Higher Serum Testosterone Concentration in Older Women is Associated with Insulin Resistance, Metabolic Syndrome, and Cardiovascular Disease

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Context: Early postmenopausal women with higher testosterone (T) levels have increased insulin resistance (IR) and cardiovascular risk factors, but whether this translates into increased cardiovascular disease later in life is unknown.

Objective: The objective of the study was to determine whether higher T levels are associated with IR, the metabolic syndrome (MetSyn), and coronary heart disease (CHD) in elderly women.

Design: Total T and free T by equilibrium dialysis were measured using ultrasensitive assays in 344 women aged 65–98 yr enrolled in the Cardiovascular Health Study. Cross-sectional analyses were performed to examine the associations between total and free T and IR, MetSyn, and CHD.

Results: There was a stepwise increase in the homeostasis model assessment of insulin resistance with increasing total ($P = 0.0.003$) and free T ($P = 0.02$) level and a corresponding decrease in Quantitative Insulin Sensitivity Check Index ($P < 0.001$ and $P = 0.002$, respectively). In adjusted models, higher levels of both total and free T were strongly associated with abdominal obesity and high fasting glucose, the two MetSyn components most strongly linked to IR. After adjustment, women in the top quartile of total T levels had a 3-fold greater odds of MetSyn (odds ratio 3.15, 95% confidence interval 1.57–6.35) than those in the bottom quartile and a 3-fold greater odds of CHD (odds ratio 2.95, 95% confidence interval 1.2–7.3) than those in second quartile, whereas free T was not significantly associated with MetSyn or CHD.

Conclusions: Higher levels of T are associated with IR, MetSyn, and CHD in elderly women. Whether T is a marker or mediator of cardiovascular disease in this population merits further investigation.
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The clinical relevance of testosterone (T) in women over the age of 65 yr is unknown. Many premenopausal women with polycystic ovary syndrome (PCOS) have hyperandrogenism and insulin resistance (IR), and numerous studies have demonstrated a high prevalence of cardiovascular risk factors and the metabolic syndrome (MetSyn) in these women (1). Additional studies in peri- and early postmenopausal women without a known his-

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Abbreviations: BMI, Body mass index; CHD, coronary heart disease; CHS, Cardiovascular Health Study; CI, confidence interval; CV, coefficient of variation; CVD, cardiovascular disease; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; IR, insulin resistance; LDL, low-density lipoprotein; MetSyn, metabolic syndrome; OR, odds ratio; PCOS, polycystic ovary syndrome; Q, quartile; QUICKI, Quantitative Insulin Sensitivity Check Index; T, testosterone.

tory of PCOS have shown that women with higher levels of T are more likely to have IR, MetSyn, and cardiovascular disease (CVD) (2–11).

The potential link between higher levels of T and IR has not been extensively examined in elderly women, the group of women with the highest prevalence of MetSyn and the greatest cardiovascular risk (12, 13). Using data from the Cardiovascular Health Study (CHS), we have previously shown that levels of T are significantly lower in women aged 65 yr and older than in a reference population of younger women but that the same factors that negatively affect the production of T in younger women (bilateral oophorectomy, estrogen use, and corticosteroid use) also are associated with lower levels of T in women aged 65 yr and older (14). These data suggest that a population shift in T levels occurs with age and that the association between T and cardiovascular risk factors found in younger populations of women should track into later life. However, it is also possible that there is a threshold relationship between T and these factors and that the lower levels found in older women are biologically irrelevant. Finally, it is also possible that the association between T and IR in these older women is similar to that in older men, in whom lower T levels are associated with a greater incidence of MetSyn and CVD (15–18). Our hypothesis is consistent with the first of these scenarios, that women aged 65 yr and older with higher total and free T levels are more insulin resistant, have a greater prevalence of MetSyn, and more likely to have CVD than those with lower T levels.

