Relationship Between Testosterone Levels, Insulin Sensitivity, and Mitochondrial Function in Men

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OBJECTIVE — The goal of this study was to examine the relationship between serum testosterone levels and insulin sensitivity and mitochondrial function in men.

RESEARCH DESIGN AND METHODS — A total of 60 men (mean age 60.5 ± 1.2 years) had a detailed hormonal and metabolic evaluation. Insulin sensitivity was measured using a hyperinsulinemic-euglycemic clamp. Mitochondrial function was assessed by measuring maximal aerobic capacity ($V_{O_{2max}}$) and expression of oxidative phosphorylation genes in skeletal muscle.

RESULTS — A total of 45% of subjects had normal glucose tolerance, 20% had impaired glucose tolerance, and 35% had type 2 diabetes. Testosterone levels were positively correlated with insulin sensitivity ($r = 0.4$, $P < 0.005$). Subjects with hypogonadal testosterone levels ($n = 10$) had a BMI >25 kg/m^2 and a threefold higher prevalence of the metabolic syndrome than their eugonadal counterparts ($n = 50$); this relationship held true after adjusting for age and sex hormone–binding globulin but not BMI. Testosterone levels also correlated with $V_{O_{2max}}$ ($r = 0.43$, $P < 0.05$) and oxidative phosphorylation gene expression ($r = 0.57$, $P < 0.0001$).

CONCLUSIONS — These data indicate that low serum testosterone levels are associated with an adverse metabolic profile and suggest a novel unifying mechanism for the previously independent observations that low testosterone levels and impaired mitochondrial function promote insulin resistance in men.

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Insulin resistance has assumed increasing importance as a risk factor for cardiovascular disease coincident with the dramatic increase in the prevalence of obesity in the Western world. Recent studies using genetic analysis (1,2), functional imaging (3,4), and animal models of differential aerobic capacity (5) have shed light on the role of mitochondrial function in inducing the metabolic disturbances characteristic of insulin-resistant states. Using gene set enrichment analysis to look for changes in sets of genes compiled on the basis of function, Mootha et al. (1) showed decreased maximal aerobic capacity ($V_{O_{2max}}$) and decreased expression of mitochondrial genes involved in oxidative phosphorylation (OXPHOS) in men of Northern European descent with impaired glucose tolerance (IGT) and type 2 diabetes. Using quantitative real-time PCR, Patti et al. (2) reported decreased OXPHOS gene expression in a Mexican-American population of type 2 diabetic subjects as well as insulin-resistant first-degree relatives of type 2 diabetic subjects with normal glucose tolerance (NGT). Magnetic resonance spectroscopy has shown that decreased mitochondrial oxidative and phosphorylation activity underlies the insulin resistance seen with aging (3) and in lean offspring of patients with type 2 diabetes (4). Recent data from rats bred to have low aerobic capacity also implicate impaired mitochondrial function in the development of the cardiovascular risk profile characteristic of the metabolic syndrome (5).

Little is known about the interaction between testosterone levels and insulin sensitivity in men, in contrast to the abundant literature on this relationship in women (6). Cross-sectional studies demonstrate an inverse relationship between testosterone and fasting insulin levels in men independent of age, obesity, and body fat distribution (7–11). A link between testosterone deficiency and diabetes has also been suggested with the demonstration that men with type 2 diabetes have lower testosterone levels than

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Abbreviations: E_2, estradiol; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; PGC-1α, peroxisome proliferator–activated receptor-γ coactivator; OXPHOS, oxidative phosphorylation; SHBG, sex hormone–binding globulin; UQCRB, ubiquinol cytochrome c reductase–binding protein; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

weight-matched nondiabetic control subjects (12,13). In addition, six large prospective studies have shown that low testosterone levels predict development of type 2 diabetes in men (14–19). Two studies demonstrate a positive relationship between total testosterone levels and insulin sensitivity in normal (20) and diabetic men (21). In contrast, data on the relationship between free testosterone levels and insulin sensitivity are conflicting, with two studies showing no correlation (21,22), whereas a third study demonstrates a weak positive relationship (20).

