Androgens and Diabetes in Men

Results from the Third National Health and Nutrition Examination Survey (NHANES III)

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OBJECTIVE — Low levels of androgens in men may play a role in the development of diabetes; however, few studies have examined the association between androgen concentration and diabetes in men in the general population. The objective of this study is to test the hypothesis that low normal levels of total, free, and bioavailable testosterone are associated with prevalent diabetes in men.

RESEARCH DESIGN AND METHODS — The study sample included 1,413 adult men aged ≥20 years who participated in the morning session of the first phase of the Third National Health and Nutrition Examination Survey, a cross-sectional survey of the civilian, noninstitutionalized population of the U.S. Bioavailable and free testosterone levels were calculated from serum total testosterone, sex hormone–binding globulin, and albumin concentrations.

RESULTS — In multivariable models adjusted for age, race/ethnicity, and adiposity, men in the first tertile (lowest) of free testosterone level were four times more likely to have prevalent diabetes compared with men in the third tertile (odds ratio 4.12 [95% CI 1.25–13.55]). Similarly, men in the first tertile of bioavailable testosterone also were approximately four times as likely to have prevalent diabetes compared wth men in the third tertile (3.93 [1.39–11.13]). These associations persisted even after excluding men with clinically abnormal testosterone concentrations defined as total testosterone <3.25 ng/ml or free testosterone <0.07 ng/ml. No clear association was observed for total testosterone after multivariable adjustment (*P* for trend across tertiles = 0.27).

CONCLUSIONS — Low free and bioavailable testosterone concentrations in the normal range were associated with diabetes, independent of adiposity. These data suggest that low androgen levels may be a risk factor for diabetes in men.

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Abbreviations: AAG, androstanediol glucuronide; NHANES III, Third National Health and Nutrition Examination Survey; SHBG, sex hormone–binding globulin.

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

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It has been suggested that sex steroid hormones may play a causal role in the development of insulin resistance and type 2 diabetes (1,2). There is a growing amount of literature examining the role sex steroid hormones may play in the development of diabetes and cardiovascular disease in women, but there has been relatively less attention paid to this association in men. Men with endocrine disorders that are associated with low testosterone levels (hypogonadism), such as Klinefelter's and Wolfram's syndromes, have an elevated risk of developing insulin resistance and diabetes (3,4). However, the association between sex steroid concentrations within the normal range and diabetes risk in men in the general population has not been well characterized.

Obesity, one of the most important underlying causes of insulin resistance, is associated with low testosterone levels in men (5–7) and may partially or wholly mediate the process by which endogenous sex hormones influence diabetes risk. Many previous studies have been conducted in small, highly selected populations or convenience samples and/or did not rigorously measure or control for the possible effects of adiposity. The present study was undertaken to investigate the association between sex steroid hormones and diabetes in the general adult male population in the U.S. Specifically, we hypothesized that low levels of total, free, and bioavailable testosterone would be associated with prevalent diabetes. We also hypothesized that this association would persist after controlling for the potentially confounding effects of adiposity. Estimates from this study are nationally representative of the U.S. adult male population in 1988–1991.

RESEARCH DESIGN AND METHODS

— Between 1988 and 1994, the National Center for Health Statistics conducted the Third National Health and Nutrition Examination Survey (NHANES III). NHANES III was designed as a cross-sectional study using a multistage-stratified, clustered probability sample of the U.S. civilian noninstitutionalized population. To ensure adequate

sample sizes of specific subgroups of the U.S. population, Mexican Americans, non-Hispanic blacks, and the elderly were oversampled. Subjects participated in an interview that was conducted at home, as well as an extensive physical examination. This examination was performed at a mobile examination center and included collection of a blood sample.

NHANES III was conducted in two phases (1988–1991 and 1991–1994). Unbiased national estimates of health and nutrition characteristics can be independently produced for each phase. Within each phase, subjects were randomly assigned to participate in either the morning or afternoon/evening examination session. In total, 33,944 subjects were interviewed in NHANES III, of which 30,818 had had a physical examination at the medical examination center. Of 14,781 male subjects with an examination, 9,282 were at least 12 years old, of whom 2,205 participated in the morning session of phase I. Morning sample participants were chosen for this hormone study to reduce extraneous variation due to diurnal production of sex hormones. Serum was still available in the main NHANES III repository for 1,637 of these men: 716 non-Hispanic white, 411 non-Hispanic black, 448 Mexican-American, and 62 other race/ethnicity subjects. The men of other racial/ethnic groups were excluded from the statistical analysis because of small sample size. The present study population was limited to men aged ≥20 years ($n = 1,413$).

