American Journal of Epidemiology

Vol. 143, No. 9
Printed in U.S.A.

Low Levels of Sex Hormone-binding Globulin and Testosterone Predict the Development of Non-Insulin-dependent Diabetes Mellitus in Men

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Few prospective data are available regarding the association of sex hormone-binding globulin (SHBG), testosterone, and the risk of developing diabetes. Stored fasting serum samples from participants enrolled in the Multiple Risk Factor Intervention Trial (MRFIT) at 22 different centers throughout the United States from December 1973 through February 1976 were used to perform a nested case-control study. For 176 initially nondiabetic men who developed diabetes during 5 years of follow-up, two controls were selected, one matched only for randomization date, treatment group, and clinic ("loose controls") and the other matched additionally for fasting glucose and body mass index ("tight controls"). When cases were compared with loose controls, higher levels of fasting insulin and lower levels of total and free testosterone and SHBG were significantly associated with increased development of diabetes. However, when cases were compared with tightly matched controls, these associations weakened considerably. Low SHBG and testosterone may constitute part of the prediabetic state in men along with previously reported variables, such as higher glucose and insulin levels and obesity. *Am J Epidemiol* 1996;143:889–97.

diabetes mellitus; insulin; prasterone; sex hormone-binding globulin; testosterone

Established risk factors for the development of non-insulin-dependent diabetes mellitus include obesity (1–6), an unfavorable body fat distribution (1, 3, 5, 6), glucose (1–6) and insulin concentrations (1–6), and insulin resistance (6).

Administration of anabolic steroids in men (7) has been reported to cause glucose intolerance and hyperinsulinemia. Total testosterone has been inversely related to insulin concentrations and insulin resistance in men (8–15). In cross-sectional analysis, total testosterone concentrations have been reported to be lower in men with non-insulin-dependent diabetes mellitus than in normoglycemic men (16–18). Moreover, administration of testosterone (unlike anabolic steroids (7, 19)) was not associated with deterioration in glucose tolerance in men (20).

Many of the studies of sex hormones and diabetes have been limited by small sample size and by cross-sectional study design. Only one study has examined the relation of sex hormone-binding globulin (an indirect index of androgenicity (21)) to the development of non-insulin-dependent diabetes mellitus in men (22). Sex hormone-binding globulin was not significantly related to the development of non-insulin-dependent diabetes mellitus in men, but the sample size was small (22). Furthermore, the association of other sex hormones with the development of non-insulin-dependent diabetes mellitus in this study was not explored. We hypothesized on the basis of cross-sectional studies that testosterone would be related to the development of non-insulin-dependent diabetes mellitus prospectively. In the current report, we examine the association of total testosterone, free testosterone, estradiol, dehydroepiandrosterone sulfate, and sex hormone-binding globulin in 176 male subjects who developed diabetes in the Multiple Risk Factor Intervention Trial (MRFIT) and two matched control groups (each n = 176) that remained normoglycemic.

Received for publication February 8, 1995, and in final form February 26, 1996.

Abbreviations: DHEA, dehydroepiandrosterone; MRFIT, Multiple Risk Factor Intervention Trial; SHBG, sex hormone-binding globulin.

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MATERIALS AND METHODS

MRFIT procedures

Detailed descriptions of the MRFIT have been published previously (23–27). Briefly, the MRFIT was a primary prevention trial to determine the effects of multifactor intervention on coronary heart disease mortality. Over 360,000 men were screened for eligibility at 22 clinical centers throughout the United States; 12,886 men aged 35–57 years were enrolled in the trial from December 1973 through February 1976. Of these, 6,428 were randomized to a special intervention group, and 6,438 were randomized to their usual sources of medical care.

Definition of diabetes

A complete description of the MRFIT definition of diabetes has been given previously (28). Briefly, diabetes was defined to have occurred the year in which the participant first reported using insulin or oral hypoglycemics, or the first of two consecutive years in which fasting glucose values exceeded 140 mg/dl, or the year in which fasting glucose exceeded 140 mg/dl if in the following year the participant reported using insulin or hypoglycemic drugs.

