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Normalization of Testosterone Levels After Testosterone Replacement Therapy Is Not Associated With Reduced Myocardial Infarction in Smokers

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Abstract

Objective: To examine the effect of cigarette smoking (CS) status and total testosterone (TT) levels after testosterone replacement therapy (TRT) on all-cause mortality, myocardial infarction (MI), and stroke in male smokers and nonsmokers without history of MI and stroke.

Participants and Methods: Data from 18,055 males with known CS status and low TT levels who received TRT at the Veterans Health Administration between December 1, 1999, and May 31, 2014, were grouped into (1) current smokers with normalized TT, (2) current smokers with nonnormalized TT, (3) nonsmokers with normalized TT, and (4) nonsmokers with nonnormalized TT. Combined effect of CS status and TT level normalization after TRT on all-cause mortality, MI, and stroke was compared using propensity score–weighted Cox proportional hazard models.

Results: Normalization of serum TT levels in nonsmokers was associated with a significant decrease in all-cause mortality (hazard ratio [HR]=0.526; 95% CI, 0.477-0.581; $P<.001$) and MI (HR=0.717; 95% CI, 0.522-0.986; $P<.001$). Among current smokers, normalization of serum TT levels was associated with a significant decrease in only all-cause mortality (HR=0.563; 95% CI, 0.488-0.649; $P<.001$) without benefit in MI (HR=1.096; 95% CI, 0.698-1.720; $P=.69$). Importantly, compared with nonsmokers with normalized TT, all-cause mortality (HR=1.242; 95% CI, 1.104-1.396; $P<.001$), MI (HR=1.706; 95% CI, 1.242-2.342; $P=.001$), and stroke (HR=1.590; 95% CI, 1.013-2.495; $P=.04$) were significantly higher in current smokers with normalized TT.

Conclusion: We conclude that active CS may negate the protective effect of testosterone level normalization on all-cause mortality and MI after TRT.

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At any given age, men are more vulnerable than women to death from cardiovascular disease (CVD).[1] Male gender is thought to be a strong independent risk factor for coronary artery disease (CAD), myocardial infarction (MI), and stroke.[2] The male sex hormone testosterone is postulated to play a role in the observed incidence of CAD, MI, and stroke in men.[2-4] *Ex vivo* studies showing increased human platelet thromboxane A2 receptor density and aggregation in response to testosterone lend support to this hypothesis.[5] However, other studies have provided data that highlight the importance of normal testosterone levels for cardiovascular (CV) health specifically in symptomatic subjects with persistently low testosterone levels.[6-9] The significance of testosterone replacement therapy (TRT) in CV health is a topic of ongoing debate as

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several studies have reported higher CV events with TRT.[10,11] In contrast, we and others have found that normalization of testosterone levels after TRT is associated with a significant decrease in all-cause mortality and MI.[6,7]

Lifestyle choices such as cigarette smoking (CS) could also contribute to this disparity in CVD between men and women. Historically, CS rates are higher in men than in women.[12] Our group and others have shown that CS is associated with increased atherothrombotic events.[13-18] In an experimental study using rabbit aorta it was reported that physiologic levels of testosterone augmented the endothelial dysfunction associated with environmental tobacco smoke exposure.[19] Therefore, it is conceivable that a combination of testosterone and CS could additively increase the risk of MI and stroke in male smokers *via* their reported prothrombotic effects. However, to our knowledge, no study has specifically examined the combined effect of CS and testosterone level on mortality, MI, or stroke in humans after TRT. It is also unclear whether normal testosterone levels indeed potentiate the active CS-related adverse effect on the mortality and CV events as suggested by some *ex vivo* and preclinical data.[5,19]

In this study, we examined whether CS status modulated the risk for all-cause mortality, MI, and stroke in men in relation to normalized testosterone level after TRT.