Height, weight, and waist and hip circumferences were measured as part of the NHANES examination. BMI was calculated as weight in kilograms divided by the square of height in meters. Participants were defined as having diabetes if they answered "yes" to the question, "Have you ever been told by a doctor or other health professional you had diabetes or sugar diabetes?" Information on age and race/ethnicity were self-reported. Detailed information regarding the collection of data in NHANES III is available elsewhere (8).

Table 1—*Selected characteristics of the study population in men aged ≥20 years by diabetes status, U.S. 1988–1991, NHANES III*

	Overall	Diabetes	No diabetes
n	1,413	101	1,312
Age (years)	42.4 ± 0.8	41.8 ± 0.8	57.0 ± 2.8
Race/ethnicity			
Non-Hispanic white	84.2 ± 2.6	84.6 ± 2.5	74.5 ± 6.6
Non-Hispanic black	10.3 ± 2.0	9.9 ± 1.9	19.6 ± 5.9
Mexican American	5.5 ± 1.5	5.5 ± 1.5	5.9 ± 2.3
BMI (kg/m^2)	26.4 ± 0.2	26.2 ± 0.2	29.5 ± 1.4
Waist-to-hip ratio	0.95 ± 0.003	0.95 ± 0.003	1.02 ± 0.01

Data are means or proportions ± SE.

Sex steroid hormones

The main hormones of interest in this study were measured total testosterone (serum), estimated bioavailable testosterone, and estimated free testosterone. Testosterone is the major male androgen, and its free circulating levels are primarily determined by sex hormone–binding globulin (SHBG). Free (unbound) testosterone accounts for a relatively small circulating concentration of total testosterone (2–3%). Bioavailable testosterone is the concentration of non–SHBG-bound testosterone and is comprised of both free and albumin-bound (20–40%) testosterone levels. Total testosterone is the combination of circulating bioavailable levels and SHBG-bound levels (considered biologically inactive). Measurements of free and bioavailable testosterone levels more accurately represent concentrations readily available to tissues and metabolic processes.

Serum testosterone, estradiol, androstanediol glucuronide (AAG), and SHBG concentrations were measured as part of the larger Hormone Demonstration Project, and all are included in the present study for comprehensiveness. Estradiol is the major estrogen in men; AAG is an indicator of the conversion of testosterone to dihydrotestosterone, the major intraprostatic androgen; and, as mentioned above, SHBG is the major carrier of testosterone and estradiol in circulation.

Blood was drawn after an overnight fast for participants in the morning sample during either an examination at the medical examination center or during an abbreviated examination at home. After centrifugation, the serum was aliquotted and stored at −70°C until spring 2005. Levels of sex steroid hormones and SHBG are stable after multiple freeze-thaw cycles (9,10). Serum concentrations of testosterone, estradiol, AAG, and SHBG were measured in the laboratory of N.R. at Children's Hospital in Boston, Massachusetts. We used competitive electrochemiluminescence immunoassays on the 2010 Elecsys autoanalyzer (Roche Diagnostics, Indianapolis, IN) to quantify serum testosterone, estradiol, and SHBG concentrations. AAG was measured by an enzyme immunoassay (Diagnostic Systems Laboratories, Webster, TX). The participant samples were randomly ordered for testing, and the laboratory technicians were blinded to participant characteristics. The lowest detection limits of the assays were 0.02 ng/ml testosterone, 5 pg/ml estradiol, 0.33 ng/ml AAG, and 3 nmol/l SHBG. For quality control specimens included during the analyses of NHANES III specimens, the coefficient of variation percentages were as follows: testosterone 5.9 and 5.8% at 2.5 and 5.5 ng/ml, estradiol 6.5 and 6.7% at 102.7 and 474.1 pg/ml, AAG 9.5 and 5.0% at 2.9 and 10.1 ng/ml, and SHBG 5.3 and 5.9% at 5.3 and 16.6 nmol/l. Bioavailable and free testosterone levels were calculated from serum total testosterone, SHBG, and albumin concentrations (11).

