Low Free Testosterone Levels Are Associated With All-Cause and Cardiovascular Mortality in Postmenopausal Diabetic Women

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OBJECTIVE—Hyperandrogenemia is associated with cardiovascular risk factors in women but evidence about the relationship of testosterone levels with mortality is sparse. We aimed to evaluate whether total testosterone (TT), free testosterone (FT), and sex hormone–binding globulin (SHBG) are associated with all-cause and cardiovascular mortality in a cohort of postmenopausal women.

RESEARCH DESIGN AND METHODS—We measured TT and SHBG levels in 875 postmenopausal women who were referred for coronary angiography (during 1997–2000). FT was calculated according to the Vermeulen method. The main outcome measures were Cox proportional hazard ratios (HRs) for mortality from all causes and from cardiovascular causes.

RESULTS—After a median follow-up time of 7.7 years, 179 women (20.5%) had died. There were 101 deaths due to cardiovascular disease (56.4% of all deaths). We found no association of FT, TT, and SHBG levels with mortality in all postmenopausal women. In postmenopausal diabetic women, multivariable-adjusted HRs (with 95% CIs) in the fourth compared with the first FT quartile for all-cause and cardiovascular mortality were 0.38 (0.08–0.90), $P = 0.025$, and 0.28 (0.08–0.90), $P = 0.032$, respectively. We found no association of TT and SHBG with mortality in diabetic postmenopausal women.

CONCLUSIONS—In postmenopausal diabetic women referred for coronary angiography, low FT levels are independently associated with increased all-cause and cardiovascular mortality.

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Hyperandrogenemia is associated with adverse metabolic features, including insulin resistance, central obesity, dyslipidemia, and chronic inflammation in premenopausal women, which might lead to an increased cardiovascular risk (1). Hyperandrogenemia in premenopausal women is most frequently caused by polycystic ovary syndrome (PCOS). In a recent meta-analysis (2), a twofold increased risk for arterial disease was observed in patients with PCOS relative to women without PCOS. BMI adjustment did not affect this finding, suggesting that the increased risk for cardiovascular events in PCOS might rather be a consequence of hyperandrogenemia than of obesity per se. High testosterone levels, which indicate an increased risk of type 2 diabetes in postmenopausal women (3), may underlie this association when considering that type 2 diabetes is associated with a 3.5-fold increased mortality (4). On the other hand, low levels of testosterone have been reported in women with atherosclerotic disease (5). Little is known about the association of androgen levels with mortality in pre- as well as postmenopausal women. The few studies conducted so far revealed conflicting results. Results from the Rancho Bernardo Study (6) indicate no association of testosterone levels with mortality, whereas Shaw et al. (7) demonstrated that hyperandrogenemia and a history of irregular menses were associated with angiographic coronary artery disease and increased mortality. In contrast, Sievers et al. (8) demonstrated that low total testosterone (TT) levels were associated with increased all-cause mortality and incident cardiovascular events in a primary care cohort of 2,914 female patients. These latter studies were, however, limited by use of TT for assessment of androgen status. This may not be the best approach because free testosterone (FT), and not TT, is the biologically active form.

Hence, our aim was to study the association of TT, sex hormone–binding globulin (SHBG), and FT levels with all-cause as well as cardiovascular mortality in postmenopausal women referred for coronary angiography. Considering previous data suggesting a possible association of testosterone status and type 2 diabetes, we performed analyses stratified by diabetes status.

RESEARCH DESIGN AND METHODS

Study population

The LUdwigshafen RIsk and Cardiovascular Health (LURIC) study is a prospective study that included 3,316 patients (2,310 men and 1,006 women) who were referred for coronary angiography at Ludwigshafen Heart Centre between July 1997 and January 2000. Our analyses were restricted to postmenopausal women

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of that cohort. Serum concentrations of TT and SHBG and follow-up data were available in 875 postmenopausal women included in the LURIC study. The study was performed at a cardiology unit in a tertiary care medical center in southwest Germany. Inclusion criteria were the availability of a coronary angiogram, clinical stability with the exception of acute coronary syndromes, and Caucasian origin, to limit genetic heterogeneity. Individuals suffering from acute illness other than acute coronary syndromes, any chronic disease where noncardiac disease predominated, or malignancy within the past 5 years (and those unable to understand the purpose of the study) were excluded. All study participants gave written informed consent, and the study was approved by the ethics committee at the Ärztekammer Rheinland-Pfalz.