Subjects and Methods

Study population

The CHS is a population-based, longitudinal study of risk factors for developing CVDs in 5888 adults aged 65 yr and older (19). Enrollment of an original cohort of 5201 adults occurred between May 1989 and June 1990 and an additional cohort of 687 predominantly African-Americans enrolled in 1992–1993. Eligible individuals were identified from an age- and gender-stratified random sample of the Medicare eligibility rosters in four U.S. communities: Washington County, MD; Pittsburgh, PA; Sacramento County, CA; and Forsyth County, NC. To be eligible, individuals had to be noninstitutionalized, expecting to remain in the area for the following 3 yr, not under active cancer treatment, not wheelchair bound in the home, not requiring a proxy respondent at entry, and capable of providing consent. Household members of the sampled individuals were recruited if eligible. The institutional review boards of all four sites and the coordinating center at the University of Washington (Seattle, WA) approved the study, and all participants gave informed consent.

These analyses are based on data from a random sample of 368 women seen at the 1992–1993 visit. This assessment included a detailed medical history, physical examination, and assessment of health status. Blood was drawn in the morning after a 12-h fast and serum was frozen in −70 C freezers for future investigations (20).

Of 368 women in the original sample, six women were excluded due to insufficient serum for analyzing free T and three extreme outliers were excluded. Nine women taking oral corticosteroids were excluded due to their extremely low T values (14), as were six other women with incomplete data on covariates, yielding 344 subjects for analysis.

Assessment of biochemical measures

Total T concentrations were measured by RIA using iodinated T as a tracer (21). This assay was developed and validated for the low range of T prevalent in HIV-infected (21–23) and postmenopausal women (24). The sensitivity, defined as hormone concentration corresponding to 90% percent bound in presence and absence of analyte point, was 0.22 ng/dl (0.008 nmol/liter). The intraassay coefficient of variation (CV) was 8.2%. Interassay CVs were measured in the low, medium, and high female pool and were 13.2% in the medium pool. In other pools, interassay CVs ranged from 13 to 15%. This assay was validated against liquid chromatography-tandem mass spectrometry (23). These measurements demonstrated a correlation of 0.997 between the RIA and liquid chromatography-tandem mass spectrometry measurements. Free T concentrations were measured by a sensitive equilibrium dialysis assay (21), optimized to precisely and accurately measure low concentrations. The sensitivity of this assay is 0.3 pg/ml (1.0 pmol/liter). Ten replicates of low, medium, and high female pools were used to generate intra- and interassay CVs. The respective intraassay CVs for the low, medium, and high female pools were +5.6% for the low pool, +4.6% for the medium pool, and 2.6% for the high pool (21). The interassay CVs were in the 12–15% range. Cross-reactivity of the major steroids (dehydroepiandrosterone, dehydroepiandrosterone sulfate, dihydrotestosterone, and androstenedione) was less than 1%.

Fasting glucose levels were performed on the Kodak Ektachem 700 Analyzer (Eastman Kodak Corp., Rochester, NY). Fasting serum insulin was measured by solid-phase RIA, using serum-based standards (Diagnostic Products Corp., Los Angeles, CA). As previously described (20), fasting total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured directly and standardized according to Centers for Disease Control and Prevention guidelines, with low-density lipoprotein (LDL) cholesterol calculated according to the Friedewald equation (25).

Assessment of covariates

Sociodemographic characteristics included age, race, the number of ovaries removed, and estrogen use. Medication use was determined from examination of medication bottles at the time of the study visit. Blood pressure was determined by the average of duplicate measurements of sitting blood pressure evaluated in the right arm after a 5-min rest. Diabetes was defined as a fasting glucose of 126 mg/dl or greater, 2-h postload glucose of 200 mg/dl or greater, medical history of diabetes, or use of insulin or an oral hypoglycemic medication. Hypertension was defined as systolic blood pressure of 140 mm Hg or greater, diastolic blood pressure of 90 mm Hg or greater, or taking one or more antihypertensive medications.

Ascertainment of outcomes

Insulin resistance was assessed via calculation of homeostasis model assessment insulin resistance (HOMA-IR): (fasting glucose in milligrams per deciliter × insulin in microunits per milliliter)/405 (26) and insulin sensitivity by calculation of the Quantitative Insulin Sensitivity Check Index (QUICKI): 1/(log-[fasting glucose in milligrams per deciliter] + log [insulin in microunits per milliliter]) (27).

As initially detailed in the National Cholesterol Education Program Adult Treatment Panel III report (28) and later modified (29), women with three or more of the following criteria were categorized as having the MetSyn: abdominal obesity (waist circumference > 88 cm); hypertriglyceridemia (fasting triglycerides ≥ 150 mg/dl); low HDL cholesterol (HDL < 50 mg/dl); high blood pressure (blood pressure ≥ 130/85 mm Hg or using antihypertensive medication); and high fasting glucose (also known as impaired fasting glucose; fasting glucose ≥ 100 mg or using antidiabetic medication).

