which avoids imprecise estimates due to overly simplistic exposure definition. Loo *et al.* [33] mentioned another two cohort studies in

a systematic review that investigated the treatment effect of testosterone on the stroke risk also mentioned including testosterone treatment as a time-varying variable. However, only testosterone therapy exposure at baseline was used in the analysis, and therefore no “time-varying” factor was actually accounted for in the analysis. In our study, we only included patients who did not take testosterone therapy, and no patients switched to the treatment group, and therefore only testosterone level was included as a time-varying variable, not the treatment status.

The effects of testosterone might be different among patients with prior cardiovascular events and those without, given the different health conditions in the two populations. Patients with prior cardiovascular events might be more likely to have comorbidities and pathological changes of their arteries, and therefore more vulnerable to cardiovascular events when exposed to risk factors such as testosterone deficiency. Srinath *et al.* [22] investigated the association between endogenous testosterone level and cardiovascular event occurrence among patients without prior cardiovascular events and did not find any association. Collet *et al.* [23] assessed whether testosterone level and sex hormone-binding globulin (SHBG) affected the risk of incident cardiovascular events among patients free of prior cardiovascular events and did not find any association. Chmiel *et al.* [34] found an increased risk of major cardiovascular events associated with low testosterone levels among patients with prior cardiovascular events. However, there is no easy way to compare these associations as they were assessed in different source populations. In this study, we used the same source population from a registry study in Germany and compared the effects of testosterone level on the risk of cardiovascular events among patients with and without prior cardiovascular events. However, due to the limited number of participants in the stratified analysis, we were underpowered to detect the potential difference. Future studies are warranted to identify the more susceptible population and provide targeted intervention if necessary.

There is growing literature on the importance of normal lipid panel and glucose level in maintaining cardiovascular health, with little known about the role of testosterone. The relationship between testosterone deficiency and cardiovascular disease is complex and remains controversial. It has been suggested that testosterone deficiency modulates lipid profiles and glucose metabolism and contributes to atherosclerosis development or progression; androgen deprivation therapy that is previously used to treat prostate cancer increases the risk of coronary heart disease, diabetes, and cardiovascular mortality [35]. Animal experiments have demonstrated that the impaired lipid and glucose profile due to testosterone deficiency or androgen deprivation could be reversed by testosterone therapy. A research team reported that low testosterone in the testicular feminized mice had increased lipid deposition in the aortic root and liver when fed a high-cholesterol diet; testos-

terone therapy to return levels to those in wild-type counterparts reduced aortic fatty streaks and hepatic lipid accumulation [36]. In clinical trials, testosterone administration increased coronary artery diameter and flow, improved cardiac ischemia, and reduced peripheral vascular resistance in chronic heart failure. This might be explained by testosterone being an L-calcium channel blocker and inducing potassium channel activation in vascular smooth muscle [13]. In a review study, Lorigo *et al.* [8] pointed that testosterone triggers both genomic and non-genomic pathways that lead to an improvement of various risk factors for the cardiovascular disease development or progression. In our recent study that investigated the treatment effect of testosterone therapy on Framingham Global Cardiovascular Disease Risk Score among the same source of patients (but including both treatment and control groups), we found improved risk score in the treatment group and worsening situation in the control group. During the follow-up, 45 cardiovascular events occurred in the control group versus 0 cases in the treatment group, indicating a beneficial effect of testosterone therapy on reducing cardiovascular risk.

This study has several strengths. First, the follow-up duration is longer than most prior studies, which should add to the validity of our results since more observations contribute to a higher precision of our estimates. Second, the repeated measurement of testosterone levels enables us to investigate the association of time-varying testosterone level at each visit with the risk of cardiovascular events, which might be more relevant as compared to the baseline testosterone level. Limitations should also be noted. First, the study results are specific to the population studied and may not be generalizable. Second, because of the nature of observational study design, residual confounding cannot be entirely ruled out. Third, there are several types of strokes, among which ischemic stroke is more closely related to atherosclerosis and excess lipid accumulation that testosterone deficiency may contribute to. However, in this dataset, we did not have information on the specific type of stroke that the patient had, and it could be the reason why we did not find any association between testosterone and stroke in this study. Fourth, we did not have detailed information for some covariates in this study. For example, for “alcohol users”, we only knew the patients were occasionally drinking alcohol at study entry, yet we did not know the exact amount. Also, we did not have information on patients’ physical activity level, which is considered to have an impact on testosterone levels. However, given the participants’ age category (elderly men) and overall degeneration of physiological and physical condition, we believe the light difference if any on physical activity levels would not significantly impact the results. Additionally, as compared to total testosterone level, free testosterone level might be more biologically relevant in investigating the proposed association. However, by the time of this study, we only had data available on total testosterone level and therefore were

not able to explore further on the effect of time-varying free testosterone level on cardiovascular risk. Last, it is possible that no direct relationship exists between testosterone and cardiovascular risk, and that testosterone level is only a marker of illness and comorbidities, or that both low testosterone level and the development of cardiovascular disease may occur concurrently [21,22]. However, without direct measurements before and after treatment to various comorbidities, there is no clear way to assess these relationships.

5. Conclusions

In conclusion, in this cohort study with extended follow-up, we found lower time-varying testosterone level was associated with a higher risk of myocardial infarction, but not of stroke. We did not find any association between baseline testosterone level and cardiovascular risk. One single measurement of testosterone level may not be sufficient to evaluate one's cardiovascular risk, given the dynamic changes of testosterone levels over men's life course. The associations between testosterone level and cardiovascular events were not different among patients with prior cardiovascular events and those without. Future longitudinal studies are needed to confirm our study findings.

Author contributions

XZ, JH, HZ, NJ, and XX contributed to the conception and design of the study. FS, KSH, and AH provided access to the data. XZ and HZ contributed to methodology, analyses, and interpretation of data. XZ prepared the original draft. HZ, JH, NJ, FS, KSH, AH, and XX reviewed and edited the manuscript.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of German Medical Association.

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Conflict of interest

FS works as a consultant to Medical Affairs, Bayer AG, manufacturer and distributor of testosterone undecanoate injections. AH has received research support, lecture honoraria and travel grants from Bayer AG. KSH has received lecture honoraria and travel grants from Bayer AG. Other authors declare no competing interests.

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