PARTICIPANTS AND METHODS

We conducted a retrospective cohort study of male veterans who received medical care in the Veterans Health Administration (VHA) from December 1, 1999, to May 31, 2014. Data were retrieved from Veterans Administrations Corporate Data Warehouse (CDW) through the Veterans Administrations Informatics and Computing Infrastructure.[20] The VHA provides care to veterans at more than 1400 establishments across the United States and each veteran is assigned a unique identifier in the CDW database. The Institutional Review Board of the Veterans Affairs Medical Center, Kansas City, MO, approved the study. The quality of data from these sources is well documented, and the data have been widely used by investigators for retrospective longitudinal studies.[21]

Study Design

This study was designed to determine the effect of the interaction between smoking and testosterone levels on CV events by determining the incidence of MI, stroke, and all-cause mortality in subpopulations of patients who received TRT. Cardiovascular events and comorbidities in patients were identified according to *International Classification of Diseases, 9th Revision* (*ICD-9*) codes. Patients included in the study had their testosterone levels checked on at least 2 separate occasions as recommended by guidelines.[22]

Determination of Exposure to TRT and Smoking. Patients' medical records were used to ascertain prescriptions for TRT administration. For this study, patients who received any form of TRT (injection, gel, or patch) were considered as treated. Smoking status was obtained from HealthFactors files in the Veterans Administrations Informatics and Computing Infrastructure database. Only those patients whose smoking status could be verified from the database were included in the study. Patients were classified as current smokers (smoking at the beginning of the study and at every encounter throughout the study period), nonsmokers (never smoked or had quit smoking at the beginning of the study and did not resume smoking throughout the study period).

Determination of Total Testosterone Levels. Total testosterone (TT) levels were considered low when the reported serum TT values were below the lower limit of normal laboratory reference range for each test result. This approach permitted inclusion of results from most laboratories in the Veterans Administration (VA) health system over the study period ($\geq$14 years). The available test results from such a long period of follow-up lacked a uniform laboratory range for normal TT level and reporting units in the database. We chose to use this method rather than a discrete cutoff value because we found that facilities within the VHA system used different assay methods with different reference ranges and reporting units.[23,24] Even in the same hospital, the assay used could change over time. Moreover, lack of standardized values for serum testosterone levels and other stoichiometric measures made

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it difficult to select a single set of minimum and maximum values.[25,26] Thus, we categorized each test result as normal or low on the basis of their respective reference range reported with the test result, because this was the most accurate method to minimize the effect of use of multiple assays.

Outcome Measures. Primary outcome measures were (1) all-cause mortality, (2) the incidence of MI (*ICD-9* codes 410.x0 and 410.x1), and (3) the incidence of ischemic-stroke (*ICD-9* codes 433.x1 and 434 [excluding 434.x0], or 436) determined using dates of death in CDW data augmented with vital status files.

Confounding Factors Measured. Confounding measures included patients' demographic characteristics, comorbidities such as diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD), obstructive sleep apnea (OSA), congestive heart failure (CHF), peripheral vascular disease (PVD), and CAD, and other factors. Other factors included the baseline body mass index (BMI), low-density lipoprotein (LDL), and use of aspirin, β-blockers, and statins. The *International Classification of Diseases, Ninth Revision, Clinical Modification* diagnosis codes were used to capture the coexisting conditions.

Study Population

Inclusion Criteria. All male patients with TT levels lower than the corresponding normal laboratory reference range during initial laboratory assay were included.

Exclusion Criteria. (1) Females, (2) those who received TRT before the first available low-TT values, and (3) those who suffered MI or stroke before the first day of the study were excluded.

Eligible study patients were divided into 4 groups: (1) current smokers with normalized TT levels after TRT, (2) current smokers with nonnormalized TT levels after TRT, (3) nonsmokers with normalized TT levels after TRT, and (4) nonsmokers with nonnormalized TT levels after TRT. Normalized-treated are those whose TT levels normalized during follow-up visits and throughout the study. Nonnormalized-treated did not normalize throughout the study.

Statistical Analyses

Means and SDs were used to report continuous variables, whereas percentages were used for categorical variables. Nonparametric tests were used to compare nonnormally distributed baseline characteristics of study participants. Chi-square test and *t* test were used for normally distributed characteristics. First, we fitted models to determine the impact of smoking on mortality and MI. We then determined how TRT impacted the effects of smoking status on the same health outcomes. We conducted univariate and multivariable Cox proportional hazard regression analyses to determine the differences between each pair of the study groups. To correct for potential systematic differences between untreated and treated groups, propensity scores were computed. For each patient, propensity scores for receiving TRT were computed by adjusting for the covariates in a logistic regression analysis. The covariates included were age, BMI, hypertension, DM, OSA, COPD, PVD, CHF, LDL, CAD, and the use of aspirin, statins, and β-blockers. For a robust analysis of our data, propensity score–weighted stabilized inverse probability of treatment weights (IPTW) was used.[27,28] In addition, using the stabilized-IPTW approach, Kaplan-Meier survival curves were generated to compare event-free survival time between the groups. The SAS Enterprise Guide 7.1 supported on SAS 9.4 was used for statistical analyses with TRT as a time-varying factor. All hypothesis tests were 2-sided and carried out at a significance level of .05.

