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Testosterone and All-Cause Mortality in Older Men: The Role of Metabolic Syndrome

Nasser Laouali,[1] Sylvie Brailly-Tabard,[2,3,4] Catherine Helmer,[5,6] Marie-Laure Ancelin,[7] Christophe Tzourio,[5,6] Archana Singh-Manoux,[1] Aline Dugravot,[1] Alexis Elbaz,[1] Anne Guiochon-Mantel,[2,3,4] and Marianne Canonico[1]

[1]Paris-Saclay University, Paris-South University, Université de Versailles St-Quentin-en-Yvelines, Center for Research in Epidemiology and Population Health, INSERM, 78035 Versailles, France; [2]Department of Molecular Genetics, Pharmacogenetics, and Hormonology, Paris-South University, 94276 Le Kremlin-Bicêtre, France; [3]Bicêtre Hospital, Public Assistance-Paris Hospital, 94275 Le Kremlin-Bicêtre, France; [4]INSERM U693, 94276 Le Kremlin-Bicetre, France; [5]INSERM, U1219-Bordeaux Population Health Research Center, 33000 Bordeaux, France; [6]Bordeaux University, Institut de Santé Publique d'Epidémiologie et de Développement, 33000 Bordeaux, France; and [7]INSERM U1061, Montpellier University, 34093 Montpellier, France

Previous studies have shown controversial results about the role of testosterone in all-cause mortality in elderly men. We hypothesized that metabolic syndrome (MetS) could partly explain this discrepancy. We therefore examined the association of all-cause mortality with total and bioavailable testosterone, taking into account the MetS. We used data from the Three-City Cohort (3C) study with 12-year follow-up. The 3C study included 3650 men aged >65 years in three French cities. Hormone was measured in a random subsample of 444 men, and MetS was determined as stated by the International Diabetes Federation criteria. We used inverse-probability–weighted Cox regression to estimate hazard ratios (HRs) and 95% confidence intervals (95% CIs). Of 444 men included in the analysis, 106 (23.9%) had MetS at baseline, and 166 died over the follow-up. There was a significant interaction between testosterone level and MetS for all-cause mortality ($P = 0.002$ and $P = 0.008$ for total and bioavailable testosterone, respectively). Among men with MetS, a decrease in one standard deviation of testosterone was associated with higher mortality risk [HR 1.78 (95% CI 1.13 to 2.78) and HR 1.83 (95% CI 1.17 to 2.86) for total and bioavailable testosterone, respectively]. By contrast, there was no association of testosterone with mortality risk among men without MetS. Our results suggest that MetS modifies the association between testosterone and mortality in older men. If confirmed, these findings could contribute to improve risk stratification and better manage the health of older men.

Freeform/Key Words: aging, metabolic syndrome, mortality, testosterone

Testosterone is the principal male sex hormone, mainly secreted by the testes and, to a lesser extent, by adrenal glands [1]. Total testosterone consists of sex hormone-binding globulin–and cortisol-binding globulin–bound steroid, and a bioavailable component that includes free testosterone and an albumin-bound fraction. The bioavailable form of testosterone represents 30% to 50% of total testosterone and is primarily responsible for its biological effects.

Testosterone is an important anabolic hormone that controls the expression and maintenance of male sexual characteristics and promotes muscle mass, strength, and bone density [2]. Testosterone levels decline gradually with age, and low levels have been associated with

Abbreviations: 3C, Three-City Cohort; ATC, Anatomical Therapeutic Chemical; BMI, body mass index; CDE, controlled direct effect; CI, confidence interval; HDL, high-density lipoprotein; HR, hazard ratio; IPW, inverse-probability weight; LDL, low-density lipoprotein; MetS, metabolic syndrome; NDE, natural direct effect; NIE, natural indirect effect; RIA, radioimmunoassay; SD, standard deviation.

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sexual dysfunction. Low levels of testosterone are also linked to a range of physiological disorders, including reduced insulin sensitivity, diabetes, abdominal obesity, hypertension, and dyslipidemia, which can be grouped under the metabolic syndrome (MetS) [3–6].

Several epidemiological studies have examined the association of low plasma total and bioavailable testosterone with all-cause mortality in elderly men. Their results, reviewed by Araujo *et al*. [7] in a meta-analysis, are inconsistent. Although some studies show an increased risk of death among men with the lowest testosterone levels [8–12], others found no association [13–16]. More recent studies also yielded inconsistent findings [7, 17–19].

Studies that reported a noteworthy association of low testosterone with mortality were usually performed in older men who are more likely than younger men to have low high-density lipoprotein (HDL) cholesterol levels, dyslipidemia, or abdominal obesity, three important components of MetS [8–10, 12]. In addition, a previous study [20] suggested that the association of low plasma testosterone and mortality could be restricted to men with MetS.

Based on these observations, we hypothesized that the association of testosterone and mortality in men may be modified by MetS. In a random subsample of elderly men aged ≥65 years from the French Three-City Cohort (3C) study [21], we examined the association between the plasma level of total and bioavailable testosterone and the risk of 12-year all-cause mortality, while taking into account the presence of MetS status and its individual components.