Procedures

The rationale, design, and baseline examination have been published previously in detail (9). Venous blood sampling was performed in the morning after an overnight fast and before coronary angiography; routine laboratory parameters, including TT, were measured immediately after sampling on a daily or weekly basis as previously described (9). Remaining blood samples were frozen and stored at −80°C until analysis. Serum levels of SHBG were assayed in 2009. TT was measured in serum using a solid-phase chemoluminescence enzyme immunoassay (Testosterone.Immulite; DPC Biermann GmbH, Bad Nauheim, Germany) with an intra- and interassay coefficient of variation (CV) of 7.2 and 9.1%, respectively. To convert serum TT levels into nmol/L, multiply by 3.47. SHBG was measured by luminescence immunoassay (Roche, Basel, Switzerland) with an intra- and interassay CV of 1.3 and 2.1%, respectively. Albumin was measured in serum using photometric color test (ALBUMIN liquicolor/Hitachi 717; Human Gesellschaft für Biochmemica and Diagnostica GmbH, Wiesbaden, Germany). Insulin was measured in serum using immunoenzymometric assay (AIA pack IRI; Eurogenetics Germany, Eschborn, Germany). C-reactive protein (CRP) was measured in serum using mAb/nephelometric assay (Behring nephelometer II; Dade Behring GmbH, Marburg, Germany). FT values were calculated from TT, SHBG, and albumin levels according to the method described by Vermeulen et al. (10).

Coronary artery disease was defined based on angiographic criteria as the occurrence of at least one stenosis of at least 50% in at least one of 15 coronary segments, using the maximal luminal narrowing estimated by standardized visual analysis. Type 2 diabetes was determined by 2-h glucose ≥200 mg/dL or fasting glucose ≥126 mg/dL according to the ADA criteria (11) and in patients already receiving antidiabetic medication. Smoking status was assessed by questionnaires (9). The study participants were asked to self-assess the degree of their physical activity on a semiquantitative scale ranging from 1 to 11, whereby the extremes of this scale were labeled as "sedentary" (avoid walking or exertion) or "regular heavy exercise". They were grouped according to the following three categories of physical activity: below average (score, 1–3), average (score, 4–7), and above average (score, 8–11).

Follow-up

The median follow-up time was 7.7 years. Information on vital status was continuously obtained from local person registries. Death certificates were reviewed to classify causes of deaths into cardiovascular and noncardiovascular deaths. Two experienced physicians who were blinded to any data of the study probands, except for the information from death certificates, independently classified causes of death. In the case of a disagreement concerning the classification (<10% of all classifications), the final decision was made by one of the principal investigators of the study who was also blinded to any data except the death certificates.

Statistical analysis

Data for continuous variables are presented as median and interquartile range, and data for categorical variables are presented as percentages. Kolmogorov-Smirnov test and descriptive statistics were used to examine for normal distribution, and variables with a skewed distribution (TT, SHBG, FT, age, BMI, waist circumference [WC], systolic and diastolic blood pressure, total cholesterol [TC], triglycerides [TG], homeostasis model assessment–insulin resistance [HOMA-IR], and CRP) were logarithmically transformed for parametric statistical analyses. Differences between groups were calculated by AN(C)OVA with P for trend for continuous variables and by χ^2 test with P for linear by linear test for categorical variables. Prospective associations of TT, SHBG, and FT with all-cause mortality and cardiovascular mortality were examined by calculating Cox proportional hazard ratios (HRs). All parameters were examined as quartiles based on the entire population using the lowest quartile as reference. We also show the effect estimate per one SD increase in TT, SHBG, and FT levels. We present crude as well as age- and BMI-adjusted HRs, and we performed further adjustments for possible confounders, including WC, TG, TC, CRP, HOMA-IR, HbA_{1c}, systolic and diastolic blood pressure, active smoking (yes/no), physical activity (below average/average/above average), glucocorticoid use (yes/no), statin use (yes/no), ACE inhibitor use (yes/no), angiotensin II receptor blocker use (yes/no), β-blocker use (yes/no), aspirin or antiplatelet treatments (yes/no), oral antidiabetic agent use (yes/no), and insulin treatment (yes/no). We performed subgroup analyses of postmenopausal women with and without type 2 diabetes (301 and 574 women, respectively). Linearity assumptions for all Cox regression analyses were tested by log-minus-log survival plots and partial (Schönfeld) residuals versus survival time plots and found valid. A P value of < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL).