Given that low testosterone levels predict development of type 2 diabetes in men (14–19) and that aging is accompanied by insulin resistance and a decline in testosterone secretion (23–25), we hypothesized that testosterone is an important modulator of insulin sensitivity and mitochondrial function in men. Thus, the aims of this study were to *1*) examine the relationship between serum testosterone levels and insulin sensitivity in men across a wide spectrum of insulin sensitivity, *2*) dissect the role of obesity and alterations in sex hormone–binding globulin (SHBG) levels in mediating this relationship, and *3*) determine if there is an association between serum testosterone levels and mitochondrial function reflected by $V_{O_{2max}}$ and OXPHOS gene expression in skeletal muscle.

RESEARCH DESIGN AND METHODS

— A total of 60 men aged 39–69 years (mean 60.5 ± 1.2) were enrolled in this study. Subjects with a history of testicular disorders or who were taking medications known to interfere with testosterone secretion/action or glucose homeostasis were excluded from the study. Healthy normal men, obese men, and men with newly diagnosed type 2 diabetes on no hypoglycemic agents were enrolled to cover the full spectrum of insulin sensitivity. A total of 42 Caucasian men, who were participants in the Malmo Prevention Study (26), were studied in Sweden. Some of the metabolic characteristics of this Swedish cohort have already been reported (1). There were 18 subjects enrolled in the U.S., of whom 16 were Caucasian and 2 were African-American. The study was approved by the Ethics Committee at Lund University and the Human Research Committee of Massachusetts General Hospital. All subjects provided written informed consent before the initiation of any study procedures.

Anthropometric measures

Height and weight were measured by standard procedures to calculate BMI as weight (kg) divided by height squared (m^2). Waist-to-hip ratio (WHR) was calculated by measuring waist circumference at the level of the umbilicus and hip circumference at the level of the greatest hip girth. In the 42 Swedish subjects, percent body fat was measured using bioelectrical impedance analysis.

Oral glucose tolerance test

A standardized 2-h oral glucose tolerance test using 75 g glucose was performed on the basis of which subjects were classified as having NGT, IGT, or type 2 diabetes using 1985 criteria from the World Health Organization (27).

Insulin sensitivity

Insulin sensitivity was determined by the hyperinsulinemic-euglycemic clamp technique (28). For 3 days before the study, all subjects consumed a weight-maintaining diet containing 300 g carbohydrate per day. The clamp studies were performed at 7:30 A.M. after a 12-h fast. An intravenous cannula was inserted into an antecubital vein for the infusion of insulin and glucose. A second catheter was inserted retrogradely into a hand vein for blood sampling; the hand was kept heated in a warming chamber to arterialize venous samples. Blood glucose was measured every 5 min for 120 min. Forty-two clamps were performed with an insulin infusion rate of 40 mU · m^{-2} · min^{-1} with use of [3-^{3}H]glucose to correct for hepatic glucose production. There were 18 clamps performed with an insulin infusion rate of 80 mU · m^{-2} · min^{-1}, a dose sufficient to suppress hepatic glucose production. An infusion of 20% glucose was initiated and titrated to maintain blood glucose at the fasting level. The glucose disposal rate was determined during the last 30 min of the 2-h clamp. Under steady-state conditions of euglycemia, the rate of exogenous glucose infusion, corrected for glucose space and hepatic glucose production, is equal to the rate of insulin-stimulated glucose disposal, expressed as the *M* value (mg · kg^{-1} · min^{-1}), a measure of insulin sensitivity. Mean insulin levels during the clamp were 497 ± 15 pmol/l and 1,188 ± 75 pmol/l for insulin doses of 40 mU · m^{-2} · min^{-1} and 80 mU · m^{-2} · min^{-1}, respectively. All statistical analyses using insulin sensitivity as a parameter were corrected for insulin levels during the clamp.