Study design

The present study utilized a matched case-control design nested within a prospective cohort design in which cases were MRFIT participants (both usual sources of medical care and special intervention group) who had developed diabetes in years 1–5 of the trial. Two controls were matched to each case. One control (referred to as "loose") was matched only by randomization date (within 30 days), treatment group assignment, and clinic. The other control was matched additionally for fasting glucose (within 5 mg/dl) and, if more than one potential control was available, the participant whose body mass index (defined as weight (kg)/height $(m)^2$) was closest to the case was selected.

Subjects excluded from the current analysis included those who had no baseline fasting glucose value, fewer than two fasting glucose readings throughout the trial, or no sera stored in the freezer. In addition, this study excluded participants with baseline fasting glucose exceeding 140 mg/dl and/or baseline glucose 1 hour after a 75-g glucose load exceeding 300 mg/dl. The sera from 177 cases, 176 of the first set of controls, and 177 of the second set of controls were available for analysis, giving 176 complete triads.

Determinations of insulin and sex hormones

Sera drawn at baseline had been frozen and stored at −70°C for an average of 20 years. After cases and controls were identified, their sera were withdrawn from the freezer and shipped to the University of Pittsburgh. These samples remained frozen until they were thawed just prior to analysis. Upon thawing, they were separated into two aliquots. The first aliquot was analyzed for insulin at the University of Pittsburgh using a standard double antibody charcoal assay (29, 30) that has a high degree of cross-reactivity with proinsulin. The second aliquot was shipped on ice to the Division of Clinical Epidemiology, University of Texas Health Science Center at San Antonio, for analysis of sex hormones. These assays were completed within 2 weeks of arrival in San Antonio. Estradiol, total testosterone, and dehydroepiandrosterone (DHEA) sulfate were measured with solid phase commercial radioimmunoassays (Diagnostic Products Corporation, Los Angeles, California). Free testosterone was measured with a commercial double antibody system (Diagnostic Products Corporation) (14, 15). Sex hormone-binding globulin was measured by a commercial double antibody system (Diagnostic Systems Laboratory, Webster, Texas). The intraassay and interassay coefficients of variation for SHBG were 6.0 percent and 9.0 percent, respectively. Intra- and interassay coefficients of variation in the Division of Clinical Epidemiology laboratories for total testosterone were 4.1 percent and 6.0 percent, respectively; for estradiol, 5.9 percent and 7.2 percent, respectively; and for DHEA sulfate, 5.8 percent and 7.9 percent, respectively (14, 15). The lower limit of sensitivity of the free testosterone assay is 0.52 pmol/liter. The intraassay coefficient of variation for free testosterone is 5.0 percent, and the interassay coefficient of variation is 7.0 percent in the Division of Clinical Epidemiology laboratory for specimens from males.

Statistical analyses

Univariate comparisons of cases and controls were performed by subtracting values within the matched triad, averaging the differences, and using the Student t statistic to test the null hypothesis that the average difference was zero. For insulin and sex hormones, this analysis was performed on the log-transformed difference to adjust for skewness.

Multivariate comparisons of cases and controls were performed using Cox proportional hazards regression models, stratifying by case-control triad. The time until event (development of diabetes) was assigned a dummy variable of 1 for cases and 0 for controls. (This statistical method is equivalent to fitting conditional logistic regression models.) The relative risk of developing diabetes was assessed for cases and controls whose levels of insulin and sex hormones were above the median (pooled case-control population) compared

with those below the median. In figures 1 and 2, body mass index, insulin, and the sex hormones were stratified into quintiles. The cutpoints are given in the figures.

RESULTS

Table 1 shows means for selected baseline variables in diabetic cases, tight controls, and loose controls. It also shows the average paired difference between the cases and the two control groups. Diabetic cases were slightly older than were their controls (average paired difference = 0.82 years), but the difference was not statistically significant. Despite attempts to match the tight controls for both fasting glucose and body mass index, the paired difference between cases and controls for both variables was still statistically significant at $p < 0.01$. Using the controls in which no attempt was made to match according to glucose or body mass index, we found that the average level of fasting glucose was 10 mg/dl higher in cases than in controls and that the average level of body mass index was 2.2 kg/m^2 higher in cases than in controls. Cases also had higher levels of weight, systolic blood pressure, and postload glucose and lower levels of serum cholesterol.