RESULTS—Supplementary Table 1 shows characteristics of women according to FT quartiles. Women within higher FT quartiles had significantly higher BMI, WC, fasting and 2-h glucose, fasting and 2-h insulin, and HOMA-IR, CRP, TG, and TT levels and significantly lower SHBG levels. The prevalence of type 2 diabetes was higher in women within higher FT quartiles ($P = 0.001$), and women within higher FT quartiles were more likely to take hormone replacement therapy.

Follow-up

After a median follow-up time of 7.7 years, 179 women (20.5%) with available sex steroid levels had died. There were 101 deaths due to cardiovascular disease (56.4% of all deaths). In women with type 2 diabetes, 86 women died (28.1%); there were 55 deaths due to cardiovascular disease (63.9% of all deaths).

TT

In crude as well as in multivariable-adjusted models, there was no significant association of TT, as quartiles or per one SD increase, with all-cause mortality or

cardiovascular mortality (Table 1). When postmenopausal women with and without type 2 diabetes were analyzed separately, no significant associations of TT with all-cause or cardiovascular mortality were found.

SHBG

We found no significant association of SHBG, as quartiles or per one SD increase, with all-cause mortality or cardiovascular mortality (Table 1) in crude as well as in multivariable-adjusted models. No significant associations of SHBG with all-cause or cardiovascular mortality were found when postmenopausal women with and without type 2 diabetes were analyzed separately.

Table 1—*HRs according to testosterone, SHBG, and FT levels for all-cause mortality and cardiovascular mortality in postmenopausal women*