1. Methods

A. Study Population and Follow-up

Men included in the present analysis are a random subsample of the 3C study, a large ongoing French prospective cohort study that aimed at studying the relation between vascular factors and dementia. The study protocol was approved by the Ethics Committee of the University Hospital of Kremlin-Bicêtre, and all participants gave written informed consent. The detailed protocol has been described elsewhere [21]. Briefly, 9294 noninstitutionalized subjects (3650 men and 5644 women) >65 years of age were selected from electoral rolls of three French cities (Bordeaux, Dijon, and Montpellier) between 1999 and 2001. Data were collected by trained psychologists or nurses during face-to-face interviews using standardized questionnaires. Information recorded included sociodemographic characteristics (education, occupational position, and marital status), medical history, cognitive function (Mini-Mental State Examination scores), drugs use in the past month [coded with the Anatomical Therapeutic Chemical (ATC) Classification], diet, alcohol drinking, and smoking. Systolic and diastolic blood pressure, weight, and height were assessed during a clinical examination.

At baseline, fasting blood samples were collected, and plasma aliquots were immediately stored at −80°C after centrifugation at 3000*g*. Fasting glycemia, different fractions of cholesterol [total, HDL, and low-density lipoprotein (LDL)], and triglycerides were measured for all participants.

Participants were then interviewed five times every 2 to 3 years. Over the follow-up, the vital status of each participant was determined through death certificates, contact with family members and physicians, and hospital records. Our analyses are based on mortality data collected over 12 years of follow-up.

For the biological investigations including the present analysis, a random subcohort representing one-seventh of the original cohort was selected within participants with blood samples, after stratification on sex, center, and age. The present analyses are based on men included in this subcohort, after excluding those who used treatments that might change testosterone levels [antiandrogens (ATC code G03H), hormones and related agents (ATC code L02A), hormone antagonists and related agents (ATC code L02AB)], men with prostate cancer or prostatitis, and those with missing data on measures used to define MetS.

B. Hormone Measurement

Frozen EDTA plasmas were used to determine hormone concentrations. Total testosterone was assessed by a radioimmunoassay (RIA) method with an Orion Diagnostica device (Spectria, Espoo, Finland) and a minimum detectable concentration of 0.06 nmol/L; all testosterone assays were detectable. The intra- and interassay coefficients of variation were 3.8% and 4.8%, respectively, for a total testosterone concentration of 10.2 nmol/L and 4.8% and 5.5%, respectively, for a total testosterone concentration of 21.3 nmol/L. Bioavailable testosterone concentration was measured by an indirect method after differential precipitations of testosterone bound to globulins with 50% ammonium sulfate and equilibration of the plasma sample with testosterone labeled with tritium [(3H)-testosterone]. The intra- and interassay coefficients of variation were, respectively, 7.0% and 8.5% for a bioavailable testosterone concentration of 4.5 nmol/L.

C. MetS

Baseline MetS was defined according to the International Diabetes Federation criteria, which require the presence of central obesity [defined as waist circumference ≥94 cm or body mass index (BMI) >30 kg/m^2] plus any two of the following four characteristics (dichotomized as present or absent): (1) raised triglyceride levels (>1.7 mmol/L) or treatment of this lipid abnormality, (2) reduced HDL cholesterol (<1.03 mmol/L) or treatment of this lipid abnormality, (3) raised blood pressure (systolic ≥130 mm Hg and diastolic ≥85 mm Hg) or treatment of previously diagnosed hypertension, and (4) raised fasting plasma glucose (≥5.6 mmol/L) or treatment of previously diagnosed type 2 diabetes [22].

D. Statistical Analysis

Baseline characteristics were reported as means [standard deviation (SD)] for normally distributed continuous variables or otherwise as medians (interquartile range) and as numbers (percentages) for categorical variables. Characteristics were compared according to baseline MetS status and vital status at the end of follow up using Pearson χ^2 tests for categorical variables and Student *t* test for continuous variables after normalization by log-transformation where appropriate.

BMI was calculated by dividing weight in kilograms by height in meters squared. Baseline smoking status and daily alcohol consumption were considered in three categories (never, former, and current) as well as education level (less than lower secondary school, secondary school, high school diploma, or university). Hypothyroidism was defined by self-report or use of thyroid medications (ATC code H03A), and statin use was identified by ATC code C10A.

We used weighted Cox proportional hazards models with age as the time scale to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). Participants were followed from age at recruitment until death or end of follow-up, whichever occurred first. Schoenfeld residuals and log-minus-log plots were examined to confirm the proportional hazards assumption.

The interaction between MetS and testosterone on the risk of all-cause mortality was estimated by including a multiplicative term between the two variables in the Cox model. As it was statistically significant, we examined the association of testosterone with all-cause mortality in men with and without MetS status. Other interactions were also systematically tested but were not statistically significant.