The protocols for conduct of NHANES III were approved by the institutional review board of the National Center for Health Statistics, Centers for Disease Control and Prevention. Informed consent was obtained from all participants. The assay of stored serum specimens for the Hormone Demonstration Project was approved by institutional review boards at the Johns Hopkins Bloomberg School of Public Health and the National Center for Health Statistics, Centers for Disease Control and Prevention.

Statistical analysis

All statistical analyses were performed using SUDAAN, as implemented in SAS v. 8.1 (Cary, NC) software. In each analysis, we applied sampling weights to take into account the specific probabilities of selection for the individual domains that were oversampled, nonresponse, and differences between the sample and the total U.S. population. Because the serum concentrations were not normally distributed, we compared geometric means by

Table 2—*Crude and adjusted geometric means (95% CI) of sex steroid hormone concentrations in men aged ≥20 years by diabetes status, U.S. 1988–1994*

	Crude geometric mean (95% CI)			Adjusted geometric mean (95% CI)*		
	Overall	No diabetes	Diabetes	Overall	No diabetes	Diabetes
n	1,413	1,312	101	1,413	1,312	101
Total testosterone (ng/ml)	5.11 (4.93–5.29)	5.15 (4.97–5.34)	4.12 (3.78–4.49)	5.11 (4.94–5.28)	5.12 (4.95–5.30)	4.72 (4.31–5.18)
Estradiol (E2) (pg/ml)	35.68 (34.27–37.15)	35.67 (34.22–37.18)	35.93 (33.03–39.09)	35.68 (34.31–37.11)	35.64 (34.24–37.10)	36.73 (33.46–40.32)
SHBG (nmol/l)	35.12 (33.59–36.72)	34.97 (33.41–36.62)	38.92 (35.45–42.73)	35.12 (33.79–36.50)	35.18 (33.84–36.57)	33.68 (30.41–37.31)
AAG (ng/ml)	12.02 (11.44–12..63)	12.12 (11.52–12.76)	9.78 (8.87–10.78)	12.08 (11.48–12.58)	12.04 (11.50–12.61)	11.46 (9.97–13.18)
Estimated free testosterone (ng/ml)	0.10 (0.10–0.11)	0.10 (0.10–0.11)	0.07 (0.07–0.08)	0.10 (0.10–0.10)	0.10 (0.10–0.11)	0.10 (0.09–0.11)
Estimated bioavailable testosterone (ng/ml)	2.38 (2.28–2.49)	2.41 (2.31–2.52)	1.74 (1.57–1.93)	2.38 (2.31–2.46)	2.39 (2.31–2.47)	2.24 (2.02–2.47)
Estradiol:total testosterone (1,000)*	7.40 (7.01–7.81)	7.33 (6.94–7.74)	9.24 (8.17–10.44)	7.40 (7.01–7.80)	7.36 (6.98–7.77)	8.23 (7.35–9.22)
Total testosterone:SHBG	0.50 (0.48–0.53)	0.51 (0.49–0.54)	0.37 (0.33–0.41)	0.50 (0.49–0.52)	0.50 (0.49–0.52)	0.49 (0.43–0.55)
Estradiol:SHBG (1,000)*	3.73 (3.47–4.01)	3.74 (3.48–4.03)	3.39 (3.11–3.69)	3.73 (3.49–3.99)	3.72 (3.48–3.98)	4.00 (3.50–4.59)

*Adjusted for age and race/ethnicity.

diabetes status. Molar ratios of testosterone to SHBG, estradiol to SHBG, and estradiol to testosterone were calculated and analyzed in the same way.

In logistic regression models of prevalent diabetes, the highest (third) tertile of each hormone was used as the reference group and we adjusted for age, race/ethnicity, BMI, and waist-to-hip ratio, as these factors may both influence hormone concentrations and the distributions of which vary by diabetes status. In additional logistic regression models, we also examined the association of total testosterone, estradiol, and SHBG with diabetes after simultaneously adjusting for the other two hormones and the risk factors listed above. Simultaneous adjustment for testosterone, estradiol, and SHBG allowed us to observe whether an association was present with one hormone while holding constant the concentrations of the other two.