	HR	*P* value	HR	*P* value	HR	*P* value	HR	*P* value
	Model 1		Model 2		Model 3		Model 4	
All-cause mortality								
Testosterone								
Q1 (<1.74 nmol/L)	1.0 (reference)		1.0 (reference)		1.0 (reference)		1.0 (reference)	
Q2 (1.74–2.43 nmol/L)	0.92 (0.61–1.40)	0.710	0.87 (0.57–1.31)	0.499	0.82 (0.54–1.25)	0.351	0.76 (0.49–1.17)	0.212
Q3 (2.44–3.47 nmol/L)	1.02 (0.65–1.59)	0.944	0.99 (0.63–1.55)	0.964	0.91 (0.57–1.43)	0.671	0.83 (0.51–1.34)	0.436
Q4 (>3.47 nmol/L)	1.12 (0.72–1.71)	0.618	0.96 (0.62–1.49)	0.86	0.84 (0.54–1.32)	0.457	0.80 (0.50–1.72)	0.346
Risk per one SD increase in TT	1.07 (0.88–1.30)	0.515	1.07 (0.86–1.34)	0.552	1.02 (0.79–1.31)	0.79	0.99 (0.87–1.14)	0.921
SHBG								
Q1 (<37.9 nmol/L)	1.0 (reference)		1.0 (reference)		1.0 (reference)		1.0 (reference)	
Q2 (37.9–56.4 nmol/L)	0.98 (0.62–1.57)	0.952	0.85 (0.53–1.35)	0.479	0.95 (0.59–1.53)	0.825	1.10 (0.66–1.82)	0.723
Q3 (56.5–79.2 nmol/L)	1.25 (0.80–1.94)	0.335	0.93 (0.59–1.47)	0.754	1.19 (0.74–1.92)	0.471	1.20 (0.72–1.98)	0.488
Q4 (>79.2 nmol/L)	1.37 (0.89–2.12)	0.156	0.99 (0.62–1.59)	0.984	1.34 (0.82–2.18)	0.243	1.57 (0.92–2.67)	0.095
Risk per one SD increase in SHBG	1.01 (1.00–1.01)	0.097	1.00 (0.99–1.01)	0.966	1.00 (0.99–1.01)	0.47	1.00 (0.99–1.01)	0.298
FT								
Q1 (<0.57 ng/mL)	1.0 (reference)		1.0 (reference)		1.0 (reference)		1.0 (reference)	
Q2 (0.57–0.93 ng/mL)	1.05 (0.62–1.79)	0.853	0.90 (0.53–1.55)	0.712	0.87 (0.51–1.50)	0.619	0.66 (0.37–1.18)	0.660
Q3 (0.93–1.38 ng/mL)	1.00 (0.58–1.72)	0.999	0.99 (0.57–1.71)	0.97	0.88 (0.50–1.54)	0.655	0.78 (0.44–1.41)	0.784
Q4 (>1.38 ng/mL)	0.83 (0.47–1.46)	0.511	0.89 (0.50–1.58)	0.685	0.73 (0.40–1.32)	0.293	0.69 (0.37–1.27)	0.690
Risk per one SD increase in FT	0.99 (0.93–1.06)	0.812	0.98 (0.89–1.09)	0.74	0.97 (0.89–1.07)	0.583	0.97 (0.87–1.08)	0.576
Cardiovascular mortality								
Testosterone								
Q1 (<1.74 nmol/L)	1.0 (reference)		1.0 (reference)		1.0 (reference)		1.0 (reference)	
Q2 (1.74–2.43 nmol/L)	1.43 (0.84–2.44)	0.188	0.87 (0.57–1.31)	0.499	1.22 (0.71–2.10)	0.468	1.04 (0.60–1.81)	0.881
Q3 (2.44–3.47 nmol/L)	1.07 (0.57–2.01)	0.834	0.99 (0.63–1.55)	0.964	0.94 (0.50–1.79)	0.858	0.76 (0.39–1.50)	0.432
Q4 (>3.47 nmol/L)	1.30 (0.72–2.36)	0.384	0.96 (0.62–1.49)	0.860	0.94 (0.51–1.73)	0.836	0.92 (0.50–1.72)	0.803
Risk per one SD increase in TT	1.11 (0.90–1.39)	0.332	1.10 (0.86–1.40)	0.442	1.06 (0.80–1.39)	0.701	1.03 (0.78–1.37)	0.817
SHBG								
Q1 (<37.9 nmol/L)	1.0 (reference)		1.0 (reference)		1.0 (reference)		1.0 (reference)	
Q2 (37.9–56.4 nmol/L)	0.72 (0.39–1.34)	0.297	0.63 (0.34–1.17)	0.140	0.69 (0.36–1.32)	0.264	0.75 (0.38–1.48)	0.411
Q3 (56.5–79.2 nmol/L)	0.97 (0.55–1.74)	0.930	0.76 (0.42–1.37)	0.359	1.01 (0.54–1.88)	0.974	0.91 (0.48–1.74)	0.781
Q4 (>79.2 nmol/L)	1.16 (0.67–2.02)	0.601	0.91 (0.50–1.66)	0.758	1.30 (0.69–2.43)	0.415	1.27 (0.65–2.48)	0.486
Risk per one SD increase in SHBG	1.00 (0.99–1.01)	0.723	1.00 (0.99–1.01)	0.571	1.00 (0.99–1.01)	0.903	1.00 (0.99–1.01)	0.835
FT								
Q1 (<0.57 ng/mL)	1.0 (reference)		1.0 (reference)		1.0 (reference)		1.0 (reference)	
Q2 (0.57–0.93 ng/mL)	1.13 (0.54–2.37)	0.749	0.96 (0.46–2.03)	0.916	0.93 (0.44–1.96)	0.847	0.83 (0.37–1.86)	0.655
Q3 (0.93–1.38 ng/mL)	1.54 (0.77–3.09)	0.226	1.49 (0.74–3.01)	0.261	1.33 (0.65–2.73)	0.435	1.36 (0.64–2.89)	0.427
Q4 (>1.38 ng/mL)	0.91 (0.41–1.99)	0.806	0.92 (0.41–2.04)	0.827	0.73 (0.32–1.63)	0.440	0.75 (0.32–1.74)	0.499
Risk per one SD increase in FT	1.01 (0.92–1.11)	0.811	1.02 (0.90–1.17)	0.735	1.01 (0.88–1.16)	0.906	1.01 (0.89–1.15)	0.867

Model 1, crude; model 2, adjusted for age and BMI; model 3, adjusted for age, BMI, CRP, TC, TG, HOMA-IR, systolic and diastolic blood pressure, and active smoking; model 4, adjusted for age, BMI, CRP, TC, TG, HOMA-IR, systolic and diastolic blood pressure, active smoking, WC, HbA_{1c}, physical activity, glucocorticoid use, statin use, ACE inhibitor use, angiotensin II receptor blocker use, β-blocker use, aspirin or antiplatelet treatments, oral antidiabetic agents, and insulin treatment. Q, quartile.