RESULTS

Cohort Description

From our database, smoking status could be verified in 18,055 patients who received TRT. They were categorized into current smokers (5500) and nonsmokers (12,555). Each smoking status was further divided into normalized-treated and nonnormalized-treated depending on whether their testosterone levels normalized after receiving TRT. In the current smokers group, 3414 patients had normalized TT levels and 2086 patients had nonnormalized TT levels after TRT. In

TABLE 1. All Treated Patients: Current Smokers vs Nonsmokers (Ref = Nonsmokers)[a,b]

Characteristic	Unmatched cohort			Propensity-matched cohort (stabilized IPTW)		
	Current smokers (N=5500)	Nonsmokers (N=12,555)	*P* value	Current smokers (N=5509)	Nonsmokers (N=12,549)	*P* value
Age ≥50 y	4824 (87.7)	11,558 (92.1)	<.001	4990 (90.7)	11,386 (90.7)	1.00
Age (y), median	63.0	67.0		63.0	67.0	
Body mass index ≥30 kg/m^2	3276 (59.6)	8,661 (69.0)	<.001	3658 (66.4)	8,312 (66.2)	.84
Body mass index (kg/m^2), mean ± SD	32.0±6.6	33.6±6.7		32.8±6.6	33.3±6.8	
Follow-up time (y), mean ± SD	5.3±3.1	6.0±3.3		5.4±3.1	6.0±3.4	
Hypertension	1312 (23.9)	2,571 (20.5)	<.001	1196 (21.7)	2,704 (21.6)	.81
Diabetes mellitus	1574 (28.6)	4,632 (36.8)	<.001	1906 (34.6)	4,320 (34.4)	.83
Chronic obstructive pulmonary disease	155 (2.8)	162 (1.3)	<.001	98 (1.8)	222 (1.8)	.94
Obstructive sleep apnea	123 (2.2)	299 (2.4)	.55	134 (2.4)	297 (2.4)	.79
Congestive heart failure	111 (2.0)	323 (2.6)	.03	141 (2.6)	304 (2.4)	.58
Peripheral vascular disease	96 (1.8)	114 (0.9)	<.001	63 (1.2)	145 (1.2)	.97
Coronary artery disease	358 (6.5)	827 (6.6)	.85	370 (6.7)	832 (6.6)	.82
Depression	854 (15.5)	1,023 (8.2)	<.001	567 (10.3)	1,297 (10.3)	.93
LDL >100 mg/dL	2851 (51.8)	6,158 (49.1)	<.001	2751 (49.9)	6,263 (49.9)	.97
Concomitant therapy with						
Antiplatelet agents (ASA)	1869 (34.0)	4,148 (33.0)	.22	1841 (33.4)	4,186 (33.4)	.94
β-Blockers	2242 (40.8)	5,345 (42.6)	.02	2325 (42.2)	5,281 (42.1)	.88
Statins	3296 (59.9)	8,158 (65.0)	<.001	3504 (63.6)	7,967 (63.5)	.89

	All-cause mortality			Myocardial infarction			Stroke		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Univariate N=5500 *vs* 12,555	1.281	1.175-1.397	<.001	1.358	1.047-1.760	.02	1.693	0.973-2.946	.06
Propensity matched (stabilized IPTW) N=5509 vs 12,549	1.217	1.115-1.327	<.001	1.396	1.079-1.806	.01	1.739	1.201-2.519	.003

[a]ASA = aspirin; HR = hazard ratio; IPTW = inverse probability of treatment weights; LDL = low-density lipoprotein; Ref = reference.
[b]Values represent n (%) unless otherwise indicated.

the nonsmokers group, 8137 patients had normalized TT levels and 4418 had nonnormalized TT levels after TRT.

Study Subjects

Tables 1 to 4 present the characteristics at baseline for different groups of patients: all patients, smokers, nonsmokers, and the normalized cohorts. Differences with respect to age, BMI, DM, hypertension, OSA, COPD, PVD, CHF, LDL, CAD, and use of aspirin, β-blockers, and statins between the study groups were normalized by using IPTW ($P>.05$).

