Association of Bioavailable, Free, and Total Testosterone With Insulin Resistance

Influence of sex hormone–binding globulin and body fat

ELAINE C. TSAI, MD, MPH
ALVIN M. MATSUMOTO, MD
WILFRED Y. FUJIMOTO, MD
EDWARD J. BOYKO, MD, MPH

OBJECTIVE — Previous reports of an association between low testosterone levels and diabetes risk were often confounded by covariation of sex hormone–binding globulin (SHBG) and testosterone measurements. Measurements of bioavailable and free testosterone, more reliable indexes of biologically active testosterone, were examined for their associations with markers of insulin resistance and body fat measures in 221 middle-aged nondiabetic men.

RESEARCH DESIGN AND METHODS — Bioavailable and free testosterone were calculated from the concentrations of total testosterone, SHBG, and albumin, and they were not significantly correlated with SHBG (r = 0.07–0.1). In contrast, total testosterone correlated significantly with SHBG (r = 0.63). We evaluated the relationship between these measures of circulating testosterone and markers for insulin resistance (i.e., fasting insulin, C-peptide, and homeostasis model assessment for insulin resistance [HOMA-IR]) as well as total body fat (assessed by dual-energy X-ray absorptiometry [DEXA]) and abdominal fat distribution (assessed by single-slice computed tomography [CT]).

RESULTS — Bioavailable, free, and total testosterone and SHBG all correlated significantly with fasting insulin (age-adjusted $r = -0.15$ [$P = 0.03$], -0.14 [$P = 0.03$], -0.32 [$P < 0.0001$], and -0.38 [$P < 0.0001$], respectively), fasting C-peptide ($r = -0.18$ [$P = 0.009$] to -0.41 [$P < 0.0001$]), HOMA-IR ($r = -0.15$ [$P = 0.03$] to -0.39 [$P < 0.0001$]), and body fat measures ($r = -0.17$ [$P = 0.008$] to -0.44 [$P < 0.0001$]). Only SHBG and total testosterone were significantly associated with fasting glucose ($r = -0.20$ [$P = 0.003$] to -0.21 [$P = 0.002$]). In multivariate analysis, bioavailable or free testosterone was significantly and inversely associated with insulin, C-peptide, and HOMA-IR, but this was not independent of total body or abdominal fat. SHBG was a significant determinant of insulin, C-peptide, and HOMA-IR, independent of body fat. The associations between total testosterone and insulin resistance were confounded by SHBG.

CONCLUSIONS — The inverse association between testosterone and insulin resistance, independent of SHBG, was mediated through body fat.

Diabetes Care 27:861–868, 2004

Several observational studies have reported an association of insulin resistance with low serum testosterone concentrations in men (1–7). This is consistent with a short-term trial of testosterone supplementation in obese men showing improved insulin sensitivity (8). The Multiple Risk Factor Intervention Trial (MRFIT) (9), the Rancho Bernardo Study (2), the Massachusetts Male Aging Study (10), and a study on men from Gothenburg, Sweden (11), showed low testosterone (total or the bioavailable fractions) to be a predictor of type 2 diabetes. However, there are inconsistencies in the strength and significance of the association between testosterone and markers of insulin resistance or diabetes risk because of differences in study design, characteristics of the populations studied, testosterone assay methodology, and covariates used for adjustment. Moreover, sex hormone–binding globulin (SHBG), which binds tightly to testosterone and transports it in the circulation, has emerged as a stronger correlate with insulin resistance, central adiposity, and dyslipidemia. Low SHBG level was independently associated with increased risk of diabetes in men in two of the studies mentioned (9,10), it was borderline in one study (11), and it was unrelated in the San Antonio Heart Study (12). Therefore, it is likely that SHBG confounds the association between testosterone and insulin resistance. However, this has not been adequately addressed in published research.

Low levels of androgens are significantly associated with measures of central adiposity in men, cross-sectionally and prospectively (3,5,13–16). In several studies, the relationship between low testosterone and measures of insulin resistance or glucose metabolism was independent of BMI or waist-to-hip ratio (WHR), indirect measures of overall or central obesity (2,3,5,7,11). In middle-aged obese men, testosterone administration decreased intra-abdominal fat assessed by computed tomography (CT) and also improved their insulin sensitivity (8). Thus, visceral adiposity may be an intermediate step in the association between testosterone and altered glucose metabolism. Seidell et al. (5) found that the inverse association between free testosterone and fasting insulin was independent of CT-assessed visceral fat, whereas Abate et al. (16) found that the association between SHBG and peripheral

From the Veterans Affairs Puget Sound Health Care System and the University of Washington, Seattle, Washington.

Address correspondence and reprint requests to Elaine Tsai, MD, MPH, VA Puget Sound Health Care System (152E), 1660 S. Columbian Way, Seattle, WA 98108. E-mail: tsaichin@u.washington.edu.

Received for publication 4 August 2003 and accepted in revised form 20 December 2003.

Abbreviations: CT, computed tomography; DEXA, dual-energy X-ray absorptiometry; HOMA-IR, homeostasis model assessment for insulin resistance; MRFIT, Multiple Risk Factor Intervention Trial; SHBG, sex hormone–binding globulin; WHR, waist-to-hip ratio.

glucose disposal was totally dependent on the magnetic resonance imaging–assessed regional fat. Recently, studies examining dual-energy X-ray absorptiometry (DEXA)-assessed body fat distribution suggested that total body fat might be more strongly correlated with insulin resistance than regional abdominal fat mass in the association with insulin resistance (17,18). Whether total body fat mass by DEXA (or other means) contributes to the relationship between testosterone and insulin resistance remains to be elucidated.