The level of insulin for cases was an average of 6.35 microunits/ml ($p < 0.001$) higher than in loose controls but only 2.96 microunits/ml higher when cases were compared with controls matched by glucose and body mass index. Levels of DHEA sulfate, free testosterone, total testosterone, and SHBG were lowest for diabetic cases and highest for the loose controls. The paired differences between cases and loose controls were statistically significant for free testosterone, total testosterone, and SHBG concentrations. Levels of estradiol were highest among the cases and lowest among loose controls, but these differences were not statistically significant. None of the paired differences in log-transformed values of sex hormones between cases and tight controls were statistically significant except for free testosterone.

Table 2 further explores the associations between insulin, sex hormones, and diabetes by showing the relative risk of developing diabetes among participants having above-median levels of selected variables compared with participants having below-median levels. This analytical method is unaffected by skewness in the data. Relative risks were assessed using proportional hazards models stratified by case-control triad, with increasingly complete adjustments. Compared with loose controls, the age-adjusted relative risk of developing diabetes is significantly associated with having insulin values above the median and having values of free testosterone and SHBG below the median. When compared with tight controls, these associations remain but weaken to become marginally statistically significant ($0.05 < p < 0.10$). For total testosterone, the relative risk of developing diabetes is not significantly different from 1 when participants having values above and below the median are compared, and this is true for both loose and tight controls. The relative risk for developing diabetes in cases compared with either loose or tight controls is not significantly different from 1 for either estradiol or DHEA sulfate (data not shown).

Adjusting the models further by other baseline risk factors and by other sex hormones does not appreciably change the pattern of associations for insulin and SHBG from what they are when the models are adjusted only by age. However, further adjustment weakens the association between developing diabetes and free testosterone to the point of statistical nonsignificance.

Tables 1 and 2 may be used to determine if associations between sex hormones and diabetes exist and, if so, the direction of the association. Figure 1 shows the age-adjusted relative risk of developing diabetes in the upper four quintiles of insulin and of each sex hormone compared with the lowest quintile; it may be used to determine if there are trends in the association. The age-adjusted relative risk of developing diabetes increases steadily with increasing levels of insulin when cases are compared with loose controls, and it increases, though to a much lesser extent, when cases are compared with tight controls. The age-adjusted relative risk of developing diabetes decreases steadily with increasing levels of SHBG and with increasing levels of free testosterone. Except for quintile III of free testosterone, the association is stronger when cases are compared with loose controls rather than tight controls, though the difference in the association is not nearly as pronounced as it is for insulin. The relative risk for developing diabetes generally declines with increasing levels of total testosterone; however, the pattern is somewhat erratic.

Table 3 and figure 2 reveal some of the cross-sectional age-adjusted interrelation between insulin, sex hormones, body mass index, and glucose. Insulin shows strong positive correlations with body mass index and fasting glucose and less strong but still statistically significant inverse correlations with SHBG, free testosterone, and total testosterone. Free testosterone and total testosterone are positively correlated with each other, but SHBG is more strongly correlated with total than free testosterone. All three sex hormones are inversely correlated with body mass index, though these associations are not as strong as the positive association between insulin and body

TABLE 1. Levels of selected baseline variables, insulin, and sex hormones for diabetic cases and matched controls enrolled in the Multiple Risk Factor Intervention Trial (MRFIT) between 1973 and 1976

	Age (years)		Body mass index (kg/m²)		Black race (%)		Systolic blood pressure (mmHg)		Serum cholesterol (mg/dl)		% smokers	
	Average	*p* value for paired difference	Average	*p* value for paired difference	Average	*p* value for paired difference	Average	*p* value for paired difference	Average	*p* value for paired difference	Average	*p* value for paired difference
Cases	46.3 (5.6)‡		29.8 (3.8)		14.1		139.1 (14.7)		247.3 (36.9)		65.5	
Tight controls	45.3 (6.1)	0.12	29.0 (3.4)	<0.001	19.8	0.16	136.4 (13.8)	0.07	256.7 (35.1)	0.02	58.8	§
Loose controls	45.7 (5.9)	0.33	27.6 (3.4)	<0.001	10.2	0.26	135.1 (13.2)	<0.01	252.4 (33.3)	0.15	60.5	§