Primary Outcome Based on Smoking Status in the Final Cohort

There were 18,055 patients who received TRT (Table 1). Of these, 5500 (30.5%) were smokers. Mean follow-up time (years) was 5.4±3.1 and 6.0±3.4 in the current smokers and nonsmokers groups, respectively. There were 2611 (95% CI, 2512-2713) *vs* 2118 (95% CI, 2029-2210) mortalities per 100,000 person-years; 302 (95% CI, 269-337) vs 218 (95% CI, 190-248) MI events per 100,000 person-years; and 67 (95% CI 52-85) *vs* 45 (95% CI 33-60) stroke events per 100,000 person-years in the current smokers and nonsmokers groups, respectively. Of the treated population, current smokers had a significantly higher mortality risk (hazard ratio [HR]=1.217; 95% CI, 1.115-1.327; $P<.001$), significantly higher MI risk (HR=1.396; 95% CI, 1.079-1.806; $P=.011$), and significantly higher stroke risk (HR=1.739; 95% CI, 1.201-2.519; $P=.003$) than did nonsmokers.

Effect of TRT on Current Smokers

In the treated group, there were 5500 current smokers of whom 3414 (62.07%) had their

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TABLE 2. Only Current Smokers[a,b]

Characteristic	Normalized-treated *vs* nonnormalized-treated (ref = nonnormalized-treated)					
	Unmatched cohort			Propensity-matched cohort (stabilized IPTW)		
	Normalized-treated (N=3414)	Nonnormalized-treated (N=2086)	*P* value	Normalized-treated (N=3414)	Nonnormalized-treated (N=2086)	*P* value
Age ≥50 y	2988 (87.5)	1836 (88.0)	.59	2995 (87.7)	1830 (87.7)	1.00
Age (y), median	63.0	63.0		63.0	63.0	
Body mass index ≥30 kg/m^2	1992 (58.4)	1284 (61.6)	.02	2034 (59.6)	1242 (59.6)	1.00
Body mass index (kg/m^2), mean ± SD	31.8±6.6	32.3±6.7		31.8±6.6	32.3±6.7	
Follow-up time (y), mean ± SD	5.8±3.0	4.5±3.0		5.8±3.0	4.5±3.0	
Hypertension	775 (22.7)	537 (25.7)	.01	815 (23.9)	498 (23.9)	.99
Diabetes mellitus	920 (27.0)	654 (31.4)	<.001	978 (28.6)	598 (28.7)	1.00
Chronic obstructive pulmonary disease	94 (2.8)	61 (2.9)	.71	97 (2.8)	60 (2.9)	.96
Obstructive sleep apnea	63 (1.9)	60 (2.9)	.01	77 (2.3)	47 (2.3)	1.00
Congestive heart failure	63 (1.9)	48 (2.3)	.24	70 (2.0)	43 (2.0)	.99
Peripheral vascular disease	52 (1.5)	44 (2.1)	.11	60 (1.7)	36 (1.7)	1.00
Coronary artery disease	213 (6.2)	145 (7.0)	.30	223 (6.5)	137 (6.6)	.96
Depression	514 (15.1)	340 (16.3)	.22	530 (15.5)	324 (15.5)	1.00
LDL >100 mg/dL	1830 (53.6)	1021 (49.0)	<.001	1769 (51.8)	1080 (51.8)	.98
Concomitant therapy with						
Antiplatelet agents (ASA)	1104 (32.3)	765 (36.7)	.001	1162 (34.0)	711 (34.1)	.98
β-Blockers	1337 (39.2)	905 (43.4)	.002	1391 (40.7)	849 (40.7)	.98
Statins	1998 (58.5)	1298 (62.2)	.007	2047 (60.0)	1250 (60.0)	1.00

	All-cause mortality			Myocardial infarction			Stroke		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Univariate N=3414 *vs* 2086	0.534	0.462-0.616	<.001	1.051	0.670-1.649	.83	0.748	0.298-1.878	.54
Propensity-matched (stabilized IPTW) N= 3414 vs 2086	0.563	0.488-0.649	<.001	1.096	0.698-1.720	.69	0.792	0.424-1.481	.47

[a]ASA = aspirin; HR = hazard ratio; IPTW = inverse probability of treatment weights; LDL = low-density lipoprotein; ref = reference.
[b]Values represent n (%) unless otherwise indicated.