In this report, we analyzed baseline cross-sectional measurements in a longitudinal cohort study of central obesity, metabolic syndrome, and testosterone, including measurements of total testosterone and SHBG-independent bioavailable testosterone (bioavailable testosterone, or non–SHBG-bound testosterone) and free testosterone. The highly correlated relationship between SHBG and total testosterone was carefully explored in the analysis, and adjustments were made to remove potential confounding effects of SHBG in the association between testosterone and insulin resistance. Furthermore, we examined the potential of body adiposity as an intermediate step between low testosterone and insulin resistance using DEXA and CT assessment of total and regional body fat.

RESEARCH DESIGN AND METHODS

— Nondiabetic men between 45 and 65 years old who had received any care at the Veterans Affairs Puget Sound Health Care facility within the preceding year were screened with a computerized algorithm using the Consumer Health Information and Performance Set (CHIPS) database. ICD-9 codes were used to exclude those with cardiac (including congestive heart failure), hepatic (including chronic hepatitis and cirrhosis), renal (including renal failure), or endocrine (including hypogonadism, Cushing's, adrenal insufficiency, and untreated hypo- or hyperthyroid) disorders; organic psychoses (including schizophrenia and dementia); drug or alcohol dependence; active cancers (except nonmelanomatous skin and stage A prostate cancers); AIDS; and malnutrition. We excluded those undergoing voluntary weight reduction within the preceding year or who were not able to participate in the study because of other debilitating conditions or invalid contact information. Pharmacy data were used to exclude those on insulin, oral hypoglycemic agents, androgens/anabolic steroids, glucocorticoids, antidepressants, antineoplastic agents, anticoagulants, and antilipemic agents. Of the 6,183 eligible subjects, 744 were randomly selected, and of those, 152 (20%) did not respond, 363 (49%) declined, and 229 (31%) agreed to participate. A total of 8 subjects were subsequently excluded, including 7 subjects with fasting plasma glucose ≥126 mg/dl (7 nmol/l) at the baseline visit and 1 subject on exogenous testosterone supplement, leaving a study population of 221 men who are being followed annually for a total of 3 years. The research protocol and consent form were approved by the University of Washington and Veterans Affairs Puget Sound Health Care System institutional review boards. All subject visits were conducted at the Veterans Affairs Clinical Research Unit.

Measurements

Insulin, C-peptide, and glucose. Fasting plasma insulin and C-peptide were used as surrogate measurements of insulin resistance. Insulin resistance was also estimated by homeostasis model assessment for insulin resistance (HOMA-IR), defined as [fasting insulin (pmol/l)/6 (μU/ml)/(pmol/l)] × fasting glucose (mmol/l)/22.5. Insulin, C-peptide, and glucose assays were performed by the Diabetes Endocrinology Research Center (DERC) Immunoassay Lab at the University of Washington.

Testosterone and SHBG. Total testosterone and SHBG were measured using a time-resolved DELFIA fluoroimmunoassay (Perkin Elmer Wallac, Norton, OH). For total testosterone, the intra- and interassay coefficients of variation (CVs) were 3.7 and 8.2%, respectively, and the lower limit of detection was 0.4 nmol/l. For SHBG, the intra- and interassay CVs were 1.3 and 5.1%, respectively, and the lower limit of detection was 0.5 nmol/l. Bioavailable and free testosterone were calculated from total testosterone, SHBG, and albumin using the Sodergard equation (19). Calculated bioavailable and free testosterone provide accurate estimates of these testosterone fractions, measured directly by ammonium sulfate precipitation and equilibrium dialysis, respectively (20).

Vital signs and anthropometric measurements. The following duplicate measurements were averaged for the analysis: blood pressure (with subjects sitting, measuring Korotkoff first and fifth sounds as systolic and diastolic blood pressures, respectively), heart rate, weight (kg), height (cm), waist circumference (cm, at the level just above the iliac crest while standing), and hip circumference (cm, largest measurement of buttocks while standing). BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2), and WHR was calculated from the waist and hip circumferences.

Body fat distribution. Single CT (Picker PQ 6000; Philips Medical System, Andover, MA) scans of the abdomen were used to measure cross-sectional total abdominal and intra-abdominal fat areas (cm^2) at the level of L4–5. Subcutaneous abdominal fat area was defined as the difference between total and intra-abdominal fat. Adipose tissue was captured within an attenuation range of −50 to −250 Hounsfield units (21). Intra-abdominal fat area (cm^2) was obtained by drawing a line at the transversalis fascia between the abdominal muscle wall and the abdominal cavity and measuring the area of fat contained within this boundary. The area was calculated by counting pixels that had intensities within the selected range of Hounsfield units using software provided by the manufacturer (Voxel Q version 4.1). Subcutaneous abdominal fat was calculated by subtracting intra- from total abdominal fat. Total body fat (kg and percentage) was assessed using DEXA (QDR-4500A version 9.03; Hologic, Waltham, MA).

Dietary fat intake and physical activity. Information on dietary fat intake was ascertained using the Northwest Lipid Research Clinic Fat Intake Scale, which consisted of 12 questions on intake of foods high in fat, saturated fat, and cholesterol (22). Leisure physical activity information is captured by the Godin Leisure-Time Exercise Activity Questionnaire, which asked about the frequency and intensity of the leisure physical activities (strenuous, moderate, and mild) and the frequency of sweating associated with the activities (23,24). METs were derived from the sum of strenuous activity frequency × 9, moderate activity frequency × 5, and mild activity frequency × 3 (23).