Total and bioavailable testosterone were modeled in three different ways. For our main analysis, hormones were included as continuous variables, and we report effect estimates for a 1-SD decrease (*z*-score) in hormone levels. This approach assumes a linear association between testosterone and mortality; to check this assumption, hormones were categorized into tertiles based on the subcohort distribution, and we computed tests for linear trend across tertiles by including the median of each category as an ordinal variable in the models. Finally, we used a clinically relevant cutoff (11 nmol/L and 4.4 nmol/L for total testosterone and

bioavailable testosterone, respectively) characterizing hormone deficiency in aging men [23, 24], and we compared mortality in men with low and high (reference group) levels.

Two models are presented: model 1 is adjusted for age (time scale) and center; and model 2 is further adjusted for smoking, alcohol drinking, and history of stroke and coronary heart disease.

To estimate the impact of each MetS component as potential modifiers of the association between testosterone and mortality, we conducted separate analyses among men with MetS by defining subgroups of men with each MetS component. We then compared HRs associated with a 1-SD decrease in testosterone levels in men with each MetS component to HRs in those free of MetS. According to this approach, for each MetS component, men with MetS and without that specific component were excluded from the analysis. Central obesity, which represents a mandatory criteria for our MetS definition, was therefore not studied alone as a component.

All analyses were weighted using inverse-probability weights (IPWs) [25] to take into account the sampling of the subcohort in which hormones were measured (one-seventh of the overall men cohort). Weights were estimated for each participant using data on all men in the 3C study (n = 3650). The probability of remaining in the subcohort was estimated by logistic regression based on baseline sociodemographic characteristics (age, education, height, occupational position, and marital status), health behaviors (smoking and alcohol, fruit, and vegetable consumption), cardiometabolic risk factors (BMI, hypertension, diabetes, and cholesterol), chronic conditions (coronary heart disease and stroke), and cognitive function (Mini-Mental State Examination scores) and 12-year mortality.

We conducted several sensitivity analyses. First, we excluded participants who died during the first 2 and 4 years of the follow-up to examine whether reverse causation may partly explain our findings. Second, we performed further adjustments on (1) MetS components (central obesity, hypertension, hypercholesterolemia, and diabetes) to consider the association of each of these parameters with testosterone and (2) statin use and hypothyroidism, as these conditions can influence MetS and mortality. Finally, we conducted similar analyses using two other MetS definitions: (1) National Cholesterol Education Program Adult Treatment Panel III criteria, according to which MetS is defined by the presence of any three of the following five features: (1) central obesity (defined as waist circumference ≥102 cm), (2) raised triglyceride levels (≥1.69 mmol/L), (3) reduced HDL cholesterol (<1.03 mmol/L), (4) raised blood pressure [systolic (≥130 mm Hg) or diastolic (≥ 85 mm Hg)], or (5) raised fasting plasma glucose ≥6.11 mmol/L or diagnosed diabetes [26]; and (2) European Group for the Study of Insulin Resistance criteria that require the presence of insulin resistance (defined as insulin levels >75th percentile of patients without diabetes) plus any two of the following: (1) waist circumference ≥94 cm, (2) raised triglyceride levels (≥2.0 mmol/L) and/or reduced HDL cholesterol (<1.01 mmol/L) or specific treatment of lipid abnormality, (3) raised blood pressure [systolic (≥140 mm Hg) or diastolic (≥90 mm Hg)] or treatment of previously diagnosed hypertension, and (4) raised fasting plasma glucose ≥6.11 mmol/L [27].

In addition to our main analysis described previously and to better understand the complex relationship among testosterone, MetS, and mortality, we used counterfactual mediation models allowing for exposure-mediation interaction, according to which MetS represents both a mediator and an effect modifier of the relation between testosterone and mortality. This approach allows us to estimate several quantities for a change of testosterone from high to low: the controlled direct effect (CDE) is the average effect of a decrease in testosterone in the absence of MetS; the natural direct effect (NDE) represents the average effect of a decrease in testosterone when MetS is fixed at the level it would take when testosterone is high; and the natural indirect effect (NIE) expresses how much the outcome would change on average when testosterone is controlled at a low level, but the mediator is changed from the level it would take if testosterone is high to the level it would take if testosterone is low. The total effect is the product of the NDE and NIE. In the absence of interaction, the CDE is equal to the NDE [28–30].

Statistical analyses were performed with Statistical Analysis System software, version 9.4 (SAS Institute, Inc., Cary, NC).

2. Results

A. General Characteristics

The characteristics of the 495 men included in the random subcohort were similar to those of other men in the study (n = 3155) (Supplemental Table 1). To validate the random sampling and the adequacy of the IPW procedure, we compared the association of cardiometabolic risk factors and personal history of cardiovascular disease with mortality in the overall men cohort and in the subcohort with and without IPW. Results showed that the three different samples yielded similar findings (Supplemental Table 2).

From the random subcohort, we excluded 29 men who did not have data on MetS and 22 men who had treatments that might change hormone levels. These 51 men did not differ from the others in term of age, sociodemographic characteristics, and risk factors. The remaining 444 subjects constitute the sample for our analysis (Fig. 1).