FT

In crude as well as in multivariable-adjusted models, there was no significant association of FT, as quartiles or per one SD increase, with all-cause mortality or cardiovascular mortality (Table 1). When postmenopausal women with type 2 diabetes were analyzed separately, we found a significant association of FT quartiles with all-cause mortality in crude as well as in age- and BMI-adjusted analysis and multivariate-adjusted analyses (Table 2; Fig. 1*A* and *B*). Moreover, we found a significant association of FT as quartiles with increased cardiovascular mortality in crude and multivariate-adjusted analyses (Table 2; Fig. 1*C* and *D*). In crude as well as in multivariable-adjusted models, there was no significant association per one SD increase of FT with all-cause mortality or cardiovascular mortality (Table 2). When postmenopausal women taking hormone replacement therapy and women with previous oophorectomy were excluded from the analyses, results did not materially change. To examine whether the significant associations are a result of SHBG directly, we performed further adjustment for SHBG.

Moreover, we performed additional analyses after exclusion of deaths within the first 2 years of follow-up and results remained materially unchanged. Multivariate-adjusted HRs (adjusted for age, BMI, CRP, TC, TG, HOMA-IR, systolic and diastolic blood pressure, and active smoking) for all-cause and cardiovascular mortality for the highest versus the lowest quartile were 0.26 (0.09–0.72), $P = 0.010$, and 0.21 (0.05–0.92), $P = 0.038$, in diabetic postmenopausal women after exclusion of deaths within the first 2 years. We found no significant association of FT with mortality in postmenopausal women without type 2 diabetes.

CONCLUSIONS—We found no association of FT, TT, and SHBG levels with mortality in all postmenopausal women. In diabetic postmenopausal women referred for coronary angiography, we observed a significant association of low FT levels with increased all-cause as well as cardiovascular mortality after adjustment for various cardiovascular risk factors. Moreover, high FT levels were associated with cardiovascular risk factors including obesity, insulin resistance, and type 2 diabetes at baseline.

High testosterone levels have been shown to be associated with insulin resistance, metabolic syndrome, obesity, type 2 diabetes, and chronic inflammation in pre- as well as in postmenopausal women (1,3). Our cross-sectional analyses are in line with these previous reports. In contrast, Debing et al. (12) found a positive association between low serum androgen levels and severe carotid artery atherosclerosis in postmenopausal women. Interestingly, TT levels were positively associated with carotid artery intima-media thickness but negatively associated with high coronary calcium scores (13). Besides the association of high androgen levels with cardiovascular risk factors, evidence regarding the association of androgen levels with mortality in women is sparse. In our cohort, we did not observe an association of TT, SHBG, and FT levels with all-cause mortality and cardiovascular mortality, which is in line with previous results from the Rancho Bernardo Study (6). In contrast, Sievers et al. (8) found a significant association of low TT levels with increased all-cause mortality and incident cardiovascular events in a population aged 18–75 years (mean, 58 years), which is somewhat surprising considering the fact that high testosterone levels are associated with several traditional cardiovascular risk factors, including type 2 diabetes (3). Further, high levels of TT might increase the risk of developing breast cancer in postmenopausal women (14). Moreover, Laughlin et al. (15) reported that low levels of testosterone are associated with an increased risk of coronary heart disease events prospectively in a population-based study including 639 postmenopausal women, aged 50–91 years (mean, 73.8 years).