Table 1 —*Baseline characteristics: age, anthropometrics, body fat distribution, and CT fat by HOMA-IR tertiles*

	HOMA-IR tertiles			
	I 0.33–2.85	II 2.86–4.89	III 4.92–17.79	Total 0.33–17.79
n	74	73	74	221
Age (years)	57 (46–65)	57 (48–65)	57 (47–66)	57 (46–66)
BMI (kg/m^2)	26.6 (17.9–38.4)	28.1 (18.7–34.7)	32.2 (21.8–43.3)	29.0 (17.9–43.3)
Waist (cm)	95.1 (76.2–119.4)	99.4 (80.5–120.2)	110.6 (86.4–134.4)	101.7 (76.2–134.4)
WHR	0.94 (0.79–1.08)	0.97 (0.85–1.11)	1.01 (0.87–1.15)	0.97 (0.79–1.15)
Mean arterial pressure (mmHg)	95 (79–115)	99 (80–129)	100 (77–127)	98 (77–129)
Fasting insulin (pmol/l)	52.0 (8.4–79.2)	93.2 (61.2–147.0)	187.5 (111.6–366.6)	111.0 (8.4–366.6)
Fasting C-peptide (nmol/l)	0.43 (0.17–0.96)	0.64 (0.33–1.06)	1.04 (0.40–1.82)	0.7 (0.17–1.82)
Glucose (mmol/l)	5 (3.7–6.2)	5.4 (4.3–6.6)	5.6 (4.6–6.8)	5.3 (3.7–6.8)
BAT (nmol/l)	8.4 (1.7–22.9)	7.8 (3.4–16.5)	7.5 (3.8–25.3)	7.9 (1.7–25.3)
FT (nmol/l)	0.35 (0.06–0.98)	0.31 (0.15–0.6)	0.31 (0.14–1.18)	0.32 (0.06–1.18)
TT (mmol/l)	20.6 (2.7–58.5)	17.9 (6.8–36.4)	15.4 (5.6–43.2)	18.0 (2.7–58.5)
SHBG (nmol/l)	48.5 (18.4–95.1)	43.4 (13.6–81.2)	34.6 (9–77.4)	42.2 (9–95.1)
CT-TAF (cm^2)	344 (70–726)	404 (208–676)	536 (277–921)	428 (70–921)
CT-SAF (cm^2)	226 (48–494)	242 (115–445)	317 (151–596)	262 (48–596)
CT-IAF (cm^2)	118 (21–245)	162 (71–395)	219 (79–483)	166 (21–483)
DEXA-TBF (kg)	19.8 (6.3–35.8)	22.6 (11.5–35.5)	29.2 (14.1–49.4)	23.9 (6.3–49.4)
DEXA-TBF (%)	24 (10–34)	26 (17–35)	30 (17–43)	27 (10–43)
Dietary fat intake scale	29 (14–40)	31 (16–44)	31 (20–42)	30 (14–44)
Leisure physical activity				
MET*	37 (0–101)	36 (0–170)	29 (0–153)	34 (0–170)
Sweat†	2.2 (1–3)	2.0 (1–3)	2.0 (1–3)	2.1 (1–3)

Data are means (minimum–maximum). *METs were calculated as followed: (strenuous activity frequency × 9) + (moderate activity frequency × 5) + (mild activity frequency × 3). †Coded as "1" (never/rare), "2" (sometimes), or "3" (often). BAT, bioavailable testosterone; FT, free testosterone; IAF, intra-abdominal fat; MET, metabolic equivalent; SAF, subcutaneous abdominal fat; TAF, total abdominal fat; TBF, total body fat; TT, total testosterone.

Statistical analysis

Data were analyzed primarily with SPSS (version 11.5; SPSS, Chicago, IL) and STATA (version 7; Stata, College Station, TX). Because of slightly skewed distributions of insulin, C-peptide, HOMA-IR, testosterone, SHBG, and CT fat areas, analyses were performed using natural log-transformed data for correlations, trends (ANOVA), and linear regression (dependent variables only). However, mean values were reported for untransformed data (Table 1). First-order interaction terms were used to test for the presence of interactions in multivariate linear regression models.

RESULTS — Table 1 shows baseline characteristics of the cohort by tertiles of HOMA-IR. A significant trend with increasing HOMA-IR tertiles was detected for all parameters except age, bioavailable and free testosterone, and leisure physical activity. Of the subjects, >52% were overweight (25 ≤ BMI <30), 31% were obese (BMI ≥30), and 43% were classified as centrally obese using the waist circumference cutoff of >102 cm.

Insulin resistance and body fat

Total body fat by DEXA (kg and percentage) correlated significantly (all *P* values <0.0001) with insulin resistance measures: fasting insulin (age-adjusted r = 0.52–0.63), fasting C-peptide (0.51–0.63), and HOMA-IR (0.49–0.61). Similarly, abdominal fat distribution by CT (intra-, total, and subcutaneous abdominal fat) was significantly (all *P* values <0.0001) associated with fasting insulin (age-adjusted r = 0.48–0.5), C-peptide (0.44–0.62), and HOMA-IR (0.47–0.5). The age-adjusted correlations of glucose with CT–intra-abdominal fat and with CT–total abdominal fat areas were also significant (r = 0.22 [$P < 0.0001$] and 0.16 [P = 0.01], respectively), but not with CT–subcutaneous abdominal fat area or DEXA–total body fat.

Testosterone and body fat

All CT fat areas correlated significantly with bioavailable testosterone (age-adjusted $r = -0.14$ [$P = 0.008$] to -0.29 [$P < 0.0001$]), free testosterone ($r = -0.14$ [$P = 0.008$] to -0.26 [$P < 0.0001$]), total testosterone ($r = -0.33$ [$P < 0.0001$] to -0.40 [$P < 0.0001$]), and SHBG ($r = -0.29$ [$P < 0.0001$] to -0.39 [$P < 0.0001$]). Similarly, DEXA–total body fat (kg and percentage) correlated significantly with bioavailable testosterone (age-adjusted $r = -0.31$ to -0.34; all $P < 0.0001$), free testosterone ($r = -0.27$ to -0.31; all $P < 0.0001$), total testosterone ($r = -0.37$ to -0.44; all $P < 0.0001$), and SHBG ($r = -0.29$ to -0.38; all $P < 0.0001$).