The prevalence of MetS was 23.9% (n = 106). Table 1 presents the baseline characteristics of the study sample, overall and according to baseline MetS status. Mean age of the subjects was 73.6 years (SD 5.1). Men with MetS were less likely to be current smokers and former/current drinkers than those without. By contrast, they were more likely to have a history of stroke. As expected, they had also a higher BMI and lower levels of total and bioavailable testosterone. Supplemental Table 3 presents participants' characteristics by tertiles of total testosterone. Total testosterone was inversely associated with BMI (Pearson correlation −0.20; $P < 0.01$), diabetes (P for trend <0.01), stroke (P for trend <0.05), and triglycerides (Pearson correlation −0.13; $P < 0.01$).

There were 166 deaths (37.5%) over the 12-year follow-up. Table 2 shows baseline characteristics according to vital status at the end of study. Mean age, waist circumference, hypertension, current alcohol drinking, and coronary heart disease and stroke were significantly associated with mortality. These associations were explained by the age differences between the two groups. Overall, mean testosterone levels did not differ between the two groups.

There was a strong positive correlation between total and bioavailable testosterone ($r = 0.90$; $P < 0.001$).

B. Hormone Levels and All-Cause Mortality Stratified by MetS Status

Overall, total and bioavailable testosterone levels were not significantly associated with all-cause mortality in an adjusted model including MetS. For 1-SD decrease of hormones, the HR was 0.97 (95% CI 0.82 to 1.14) for total testosterone and 1.08 (95% CI 0.92 to 1.26) for bioavailable testosterone. However, there was a significant interaction between hormone levels and MetS on the risk of mortality ($P = 0.002$ and $P = 0.008$ for total and bioavailable testosterone, respectively), and we stratified the analyses on MetS status. Figure 2 shows the association of total and bioavailable testosterone with all-cause mortality stratified by MetS status. In subjects without MetS, there was no association of total and bioavailable testosterone with mortality. On the contrary, lower total and bioavailable testosterone were associated with higher mortality in men with MetS [HR for 1-SD decrease: 1.78 (95% CI 1.13 to 2.78) and 1.83 (95% CI 1.17 to 2.86) for total and bioavailable testosterone, respectively]. When hormones were categorized into tertiles, we observed a significant linear trend among those with MetS ($P = 0.033$ and $P = 0.047$ for total and bioavailable testosterone, respectively), but not among those without MetS. When hormones levels were categorized using a clinically relevant cutoff, we found an increased risk of death associated with low hormones levels for men with MetS [HR 3.14 (95% CI 1.33 to 7.40) and 3.47 (95% CI 1.12 to 10.8) for total and bioavailable testosterone, respectively] (Supplemental Table 4), but not for men without MetS for total testosterone; for bioavailable testosterone, the small number of men with low levels without MetS precluded the analysis.