RESULTS—Table 1 shows selected (crude) characteristics of this study population of men aged ≥20 years by diabetes status. Men with diabetes were substantially older, more likely to be non-Hispanic black or Mexican American, and had higher BMI and waist-to-hip ratio, highlighting the importance of adjustment for these factors in subsequent analyses.

Table 2 displays the crude and age- and race/ethnicity-adjusted geometric means of each hormone and molar ratios by diabetes status. Total testosterone and estimated bioavailable testosterone concentrations were lower in men with diabetes compared with men without diabetes. Estradiol levels were similar in men with and without diabetes. SHBG levels appeared higher in men with diabetes but not after age and race/ethnicity adjustment. Crude estimated free testosterone was lower in men with diabetes, but this did not persist following adjustment.

The results from our adjusted logistic regression models are displayed in Tables 2 and 3. Total testosterone, estradiol, SHBG, and AAG were not significantly associated with diabetes status after multivariable adjustment (Table 3). However, as shown in Table 4, there was some evidence of an association between total testosterone and diabetes after further adjustment for estradiol and SHBG (odds ratio [OR] 1.99 [95% CI 0.76–5.19]; *P* value for trend = 0.014). Estimated free testosterone and bioavailable testosterone were highly inversely associated with diabetes status, even after multivariable adjustment (Table 3). Men in the lowest tertile of free testosterone level were four times more likely to have prevalent diabetes compared with men in the third tertile (4.12 [1.25–13.55]; *P* value for trend = 0.04). Similarly, men in the first tertile of bioavailable testosterone were also approximately four times as likely to have prevalent diabetes compared with men in the third tertile (3.93 [1.39–11.13]; *P* value for trend = 0.01). These associations persisted even after further adjustment for total cholesterol, triglycerides, and systolic blood pressure (analyses not shown).

We also conducted sensitivity analyses including cases of undiagnosed diabetes (diabetes defined on the basis of a fasting glucose alone; $n = 58$) and excluding men with low total testosterone (<3.25 ng/ml; $n = 211$) or low free testosterone (<0.07 ng/ml; $n = 339$). The results for estimated free testosterone and total testosterone were essentially unchanged after excluding men with clinically low levels: OR 3.23 (95% CI 1.18–8.86) for free testosterone and 1.03 (0.41–2.58) for total testosterone comparing men in the first tertile with the third. The results for all models also were not altered appreciably by the inclusion of undiagnosed diabetes in our case definition (analyses not shown).

CONCLUSIONS— The independent association of low free and bioavailable testosterone levels in our adjusted models suggest that testosterone insufficiency may be a risk factor for diabetes. Associations of low free and bioavailable testosterone levels with diabetes remained even after adjustment for age and known confounding factors including race/ethnicity and adiposity, as measured by BMI and waist-to-hip ratio. The association with low free testosterone persisted even after the exclusion of men with clinically low total and/or free testosterone levels, suggesting that this association was not entirely driven by hypogonadal men.

While the directionality of the associations between low androgen levels and adiposity remain unclear, our data are consistent with the hypothesis that an-

Table 3—*Adjusted* OR (95% CI) of diabetes by tertiles of sex steroid hormone concentrations, NHANES III*

	Q1 (lowest)	Q2	Q3 (highest)	*P* trend
Total testosterone (≤4.54, 4.55–6.27, >6.27 ng/ml)	1.27 (0.61–2.65)	0.51 (0.15–1.72)	1.00 (reference)	0.27
Estradiol (E2) (≤31.90, 31.91–40.26, >40.26 pg/ml)	0.88 (0.36–2.18)	0.96 (0.36–2.57)	1.00 (reference)	0.79
SHBG (≤28.03, 28.04–43.50, >43.50 nmol/l)	0.68 (0.29–1.59)	0.90 (0.43–1.87)	1.00 (reference)	0.42
AAG (≤9.57, 9.58–15.44, >15.44 ng/ml)	2.10 (0.79–5.58)	1.69 (0.64–4.46)	1.00 (reference)	0.12
Estimated free testosterone (≤0.09, 0.10–0.14, >0.14 ng/ml)	4.12 (1.25–13.55)	2.86 (0.78–10.45)	1.00 (reference)	0.04
Estimated bioavailable testosterone (≤2.11, 2.12–3.02, >3.02 ng/ml)	3.93 (1.39–11.13)	3.05 (0.85–10.88)	1.00 (reference)	0.01
Estradiol:total testosterone (1,000) (≤6.24, 6.25–8.23, >8.23)*	0.90 (0.43–1.89)	0.99 (0.43–2.26)	1.00 (reference)	0.82
Total testosterone:SHBG (≤0.45, 0.46–0.63, >0.63)	2.19 (0.83–5.73)	1.65 (0.71–3.83)	1.00 (reference)	0.11
Estradiol:SHBG (1,000) (≤2.92, 2.93–4.75, >4.75)*	2.10 (0.79–5.58)	1.50 (0.61–3.65)	1.00 (reference)	0.14