Maximal aerobic capacity

$V_{O_{2max}}$ was measured in the Swedish subjects using an incremental work-conducted upright exercise test with a bicycle ergometer (Monark Varberg, Sweden) combined with continuous analysis of expiratory gases and minute ventilation. Exercise was started at a workload varying between 30 and 100 W depending on the history of endurance training or exercise habits and increased by 20–50 W every 3 min, until a perceived exhaustion or a respiratory quotient of 1.0 was reached. Maximal aerobic capacity was defined as the V_{O_2} during the last 30 s of exercise and is expressed per lean body mass calculated by bioelectrical impedance analysis.

Percutaneous muscle biopsy

Percutaneous muscle biopsies (20–50 mg) were taken from the *vastus lateralis* muscle of the Swedish subjects. Biopsies were performed under local anesthesia (1% lidocaine) using a Bergström needle after the hyperinsulinemic-euglycemic clamp. Fiber-type composition and whole-muscle glycogen concentration were determined as previously described (29). Quantification and calculation of the fibers was performed using the COMFAS image analysis system (Scan Beam, Hadsun, Denmark).

Gene set enrichment analysis

Total RNA was isolated from the muscle biopsies, and the targets were hybridized to the Affymetrix HG-U133A chip. Marker analysis was first performed using GeneCluster as previously described (1). The software package SAM was used to identify genes on the microarrays with statistically significant changes in expression. The approach of gene set enrichment analysis was then used to analyze previously defined sets of functionally related genes and search for systematic expression differences among the genes in the set according to glucose tolerance.

Using this technique, a subset of genes involved in oxidative phosphorylation (OXPHOS-CR) was recently identified that is tightly coregulated across

Table 1—*Clinical and biochemical characteristics of the study population*

	All subjects	Testosterone <9.7 nmol/l	Testosterone ≥9.7 nmol/l	*P*
n	60	10	50	—
Age (years)	60 ± 1	57 ± 3.5	61 ± 1	NS
NGT (*n*)	27	1	26	
IGT (*n*)	12	3	9	
Type 2 diabetes (*n*)	21	6	15	
Body composition				
BMI (kg/m^2)	27 ± 0.6	32 ± 2	26 ± 0.5	0.0004
WHR	0.96 ± 0.01	1.02 ± 0.05	0.95 ± 0.01	0.008
Body fat (%)	24.0 ± 0.97	30.1 ± 2.8	23 ± 0.97	0.008
Biochemical characteristics				
Testosterone (nmol/l)	16 ± 0.8	6.8 ± 0.5	17 ± 0.6	<0.0001
SHBG (nmol/l)	36 ± 2	20 ± 3	39 ± 2	0.0008
Fasting glucose (mmol/l)	5.9 ± 0.2	7.5 ± 0.6	5.6 ± 0.3	0.017
Fasting insulin (pmol/l)	66 ± 4	97 ± 3	60 ± 4	0.04
Cholesterol (mmol/l)	5.3 ± 0.14	5.9 ± 0.4	5.2 ± 0.1	NS
Triglycerides (mmol/l)	1.4 ± 0.1	2.3 ± 0.5	1.3 ± 0.1	0.01
HDL cholesterol (mmol/l)	1.3 ± 0.05	1.0 ± 0.8	1.32 ± 0.05	NS
Free fatty acids (μmol/l)	509 ± 24	516 ± 68	507 ± 25	NS
Glucose clamp data				
Insulin sensitivity (mg · kg^{-1} · min^{-1})	6.5 ± 0.4	3.6 ± 0.6	7.3 ± 3	0.0007

Data are means ± SE. *P* values represent differences between subjects with testosterone levels <9.7 vs. ≥9.7 nmol/l.

many tissues, is highly expressed in sites of insulin-mediated glucose disposal, and for which expression is decreased by ~20% in skeletal muscle of men with IGT and type 2 diabetes (1). Of the 34 genes in the OXPHOS-CR set, the top-ranking gene (i.e., the gene with the largest expression difference between normal and diabetic muscle) was ubiquinol cytochrome c reductase–binding protein (*UQCRB*). In the present study, the data on OXPHOS-CR gene expression were analyzed to determine if there was an association with serum testosterone levels.