Despite the inverse associations of FT levels with cardiovascular risk factors, there was no significant association of FT levels with all-cause or cardiovascular mortality when all postmenopausal women

Table 2—*HRs according to FT levels for all-cause mortality and cardiovascular mortality in postmenopausal women with type 2 diabetes*

	HR	*P* value	HR	*P* value	HR	*P* value	HR	*P* value
	Model 1		Model 2		Model 3		Model 4	
All-cause mortality								
Q1 (<0.57 ng/mL)	1.0 (reference)		1.0 (reference)		1.0 (reference)		1.0 (reference)	
Q2 (0.57–0.93 ng/mL)	0.72 (0.34–1.53)	0.396	0.54 (0.25–1.16)	0.115	0.53 (0.24–1.17)	0.115	0.32 (0.13–0.79)	0.013
Q3 (0.93–1.38 ng/mL)	0.76 (0.36–1.62)	0.476	0.73 (0.34–1.56)	0.42	0.67 (0.29–1.56)	0.349	0.42 (0.16–1.10)	0.076
Q4 (>1.38 ng/mL)	0.40 (0.18–0.88)	0.023	0.43 (0.19–0.96)	0.039	0.38 (0.17–0.89)	0.025	0.31 (0.13–0.78)	0.012
Risk per one SD increase in FT	0.98 (0.92–1.05)	0.560	0.96 (0.87–1.06)	l0.433	0.96 (0.86–1.06)	0.421	0.91 (0.78–1.06)	0.211
Cardiovascular mortality								
Q1 (<0.57 ng/mL)	1.0 (reference)		1.0 (reference)		1.0 (reference)		1.0 (reference)	
Q2 (0.57–0.93 ng/mL)	0.68 (0.25–1.80)	0.431	0.53 (0.20–1.43)	0.210	0.52 (0.19–1.46)	0.215	0.37 (0.12–1.14)	0.083
Q3 (0.93–1.38 ng/mL)	0.98 (0.39–2.43)	0.978	0.96 (0.39–2.40)	0.933	0.78 (0.27–2.20)	0.633	0.60 (0.18–2.03)	0.410
Q4 (>1.38 ng/mL)	0.30 (0.10–0.90)	0.033	0.32 (0.11–1.00)	0.050	0.28 (0.08–0.90)	0.032	0.28 (0.07–0.98)	0.047
Risk per one SD increase in FT	0.98 (0.90–1.07)	0.657	0.97 (0.85–1.10)	0.611	0.95 (0.81–1.10)	0.479	0.92 (0.76–1.11)	0.384

Model 1, crude; model 2, adjusted for age and BMI; model 3, adjusted for age, BMI, CRP, TC, TG, HOMA-IR, systolic and diastolic blood pressure, and active smoking; model 4, adjusted for age, BMI, CRP, TC, TG, HOMA-IR, systolic and diastolic blood pressure, active smoking, WC, HbA_{1c}, physical activity, glucocorticoid use, statin use, ACE inhibitor use, angiotensin II receptor blocker use, β-blocker use, aspirin or antiplatelet treatments, oral antidiabetic agents, and insulin treatment. Q, quartile.