Prevalent coronary heart disease (CHD) was defined by the occurrence of angina, myocardial infarction, coronary angioplasty, or coronary artery bypass graft surgery occurring before the blood draw from which T was measured. Provisional diagnoses of CHD were reviewed and adjudicated at periodic meetings of the study's morbidity and mortality subcommittee, including investigators from each field center and the coordinating center. The full details of the surveillance and ascertainment of events in CHS have been published previously (30).

Statistical analysis

For analytic purposes, T levels were categorized into quartiles. Quartiles were selected for two reasons: there is no reference range for T levels in older women to define high levels, and we wanted to allow for the possibility of a nonlinear relationship. For total T, quartile (Q) 1 was 7.0 ng/dl or less, Q2 was 7.1–15.0 ng/dl, Q3 was 15.1–27.0 ng/dl, and Q4 was 27.0 ng/dl or greater. For free T, Q1 was 1.4 pg/ml or less, Q2 was 1.5–2.0 pg/ml, Q3 was 2.1–3.2 pg/ml, and Q4 was 3.3 pg/ml or greater.

Median HOMA and QUICKI measurements were calculated by quartile of total and free T level, and a trend test was performed to assess statistical significance. Women using insulin or with glucose greater than 200 mg/dl were excluded, yielding 328 women for inclusion in these analyses.

Total and free T levels were compared between women with MetSyn and women without MetSyn in the entire group of 344 women as well as between women with and without each of the individual MetSyn criteria. Student *t* tests were performed to assess the association between log-transformed total and free T levels and the presence or absence of MetSyn. The prevalence of MetSyn and each of its components were also examined by quartile of T and free T. Age, race, estrogen use, and bilateral ovarian removal, factors that were previously found to be important determinants of total and free T levels in women in this cohort (14), were included in multivariable logistic regression models of total and free T quartiles predicting MetSyn and each of its components. All analyses were initially performed in the total sample and were subsequently repeated excluding women with diabetes mellitus.

Multivariable logistic regression models of total and free T quartiles predicting CHD were adjusted for age, race, smoking, estrogen use, hypertension, and LDL cholesterol. Additional models including log HOMA-IR were performed.

All analyses were performed using SAS (version 8.2; SAS Institute, Cary, NC).

Results

The 344 women ranged in age from 65 to 98 yr, with a mean age of 74.4 yr (Table 1). The mean body mass index (BMI) in our study cohort was 26.9 kg/m^2. Nineteen percent were current oral estrogen users. Thirteen percent had diabetes and 30% had impaired fasting glucose. Forty-five percent of our sample met the National Cholesterol Education Program criteria for the MetSyn. Mean total testosterone levels were 19.1 ng/dl (range 1–133 ng/dl) and mean free testosterone levels were 2.8 pg/ml (range 0.3–20.6 pg/ml).

IR

With increasing total and free T levels, there was a stepwise increase in IR, as assessed by HOMA-IR ($P = 0.003$ and $P = 0.02$, respectively; Fig. 1A). Likewise, there was a stepwise decrease in insulin sensitivity, as assessed by QUICKI, by quartile of total and free T ($P \leq 0.001$ and $P = 0.002$, respectively; Fig. 1B).

TABLE 1. Selected characteristics of the study sample (n = 344)

Variable	
Age (yr)	74.4 (5.1)
Education (yr)	14.6 (4.0)
White (%)	84
Bilateral oophorectomy (%)	17
BMI (kg/m^2)	26.9 (5.3)
Current estrogen use (%)	19
Current smoking (%)	11
Total cholesterol (md/dl)	217 (39)
LDL cholesterol (mg/dl)	131 (37)
HDL cholesterol (mg/dl)	59 (15)
Triglycerides (mg/dl)	137 (71)
Total T (ng/dl)	19.1 (17.6)
Free T (pg/ml)	2.8 (2.5)
Fasting glucose (mg/dl)	107 (35)
Fasting insulin (μIU/ml)	13 (15)
Diabetes (%)	
Nondiabetic	57
Impaired fasting glucose	30
Diabetes	13
Systolic blood pressure (mm Hg)	136 (21)
Diastolic blood pressure (mm Hg)	71 (13)
MetSyn (%)	45
Abdominal obesity	65
Hypertriglyceridemia	33
Low HDL cholesterol	29
High blood pressure	68
High fasting glucose	13

Data are presented as mean (SD) unless otherwise indicated.