TT levels normalized after TRT (Table 2). Mean follow-up time (years) was 5.8±3.0 and 4.5±3.0 in the normalized-treated and nonnormalized groups, respectively. There were 2101 (95% CI, 2012-2193) *vs* 3675 (95% CI, 3557-3796) mortalities per 100,000 person-years; 303 (95% CI, 270-339) *vs* 298 (95% CI, 265-334) MI events per 100,000 person-years; and 66 (95% CI, 51-83) *vs* 75 (95% CI, 59-94) stroke events per 100,000 person-years in the normalized-treated and nonnormalized groups, respectively. In current smokers, normalization of testosterone with TRT resulted in a lower mortality risk compared with those who did not reach normalized testosterone levels (HR=0.563; 95% CI, 0.488-0.649; *P*<.001). However, there was no significant difference in the risk of MI (HR=1.096; 95% CI, 0.698-1.720; *P*=.69) and stroke (HR=0.792; 95% CI, 0.424-1.481; *P*=.47) between the normalized and nonnormalized current smokers.

Effect of TRT on Nonsmokers

In the treated group, there were 12,555 nonsmokers of whom 8137 (64.8%) had their TT levels normalized after TRT (Table 3). Mean follow-up time (years) was 6.6±3.3 and 5.0±3.2 in the normalized-treated and nonnormalized groups, respectively. There were 1687 (95% CI, 1607-1769) *vs* 3110 (95% CI, 3002-3221) mortalities per 100,000 person-years; 183 (95% CI, 157-211) *vs* 299 (95% CI, 266-334) MI events per 100,000 person-years; and 47 (95% CI, 35-63) *vs* 41 (95% CI, 29-56) stroke events per 100,000 person-years in the normalized-treated and nonnormalized groups, respectively. In the nonsmoking groups, normalization of testosterone with TRT resulted in a significantly

TABLE 3. Only Nonsmokers[a,b]

Characteristic	Normalized-treated *vs* nonnormalized-treated (ref = nonnormalized-treated)					
	Unmatched cohort			Propensity-matched cohort (stabilized IPTW)		
	Normalized-treated (N=8137)	Nonnormalized-treated (N=4418)	*P* value	Normalized-treated (N=8137)	Nonnormalized-treated (N=4418)	*P* value
Age ≥50 y	7508 (92.3)	4050 (91.7)	.24	7491 (92.1)	4068 (92.1)	.99
Age (y), median	67.0	67.0		67.0	67.0	
Body mass index ≥30 kg/m^2	5518 (67.8)	3143 (71.1)	<.001	5613 (69.0)	3047 (69.0)	.99
Body mass index (kg/m^2), mean ± SD	33.3±6.5	34.1±7.1		33.3±6.5	34.1±7.1	
Follow-up time (y), mean ± SD	6.6±3.3	5.0±3.2		6.6±3.3	5.0±3.2	
Hypertension	1549 (19.0)	1022 (23.1)	<.001	1667 (20.5)	905 (20.5)	.99
Diabetes mellitus	2773 (34.1)	1859 (42.1)	<.001	3001 (36.9)	1628 (36.9)	.97
Chronic obstructive pulmonary disease	83 (1.0)	79 (1.8)	<.001	105 (1.3)	57 (1.3)	1.00
Obstructive sleep apnea	153 (1.9)	146 (3.3)	<.001	105 (2.4)	194 (2.4)	.98
Congestive heart failure	166 (2.0)	157 (3.6)	<.001	209 (2.6)	113 (2.6)	.99
Peripheral vascular disease	58 (0.7)	56 (1.3)	.002	74 (0.9)	40 (0.9)	.98
Coronary artery disease	469 (5.8)	358 (8.1)	<.001	538 (6.6)	292 (6.6)	.97
Depression	632 (7.8)	391 (8.9)	.03	665 (8.2)	363 (8.2)	.95
LDL >100 mg/dL	4177 (51.3)	1981 (44.8)	<.001	3990 (49.0)	2166 (49.0)	.99
Concomitant therapy with						
Antiplatelet agents (ASA)	2594 (31.9)	1554 (35.2)	<.001	2688 (33.0)	1461 (33.0)	.98
β-Blockers	3354 (41.2)	1991 (45.1)	<.001	3465 (42.6)	1883 (42.6)	.98
Statins	5141 (63.2)	3017 (68.3)	<.001	5289 (65.0)	2872 (65.0)	.99