Testosterone and SHBG

A very strong correlation was observed, as expected, between bioavailable and free testosterone ($r = 0.96$, $P < 0.0001$), bioavailable and total testosterone ($r = 0.81$, $P < 0.0001$), and free and total testosterone ($r = 0.82$, $P < 0.0001$). SHBG correlated well with total testosterone (r = 0.63, $P < 0.0001$), consistent with the biological role of SHBG as the major sex hormone–binding protein. This is in strong contrast to the lack of significant correlations between SHBG concentration and bioavailable and free testosterone (r = 0.07 and 0.1, respectively).

Insulin and glucose metabolism and testosterone

Age-adjusted fasting insulin, C-peptide, and HOMA-IR were all significantly and inversely correlated with bioavailable testosterone ($r = -0.15$ [$P = 0.03$], -0.18 [$P = 0.009$], and -0.15 [$P = 0.03$], respectively), free testosterone ($r = -0.14$ [$P = 0.03$], -0.20 [$P = 0.004$], and -0.15 [$P = 0.024$], respectively), total testosterone ($r = -0.32$ [$P < 0.0001$], -0.37 [$P < 0.0001$], and -0.33 [$P < 0.0001$], respectively), and SHBG ($r = -0.38$ [$P < 0.0001$], -0.41 [$P < 0.0001$], and -0.39 [$P < 0.0001$], respectively). However, only total testosterone and SHBG were significantly associated with fasting glucose ($r = -0.20$ [$P = 0.003$] and -0.21 [$P = 0.002$], respectively). Those results clearly demonstrated a stronger inverse relationship between markers of insulin resistance and total testosterone than that with the bioactive fractions (bioavailable and free testosterone). However, after adjustment for SHBG concentration, all three testosterone measures were correlated similarly with insulin resistance (data not shown). Scatter plots depicting the correlations of bioavailable, free, and total testosterone and SHBG with HOMA-IR are presented in Fig. 1.

Multivariate regression

Multiple regression models were used to examine relationships between measures of insulin resistance as the dependent variable and testosterone and SHBG as independent variables while adjusting for the potential confounding effects of age and CT body fat areas. In addition, testosterone models were adjusted for SHBG. Multiple regression models of fasting insulin and HOMA-IR are shown in Table 2. Bioavailable and total testosterone were both significant predictors of fasting insulin, HOMA-IR, and fasting C-peptide (data not shown in Table 2), adjusted for age. Adjusting for SHBG in the linear regressions did not alter the association of fasting insulin with bioavailable or total testosterone or change the relationship of HOMA-IR or C-peptide with bioavailable or total testosterone.

Adjustment for body fat (CT–total abdominal fat, intra- and subcutaneous abdominal fat, and DEXA–total body fat) in the linear regression, however, rendered bioavailable testosterone insignificant as an independent predictor of insulin, HOMA-IR, or C-peptide. Total testosterone, on the other hand, remained a significant predictor in all models adjusted for body fat. However, further adjustment with SHBG in those models attenuated the significance of total testosterone. Furthermore, total testosterone was no longer a significant predictor of fasting glucose when adjusted for SHBG, with or without further adjustment for body fat (data not shown). On the other hand, SHBG was significantly associated with insulin, HOMA-IR, C-peptide, and glucose, independent of all measures of body fat and testosterone. The associations of free testosterone with insulin, HOMA-IR, C-peptide, and glucose were generally similar to those of bioavailable testosterone. First-order interaction terms, such as "age × CT–intra-abdominal fat," "bioavailable testosterone × CT–intra-abdominal fat," etc., were tested in the multivariate regression models if residual plots of the models suggested potential