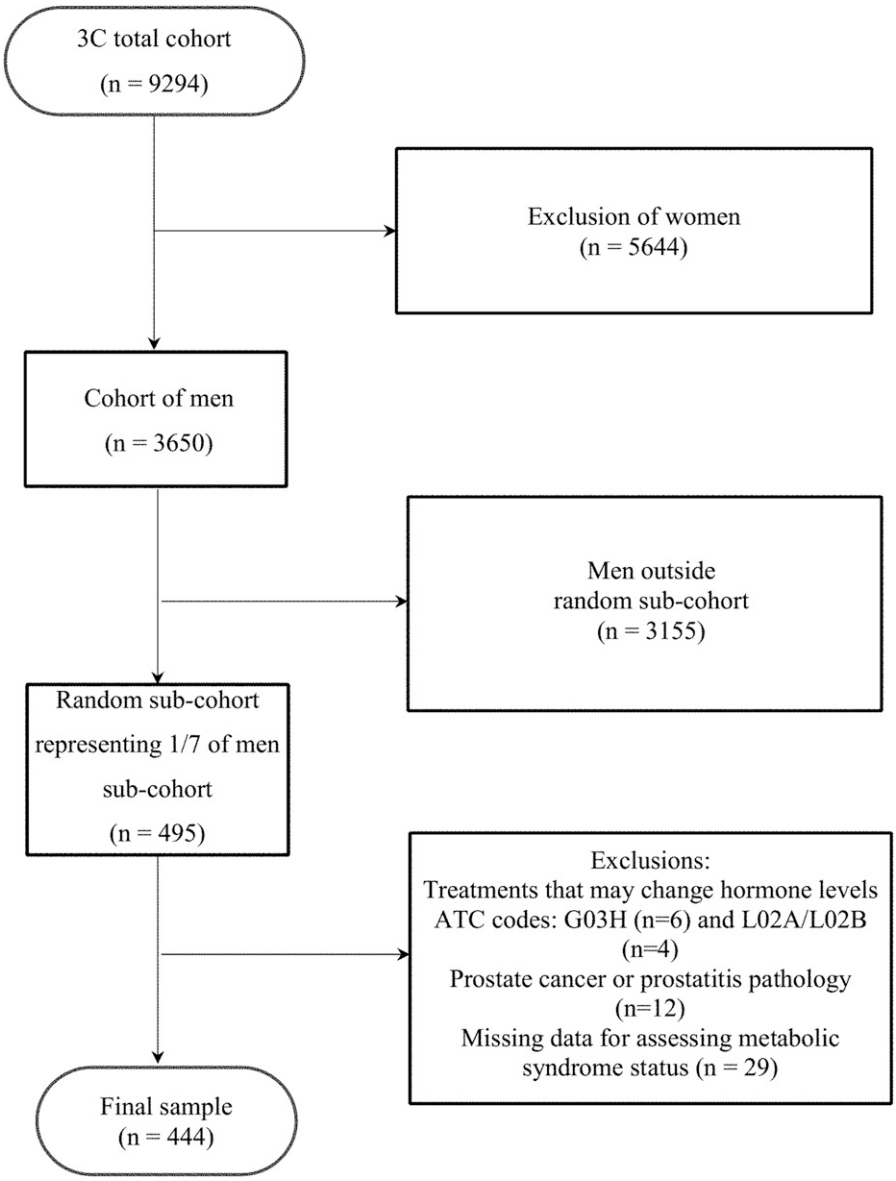

Figure 1. Flow chart representing the constitution of the sample for the investigation of all-cause mortality in relation to testosterone level.

C. Hormone Levels and All-Cause Mortality by MetS Components

Table 3 shows the results of multivariate analyses when MetS components were analyzed separately in men with MetS as compared with those without MetS. Interaction terms of each MetS component with total and bioavailable testosterone on all-cause mortality were statistically significant or borderline noteworthy.

D. Mediation Models Allowing for Testosterone-MetS Interaction

The results of these analyses are summarized in Supplemental Table 5. The CDE was different from the NDE, in agreement with the interaction between testosterone and MetS. Both for total and bioavailable testosterone, decreasing testosterone level was associated with higher mortality risk, and an important proportion of this effect (total, 56%; bioavailable,

Table 1. Baseline Characteristics of the Sample, Overall and According to MetS Status, in the 3C Cohort Study

Characteristics	Whole Sample (n = 444)	Without MetS (n = 338)	With MetS (n = 106)	*P* Value
Sociodemographic				
Age (y)	73.6 (5.1)	73.6 (5.1)	73.5 (5.2)	0.873
Center				0.249
Bordeaux	94 (21.2)	69 (20.4)	25 (23.8)	
Dijon	233 (52.6)	174 (51.5)	60 (56.2)	
Montpellier	116 (26.2)	95 (28.1)	21 (20.0)	
Education level				0.053
No education or primary school	120 (27.0)	86 (25.4)	34 (32.4)	
Secondary school	130 (29.4)	94 (27.8)	37 (34.3)	
High-school or university degree	193 (43.6)	158 (46.8)	35 (33.3)	
Anthropometric measures				
Waist (cm)	95.9 (9.8)	93.6 (9.4)	103.5 (7.1)	<0.001
BMI (kg/m^2)[a]	26.2 (3.2)	25.5 (2.9)	28.5 (2.9)	<0.001
Cardiovascular risk factors				
Smoking				0.002
Never	136 (30.7)	104 (30.8)	32 (30.5)	
Former	274 (61.9)	204 (60.4)	70 (66.7)	
Current	33 (7.4)	30 (8.9)	3 (2.9)	
Daily alcohol consumption				0.019
Never	415 (93.7)	313 (92.6)	103 (97.1)	
Former	15 (3.4)	13 (3.9)	2 (1.9)	
Current	13 (2.9)	12 (3.6)	1 (0.9)	
Hypertension	352 (79.5)	248 (73.4)	105 (99.0)	<0.001
Hypercholesterolemia	199 (44.9)	138 (40.8)	61 (58.1)	0.002
Diabetes	53 (11.9)	19 (5.6)	34 (32.1)	<0.001
Hypothyroidism	10 (2.3)	9 (2.7)	1 (0.9)	0.937
Statin use	118 (26.6)	78 (23.1)	40 (37.7)	0.003
Medical history				
Coronary heart disease	56 (12.6)	42 (12.4)	14 (13.2)	0.806
Stroke[b]	19 (4.4)	10 (3.0)	9 (8.5)	0.014
Biological parameters				
Total cholesterol (mmol/L)	5.6 (0.9)	5.6 (0.8)	5.6 (0.9)	0.474
LDL cholesterol (mmol/L)[c]	3.6 (0.8)	3.6 (0.8)	3.6 (0.8)	0.695
HDL cholesterol (mmol/L)	1.5 (0.3)	1.5 (0.3)	1.3 (0.3)	<0.001
Triglycerides (mmol/L)	1.1 (0.8–1.5)	1.0 (0.8–1.3)	1.5 (1.2–2.0)	<0.001
Glucose (g/L)	5.0 (4.7–5.5)	4.9 (4.6–5.2)	5.7 (5.1–6.4)	<0.001
Total testosterone (nmol/L)	17.4 (6.2)	18.0 (6.2)	15.4 (5.9)	<0.001
Bioavailable testosterone (nmol/L)	10.0 (3.5)	10.4 (3.4)	9.0 (3.6)	<0.001

Data are expressed as n (%) or means (SD), except for triglycerides and glucose expressed as median (interquartile range). *P* values from *t* or χ^2 test.
[a]n = 1, missing data.
[b]n = 8, missing data.
[c]n = 3, missing data.