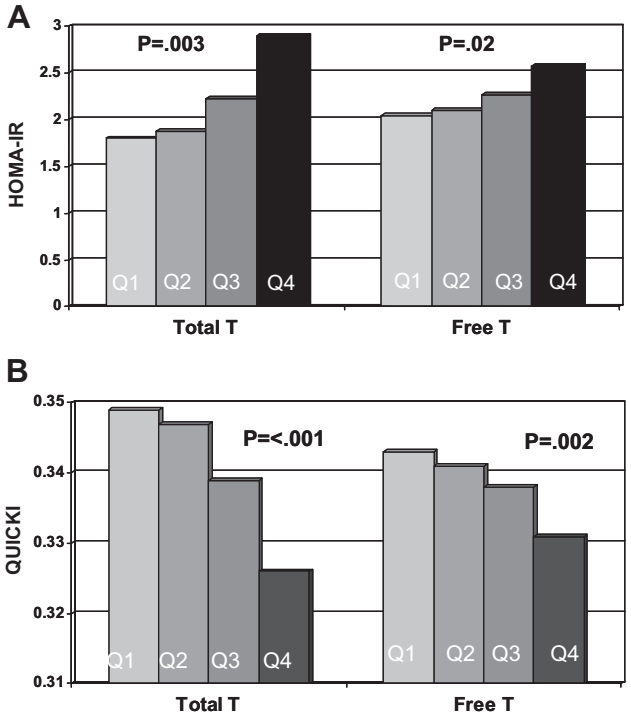

FIG. 1. A, Median HOMA-IR by quartile of total and free T. B, Median QUICKI by total and free T quartiles. Higher HOMA-IR indicates greater insulin resistance, whereas higher QUICKI indicates greater insulin sensitivity. Women using insulin or with glucose greater than 200 mg/dl were excluded; 328 women were included in this analysis.

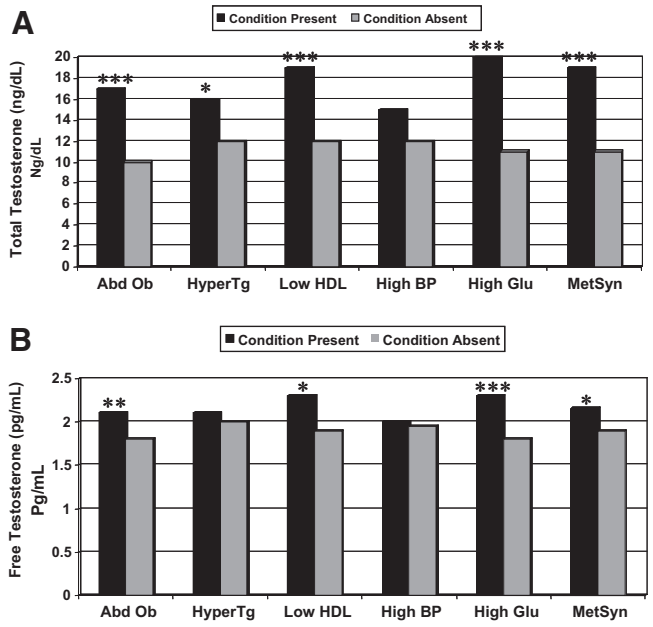

FIG. 2. A, Median total T levels in women with and without MetSyn. B, Median free T levels in women with and without MetSyn. Abd Ob, Abdominal obesity (waist circumference > 88 cm); HyperTG, hypertriglyceridemia (fasting triglycerides ≥ 150 mg/dl); low HDL (HDL cholesterol < 50 mg/dl); high BP, high blood pressure (blood pressure ≥ 130/85 mm Hg or using antihypertensive medication); high Glu, high fasting glucose (fasting glucose ≥ 100 mg or using antidiabetic medication). Multiply by 0.0347 to convert total T concentrations from nanograms per deciliter to nanomoles per liter. Multiply by 3.47 to convert free T concentrations from picograms per milliliter to picomoles per liter. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