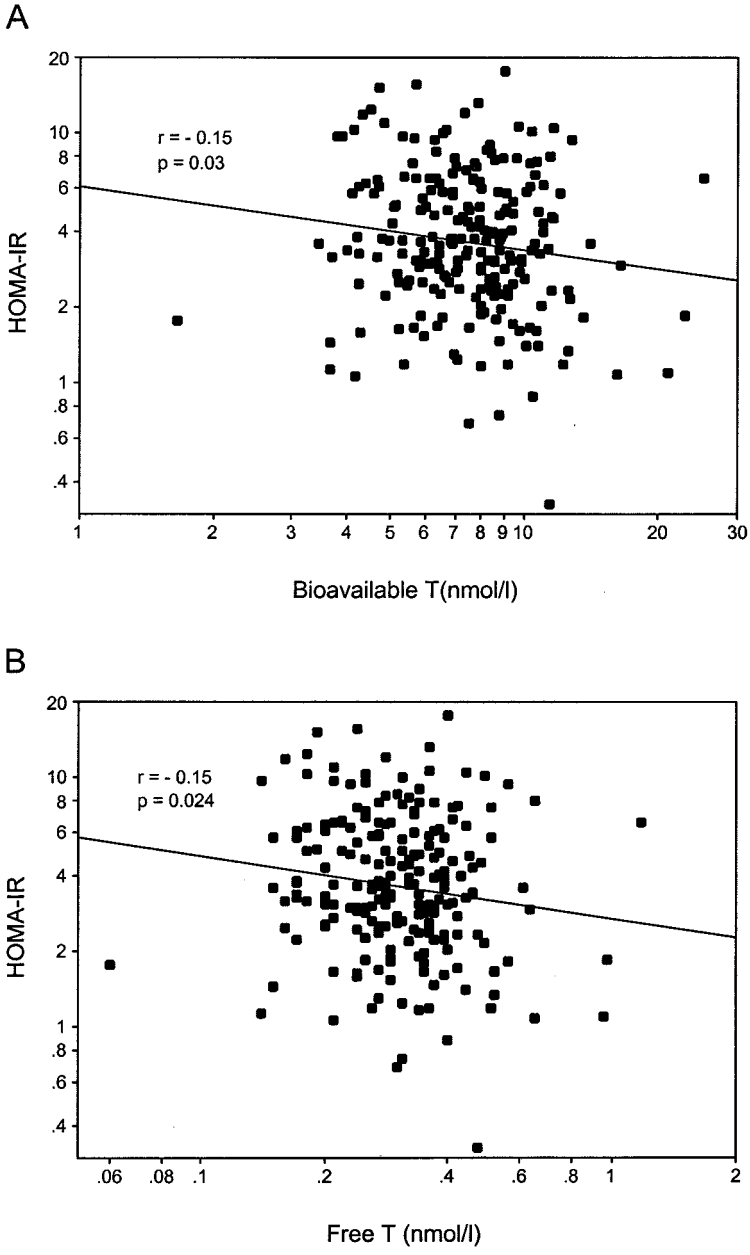

Figure 1—*Correlation plots for testosterone measurements and SHBG with HOMA-IR. All values were plotted on log-log scales.* A*: HOMA-IR and bioavailable testosterone.* B*: HOMA-IR and free testosterone.* C*: HOMA-IR and total testosterone.* D*: HOMA-IR and SHBG. T, testosterone.*

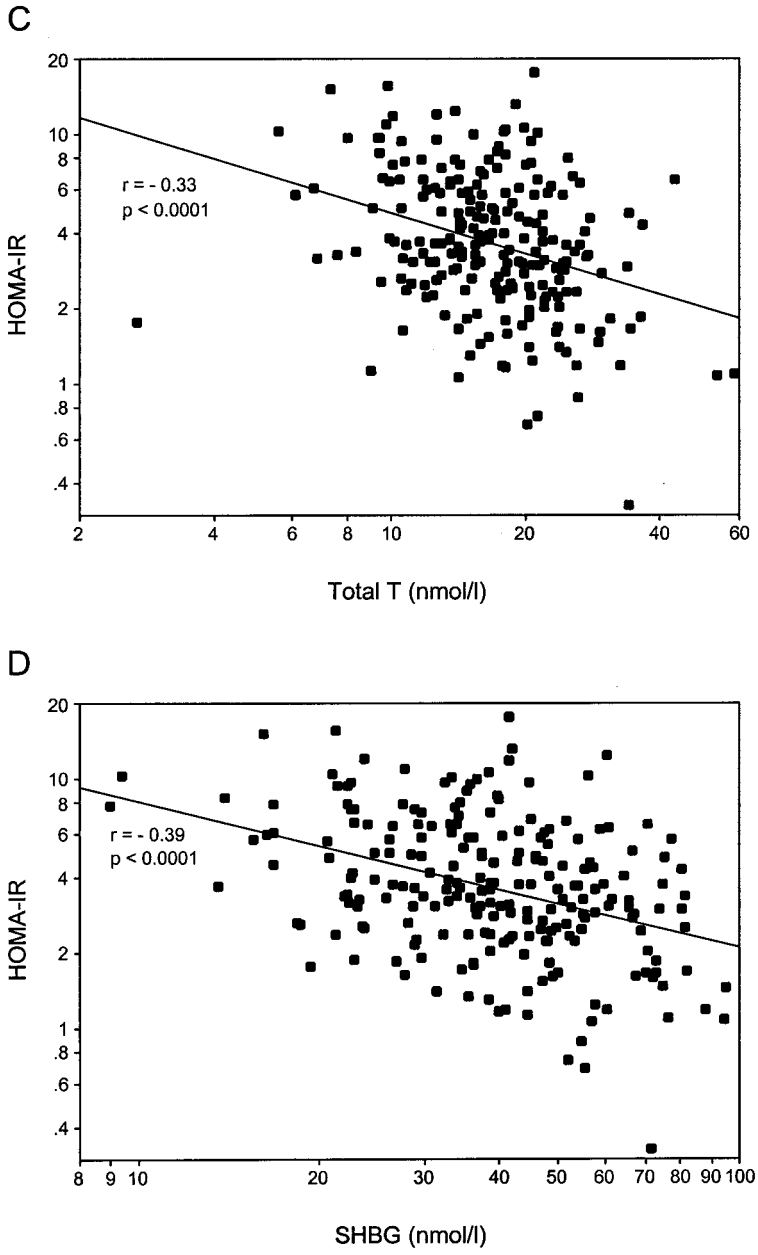

FIG. 1—*Continued.*

effect modification from the independent variables. Additional adjustment for dietary fat intake ("Fat Intake Scale") and physical activity ("MET" and "Sweat") did not significantly alter the coefficients of the independent variables for the models presented in Table 2.

CONCLUSIONS — In this cross-sectional cohort analysis of nondiabetic middle-aged men, we found that free and bioavailable testosterone (free plus albumin bound or non-SHBG bound), the biologically active fractions of circulating testosterone, correlated inversely with measures of insulin resistance (insulin, C-peptide, and HOMA-IR) and body fat measurements. However, this inverse association of endogenous free testosterone and bioavailable testosterone levels with insulin resistance is not present when adjustments are made for regional and overall body fat.