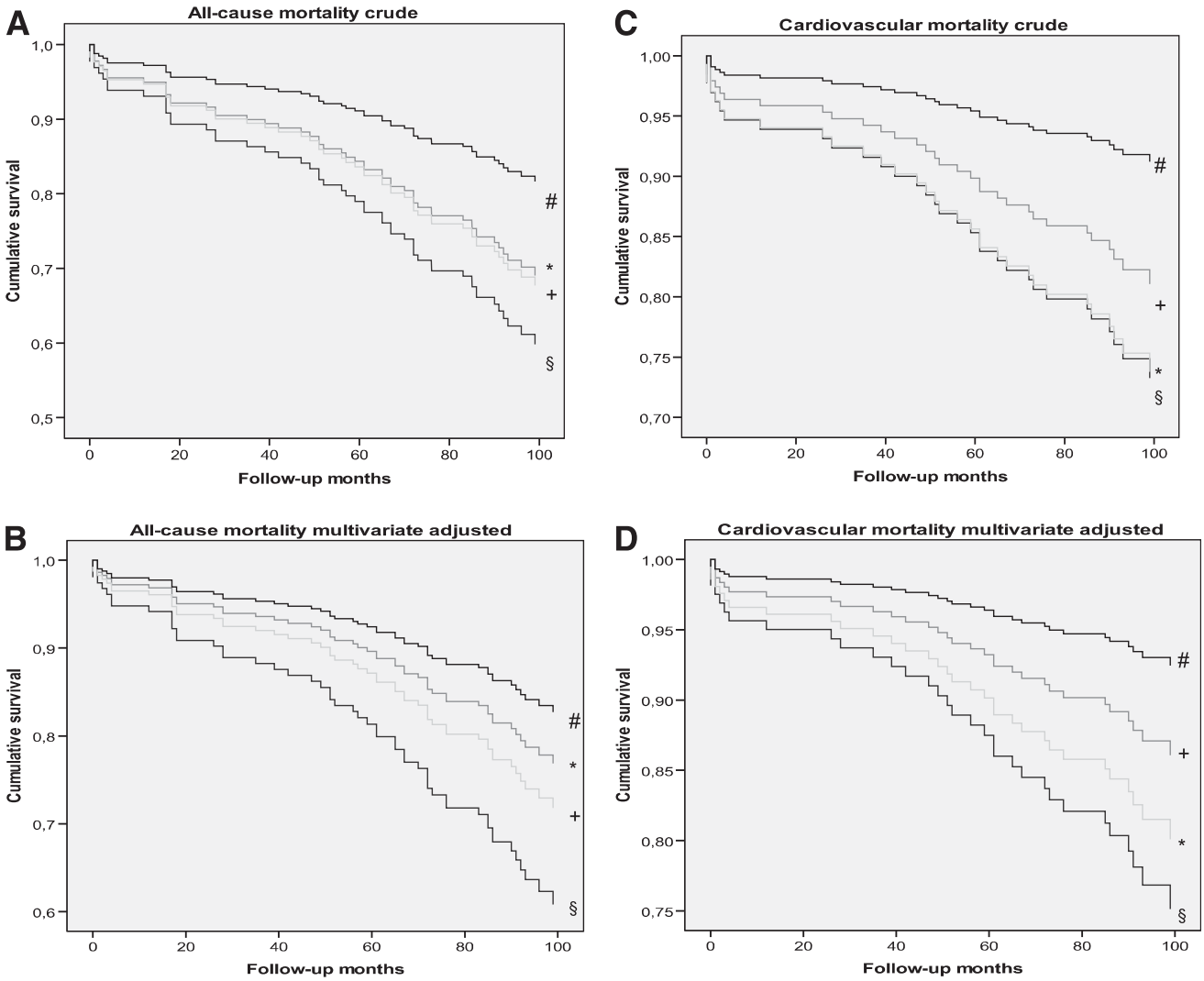

Figure 1—*Unadjusted and adjusted (age, BMI, CRP, TC, TG, HOMA-IR, systolic and diastolic blood pressure, active smoking) Kaplan-Meier curves for all-cause mortality and cardiovascular mortality in diabetic postmenopausal women.* A*: Unadjusted Kaplan-Meier curves for all-cause mortality in diabetic postmenopausal women.* B*: Adjusted (age, BMI, CRP, TC, TG, HOMA-IR, systolic and diastolic blood pressure, active smoking) Kaplan-Meier curves for all-cause mortality in diabetic postmenopausal women.* C*: Unadjusted Kaplan-Meier curves for cardiovascular mortality in diabetic postmenopausal women.* D*: Adjusted (age, BMI, CRP, TC, TG, HOMA-IR, systolic and diastolic blood pressure, active smoking) Kaplan-Meier curves for cardiovascular mortality in diabetic postmenopausal women. §Quartile 1. +Quartile 2. *Quartile 3. #Quartile 4.*

in our cohort were analyzed. Interestingly, we observed a robust association of low FT with increased all-cause and cardiovascular mortality in diabetic postmenopausal women. Underlying pathophysiological pathways remain to be explored, but there are several mechanisms that might explain our results. First, low androgen levels might be caused by a pre-existing disease and might simply be a marker of disease or poor health. Thus, low FT levels might reflect one aspect of anabolic insufficiency, which might have an adverse impact on mortality in diabetic postmenopausal women at high cardiovascular risk. However, when deaths occurring during the first 2 years were excluded, our results did not materially change. Second, low testosterone may cause or worsen disease and therefore lead to increased mortality. This hypothesis might be supported by observations in men. Several studies (16,17) indicate that low testosterone levels are associated with increased mortality, which might be attributable to direct and indirect effects of androgens on vascular tone, glucose, and lipid metabolism. In postmenopausal women, the effects of low androgens are less clear. Similar to the gradual decline of androgens observed within aging in men, an age-dependent decline in androgens also occurs in women. In postmenopausal women, androgen insufficiency may have implications for maintenance of bone density and overall quality of life (18). Potential signs or symptoms attributable to low androgen levels in postmenopausal women are vasomotor instability or decreased vaginal lubrication, bone loss, decreased muscle strength, and changes in cognition or memory (19). Moreover, a diminished sense of well-being or dysphoric mood, a lack of energy, as well as persistent, unexplained fatigue might be associated with androgen insufficiency in postmenopausal women (18). However, the diagnosis of female "androgen insufficiency" might be difficult because of the lack of normative data on TT or FT levels across the life span and the poor accuracy and precision of testosterone assays available at that time (18).