31%) was explained by a substantial indirect effect through MetS [NIE of 0.97 (95% CI 0.94 to 0.99) and 0.93 (95% CI 0.86 to 0.99) for total and bioavailable testosterone, respectively].

E. Sensitivity Analyses

Results were not substantially modified after exclusion of participants who died during the first 2 (n = 14) or 4 (n = 33) years of the follow-up (data not tabulated). Further adjustment for MetS components, statins use, or hypothyroidism did not change the results (Supplemental Table 6). The same patterns of association were observed when two other MetS definitions were used (Supplemental Tables 7 and 8).

Table 2. Baseline Characteristics of Men According to the Vital Status After a 12-Year Follow-up in the 3C Cohort Study

Characteristics	Alive (n = 278)	Died (n = 166)	*P* Value[a]	*P* Value[b]
Sociodemographic				
Age (y)	71.7 (4.2)	76.6 (4.9)	<0.001	—
Center			0.342	0.717
Bordeaux	54 (19.5)	40 (24.1)		
Dijon	145 (52.4)	88 (53.0)		
Montpellier	78 (28.1)	38 (22.9)		
Education level			0.190	0.358
No education or primary school	74 (26.7)	46 (27.7)		
Secondary school	74 (26.7)	56 (33.7)		
High-school or university degree	129 (46.6)	64 (38.6)		
Anthropometric measures				
Waist (cm)	95.2 (9.6)	97.2 (10.1)	0.045	0.722
BMI (kg/m^2)[c]	26.2 (3.1)	26.1 (3.3)	0.795	0.329
Cardiovascular risk factors				
Smoking			0.221	0.452
Never	86 (31.0)	50 (30.1)		
Former	175 (63.2)	99 (59.6)		
Current	16 (5.8)	17 (10.2)		
Daily alcohol consumption			0.055	0.001
Never	264 (95.3)	151 (91.0)		
Former	9 (3.3)	6 (3.6)		
Current	4 (1.4)	9 (5.4)		
Hypertension	212 (76.5)	140 (84.3)	0.049	0.815
Hypercholesterolemia	136 (49.1)	63 (37.9)	0.022	0.268
Diabetes	68 (24.6)	44 (26.5)	0.646	0.322
MetS-IDF	69 (24.9)	36 (21.7)	0.440	0.742
Hypothyroidism	6 (2.2)	4 (2.4)	0.698	0.909
Statin use	78 (28.1)	40 (24.1)	0.361	0.699
Medical history				
Coronary heart disease	28 (10.1)	28 (16.9)	0.038	0.321
Stroke[d]	6 (2.2)	13 (8.0)	0.006	0.032
Biological parameters				
Total cholesterol (mmol/L)	5.6 (0.9)	5.5 (0.9)	0.225	0.943
LDL cholesterol (mmol/L)[e]	3.6 (0.8)	3.5 (0.8)	0.208	0.850
HDL cholesterol (mmol/L)	1.5 (0.3)	1.4 (0.3)	0.594	0.711
Triglycerides (mmol/L)	1.1 (0.8–1.5)	1.2 (0.9–1.5)	0.679	0.877
Glucose (g/L)	5.0 (4.7–5.5)	5.0 (4.6–5.5)	0.796	0.430
Total testosterone (nmol/L)	14.4 (5.7)	17.4 (7.0)	0.919	0.640
Bioavailable testosterone (nmol/L)	10.3 (3.4)	9.8 (3.7)	0.163	0.979

Data are expressed as n (%) or means (SD), except for triglycerides and glucose expressed as median (interquartile range).
Abbreviation: MetS-IDF, MetS according to the International Diabetes Federation.
[a] *P* value from *t* test or χ^2.
[b] *P* value from a Cox model adjusted for age.
[c] n = 1, missing data.
[d] n = 8, missing data.
[e] n = 3, missing data.

3. Discussion

In this study, MetS modified the association of total and bioavailable testosterone with the risk of all-cause mortality among elderly men. Among men without MetS, we found no association between testosterone and all-cause mortality. On the contrary, lower total and bioavailable testosterone were associated with higher risk of all-cause mortality among men with MetS. This pattern was observed for most of the MetS components, except for low HDL cholesterol. These results are in agreement with the hypothesis that differences in MetS