Previous studies support an inverse association of total testosterone with insulin resistance (1–6) and body fat (25,26), particularly abdominal fat (5,13,15,27). However, total testosterone levels measure both bioavailable testosterone and SHBG-bound testosterone. The latter is not bioavailable to all target tissues and is influenced directly by SHBG concentration (28), which correlates strongly and inversely with body fat (29,30), insulin levels, and insulin resistance (5,28,31,32). This may underlie the conflicting findings from studies investigating the relationship between bioactive testosterone (free or bioavailable testosterone) and insulin resistance and risks for type 2 diabetes (3,4,7,9–11,16,33). Interpretation of these studies is made difficult by the use of free testosterone measurements, using direct analog methods that, like total testosterone assays, are influenced directly by SHBG concentrations (34,35). In our study, free and bioavailable testosterone levels were calculated from measurements of total testosterone, SHBG, and albumin, using established association constants of testosterone with these major binding proteins and the Sodergard equation (19), according to the methods of Vermeulen et al. (20) and Rosner (34). In contrast to free testosterone levels measured by direct analog assays, free and bioavailable testosterone calculated by this method correlate extremely well with estimates of free testosterone from equilibrium dialysis and bioavailable testosterone measured by the ammonium sulfate precipitation method (20). Using reliable measurements of free and bioavailable testosterone, independent of SHBG concentrations, we confirmed inverse relationships between testosterone and body fat and insulin resistance in men. To our knowledge, no other studies have systematically evaluated the differences between total testosterone and the calculated bioactive testosterone fractions in their relationships with body fat and insulin resistance.

Because adiposity is correlated with testosterone and SHBG variables, potential confounding effects of total and regional adiposity in the association between testosterone or SHBG and the markers of insulin resistance were carefully explored using multivariate regression analysis. This analysis revealed that the associations between bioactive testosterone fractions (free and bioavailable testosterone) and fasting insulin, C-peptide, and HOMA-IR were not independent of body fat measurements by DEXA or CT. Total testosterone remained associated with the insulin resistance markers inde-

Table 2—*Multivariate regression models showing coefficients (P value) of testosterone (bioavailable and total) and SHBG as independent variables*

Models (age included in all models)	Independent variable		
	BAT*	TT†	SHBG‡
Dependent: fasting insulin	−0.035 (0.03)	−0.029 (<0.0001)	−0.013 (<0.0001)
Adjusting for SHBG	−0.028 (0.04)	−0.016 (0.02)	—
Adjusting for CT-TAF	−0.004 (NS)	−0.013 (0.01)	−0.008 (<0.0001)
Adjusting for CT-TAF and SHBG	−0.004 (NS)	−0.003 (NS)	—
Adjusted for CT-IAF	−0.018 (NS)	−0.017 (0.01)	−0.008 (<0.0001)
Adjusted for CT-IAF and SHBG	−0.016 (NS)	−0.009 (NS)	—
Adjusted for DEXA-TBF (kg)§	−0.010 (NS)	−0.012 (0.02)	−0.009 (<0.0001)
Adjusted for DEXA-TBF and SHBG	−0.006 (NS)	0.002 (NS)	—
Dependent: HOMA-IR	−0.039 (0.01)	−0.033 (<0.0001)	−0.015 (<0.0001)
Adjusting for SHBG	−0.031 (0.03)	−0.017 (0.02)	—
Adjusting for CT-TAF	−0.007 (NS)	−0.016 (<0.0001)	−0.009 (<0.0001)
Adjusting for CT-TAF and SHBG	−0.006 (NS)	−0.005 (NS)	—
Adjusted for CT-IAF	−0.022 (NS)	−0.019 (<0.0001)	−0.009 (<0.0001)
Adjusted for CT-IAF and SHBG	−0.009 (NS)	−0.011 (NS)	—
Adjusted for DEXA-TBF (kg)§	0.001 (NS)	−0.015 (0.006)	−0.01 (<0.0001)
Adjusted for DEXA-TBF and SHBG	0.002 (NS)	0.0001 (NS)	—

Data are β (*P* value). *Bioavailable testosterone was a significant predictor of fasting insulin and HOMA-IR, but not independent of abdominal fat by CT or total body fat by DEXA; †the associations between total testosterone and fasting insulin and HOMA-IR were no longer independent of body fat after adjusting for confounding by SHBG; ‡inclusion of testosterone (total, bioavailable, or free) in the models did not diminish the significance of SHBG as a predictor of fasting insulin and HOMA-IR; §DEXA-TBF (kg) denotes total body fat in kilograms by DEXA. Using TBF expressed in percentage (%) yielded similar β and *P* values for the independent variables in the models. BAT, bioavailable testosterone; IAF, intra-abdominal fat, TAF, total abdominal fat, TT, total testosterone.

pendent of body fat measurements, but mainly as a result of its significant correlation with SHBG. Inclusion of SHBG in multivariate models already adjusted for body fat measures completely attenuated the significance of total testosterone as an independent variable in the linear association with fasting insulin, C-peptide, and HOMA-IR. A similar relationship was also reported in young healthy men aged 29–42 years, in whom total body fat and visceral abdominal fat accounted for the significant association between total testosterone and indexes of insulin-glucose homeostasis (29). Bioavailable testosterone fractions were not measured in that study. Interestingly, SHBG did not correlate with the fasting insulin level in that study, in contrast to our finding in middle-aged men. Overall, our results are consistent with the hypothesis that the association between biologically active testosterone (free and bioavailable testosterone) and insulin resistance in men is mediated through total adiposity as well as regional abdominal adiposity.

In several reports of low testosterone and risk of the metabolic syndrome and diabetes in men, SHBG is a stronger correlate of insulin resistance parameters than plasma testosterone (1,9,10,31). SHBG correlated strongly with insulin sensitivity ($r = 0.74$) in men with type 2 diabetes (31). In nondiabetic men, SHBG was shown to be significantly associated with insulin secretory pulse ($r = 0.86$) (32) and with total and nonoxidative glucose disposal (3). In population studies, SHBG is a significant predictor of type 2 diabetes in MRFIT (9) and the Massachusetts Male Aging Study (10). Adjusting for the confounding effect of SHBG in our analysis attenuated the strengths of association between total testosterone and fasting insulin, C-peptide, HOMA-IR, and body fat measures, such that they were similar to those of bioavailable and free testosterone, the biologically active fractions of testosterone. This finding is consistent with the influence of SHBG on total testosterone concentration, and it helps to explain the greater magnitude of associations of total testosterone (compared with bioavailable or free testosterone) with markers of insulin resistance in our study as well as in other studies.