Biochemical analysis

Fasting blood samples collected before the start of the glucose clamp at −30, −20, and −10 min were pooled and used to determine serum levels of testosterone, estradiol (E_2), SHBG, total and HDL cholesterol, triglycerides, and free fatty acids.

Immunoassays

Serum testosterone concentrations were measured using the DPC Coat-A-Count RIA kit, which has an intra- and interassay coefficient of variation (CV) of <10%. E_2 was measured with an RIA using hexane ethylacetate extraction and LH-20 chromatography (Esoterix, Calabasas Hills, CA). The E_2 assay has a sensitivity of 18 pmol/l and, based on a male serum pool, has an intra-assay CV of 4.9% and an interassay CV of 15%. Plasma glucose was measured with the glucose oxidase method (Beckman Instruments, Fullerton, CA). Insulin was measured by RIA using ^{125}I-labeled human insulin and human insulin antiserum (Linco Research, St. Charles, MO). SHBG was measured by a chemiluminescent enzyme immunometric assay (DPC; Immulite, Los Angeles, CA), which has an intra-assay CV of <7% and an interassay CV of <8%. Free fatty acids were measured by an enzymatic colorimetric method (WAKO Chemicals, Richmond, VA).

Statistical analysis

Data are presented as means ± SE. Differences in the baseline characteristics of eugonadal and hypogonadal subjects were compared with a two-sample *t* test for continuous variables; categorical variables were compared using the Fisher's exact test. A *P* value <0.05 was considered statistically significant. Pearson's correlation coefficients were used to assess the relationship between serum concentrations of testosterone and SHBG and measures of adiposity (BMI, WHR, and percent body fat), insulin sensitivity (M), and mitochondrial function ($V_{O_{2max}}$, OXPHOS gene expression). Multiple regression analysis was performed to control for potential confounding variables including age, insulin, SHBG, BMI, WHR, and percent body fat. Testosterone levels were analyzed as both a continuous and binary variable (hypogonadal = testosterone <9.7 nmol/l and eugonadal = testosterone ≥9.7 nmol/l). All analyses used study site as a covariate to correct for differences between the Swedish and U.S. cohorts.

RESULTS — The characteristics of the study population are provided in Table 1. Of the subjects, 45% had NGT, 20% had IGT, and 35% had type 2 diabetes. Body composition varied significantly, with a range of BMI from 18.7 to 46.3 kg/m^2, WHR from 0.67 to 1.13, and percent body fat from 12.3 to 29.1. Serum testosterone levels covered the spectrum from hypogonadal to eugonadal, ranging from 3 to 31 nmol/l (normal range 9.7–34.6). Testosterone levels were lower in men with IGT and type 2 diabetes than in those with NGT (13.5 ± 6 and 13.1 ± 4 vs. 18 ± 6.5 nmol/l, respectively $P < 0.05$). The glucose clamp studies revealed a spectrum of insulin sensitivity, with insulin sensitivity values ranging from 1.4 to 13.3 mg · kg^{-1} · min^{-1}.

A negative correlation was observed between insulin sensitivity and indexes of obesity, including BMI ($r = -0.58$, $P < 0.0001$), WHR ($r = -0.6$, $P < 0.0001$), and percent body fat ($r = -0.4$, $P = 0.009$). There was an inverse relationship between testosterone and BMI ($r = -0.5$, $P < 0.0001$), testosterone and WHR ($r = -0.38$, $P < 0.005$), and testosterone and percent body fat ($r = -0.5$, $P < 0.0001$). All subjects with hypogonadal testosterone levels had a BMI >25 kg/m^2 and a WHR >0.9. An inverse relationship was also observed between SHBG and BMI ($r = -0.6$, $P < 0.001$), SHBG and WHR ($r = -0.46$, $P < 0.001$), and SHBG and percent body fat ($r = -0.4$, $P = 0.008$). A positive correlation was observed between testosterone levels and insulin sensitivity ($r = 0.4$, $P < 0.005$; Fig. 1) and between SHBG and insulin sensitivity ($r = 0.44$, $P < 0.005$; Fig. 1). In contrast to testosterone, no significant relationship was observed between E_2 levels and