	No. of cigarettes/ day for smokers		Hypertensive (%)*		Fasting glucose (mg/dl)		Glucose 1 hour after 75-g load (mg/dl)		Insulin (microunits/ml)	
	Average	*p* value for paired difference	Average	*p* value for paired difference	Average	*p* value for paired difference	Average	*p* value for paired difference	Average	*p* value for paired difference†
Cases	32.2 (15.4)		48.6		107.1 (10.6)		212.3 (40.6)		24.8 (12.5)	
Tight controls	36.6 (16.3)	0.14	55.9	0.17	106.2 (10.2)	<0.01	187.8 (47.1)	<0.001	21.7 (11.9)	<0.01
Loose controls	34.0 (14.9)	0.31	54.2	0.29	97.0 (11.1)	<0.01	159.2 (40.7)	<0.01	18.6 (10.6)	<0.001

	Dehydroepiandrosterone sulfate (μg/dl)		Free testosterone (pg/ml)		Total testosterone (ng/ml)		Sex hormone-binding globulin (nmol/liter)		Estradiol (pg/ml)	
	Average	*p* value for paired difference†	Average	*p* value for paired difference†	Average	*p* value for paired difference†	Average	*p* value for paired difference†	Average	*p* value for paired difference†
Cases	235.0 (143.3)		19.03 (6.6)		5.0 (1.5)		37.03 (14.8)		37.2 (13.6)	
Tight controls	250.1 (130.0)	0.22	20.10 (5.6)	0.02	5.2 (1.5)	0.58	41.51 (18.3)	0.08	35.2 (13.4)	0.11
Loose controls	261.4 (159.9)	0.08	21.05 (7.4)	0.01	5.6 (1.9)	<0.01	43.54 (22.3)	<0.01	36.3 (15.1)	0.47

* Hypertensive at baseline if using antihypertensive medication or if diastolic blood pressure ≥ 95 mmHg.

† *p* value for paired differences in insulin and sex hormones determined by using the log-transformed values of insulin and sex hormones.

‡ Numbers in parentheses, standard deviation.

§ Smoking status was identical for cases and tight controls in 57.4% of the triads and identical for cases and loose controls in 56.5% of the triads. In 17.6% of the triads, cases were nonsmokers and tight controls were smokers, while in 25.0% of the triads, cases were smokers and tight controls were nonsmokers. In 19.2% of the triads, cases were nonsmokers and loose controls were smokers, while in 24.3% of the triads, cases were smokers and loose controls were nonsmokers.

TABLE 2. Associations between the development of diabetes and insulin and sex hormones for cases and matched controls enrolled in the Multiple Risk Factor Intevention Trial (MRFIT) between 1973 and 1976, with 20-year follow-up

Variable	Median value*	No. of cases above median	Cases compared with					
			Loose controls			Tight controls		
			No. above median	Adjusted† RR‡,§	95% CI‡	No. above median	Adjusted RR	95% CI
Models adjusted by age								
Insulin	19.1 microunits/ml	108	63	4.42	2.47–7.91	93	1.52	0.96–2.42
Free testosterone	19.08 pg/ml	73	94	0.63	0.41–0.99	90	0.66	0.41–1.06
Total testosterone	5.2 ng/ml	77	84	0.87	0.56–1.35	86	0.82	0.52–1.30
Sex hormone-binding globulin	38.7 nmol/liter	72	92	0.59	0.38–0.93	94	0.66	0.43–1.01
Models adjusted by age and baseline covariates†								
Insulin	19.1 microunits/ml	108	63	3.99	2.14–7.42	93	1.60	0.93–2.75
Free testosterone	19.08 pg/ml	73	94	0.66	0.39–1.19	90	0.78	0.46–1.32
Total testosterone	5.2 ng/ml	77	84	0.89	0.51–1.53	86	0.82	0.47–1.42
Sex hormone-binding globulin	38.7 nmol/liter	72	92	0.45	0.25–0.80	94	0.61	0.37–0.99
Models adjusted by age, baseline covariates,† insulin, and sex hormones§								
Insulin	19.1 microunits/ml	108	63	3.61	1.83–7.15	93	1.52	0.84–2.75
Free testosterone	19.08 pg/ml	73	94	0.72	0.38–1.37	90	0.85	0.48–1.48
Total testosterone	5.2 ng/ml	77	84	1.07	0.56–2.03	86	0.94	0.52–1.70
Sex hormone-binding globulin	38.7 nmol/liter	72	92	0.47	0.24–0.90	94	0.55	0.32–0.94

* Median among the pooled case-control population.