In men, as well as in women, it has been shown that testosterone therapy augments anabolic function, leading to increased muscle mass and physical strength (20). Moreover, testosterone replacement therapy at physiological levels increased muscle mass and improved some cardiovascular risk factors, such as insulin resistance in women with androgen deficiency caused by hypopituitarism (21). As reviewed by Braunstein (22), the major adverse reactions to exogenous androgens are androgenic side effects, including hirsutism and acne; testosterone administration to postmenopausal women that results in physiological to slightly supraphysiological serum-free testosterone levels is safe for at least 2 years. Although there is substantial evidence that testosterone treatment in low-dose regimens has beneficial effects on several aspects, including bone mass, muscle mass, and quality of life, there is insufficient data concerning long-time safety and side effects of testosterone replacement therapy (19).

Little is known about the clinical and biochemical features of PCOS after menopause. The definition of postmenopausal PCOS women remains to be determined because menstrual irregularity cannot be assessed in postmenopausal women and polycystic ovary morphology might change over time. Menopausal transition involves many changes regarding women's androgen status. To the best of our knowledge, no large prospective study has been published to date investigating the association of TT or FT levels with mortality in PCOS women. Considering our results showing an inverse association of FT levels with mortality in diabetic postmenopausal women, one might question the postulated negative impact of high androgens on survival in postmenopausal women at high cardiovascular risk. Of note, there is an ongoing debate on whether the increased cardiovascular risk is caused by PCOS itself, the interaction of abdominal obesity with hyperandrogenemia, or maybe by obesity alone. In contrast, data from a recent meta-analysis indicate that the increased risk for cardiovascular events in PCOS is not completely explained by obesity (2). Similarly, Krentz et al. (23) demonstrated that among nondiabetic postmenopausal women with intact ovaries, prevalent atherosclerotic disease is associated with features of a putative PCOS phenotype, which includes biochemical hyperandrogenism. Because no prospective study with a long-term follow-up in PCOS women is available, evidence on mortality in PCOS women is lacking.

The results of this study should be evaluated in the context of its limitations. The data provided are restricted to women referred for coronary angiography and may therefore not be generalizable to patients at lower cardiovascular risk, population-based cohorts, and younger age-groups. Thus, the external validity of the study is limited and larger studies on more diverse populations are needed to further establish the association of FT with mortality. Furthermore, we investigated a cohort of Caucasians living in Germany, and results might not relate to other ethnicities. Because direct measurement of FT by equilibrium dialyses is impractical in routine practice, several methods such as Vermeulen, Sodergard, Nanjee-Wheeler, and Ly-Handelsman equations are used to provide clinically useful estimates of FT concentration. However, the Vermeulen equation used to calculate FT is a reasonable approximation of actual values (10). Furthermore, TT was measured by immunoassay, and different testosterone immunoassays may give varying results. However, these methods are frequently used in large-scale studies in which assay of TT by mass spectrometry and FT via equilibrium dialysis might be impractical. Moreover, this technique has been calibrated against mass spectrometry showing a strong positive correlation. SHBG genotype (24) and coffee consumption (25) are known to influence SHBG levels but data concerning these aspects were not available in the cohort. Further, there was no power calculation with a priori established primary end points.

In summary, we present evidence that high FT levels are associated with cardiovascular risk factors, including obesity, insulin resistance, and type 2 diabetes. We found no association of TT or SHBG with mortality, whereas low FT levels were associated with increased all-cause and cardiovascular mortality in diabetic postmenopausal women referred for coronary angiography. The underlying mechanisms remain to be explored. Large prospective studies are warranted to explore the effect of androgens on mortality in women, with a special focus on women with PCOS.

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