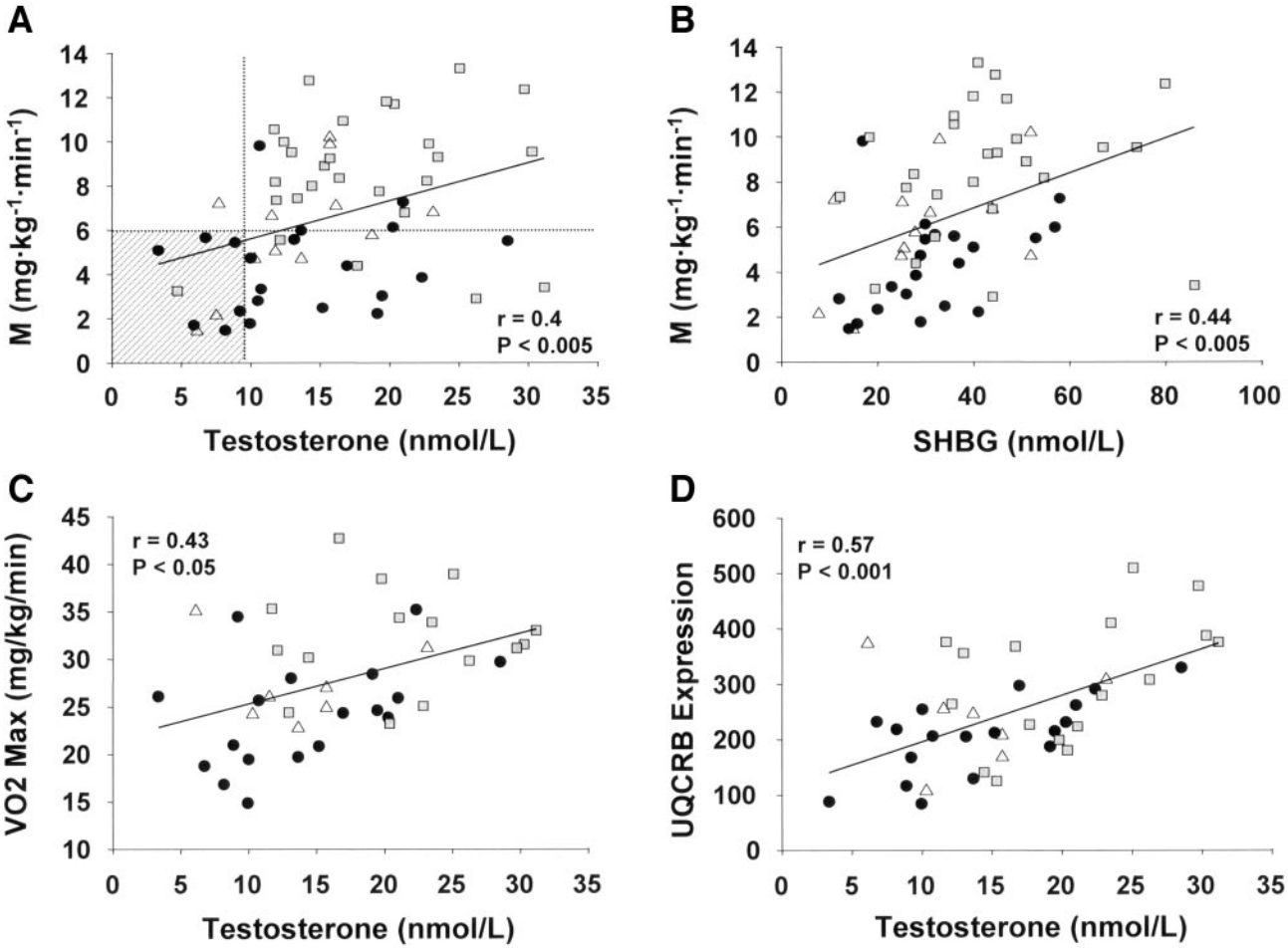

Figure 1—*Correlation between insulin sensitivity (M) and serum testosterone (T) levels* (A) *and SHBG levels* (B) *in 60 men; 27 had NGT (□), 12 had IGT (△), and 21 had type 2 diabetes (●). Shaded area represents values for subjects with hypogonadal testosterone levels, i.e., <9.7 nmol/l. In a subset of 42 men, serum testosterone levels were correlated with maximal aerobic capacity (Vo_{2max})* (C) *and expression of* UQCRB *in skeletal muscle* (D)*; 17 men had NGT (□), 7 had IGT (△), and 18 had type 2 diabetes (●).*

BMI ($r = 0.19$), WHR ($r = 0.1$), or insulin sensitivity ($r = 0.14$).

As depicted in Fig. 1, the relationship between testosterone levels and insulin sensitivity suggests the presence of two distinct populations. All subjects with a serum testosterone level <9.7 nmol/l had a low insulin sensitivity value. In contrast, men with a low insulin sensitivity value exhibited a full range of serum testosterone levels. Subjects with hypogonadal testosterone levels ($n = 10$) were more insulin resistant than their eugonadal counterparts ($n = 50$) (insulin sensitivity = 3.6 ± 0.6 vs. 7.3 ± 3 mg · kg^{-1} · min^{-1}, respectively; $P < 0.0007$) (Table 1). Using regression analysis with testosterone as the dependent variable, the association between low testosterone levels and insulin resistance held true after controlling for age, insulin levels, and SHBG ($P < 0.05$). The relationship between testosterone and insulin sensitivity was no longer significant after adjusting for BMI ($P = 