MetSyn

For each criterion, as well as for MetSyn as a whole, total T levels were higher in women in whom the criterion was present compared with those in whom it was absent, reaching statistical significance for all except hypertension (Fig. 2A). A similar pattern was present for free T, although the differences in free T levels between those with and without the criterion were of smaller magnitude than those seen for total T (Fig. 2B). Free T levels were significantly higher in women with MetSyn and in those with the individual components of abdominal obesity, low HDL, and high fasting glucose, but free T levels were not significantly different between those with hypertriglyceridemia or hypertension and those without these two conditions. The findings for total and free T persisted after exclusion of the 43 women with diabetes.

When we examined the prevalence of the MetSyn by quartile of total T levels, we found a strong relationship between total T levels and MetSyn in crude ($P < 0.001$) and adjusted ($P = 0.007$) models (Table 2). The relationship was similar at lower levels (Q1 and Q2) of total T, with a stepwise effect at higher levels (Q3 and Q4). Women in the top quartile of total T levels had a 3-fold greater odds of MetSyn than those in the bottom quartile [adjusted odds ratio (OR) 3.15; confidence interval (CI) 1.57–6.35], an effect that persisted after additional adjustment for BMI (adjusted OR 2.81; CI 1.32–5.98).

Women in the top quartile of total T were also more likely to have each of the criteria of MetSyn than those in the bottom quartile, although the magnitude of these associations varied considerably and was not statistically significant for the high blood pressure criterion (Table 2). In adjusted models, the strongest associations were for high fasting glucose (OR 5.93; CI 2.78–12.6) and abdominal obesity (OR 3.65; CI 1.68–7.93), comparing Q4 with Q1, with additional associations seen for low HDL (OR 2.29; 1.02–5.11) and hypertriglyceridemia (OR 2.19; CI 1.03–4.64).

After exclusion of women with diabetes, the relationship between total T and MetSyn persisted, with a 3-fold greater odds of the metabolic syndrome in women with total T levels in Q4 *vs.* Q1 (adjusted OR 2.89; CI 1.36–6.16) (Table 3). Of note, the group of women with high fasting glucose in this set of analyses represents exclusively women with impaired fasting glucose. Women in the top two quartiles of total T levels were very likely to have impaired fasting glucose (OR 4.95; CI 2.18–11.26 for Q4 *vs.* Q1; OR 2.77; CI 1.27–6.07 for Q3 *vs.*Q1). Likewise, women in the top two quartiles of total T were more likely to have abdominal obesity (OR 3.46; CI 1.52–7.87 for Q4 *vs.* Q1; OR 2.10; CI 1.02–4.33 for Q3 *vs.* Q1).

TABLE 2. Odds ratios of MetSyn components and MetSyn by T quartile for the entire sample (n = 344)