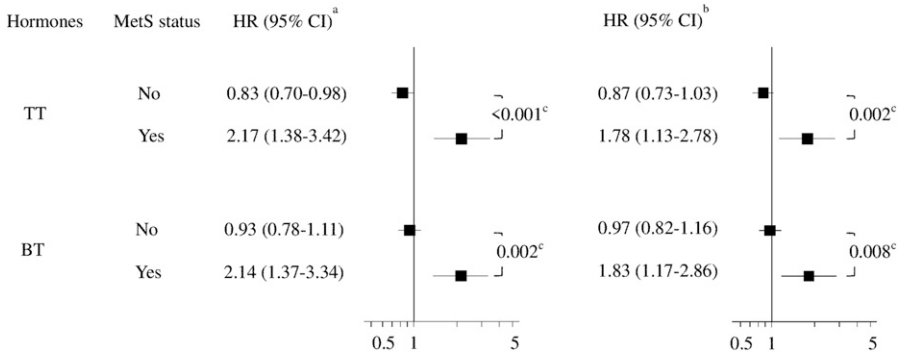

Figure 2. All-cause mortality according to baseline hormone concentrations stratified by MetS status in the 3C cohort study. HRs and 95% CIs are computed for a decrease of 1 SD [total testosterone (TT) 6.2 nmol/L and bioavailable testosterone (BT) 3.5 nmol/L] using IPW Cox models. [a]Adjusted for age and center; [b]further adjusted for smoking status, daily alcohol consumption, and personal history of coronary disease and stroke; [c]*P* value for the interaction of hormones with MetS status on all-cause mortality.

prevalence across epidemiological studies may partly explain inconsistent findings on the relation of testosterone with mortality in men.

Our finding of a differential pattern of association between testosterone and mortality in men by MetS status are in line with some previous data. On one hand, the association observed in men with MetS is consistent with some studies. In a cohort study of 596 men aged ≥40 years, 187 (31.4%) had MetS at baseline; low testosterone levels were associated with all-cause mortality only in men with MetS, whereas there was no association in men without MetS; however, the interaction was not statistically significant [20]. Other studies showed an association of low testosterone with higher all-cause mortality and acute myocardial infraction in men with type 2 diabetes [31, 32]. Our results in men with MetS are also indirectly consistent with those from studies performed in men with a high prevalence of dyslipidemia [18], hypertension [10], and elevated BMI [8] that reported an association between low testosterone and mortality. On the other hand, our findings in men without MetS are consistent with negative results of studies in which subjects were healthier and had a low

Table 3. All-Cause Mortality According to Baseline Hormone Concentrations Stratified by MetS Components in Men With MetS in the 3C Cohort Study[a]

	Total Testosterone (nmol/L)				Bioavailable Testosterone (nmol/L)			
MetS Components	**No. of Deaths**	**HR (95% CI)**	***P* Value**	***P* Interaction[b]**	**No. of Deaths**	**HR (95% CI)**	***P* Value**	
Diabetes (n = 71)								
HR for 1-SD decrease	24	1.73 (0.94–3.19)	0.080	0.015	24	1.60 (0.86–2.91)	0.138	0.059
Hypertriglyceridemia (n = 49)								
HR for 1-SD decrease	16	2.75 (1.22–6.16)	0.014	<0.001	16	3.68 (1.54–8.76)	0.003	<0.001
Low HDL-cholesterol (n = 22)								
HR for 1-SD decrease	11	2.41 (0.49–11.83)	0.279	0.056	11	9.40 (0.46–192.56)	0.146	0.058
Hypertension (n = 105)								
HR for 1-SD decrease	35	1.85 (1.15–2.98)	0.012	0.004	35	1.91 (1.18–3.07)	0.008	0.012

[a]HRs and 95% CIs computed using IPW Cox models adjusted for age, center, smoking status, daily alcohol consumption, and personal history of coronary disease and stroke plus three other MetS components.
[b]*P* for interaction compared HRs associated with 1-SD decrease in hormone levels in men with each MetS component to HRs in those without MetS [*i.e.*, HR 0.87 (95% CI 0.73–1.03) and 0.97 (0.82–1.16) for total and bioavailable testosterone, respectively; see Fig. 2]. SDs are 6.2 and 3.5 for total and bioavailable testosterone, respectively.

prevalence of diabetes and hypertension [14–16]. Finally, our results are consistent with a recent large study that found no association between endogenous testosterone and cerebrovascular disease [33], although modification by MetS was not investigated. With respect to exogenous testosterone, our results were deeply consistent with those from a study in which reduced mortality in response to testosterone supplementation was restricted to men with diabetes [34].

By contrast, our results are inconsistent with some other findings. The Fremantle Diabetes Study phase II of 788 men with diabetes showed a U-shaped association of total testosterone with all-cause mortality [35]. In addition, a recent prospective cohort study of 531 men with diabetes failed to show an association of total testosterone with the risk of death [36]. Both studies presented some differences compared with the 3C study, including the age of the participants (younger than in the 3C study), the source population (outpatients *vs.* community-based), the type of testosterone assay, and the availability of variables for multivariable analyses.