0.23$), percent body fat ($P = 0.17$), or WHR (0.38) expressed as continuous variables. However, body composition had less of an impact on this relationship when analyzed using categorical variables, i.e., BMI ≥ or <30 kg/m^2 ($P = 0.09$), body fat ≥ or <25% ($P = 0.07$), or WHR > or ≤0.9 ($P = 0.15$). Significant differences were also observed in the metabolic profile of the hypogonadal and eugonadal men (Table 1). Using the definition proposed by the Third National Health and Nutrition Examination Survey (30), 90% of subjects with low testosterone levels met criteria for the metabolic syndrome compared with 29% of those with normal testosterone levels ($P < 0.001$). Only 1 of 10 subjects with a hypogonadal testosterone level had NGT compared with 26 of 50 eugonadal men.

Expression of OXPHOS-CR genes in skeletal muscle correlated with Vo_{2max} ($r = 0.5$, $P < 0.001$) as previously reported (1) and with insulin sensitivity ($r = 0.33$, $P < 0.05$). A stronger relationship was observed between the top-ranking OXPHOS-CR gene, *UQCRB*, and both Vo_{2max} ($r = 0.57$, $P < 0.0001$) and insulin sensitivity ($r = 0.38$, $P < 0.05$). Testosterone levels correlated positively with both Vo_{2max} ($r = 0.43$, $P < 0.05$; Fig. 1) and *UQCRB* expression ($r = 0.57$, $P < 0.0001$; Fig. 1). The relationship between testosterone and expression of the entire OXPHOS-CR gene set was close to statistical significance ($r = 0.3$, $P = 0.08$). BMI correlated negatively with Vo_{2max} ($r = -0.4$, $P = 0.009$). However, there was no relationship between BMI and ex-