Our findings do not rule out the possibility that low testosterone, through changes in body fat amount or distribution, may be associated with the incidence of higher fasting or 2-h glucose level in follow-up measurements. In the Rancho Bernardo study of men between 55 and 89 years old, baseline total testosterone predicted the onset of type 2 diabetes after 8 years of follow-up, based on oral glucose tolerance tests (2). Bioavailable testosterone, by a modified ammonium sulfate precipitation method, was not a significant predictor of diabetes. Short-term (8 months) testosterone supplementation in centrally obese middle-aged men was associated with improved glucose disposal rate and fasting glucose level, accompanied by decreases in total adipose mass, including abdominal visceral fat, but not fat free mass (8). In contrast, recent reports in healthy young men with experimentally induced hypogonadism found that exogenous testosterone induced a dose-dependent decrease in fat mass and increases in fat-free mass and muscle size (36), but no change in insulin sensitivity (S_i) and glucose effectiveness (S_g). Moreover, in this population, S_i and S_g were not correlated with testosterone concentrations (37). Pretreatment lower body adiposity and lack of association of S_i and S_g with testosterone levels at baseline in those young men could possibly explain the lack of improvement in insulin sensitivity measures, in contrast to improvement in the middle-aged centrally obese men on exogenous testosterone. Furthermore, the shorter length of testosterone administration in the younger men

(20 weeks) may not have provided sufficient time for the detection of alterations in glucose tolerance.

Potential limitations of this study are *1*) the high prevalence of men who were overweight or obese, *2*) inclusion of subjects with treated hypertension, and *3*) a cross-sectional analysis. Adjusting for use of diuretics and β-blockers also did not alter the conclusions. None of our subjects had significant chronic medical conditions or were taking an exogenous testosterone supplement, as confirmed by pharmacy and medical record review and by telephone interview. Fasting insulin is a surrogate marker of insulin resistance, but it is considered to be valid in subjects without diabetes. Our finding may not necessarily be applicable to community-dwelling middle-aged men because of the Veterans Affairs medical care setting and the inclusion and exclusion criteria that we used. Nonetheless, the mean concentrations of the metabolic variables, including fasting insulin and C-peptide, and all three measures of testosterone were comparable to those reported in middle-aged men.

In summary, using testosterone measurements independent of circulating SHBG levels, our study provides additional evidence in support of a significant and inverse association between the bioactive pool of testosterone (bioavailable and free testosterone) and fasting insulin, C-peptide, and HOMA-IR (as surrogate markers for insulin resistance) in middle-aged men. However, the relationship between bioavailable or free testosterone and markers of insulin resistance was not independent of adiposity. Further studies are needed to better understand the interactions between SHBG, testosterone, and adiposity, and their contribution to insulin resistance and the onset of diabetes.

Acknowledgments— This work was supported by grants from the Veterans Affairs Cooperative Studies Program, the American Diabetes Association, and the Seattle Epidemiologic Research and Information Center. A.M.M. was supported by a Veterans Affairs Merit Review grant and the Veterans Affairs Geriatric Research Education and Clinical Center.

References

1. Endre T, Mattiasson I, Berglund G, Hulthen UL: Low testosterone and insulin resistance in hypertension-prone men. *J Hum Hypertens* 10:755–761, 1996
2. Oh JY, Barrett-Connor E, Wedick NM, Wingard DL: Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. *Diabetes Care* 25:55–60, 2002
3. Haffner SM, Karhapaa P, Mykkanen L, Laakso M: Insulin resistance, body fat distribution, and sex hormones in men. *Diabetes* 43:212–219, 1994
4. Haffner SM, Valdez RA, Mykkanen L, Stern MP, Katz MS: Decreased testosterone and dehydroepiandrosterone sulfate concentrations are associated with increased insulin and glucose concentrations in nondiabetic men. *Metabolism* 43: 599–603, 1994
5. Seidell JC, Bjorntorp P, Sjostrom L, Kvist H, Sannerstedt R: Visceral fat accumulation in men is positively associated with insulin, glucose, and C-peptide levels, but negatively with testosterone levels. *Metabolism* 39:897–901, 1990
6. Simon D, Preziosi P, Barrett-Connor E, Roger M, Saint-Paul M, Nahoul K, Papoz L: Interrelation between plasma testosterone and plasma insulin in healthy adult men: the Telecom Study. *Diabetologia* 35: 173–177, 1992
7. Phillips GB: Relationship between serum sex hormones and the glucose-insulin-lipid defect in men with obesity. *Metabolism* 42:116–120, 1993
8. Marin P, Holmang S, Jonsson L, Sjostrom L, Kvist H, Holm G, Lindstedt G, Bjorntorp P: The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. *Int J Obes Relat Metab Disord* 16:991–997, 1992
9. Haffner SM, Shaten J, Stern MP, Smith GD, Kuller L: Low levels of sex hormone-binding globulin and testosterone predict the development of non-insulin-dependent diabetes mellitus in men: MRFIT Research Group: Multiple Risk Factor Intervention Trial. *Am J Epidemiol* 143:889–897, 1996
10. Stellato RK, Feldman HA, Hamdy O, Horton ES, McKinlay JB: Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men: prospective results from the Massachusetts male aging study. *Diabetes Care* 23:490–494, 2000
11. Tibblin G, Adlerberth A, Lindstedt G, Bjorntorp P: The pituitary-gonadal axis and health in elderly men: a study of men born in 1913. *Diabetes* 45:1605–1609, 1996
12. Haffner SM, Valdez RA, Morales PA, Hazuda HP, Stern MP: Decreased sex hormone-binding globulin predicts non-insulin-dependent diabetes mellitus in women but not in men. *J Clin Endocrinol Metab* 77:56–60, 1993
13. Khaw KT, Barrett-Connor E: Lower endogenous androgens predict central adiposity in men. *Ann Epidemiol* 2:675–682, 1992
14. Haffner SM, Valdez RA, Stern MP, Katz MS: Obesity, body fat distribution and sex hormones in men. *Int J Obes Relat Metab Disord* 17:643–649, 1993
15. Tsai EC, Boyko EJ, Leonetti DL, Fujimoto WY: Low serum testosterone level as a predictor of increased visceral fat in Japanese-American men. *Int J Obes Relat Metab Disord* 24:485–491, 2000
16. Abate N, Haffner SM, Garg A, Peshock RM, Grundy SM: Sex steroid hormones, upper body obesity, and insulin resistance. *J Clin Endocrinol Metab* 87:4522–4527, 2002
17. Paradisi G, Smith L, Burtner C, Leaming R, Garvey WT, Hook G, Johnson A, Cronin J, Steinberg HO, Baron AD: Dual energy X-ray absorptiometry assessment of fat mass distribution and its association with the insulin resistance syndrome. *Diabetes Care* 22:1310–1317, 1999
18. Rattarasarn C, Leelawattana R, Soonthornpun S, Setasuban W, Thamprasit A, Lim A, Chayanunnukul W, Thamkumpee N: Relationships of body fat distribution, insulin sensitivity and cardiovascular risk factors in lean, healthy non-diabetic Thai men and women. *Diabetes Res Clin Pract* 60:87–94, 2003
19. Sodergard R, Backstrom T, Shanbhag V, Carstensen H: Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. *J Steroid Biochem* 16: 801–810, 1982
20. Vermeulen A, Verdonck L, Kaufman JM: A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 84:3666–3672, 1999
21. Rossner S, Bo WJ, Hiltbrandt E, Hinson W, Karstaedt N, Santago P, Sobol WT, Crouse JR: Adipose tissue determinations in cadavers–a comparison between cross-sectional planimetry and computed tomography. *Int J Obes* 14:893–902, 1990
22. Retzlaff BM, Dowdy AA, Walden CE, Bovbjerg VE, Knopp RH: The Northwest Lipid Research Clinic Fat Intake Scale: validation and utility. *Am J Public Health* 87:181–185, 1997
23. Godin G, Shephard RJ: A simple method to assess exercise behavior in the community. *Can J Appl Sport Sci* 10:141–146, 1985
24. Jacobs DR Jr, Ainsworth BE, Hartman TJ, Leon AS: A simultaneous evaluation of 10 commonly used physical activity questionnaires. *Med Sci Sports Exerc* 25:81–91, 1993