	All-cause mortality			Myocardial infarction			Stroke		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Univariate N=8137 *vs* 4418	0.495	0.448-0.547	<.001	0.622	0.455-0.852	.003	1.000	0.466-2.147	1.00
Propensity-matched (stabilized IPTW) N=8137 vs 4418	0.526	0.477-0.581	<.001	0.717	0.522-0.986	.04	1.069	0.652-1.754	.79

[a]ASA = aspirin; HR = hazard ratio; IPTW = inverse probability of treatment weights; LDL = low-density lipoprotein; ref = reference.
[b]Values represent n (%) unless otherwise indicated.

lower mortality risk (HR=0.526; 95% CI, 0.477-0.581; *P*<.001) and significantly lower MI risk (HR=0.717; 95% CI, 0.522-0.986; *P*=.04) compared with their nonnormalized counterparts. Stroke events were not different between normalized and nonnormalized nonsmokers (HR=1.069; 95% CI, 0.652-1.754; *P*=.79).

Effect of Testosterone Normalization After TRT on Current Smokers *vs* Nonsmokers

There were 11,551 patients whose TT level normalized after TRT (Table 4). Of these, 3414 (29.6%) were smokers. Mean follow-up time (years) was 5.8±3.0 and 6.6±3.3 in the current smokers and nonsmokers groups, respectively. There were 2101 (95% CI, 2012-2193) *vs* 1687 (95% CI, 1607-1769) mortalities per 100,000 person-years; 303 (95% CI, 270-339) *vs* 183 (95% CI, 157-211) MI events per 100,000 person-years; and 66 (95% CI 51-83) *vs* 47 (95% CI, 35-63) stroke events per 100,000 person-years in the current smokers and nonsmokers groups, respectively. In these normalized-treated groups, current smokers had a significantly higher mortality risk (HR=1.242; 95% CI, 1.104-1.396; *P*<.001), higher MI risk (HR=1.706; 95% CI, 1.242-2.342; *P*=.001), and higher stroke risk (HR=1.590; 95% CI, 1.013-2.495; *P*=.04) than did nonsmokers.

DISCUSSION

The present study compared CV events and all-cause mortality in smokers and nonsmokers after TRT. Our results showed the following: (1) There was a significant decrease in all-cause mortality and MI in nonsmokers

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TABLE 4. Subanalysis Among Treated-Normalized Patients[a,b]

Characteristic	Current smokers vs nonsmokers (ref = nonsmokers)					
	Unmatched cohort			Propensity-matched cohort (stabilized IPTW)		
	Current smokers (N=3414)	Nonsmokers (N=8137)	*P* value	Current smokers (N=3420)	Nonsmokers (N=8133)	P value
Age ≥50 y	2988 (87.5)	7508 (92.3)	<.001	3108 (90.9)	7391 (90.9)	1.00
Age (y), median	63.0	67.0		63.0	67.0	
Body mass index ≥30 kg/m^2	1992 (58.4)	5518 (67.8)	<.001	2234 (65.3)	5299 (65.2)	.85
Body mass index (kg/m^2), mean ± SD	31.8±6.6	33.3±6.5		32.6±6.6	33.0±6.6	
Follow up time (y), mean ± SD	5.8±3.0	6.6±3.3		5.9±3.0	6.5±3.3	
Hypertension	775 (22.7)	1549 (19.0)	<.001	696 (20.3)	1639 (20.2)	.82
Diabetes mellitus	920 (27.0)	2773 (34.1)	<.001	1105 (32.3)	2604 (32.0)	.76
Chronic obstructive pulmonary disease	94 (2.8)	83 (1.0)	<.001	53 (1.6)	124 (1.5)	.91
Obstructive sleep apnea	63 (1.9)	153 (1.9)	.90	68 (2.0)	155 (1.9)	.78
Congestive heart failure	63 (1.9)	166 (2.0)	.49	72 (2.1)	162 (2.0)	.69
Peripheral vascular disease	58 (0.7)	52 (1.5)	<.001	32 (0.9)	75 (0.9)	.96
Coronary artery disease	213 (6.2)	469 (5.8)	.32	206 (6.0)	484 (6.0)	.87
Depression	514 (15.1)	632 (7.8)	<.001	336 (9.8)	801 (9.9)	.98
LDL >100 mg/dL	1830 (53.6)	4177 (51.3)	.03	1781 (52.1)	4232 (52.0)	.96
Concomitant therapy with						
Antiplatelet agents (ASA)	1104 (32.3)	2594 (31.9)	.63	1104 (32.3)	2608 (32.1)	.83
β-Blockers	1337 (39.2)	3354 (41.2)	.04	1390 (40.7)	3303 (40,6)	.97
Statins	1998 (58.5)	5141 (63.2)	<.001	2121 (62.0)	5031 (61.9)	.88