	Total T		Free T	
	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
Abdominal obesity				
Q1	1.0	1.0	1.0	1.0
Q2	0.98 (0.53, 1.81)	0.92 (0.47, 1.80)	1.34 (0.72, 2.50)	1.25 (0.65, 2.40)
Q3	**2.11 (1.11, 3.99)**	2.01 (1.00, 4.05)	1.81 (0.95, 3.43)	1.56 (0.78, 3.12)
Q4	**4.26 (2.08, 8.73)**	**3.65 (1.68, 7.93)**	**2.70 (1.38, 5.30)**	**2.23 (1.08, 4.61)**
Hypertriglyceridemia				
Q1	1.0	1.0	1.0	1.0
Q2	1.22 (0.61, 2.43)	1.26 (0.60, 2.63)	0.98 (0.50, 1.91)	0.96 (0.48, 1.92)
Q3	1.57 (0.80, 3.09)	1.85 (0.89, 3.86)	1.12 (0.58, 2.17)	1.09 (0.53, 2.23)
Q4	1.95 (0.99, 3.82)	**2.19 (1.03, 4.64)**	1.30 (0.67, 2.52)	1.52 (0.74, 3.13)
Low HDL cholesterol				
Q1	1.0	1.0	1.0	1.0
Q2	1.21 (0.55, 2.67)	0.82 (0.36, 1.90)	1.02 (0.49, 2.14)	0.86 (0.39, 1.89)
Q3	**2.67 (1.28, 5.56)**	2.00 (0.91, 4.40)	1.81 (0.89, 3.66)	1.35 (0.63, 2.93)
Q4	**3.46 (1.66, 7.20)**	**2.29 (1.02, 5.11)**	**2.10 (1.04, 4.24)**	1.82 (0.84, 3.95)
High blood pressure				
Q1	1.0	1.0	1.0	1.0
Q2	0.80 (0.42, 1.53)	0.76 (0.38, 1.53)	0.72 (0.37, 1.38)	0.60 (0.30, 1.20)
Q3	0.93 (0.48, 1.77)	0.81 (0.40, 1.64)	0.84 (0.43, 1.64)	0.72 (0.35, 1.48)
Q4	1.41 (0.71, 2.80)	1.28 (0.60, 2.73)	1.04 (0.52, 2.06)	0.80 (0.38, 1.68)
High fasting glucose				
Q1	1.0	1.0	1.0	1.0
Q2	1.69 (0.83, 3.45)	1.47 (0.70, 3.09)	1.40 (0.72, 2.71)	1.28 (0.64, 2.54)
Q3	**3.38 (1.70, 6.72)**	**2.90 (1.40, 6.01)**	2.08 (0.84, 4.64)	1.77 (0.88, 3.56)
Q4	**7.13 (3.52, 14.45)**	**5.93 (2.78, 12.63)**	**3.05 (1.59, 8.27)**	**2.37 (1.17, 4.79)**
Adult Treatment Panel III MetSyn				
Q1	1.0	1.0	1.0	1.0
Q2	0.83 (0.43, 1.60)	0.71 (0.35, 1.41)	0.90 (0.48, 1.68)	0.79 (0.41, 1.51)
Q3	**2.09 (1.11, 3.92)**	1.81 (0.92, 3.54)	1.33 (0.71, 2.48)	1.09 (0.56, 2.13)
Q4	**3.87 (2.02, 7.42)**	**3.15 (1.57, 6.35)**	1.86 (0.99, 3.50)	1.48 (0.75, 2.91)

Adjusted models included age, race, estrogen use, and number of ovaries removed. Statistically significant values are indicated in *bold*.

In comparison, free T was not statistically significantly associated with MetSyn (adjusted OR 1.48; CI 0.75–2.91). However, free T levels were associated with the components of MetSyn that are most closely linked to IR: high fasting glucose (adjusted OR 2.37; CI 1.17–4.79) and abdominal obesity (adjusted OR 2.23; CI 1.08–4.61), comparing Q4 with Q1 (Table 2). These effects were attenuated after exclusion of women with diabetes, achieving statistical significance in the crude but not the adjusted analyses (Table 3).

CHD

A nonlinear, or J-shaped, relationship described the association of total T and CHD (Table 4). For ease of interpretation, Q2 was used as the reference category. Women in the top quartile of total T had a 3-fold greater odds of CHD compared with those in the reference category, independent of sociodemographic and traditional cardiovascular risk factors (Q4 *vs.* Q2 OR 2.95, CI 1.2–7.3). Free T was not associated with CHD. Adjusting for HOMA-IR decreased the magnitude of the multivariable-adjusted OR for the association between the top quartile of total T and CHD by 20% (from 2.95 to 2.35) but minimally affected the association between the top quartile of free T and CHD (from 1.41 to 1.37).

Discussion

In a cohort of women aged 65 yr and older, we demonstrated strong associations between total and free T levels and IR as well as components of MetSyn that are more closely tied to IR (high fasting glucose and abdominal obesity). Higher total T levels were also independently associated with hypertriglyceridemia, low HDL cholesterol, MetSyn, and prevalent CHD, although these associations were not found with free T.