† Baseline covariates include age, diastolic blood pressure, serum cholesterol, reported number of cigarettes smoked per day, fasting glucose, glucose 1 hour after a 75-g load, body mass index, and insulin. The model for insulin was adjusted by baseline covariates only.

‡ RR, relative risk; CI, confidence interval.

§ Relative risk for being above median compared with being below median. Relative risks for insulin, total testosterone, and sex hormone-binding globulin are not adjusted by free testosterone. The relative risk for free testosterone is not adjusted by total testosterone.

mass index. All three sex hormones are inversely correlated with fasting glucose; however, these correlations are weaker than those with body mass index and, in the case of free testosterone, statistically not significant. Figure 2 demonstrates that, although average levels of insulin rise steadily within quintiles of both body mass index and fasting glucose, average levels of the sex hormones generally decline within increasing quintiles of body mass index or fasting glucose, although these associations are more erratic. Nevertheless, age-adjusted tests for linear trend within log-transformed variables are statistically significant at conventional levels except for free testosterone with fasting glucose.

DISCUSSION

Body mass index and fasting glucose levels are strong predictors of risk of developing non-insulin-dependent diabetes mellitus in the MRFIT population (28) and other studies (1–6). In this study, matching controls to the cases on these factors leads to a considerable attenuation of the magnitude of the association between insulin and the development of diabetes. It could be argued, however, that elevated fasting glucose and insulin levels are components of the pathway through which obesity increases insulin resistance and, hence, increases diabetes risk. Attempting to adjust for a causal factor, such as obesity, may thus represent overadjustment or overmatching. For example, testosterone administration may improve insulin sensitivity in male rats (31) and men (32), and thus adjustment for this covariate (e.g., insulin) could also be considered overadjusting for variables in the causal pathway. The choice of covariates in multivariate models is not always obvious and may depend on whether one is interested in statistical prediction or alternatively in elucidating pathogenesis.

We found a consistent inverse association between SHBG concentrations at baseline and the subsequent development of diabetes. This association is present when cases are compared with both loose and tight controls, and it remains statistically significant after adjustment for insulin concentrations. In premenopausal women, low levels of sex hormone-binding globulin are thought to reflect increased androgenicity (21). In the present study of men, SHBG concentra-