25. Jankowska EA, Rogucka E, Medras M, Welon Z: Relationships between age-related changes of sex steroids, obesity and body fat distribution among healthy Polish males. *Med Sci Monit* 6:1159–1164, 2000
26. Segal KR, Dunaif A, Gutin B, Albu J, Nyman A, Pi-Sunyer FX: Body composition, not body weight, is related to cardiovascular disease risk factors and sex hormone levels in men. *J Clin Invest* 80:1050–1055, 1987
27. Garaulet M, Perex-Llamas F, Fuente T, Zamora S, Tebar FJ: Anthropometric, computed tomography and fat cell data in an obese population: relationship with insulin, leptin, tumor necrosis factor-alpha, sex hormone-binding globulin and sex hormones. *Eur J Endocrinol* 143:657–666, 2000
28. Vermeulen A, Kaufman JM, Giagulli VA: Influence of some biological indexes on sex hormone-binding globulin and androgen levels in aging or obese males. *J Clin Endocrinol Metab* 81:1821–1826, 1996
29. Tchernof A, Despres JP, Dupont A, Belanger A, Nadeau A, Prud'homme D, Moorjani S, Lupien PJ, Labrie F: Relation of steroid hormones to glucose tolerance and plasma insulin levels in men: importance of visceral adipose tissue. *Diabetes Care* 18:292–299, 1995
30. Tchernof A, Labrie F, Belanger A, Despres JP: Obesity and metabolic complications: contribution of dehydroepiandrosterone and other steroid hormones. *J Endocrinol* 150 (Suppl.):S155–S164, 1996
31. Birkeland KI, Hanssen KF, Torjesen PA, Vaaler S: Level of sex hormone-binding globulin is positively correlated with insulin sensitivity in men with type 2 diabetes. *J Clin Endocrinol Metab* 76:275–278, 1993
32. Peiris AN, Stagner JI, Plymate SR, Vogel RL, Heck M, Samols E: Relationship of insulin secretory pulses to sex hormone-binding globulin in normal men. *J Clin Endocrinol Metab* 76:279–282, 1993
33. Pasquali R, Casimirri F, Cantobelli S, Melchionda N, Morselli Labate AM, Fabbri R, Capelli M, Bortoluzzi L: Effect of obesity and body fat distribution on sex hormones and insulin in men. *Metabolism* 40: 101–104, 1991
34. Rosner W: Errors in the measurement of plasma free testosterone. *J Clin Endocrinol Metab* 82:2014–2015, 1997
35. Winters SJ, Kelley DE, Goodpaster B: The analog free testosterone assay: are the results in men clinically useful? *Clin Chem* 44:2178–2182, 1998
36. Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-Hikim I, Shen R, Storer TW: Testosterone dose-response relationships in healthy young men. *Am J Physiol Endocrinol Metab* 281: E1172–E1181, 2001
37. Singh AB, Hsia S, Alaupovic P, Sinha-Hikim I, Woodhouse L, Buchanan TA, Shen R, Bross R, Berman N, Bhasin S: The effects of varying doses of T on insulin sensitivity, plasma lipids, apolipoproteins, and C-reactive protein in healthy young men. *J Clin Endocrinol Metab* 87:136–143, 2002