As we were not able to examine the temporality of the relation between testosterone and MetS, and our analyses rely on observational data, their findings need to be interpreted with caution in terms of causality. Nevertheless, our findings are consistent with the hypothesis that the effect of low testosterone on mortality is partly mediated by MetS and their interaction. The adverse effect of low testosterone could be partly mediated by its negative impact on the risk factors that define MetS. In the elderly, the multicomponent of MetS may act synergistically and could have a causal impact on mortality risk [37]. However, further adjustment for BMI or waist circumference, hypercholesterolemia, hypertension, and diabetes did not have a substantial impact and led to similar results compared with the main analyses. Adjustment for statin use and hypothyroidism, two conditions that could influence MetS and mortality, did not change the results either. Nevertheless, we cannot exclude that this association may be explained by other risk factors that were not assessed in the 3C study. The potential causal role of testosterone on mortality has also been investigated through a Mendelian randomization approach. One study concluded that there was no evidence in favor of a causal association of testosterone with cardiometabolic risk factors and mortality [38]. However, this study had low statistical power due to the small sample size, and the authors used an indirect marker of testosterone. On the contrary, in another recent Mendelian randomization study with a larger sample size, genetically predicted anti-Müllerian hormone and testicular dysgenesis syndrome, which are both related to lower androgens in men, were inversely associated with ischemic heart disease [39]. In contrast, it is also possible that low testosterone does not play a causal role. In this case, low testosterone may be a marker for an underlying condition rather than a direct contributor to the mortality and simply reflects poor health. Low testosterone represents a biomarker of poor health in older men, as it is associated with acute and chronic medical conditions [40–42]. Testosterone may act as a biomarker of illness, similar to other hormones, such as thyroid hormone that declines with illness severity and predicts mortality [43, 44]. Different mechanisms, including low-grade inflammation, could be involved. In the 3C study, baseline fibrinogen and C-reactive protein were measured but adjustment for these variables did not attenuate the association between testosterone and mortality (data not shown). Other inflammatory parameters, especially those closely linked to adiposity, could contribute to our results. The negative impact of low testosterone could also be mediated by depressive symptoms or altered cognitive function. However, further adjustment for depression (using the Center for Epidemiologic Studies Depression scale) and Mini-Mental State Examination did not change our results.

Our study presents several strengths. First, the 3C study is a large prospective and multicenter study with a long follow-up; exclusion of deaths occurring during the first 2 and 4 years of follow-up did not change the results, which suggests that reverse causation is unlikely to explain our findings. Second, we reached similar conclusions when we used two other MetS definitions. Third, baseline data collected by standardized questionnaires included detailed

information for the definition of MetS and multivariable analyses. Fourth, data on current medication allowed us to exclude participants who used drugs susceptible to influence hormone levels. Finally, hormone measurements were conducted without knowledge of vital status and with a sensitive method that yielded no values under the detectable concentrations.

Our study also has limitations. First, our analyses were performed in a random stratified subcohort of men rather than in all men, and the number of deaths was therefore relatively small. There were minor differences between men included in the subcohort and the other men. To take into account the sampling of the subcohort and to correct for the small differences observed between the subcohort and the other men, we used IPW. Availability of data at study recruitment and over the follow-up allowed us to incorporate this information into the construction of the weights. The adequacy of the IPW procedure was demonstrated by the similarity of the associations of cardiometabolic risk factors, personal medical history, and mortality between conventional analysis (on the whole cohort) and the analytic sample with or without IPW. Therefore, although this approach led to less precise estimates (compared with using the whole cohort), the random sampling is unlikely to lead to biased estimates. Although the number of deaths was limited, several associations, including interaction tests, were statistically noteworthy, and *post hoc* power calculations are not useful in this context. Second, the testosterone measurement was carried out by RIA rather than the state-of-the-art gas chromatography mass spectrometry method. However, validation studies showed that RIA provided accurate results in terms of relative ranking and was therefore adequate for large epidemiological investigations in which population-level inferences are more of interest than subject-specific ones. This limitation of the 3C biological investigations has been already largely discussed by Soisson *et al.* [45, 46]. In addition, our analyses are based on a single measurement of testosterone, and within-subject variability of sex hormones might lead to bias associations toward the null. However, blood samples were collected in the morning from fasting men to minimize diurnal variation [47]; in addition, it is unlikely that variability is differential according to the MetS status and therefore explains the absence of association in men without MetS. Finally, MetS was assessed at baseline only, and we cannot exclude that some men developed MetS during the follow-up, leading to a misclassification of these individuals; however, this would have resulted in an attenuation of associations, making our estimates conservative.

4. Conclusion

Low total and bioavailable testosterone are associated with increased all-cause mortality in elderly men with MetS. Among them, this increase in mortality risk is also observed for men suffering for testosterone deficiency as compared with those with normal or high physiological levels of hormone. By contrast, testosterone was not associated with mortality among men without MetS. The presence of MetS could, at least partly, explain the discrepancy across previous studies. Our data suggest that the response to testosterone therapy in men may depend on the presence of MetS or its components. It would be therefore important to take MetS status into account when examining outcomes related to testosterone level. If confirmed, these results may help clinicians to detect individuals at higher risk of death and requiring intensive medical monitoring.

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Correspondence: Marianne Canonico, PhD, Centre for Research in Epidemiology and Population Health, 16 Avenue Paul Vaillant Couturier, 94807 Villejuif Cedex, France. E-mail: marianne.canonico@inserm.fr.

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