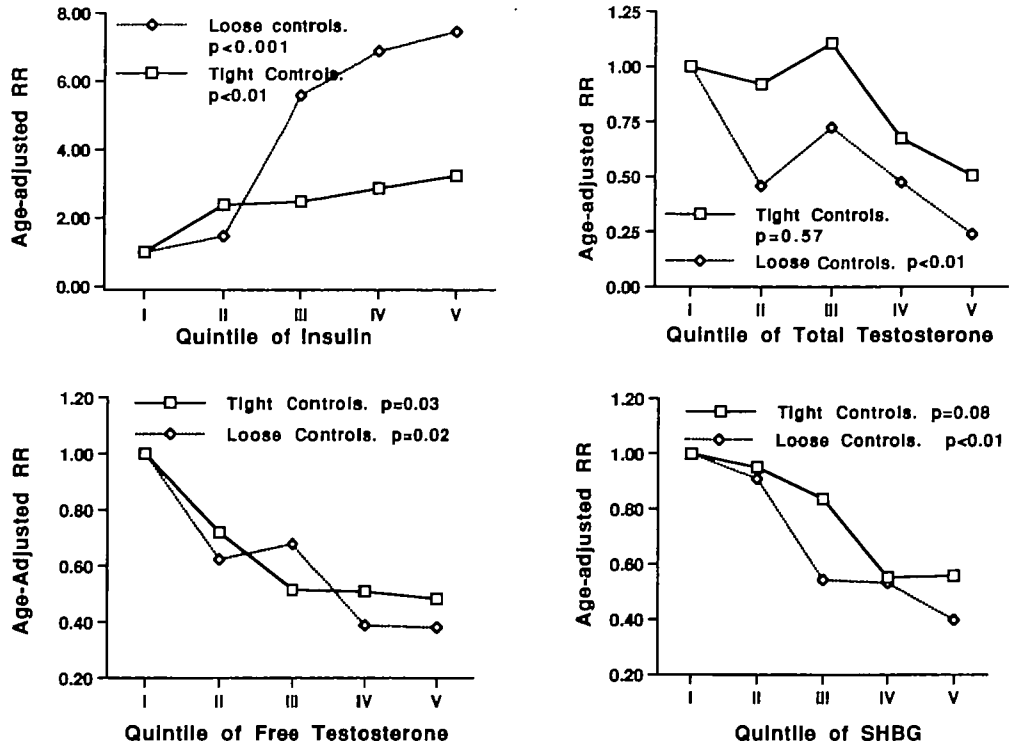

FIGURE 1. Relation of quintiles of insulin, total and free testosterone, and sex hormone-binding globulin (SHBG) to the development of diabetes. The cutpoints for insulin (microunits/ml) were for quintile 1, <11.9; quintile 2, 12.0–16.6; quintile 3, 16.7–21.5; quintile 4, 21.6–29.7; and quintile 5, >30.0. The cutpoints for total testosterone (ng/ml) were for quintile 1, <3.8; quintile 2, 3.9–4.7; quintile 3, 4.8–5.5; quintile 4, 5.6–6.5; and quintile 5, 6.6–16.2. The cutpoints for free testosterone (pg/ml) were for quintile 1, <15.23; quintile 2, 15.27–17.91; quintile 3, 17.94–20.59; quintile 4, 20.60–24.43; and quintile 5, ≥24.54. The cutpoints for SHBG (nmol/liter) were for quintile 1, <24.84; quintile 2, 24.89–34.22; quintile 3, 34.25–42.10; quintile 4, 42.11–53.62; and quintile 5, ≥53.79. RR, relative risk.

TABLE 3. Correlation matrix for insulin, sex hormones, and selected risk factors for diabetic cases and matched controls* enrolled in the Multiple Risk Factor Intervention Trial (MRFIT) between 1973 and 1976

	Insulin		Free testosterone		Total testosterone		Sex hormone-binding globulin	
	r†	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value
Insulin	1.000	0.0						
Free testosterone	–0.103	0.02	1.000	0.0				
Total testosterone	–0.086	0.05	0.41	<0.001	1.000	0.0		
Sex hormone-binding globulin	–0.159	<0.001	0.134	<0.01	0.333	<0.001	1.000	0.0
Body mass index	0.364	<0.001	–0.148	<0.001	–0.106	0.02	–0.140	<0.01
Fasting glucose	0.191	<0.001	–0.064	0.15	–0.087	0.05	–0.101	0.02

* Cases and both control groups pooled. Correlations are age adjusted and based on log-transformed values of insulin and sex hormones.
† Pearson's correlation coefficient.

tions are strongly positively associated with total (but not free) testosterone. However, in the human hepatoma (Hep G2) cell line, insulin inhibits while testosterone and estradiol both stimulate SHBG production (33). Although a large number of studies have reported conflicting results (rise, fall, or no change) on the effect of insulin on sex hormones and SHBG in women (34), few data are available on the effect of insulin on sex hormones in men. Nestler (35) has proposed that SHBG may be a marker for insulin