	All-cause mortality			Myocardial infarction			Stroke		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Univariate N=3414 *vs* 8137	1.308	1.164-1.469	<.001	1.639	1.187-2.261	.003	1.573	0.804-3.080	.19
Propensity-matched (stabilized IPTW) N=3420 vs 8133	1.242	1.104-1.396	<.001	1.706	1.242-2.342	.001	1.590	1.013-2.495	.04

[a]ASA = aspirin; HR = hazard ratio; IPTW = inverse probability of treatment weights; LDL = low-density lipoprotein; ref = reference.
[b]Values represent n (%) unless otherwise indicated.

who achieved normalized testosterone levels through TRT. (2) In current smokers, normalization of testosterone levels after TRT resulted in decreased incidence of all-cause mortality only compared with the current smokers who did not achieve normal testosterone levels after TRT. However, (3) the incidence of all-cause mortality, MI, and stroke in current smokers with normalized testosterone after TRT was significantly greater than that in nonsmokers with normalized testosterone after TRT. More importantly, (4) the incidence of MI or stroke between current smokers with normalized testosterone levels and smokers with nonnormalized testosterone levels after TRT was not different. Taken together, all these findings suggest that active CS nullifies the protective effect of normal testosterone levels after TRT on MI and all-cause mortality.

The role of testosterone and the effect of TRT in the pathophysiology of CVD remains controversial.[6-8,10,11] It has been proposed that higher levels of testosterone predispose men to a 3 times greater risk of heart disease compared with women.[3] Testosterone is considered to play a role in men developing cardiac diseases nearly a decade earlier compared with risk-matched women.[4] Anabolic steroid abuse is associated with early MI and stroke.[29] In addition, some observational studies support the notion that testosterone and TRT adversely affect CV health.[10,11,30] In the National Institutes of Health–sponsored study Osteoporotic

Fractures in Men, 697 subjects were divided into 4 groups on the basis of their testosterone levels and followed for nearly 4 years.[30] Results showed that men with TT levels of 495 ng/dL or more had more than 2-fold greater risk of coronary disease compared with men with TT levels of less than 308 ng/dL. Four studies including 1 randomized placebo-controlled trial,[11] 2 retrospective trials,[10,31] and 1 meta-analysis[32] suggest that TRT is associated with an increased risk of CV events.

However, recent research data question the conventional notion that testosterone is essentially harmful to the CV system. Accumulating data indicate the importance of optimal levels of testosterone for CV health.[6-8] Indeed, a British study of 930 individuals with angiography-confirmed CAD showed the prevalence of low testosterone levels in 24% of subjects. This study also found that mortality due to CAD doubled in subjects with low testosterone levels.[33] A Swedish study of more than 3000 men linked low testosterone levels to an increased risk of PVD.[34] In addition, TRT was found to reduce total body fat, total cholesterol, and waist circumference.[35,36] In multiple studies, testosterone or TRT was shown to improve endothelial function, decreased systemic vascular resistance, and decrease left ventricular end-diastolic pressure, leading to increased cardiac output.[7] TRT was also reported to decrease reperfusion injury via activation of signal transducer and activator of transcription 3.[7] Recently, in a large cohort of US veterans, we demonstrated decreased all-cause mortality, stroke, and MI with normalization of testosterone levels after TRT.[26]

Several studies have examined the effect of CS on testosterone levels, but the data are conflicting. A cross-sectional study including a total of 255 men failed to show any significant association between CS and total, free, and bioavailable testosterone and sex-hormone—binding globulin.[37] In contrast, a cross-sectional population-based study of 3427 men found that men who smoked had significantly higher levels of total and free testosterone compared with men had who never smoked. Testosterone levels were correlated directly with the number of cigarettes smoked daily.[38] In the present study, this potential influence of CS on testosterone levels was not likely a factor in outcome measurements because only smokers and nonsmokers with baseline low TT levels (measured on at least 2 separate occasions) were included in the study. Inclusion criteria used for the present analyses provide confidence that the observed differences in mortality, MI, or stroke between smokers and nonsmokers are likely effects of CS *per se* and not the differences in TT levels before TRT.