Our findings suggest that even at the physiologically low levels of T found in these older women, the association between T and IR found in perimenopausal and early postmenopausal women (2, 5–11) persists into old age. In this cross-sectional study, we could not determine the direction of causality of this relationship. Data from premenopausal women with PCOS suggest that the effect is largely

TABLE 3. Odds ratios of MetSyn components and MetSyn by testosterone quartile for those without diabetes (n = 301)

	Total T		Free T	
	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
Abdominal obesity				
Q1	1.0	1.0	1.0	1.0
Q2	0.92 (0.49, 1.74)	0.93 (0.47, 1.86)	1.19 (0.62, 2.26)	1.13 (0.58, 2.22)
Q3	**2.06 (1.06, 3.99)**	**2.10 (1.02, 4.33)**	1.81 (0.93, 3.54)	1.63 (0.79, 3.33)
Q4	**3.82 (1.78, 8.19)**	**3.46 (1.52, 7.87)**	**2.39 (1.17, 4.85)**	2.05 (0.96, 4.39)
Hypertriglyceridemia				
Q1	1.0	1.0	1.0	1.0
Q2	1.13 (0.56, 2.30)	1.20 (0.56, 2.55)	0.89 (0.44, 1.77)	0.91 (0.44, 1.86)
Q3	1.53 (0.76, 3.06)	1.87 (0.88, 3.98)	1.09 (0.55, 2.16)	1.08 (0.52, 2.26)
Q4	1.74 (0.85, 3.56)	1.96 (0.88, 4.33)	1.09 (0.54, 2.22)	1.27 (0.59, 2.73)
Low HDL cholesterol				
Q1	1.0	1.0	1.0	1.0
Q2	1.00 (0.44, 2.27)	0.68 (0.29, 1.65)	0.89 (0.41, 1.91)	0.76 (0.34, 1.72)
Q3	1.99 (0.93, 4.28)	1.50 (0.66, 3.40)	1.25 (0.60, 2.64)	0.89 (0.40, 2.01)
Q4	**3.01 (1.39, 6.53)**	1.97 (0.85, 4.61)	1.70 (0.80, 3.58)	1.40 (0.62, 3.17)
High blood pressure				
Q1	1.0	1.0	1.0	1.0
Q2	0.84 (0.43, 1.63)	0.80 (0.39, 1.65)	0.76 (0.39, 1.51)	0.65 (0.32, 1.33)
Q3	0.87 (0.45, 1.69)	0.79 (0.38, 1.63)	0.73 (0.37, 1.45)	0.63 (0.30, 1.33)
Q4	1.29 (0.63, 2.66)	1.26 (0.57, 2.78)	0.98 (0.47, 2.00)	0.80 (0.37, 1.74)
High fasting glucose				
Q1	1.0	1.0	1.0	1.0
Q2	1.54 (0.70, 3.35)	1.44 (0.64, 3.24)	1.18 (0.58, 2.40)	1.08 (0.52, 2.45)
Q3	**3.07 (1.45, 6.48)**	**2.77 (1.27, 6.07)**	1.70 (0.84, 3.44)	1.48 (0.71, 3.11)
Q4	**5.50 (2.55, 11.89)**	**4.95 (2.18, 11.26)**	**2.21 (1.08, 4.52)**	1.80 (0.85, 3.84)
Adult Treatment Panel III MetSyn				
Q1	1.0	1.0	1.0	1.0
Q2	0.82 (0.41, 1.65	0.72 (0.34, 1.52)	0.85 (0.44, 1.66)	0.78 (0.39, 1.54)
Q3	1.87 (0.96, 3.65)	1.74 (0.86, 3.54)	1.10 (0.57, 2.14)	0.93 (0.46, 1.89)
Q4	**3.30 (1.64, 6.65)**	**2.89 (1.36, 6.16)**	1.53 (0.78, 3.00)	1.29 (0.63, 2.67)

Adjusted models included age, race, estrogen use, and number of ovaries removed. Statistically significant values are indicated in *bold*.

a stimulatory effect of hyperinsulinemia on ovarian androgen production, although a few studies suggest that testosterone may worsen insulin resistance (31). We are aware of only two studies that examined aspects of the T-IR relationship in study populations of women with a similar mean age to ours. The Invecchiare nel CHIANTI Study reported that older women with MetSyn had higher bioavailable T, but not total T levels, and that neither measure of T was associated with MetSyn in adjusted models (32). Both T measures were assessed in linear models that did not allow for the threshold effects we saw in our study. In the Rancho Bernardo Study, bioavailable T levels were associated with fasting plasma glucose and were higher in women with impaired glucose tolerance or type 2 diabetes (33). Differences in results between these two studies and ours may in part reflect differences in assay methodology, mean BMI (lowest in the Rancho Bernardo Study and similar between inCHIANTI and CHS), and exclusion criteria (estrogen users were excluded in both inCHIANTI and the Rancho Bernardo Study, and, additionally, women with bilateral oophorectomy were excluded from the inCHIANTI analyses). The mecha-