pression of either *UQCRB* ($r = -0.18$, $P = 0.24$) or OXPHOS-CR ($r = -0.15$, $P = 0.36$). Similarly, a negative correlation was seen between percent body fat and $V_{O_{2max}}$ ($r = -0.49$, $P = 0.001$), and WHR and $V_{O_{2max}}$ ($r = -0.43$, $P = 0.008$). There was a trend for percent body fat to correlate with *UQCRB* ($r = -0.28$, $P = 0.07$), whereas there was no relationship between WHR and *UQCRB* ($r = -0.23$, $P = 0.16$). The relationship between testosterone and $V_{O_{2max}}$ remained significant when adjusted for BMI ($P < 0.05$), percent body fat ($P < 0.05$), and SHBG ($P < 0.0001$), but not WHR ($P = 0.07$). Similarly, the relationship between testosterone and *UQCRB* expression was still significant after adjusting for SHBG levels ($P < 0.0001$). Finally, no relationship was observed between serum testosterone levels and muscle glycogen content or the percentage of type 1, type 2a, or type 2b muscle fibers.

CONCLUSIONS — These data demonstrate a positive correlation between serum testosterone levels and insulin sensitivity in men across the full spectrum of glucose tolerance. Moreover, men with hypogonadal testosterone levels are twice as insulin resistant as their eugonadal counterparts, and 90% fulfill criteria for the metabolic syndrome. From a clinical perspective, these data highlight the importance of performing a comprehensive metabolic evaluation in men with hypogonadism. The demonstration that testosterone levels correlate not only with insulin sensitivity but also with genetic (OXPHOS gene expression) and functional ($V_{O_{2max}}$) markers of mitochondrial function suggests a novel molecular mechanism whereby testosterone might modulate insulin sensitivity in men.

Previous studies on the relationship between androgens and insulin sensitivity in men gave conflicting results depending on whether total or free testosterone levels were used. One explanation for this discrepancy is that SHBG is mediating the link between testosterone and insulin sensitivity. Proponents of the use of free testosterone argue that it is the best index of androgenicity in insulin-resistant men given the low SHBG levels that pertain in this setting (31). An alternative explanation for the discordant results is that the assays used to measure free testosterone have serious methodological limitations, as highlighted in studies validated by mass spectroscopy (32–34). A recent study showed no relationship between free testosterone levels and insulin sensitivity in obese and diabetic men (22). However, the commercial double antibody system used to measure free testosterone in this study correlates poorly with results obtained by equilibrium dialysis (32,35), which is considered the gold standard. In addition, there is controversy as to whether it is only free testosterone that is biologically active, given that testosterone bound to SHBG can bind to cell surface receptors in prostate tissue, leading to activation of adenylyl cyclase and generation of cAMP (36). For these reasons, we chose to use total testosterone levels as the most robust index of androgenicity and to use multiple regression analysis to assess the contribution of SHBG to any association between testosterone levels and insulin sensitivity.

The fact that the correlation between testosterone levels and insulin sensitivity is no longer significant after controlling for BMI could mean that obesity is causing both low testosterone levels and insulin resistance and that there is no direct relationship between testosterone and insulin sensitivity. However, the fact that the impact of BMI on the relationship between testosterone and insulin sensitivity is attenuated significantly when BMI is expressed as a categorical variable suggests that this may not be the case. A second possibility is that the effect of testosterone on insulin sensitivity is mediated through changes in BMI. The relationship between androgens and BMI is likely bi-directional. Morbid obesity has negative effects on the hypothalamic-pituitary-gonadal axis in men (37). In addition, low testosterone levels predispose to central (38) and visceral adiposity (39). In our analysis, all men with hypogonadal testosterone levels have a BMI >25 kg/m^2 and a WHR >0.9. Whereas visceral fat was not assessed in the present study, androgens have been shown to have important effects on visceral fat metabolism mediated largely by stimulation of lipolysis. In vitro, testosterone enhances catecholamine-induced lipolysis by increasing the number of β3-adrenergic receptors on rat adipocyte precursor cells (40). Similarly, castration of male rats reduces lipolysis, which is reversed by physiological testosterone replacement (41). In two small studies of men with central obesity, testosterone inhibits lipoprotein lipase activity in abdominal adipose tissue, leading to decreased triglyceride uptake in central fat depots (42,43). Thus, low testosterone levels may predispose to visceral obesity, leading to dysregulation of fatty acid metabolism, which in turn promotes insulin resistance (44).