Cigarette smoking is a major risk factor for MI and sudden cardiac death and it alters the interplay between prothrombotic/antithrombotic and profibrinolytic/antifibrinolytic factors.[13,15,17,18] Because the CS rate is higher among men, it is important to know how testosterone levels and TRT may affect mortality and MI in such populations. To our knowledge, the present investigation is the first study to examine the interaction between testosterone levels and CS in the context of CV events. During these analyses, we found that normalized testosterone levels after TRT in current smokers were associated with a decrease in all-cause mortality. But normalization of testosterone levels after TRT in smokers did not decrease the incidence of MI as observed in nonsmokers. In addition, a significant decrease in all-cause mortality ($P<.001$), MI ($P=.001$), and stroke ($P=.04$) among nonsmokers with normalized testosterone levels was noted compared with current smokers with normalized testosterone levels after TRT. Together, these data indicate that in current smokers with normal testosterone levels, MI or stroke events were driven by CS rather than by TT levels. Based on our previous studies,[13,17] it is reasonable to postulate that the proatherothrombotic effects of CS overwhelmed the potential beneficial effects of a normalized testosterone level. Consequently, no reduction in the incidence of MI among the current smokers was observed. Although a noticeable protective effect of testosterone normalization on all-cause-mortality was observed in current smokers after TRT, this effect was significantly lower when compared with that in nonsmokers. On the basis of these findings, we believe that it may be prudent to recommend CS cessation as an important pretreatment intervention in candidates for TRT.

An additional interesting and clinically relevant finding in our study is a lack of a significant difference in the incidence of MI ($P=.69$)

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and stroke (P=.47) between current smokers with normalized and current smokers without normalized TT levels. Thus, it appears unlikely that normal testosterone levels would potentiate CS-related adverse effects on the mortality and CV events as proposed by *ex vivo* and preclinical studies. On a similar note, in our recent study, we found that normalization of testosterone after TRT or TRT itself did not increase the incidence of deep venous thrombosis or pulmonary embolism.[39]

Study Limitations

The findings of our study have to be viewed in the context of the limitations of an observational study. Without assigning an order of significance, our study limitations include the following: (1) Unobserved confounding factors or hidden bias may be present. (2) We could not find the exact time of the day when samples for testosterone assays were collected. Samples for laboratory analysis are generally collected during morning hours in the VA health care system. Sample draw later in the day may result in lower TT values. If some subjects had their blood drawn after the morning hours, their TT levels would be underestimated. (3) Smoking status was obtained on the basis of self-reporting and documented in the HealthFactor data files in the VA database. (4) Laboratory measurements such as serum cotinine levels were not measured to confirm smoking status. (5) *ICD-9* codes were used to determine entry criteria and outcomes. The VA cohort *ICD-9* codes are valid for determining outcomes.

CONCLUSION

Our findings show that maintaining normal levels of testosterone in testosterone-deficient nonsmokers reduces all-cause mortality and MI. Cigarette smoking negates the beneficial effects of testosterone level normalization after TRT on MI and all-cause mortality. Adequately powered randomized clinical trials would be needed for conclusive determination of the effects of CS and TRT on CV risk and mortality. However, conducting such a randomized clinical trial in the United States would be prohibitive because smoking is an established risk factor for CVD. Based on the present study, counseling and treatment for smoking cessation would be an important intervention before and during TRT. Acknowledging the limitations of a retrospective analysis, results presented in the article provide insight and information that may be valuable in clinical practice.

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Abbreviations and Acronyms: **BMI** = body mass index; **DM** = diabetes mellitus; **CAD** = coronary artery disease; **CDW** = Corporate Data Warehouse; **CHF** = congestive heart failure; **COPD** = chronic obstructive pulmonary disease; **CS** = current smoking; **CV** = cardiovascular; **CVD** = cardiovascular disease; **HR** = hazard ratio; ***ICD-9*** = *International Classification of Diseases, Ninth Revision*; **IPTW** = inverse probability of treatment weights; **LDL** = low-density lipoprotein; **MI** = myocardial infarction; **OSA** = obstructive sleep apnea; **PVD** = peripheral vascular disease; **TRT** = testosterone replacement therapy; **TT** = total testosterone; **VA** = Veterans Administration; **VHA** = Veterans Health Administration

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