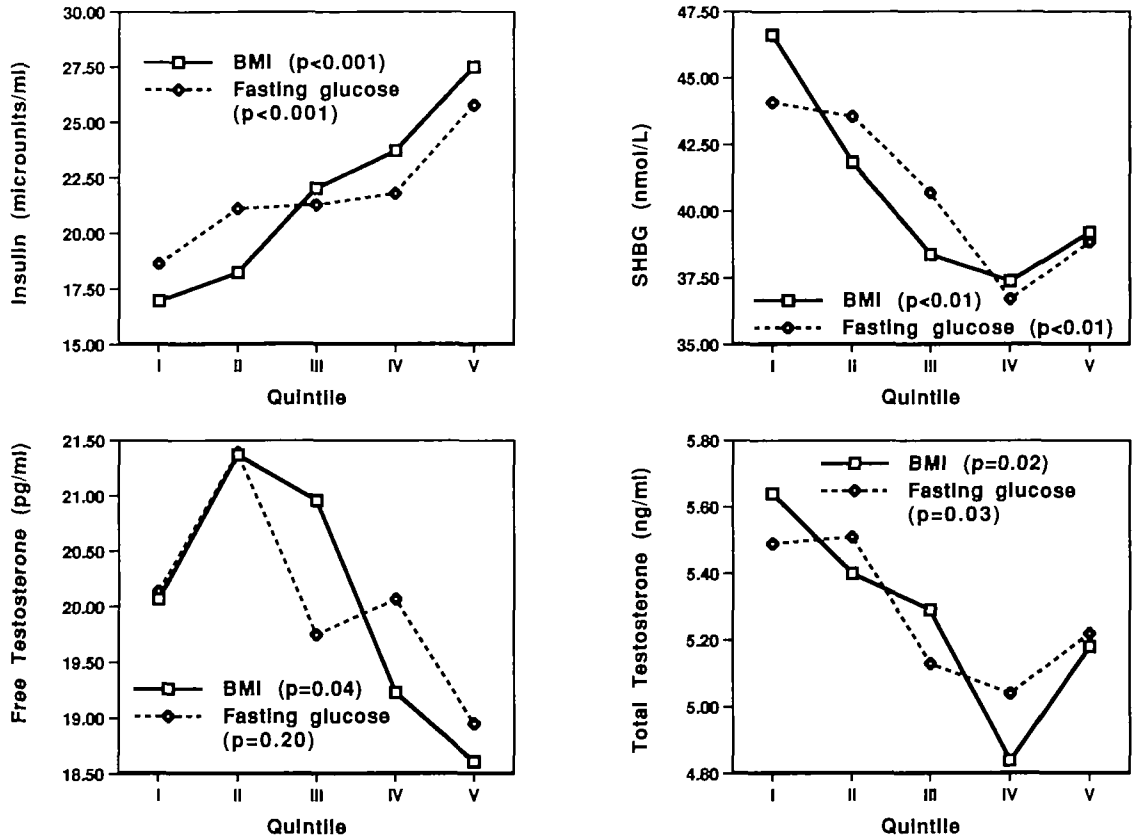

FIGURE 2. Relation of quintiles of body mass index (BMI) and glucose to insulin, sex hormone-binding globulin (SHBG), free testosterone, and total testosterone. The cutpoints for body mass index (kg/m^2) were for quintile 1, <25.7; quintile 2, 25.7–27.5; quintile 3, 27.5–29.6; quintile 4, 29.6–31.9; and quintile 5, >31.9. The cutpoints for fasting glucose (mg/dl) were for quintile 1, <94; quintile 2, 94–100; quintile 3, 101–106; quintile 4, 107–113; and quintile 5, ≥114. *p* values were calculated by linear trend across quintiles. L, liter.

resistance. In men who normally have high androgen levels, it is possible that insulin resistance or hyperinsulinemia may be a major determinant of SHBG levels.

Our results indicate that men with low levels of free testosterone are at increased risk of developing diabetes, although the association was weaker when using the tight controls. Low levels of total testosterone are associated with a more extreme increased risk of diabetes, but these associations are inconsistent over varying levels of total testosterone. A considerable body of evidence supports the cross-sectional association of lower free and total testosterone with increased insulin concentrations and insulin resistance in men (8–15). These results are consistent with animal studies in which castrated male rats have decreased insulin sensitivity that is improved by low dose (replacement) testosterone administration (31). Treatment with high dose testosterone, however, decreases insulin sensitivity (31). Mårin et al. (32) have reported that administration of testosterone to centrally obese middle-aged men reduces central adiposity and decreases insulin resistance. These animal (31) and human (32) studies are thus compatible with the inverse association between testosterone and the incidence of diabetes reported in this study.

In the present study, we found inconsistent and weak inverse associations between DHEA sulfate concentrations and the incidence of diabetes. We also did not find a significant association between estradiol and the incidence of diabetes. Most previous studies have not found a relation between estradiol concentrations and either glucose or insulin concentrations (9, 13–15) or insulin resistance (22), but Small et al. (16) did find higher estradiol levels in diabetic subjects than in nondiabetic subjects.