TABLE 4. Odds ratios of CHD by total T and free T quartiles for the entire sample

	Total T		Free T	
	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
CHD				
Q1	2.12 (0.8, 5.4)	2.12 (0.8, 5.7)	1.23 (0.5, 2.9)	1.19 (0.5, 2.8)
Q2	1.0	1.0	1.0	1.0
Q3	1.38 (0.5, 3.6)	1.26 (0.5, 3.4)	0.85 (0.4, 2.0)	0.87 (0.4, 2.1)
Q4	**3.17 (1.3, 7.6)**	**2.95 (1.2, 7.3)**	1.47 (0.7, 3.2)	1.41 (0.6, 3.2)

Adjusted models included age, race, smoking, estrogen use, hypertension, and LDL cholesterol. Statistically significant values are indicated in *bold*.

nisms that contribute to the T-IR relationship bear further investigation in older women, in light of the increase in visceral adiposity and resultant IR with increasing age (34) and the sustained ability of the ovary to produce testosterone after menopause (35).

Although MetSyn was highly prevalent among the participants in our study, at 45%, and has been widely studied as a risk factor for cardiovascular disease, our analyses of the individual MetSyn criteria suggest that the primary determinant of the relationship between T and MetSyn is IR, even after exclusion of women with diabetes. We do not know the menstrual histories of these women, and their premenopausal years preceded the first definition of PCOS. However, our analyses suggest that our findings are not solely confined to women with a prior history of PCOS, given a greater than 2-fold higher prevalence of abdominal obesity and high fasting glucose in women in the top 50% of T levels, compared with the bottom 25%.

Studies examining the relationship between endogenous T levels and CVD in postmenopausal women have yielded conflicting results. Several studies suggest that higher T levels are protective against CVD (36–38); however, these studies examined an early postmenopausal population when the prevalence of CVD is low. The Rancho Bernardo Study found no association between T and fatal CVD, although this study population had relatively low BMI values (39). On the other hand, three studies found that higher T levels are associated with increased CVD (3, 10, 40).

We found a J-shaped relationship between total T and CHD. Those in the bottom and top quartiles were more likely to have had a greater odds of CHD compared with those in the second quartile, although only the top quartile association was statistically significant. It is possible that those in the bottom quartile had lower T levels because of systemic underlying illness, although we did not specifically test this. Another possibility is that there is a target range of acceptable T levels and that both too little and too much have negative consequences. Interestingly, data in men have consistently shown that lower T levels are associated with increased CVD risk factors (41, 42), CVD (17, 18), and CVD mortality (43), suggesting a gender dimorphism.

In our study, the associations between T and MetSyn and CHD were universally stronger for total T than for free T. We do not have SHBG levels in our study population, but there are two potential explanations for our findings. The most likely explanation is that our free T assay, which was performed by equilibrium dialysis, had difficulty discriminating among the low levels found in these women, introducing additional noise into our data analyses. Alternatively, these data suggest that the increase in T had a predominant effect over the expected decrease in SHBG seen with high insulin levels in the setting of IR, and this may have contributed to the weaker association of free T levels with our outcomes.

The strengths of our study include its population-based sample of older women; the measurement of each of the Adult Treatment Panel III criteria for MetSyn; the rigorous definition of diabetes, which included an oral glucose tolerance test in those without known diabetes; the use of an adjudicated CHD outcome; and the use of highly sensitive assays. However, our analyses are cross-sectional in nature, which limits causal inference. Residual confounding is also possible despite adjustment for multiple potential confounders.

Although our study extends the finding of a T-IR relationship to a population of women of advanced age, the clinical implications of this finding are unclear. The majority of mechanistic studies performed in premenopausal women with PCOS suggest that T is simply a marker of IR (44). However, several studies support the potential for adverse effects of T on IR (31). If so, then augmented T concentrations could be an additional contributor to the age-related increase in risk of metabolic disorders in postmenopausal women. Further studies are required to determine whether a causal relationship exists between testosterone and IR and to provide more insight into the role T plays in the pathogenesis of CVD in women.

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