*Adjusted for age, race/ethnicity, BMI, and waist-to-hip ratio.

drogens may directly influence glucose metabolism and the development of insulin resistance independently of the effects of adiposity. Nonetheless, our results are not as strong in magnitude as those reported in some previous studies (12), likely because we examined these associations in the general male population with "normal" range androgens. We did not observe a clear association of total testosterone concentration with diabetes. Contrary to previous studies (13–15), we did not observe significantly lower levels of SHBG in diabetic compared with nondiabetic men before or after adjustment.

The cross-sectional design is an important limitation of this study. We cannot determine the temporality of the associations observed here between androgen levels and diabetes. However, several previous prospective analyses (13,14,16,17) suggest that decreases in testosterone level may precede the development of diabetes, lending support to a temporal if not causal relation. Additionally, including individuals with undiagnosed diabetes, a population at an earlier point in the progression of diabetes, did not change our results. This is one of the largest epidemiologic studies of androgens and diabetes in men in the published literature (12); nonetheless, we were unable to explore possible effect modification and subgroup analyses due to power limitations resulting from the relatively small number of diabetic cases ($n = 101$) in this general population.

To our knowledge, this is the first study to examine the association between sex steroid hormones and diabetes in a large, nationally representative male population. Strengths of the present study were the large sample and corresponding power to detect small differences, even after adjustment for relevant covariates including measures of adiposity. Furthermore, we have shown here relationships of each major sex steroid hormone with diabetes including differences by molar ratios and multivariable models, which included simultaneous adjustment for total testosterone, estradiol, and SHBG. This study also benefited from the rigorous and standardized measurement of demographic characteristics, laboratory analyses, and anthropometric measures in the NHANES III Study.

In men, serum levels of testosterone and bioavailable testosterone decline with age (18); however, the clinical consequences of this decline are largely uncharacterized. The literature investigating the association between androgen levels in men and the development of cardiovascular disease has been equivocal (19), with some evidence of a possible protective effect of higher testosterone levels (13,20–23). Diabetes is a known risk factor for the development of atherosclerosis and cardiovascular disease. Further studies are need to understand if the elevated risk of cardiovascular disease in hypogonadal men and in men with "low normal" androgen levels seen in some studies might be wholly or partially mediated by the development of diabetes. Additional epidemiologic and etiologic studies are needed to clarify the mechanisms by which sex steroid hormones may directly contribute to diabetes and other chronic diseases.

Table 4—*Adjusted* OR (95% CI) of diabetes by tertiles of sex steroid hormone concentrations after mutually adjusting the hormones, NHANES III*

	Q1 (lowest)	Q2	Q3 (highest)	*P* trend
Total testosterone (ng/ml) (≤4.54, 4.55–6.27, >6.27)	1.99 (0.76–5.19)	0.64 (0.15–2.65)	1.00 (reference)	0.014
Estradiol (E2) (pg/ml) (≤31.90, 31.91–40.26, >40.26)	0.71 (0.24–2.07)	0.99 (0.29–3.33)	1.00 (reference)	0.33
SHBG (nmol/l) (≤28.03, 28.04–43.50, >43.50)	0.48 (0.20–1.18)	0.76 (0.35–1.63)	1.00 (reference)	0.32

*Simultaneously adjusted for age, race/ethnicity, BMI, waist-to-hip ratio, and the other two hormones.

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