Several studies have examined the cross-sectional association of insulin resistance with sex hormones and binding proteins in men. Birkeland et al. (36) showed strong correlations between insulin sensitivity and SHBG ($r = 0.74$, $p < 0.001$) and moderate correlations between insulin sensitivity and total testosterone ($r = 0.43$, $p < 0.05$) in 23 men with non-insulin-dependent diabetes mellitus. These investiga-

tors did not observe a significant correlation between insulin sensitivity and free testosterone ($r = -0.06$), free estradiol ($r = -0.33$), or body mass index ($r = -0.39$), although the small sample size meant that it was not possible to exclude the existence of correlations of substantial magnitude. They also showed that the association between SHBG and insulin sensitivity remained statistically significant after adjustment for obesity and body fat distribution. In contrast, Peiris et al. (37) showed a significant association between SHBG and insulin secretory pulse interval ($r = 0.86$, $p < 0.05$) but not with peripheral insulin sensitivity in 10 nondiabetic men. Strong significant positive correlations between SHBG and total ($r = 0.37$) and nonoxidative ($r = 0.37$) glucose disposal and also between total testosterone and total ($r = 0.45$) and nonoxidative ($r = 0.45$) glucose disposal were found in 87 normoglycemic men (15). In contrast, glucose oxidation was not significantly related to any of the sex hormones or DHEA sulfate. The finding of a specific defect in nonoxidative glucose disposal with higher SHBG and total testosterone is consistent with previous data in nondiabetic relatives of subjects with non-insulin-dependent diabetes mellitus (38, 39). Thus, decreased total testosterone and SHBG might constitute early correlates of nonoxidative glucose disposal in the prediabetic state.

Several limitations of the current study should be noted. We did not have information on upper body adiposity, which has been associated with an increased incidence of non-insulin-dependent diabetes mellitus (1, 3, 5). In addition, we only used body mass index as a proxy for a more direct measure of overall adiposity. We have also measured insulin concentrations rather than insulin resistance, although in normoglycemic individuals, the two are moderately correlated ($r = -0.60$) (40). Lastly, this study was not based on a population-based cohort but rather on a group of men at high risk for coronary heart disease. Selection criteria for the MRFIT were based on the presence of risk factors that could distort the cross-sectional associations reported in this study. The selection criteria should have less influence on prospective risk relations, however, as is illustrated by the similarity of risk factors for diabetes in MRFIT (28) compared with other studies (1–6).

These investigations also are limited by the skewness and imprecision of measures of insulin and sex hormones. A variety of methods were used to overcome these limitations, including using log-transformed values of the measurements in tests assuming normal distributions and assessing relative risks over different points in the distribution of the data (i.e., the median in table 2 and quintiles in figure 1). Differing methods sometimes produced somewhat different levels of statistical significance, though the general pattern of observations remained the same; that is, development of diabetes is positively associated with increased levels of insulin and with decreased levels of SHBG and testosterone. For most variables, the strength of these associations is weakened when baseline levels of fasting glucose and body mass index are similar in cases and controls, although the association between SHBG and developing diabetes appears most robust. The relative risk of developing diabetes rises rather consistently with increased levels of insulin and with decreased levels of free testosterone and SHBG. However, the relative risk of developing diabetes is somewhat inconsistent over differing levels of total testosterone.

In conclusion, when diabetic cases are compared with loosely matched controls, we show a strong positive association of fasting insulin and strong inverse associations of both free testosterone and SHBG to the development of diabetes in men. These results were similar whether the data were analyzed by a paired difference in log-transformed values (table 1), by the relative risks associated with being above versus below the median (table 2), or by quintiles of sex hormones (figure 1). Our data suggest that low sex hormone-binding globulin and testosterone may form part of the prediabetic state in men in addition to previously reported factors including obesity, an unfavorable body fat distribution, impaired glucose tolerance, hyperinsulinemia, and insulin resistance (1–6).

ACKNOWLEDGMENTS

This work was supported by grants from the National Heart, Lung, and Blood Institute (R01 HL24799, R37 HL36820, and HL43232).

The authors acknowledge the efforts of the many MRFIT investigators who collected these data and the MRFIT Editorial Committee. A complete list of all MRFIT investigators has been published previously (24).

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