Although the present study demonstrates a positive correlation between testosterone levels and insulin sensitivity in men, no conclusions can be drawn about causality given the cross-sectional nature of the data. However, in male rats, castration leads to the rapid development of insulin resistance, which is corrected by physiological testosterone replacement (45). In the human, relatively little is known about the impact of androgens on insulin sensitivity. In men with prostate cancer, induction of hypogonadism with a gonadotropin-releasing hormone agonist results in a 60% increase in fasting insulin levels at 3 months (46,47). Data on the impact of androgen supplementation on insulin sensitivity in men are conflicting depending on the population studied. Androgen administration to men with central obesity and testosterone levels in the low normal range increases insulin sensitivity (48–50). However, a recent study of human chorionic gonadotropin (hCG) administration to healthy older men with low normal testosterone levels showed no change in insulin sensitivity (51). Differences in the outcome of this study may reflect the fact that the study population was less obese and that use of human chorionic gonadotropin was associated with very high E_2 levels, which may have negated the beneficial effects of androgens. In men with type 2 diabetes, one small nonrandomized study showed no beneficial effect of testosterone replacement on glycemic control (52), whereas a larger non–placebo-controlled study showed a significant reduction in HbA_{1c} (A1C) levels after 3 months of testosterone therapy (53).

Recent studies provide insight into the role of mitochondrial function in the pathogenesis of insulin resistance with the demonstration of decreased expression of peroxisome proliferator–activated receptor-γ coactivator (PGC-1α) and downregulation of OXPHOS genes in skeletal muscle of insulin-resistant subjects (1,2). A model for the pathogenesis of insulin resistance has thus been proposed whereby, in genetically susceptible

individuals, environmental risk factors such as physical inactivity contribute to decreased expression of PGC-1α. Reduced PGC-1α expression, in turn, leads to decreased transcription of metabolic and mitochondrial genes and thus decreased oxidative phosphorylation, decreased lipid oxidation, intracellular accumulation of triglycerides in skeletal muscle, and ultimately insulin resistance. In this study, we demonstrate that testosterone levels correlate positively with $V_{O_{2max}}$ and OXPHOS-CR gene expression. From a mechanistic perspective, little is known about how testosterone might influence insulin action. Induction of hypogonadism with a gonadotropin-releasing hormone (GnRH) agonist in normal men decreases lipid oxidation and resting energy expenditure (54). The insulin resistance resulting from castration in rats is associated with a decrease in glycogen synthase activity (45). Lean offspring of patients with type 2 diabetes (4) have also been shown to have decreased glycogen synthase activity, which has been attributed to intramyocellular triglyceride accumulation. Data from the Otsuka Long Evans Fatty (OLETF) rat, a genetic model of obesity and type 2 diabetes, support a role for androgens in modulating mitochondrial function (55). In this model, expression levels of uncoupling protein 1 (UCP-1) and its upstream regulators, PGC-1α and the β_3 adrenergic receptor (β_3AR), are reduced, leading to inefficient energy utilization and obesity (55). Administration of dehydroepiandrosterone (DHEA) for 14 days caused a significant increase in UCP-1, β_3AR, and PGC-1α expression and reversal of the adverse metabolic phenotype that characterizes the OLETF rat with a reduction in body weight, glucose, insulin, free fatty acids, and leptin levels (55).

Thus, it is plausible that hypogonadism may cause insulin resistance via dysregulation of fatty acid metabolism and that low testosterone levels may represent an additional environmental factor contributing to decreased expression of genes involved in oxidative metabolism. Further studies are required to test this hypothesis by examining gene expression profiles in skeletal muscle before and after androgen manipulations. If testosterone is shown to modulate OXPHOS gene expression, androgen supplementation may represent an important therapeutic modality for preventing or treating the metabolic syndrome and/or type 2 diabetes in men.

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