Relation of Serum Testosterone Levels to High Density Lipoprotein Cholesterol and Other Characteristics in Men

David S. Freedman, Thomas R. O'Brien, W. Dana Flanders, Frank DeStefano, and Joseph J. Barboriak

Although levels of high density lipoprotein (HDL) cholesterol in males decrease during adolescence and after treatment with testosterone derivatives, several studies have reported that levels of HDL cholesterol are positively associated with endogenous levels of testosterone in men. This association was further examined using data collected during 1985 and 1986 from 3,562 white and 500 black men who ranged in age from 31 to 45 years. Black men had higher mean levels of both HDL cholesterol (8 mg/dl) and total testosterone (33 ng/dl) than white men, and positive associations were observed between testosterone and HDL cholesterol levels \( r = 0.22 \) (whites); \( r = 0.26 \) (blacks). In addition, levels of testosterone were related positively to alcohol consumption and cigarette smoking and negatively to age, Quetelet index, and use of \( \beta \)-blockers. We used stratification and regression analyses to determine if any of these characteristics could account for the positive association between levels of HDL cholesterol and total testosterone. Although controlling for most factors had little influence, adjusting for Quetelet index reduced the strength of the association between levels of testosterone and HDL cholesterol by approximately 30%. These findings suggest that the positive association between levels of testosterone and HDL cholesterol may not be causal. Multivariable analyses that control for obesity and other potentially confounding characteristics should be used in studies that assess the relation of testosterone levels to coronary heart disease. (Arteriosclerosis and Thrombosis 1991;11:307–315)

Middle-aged men in industrialized countries are at a threefold to 10-fold higher risk for coronary heart disease (CHD) than are women. Several investigators have assessed possible determinants of this sex differential, and it has been suggested that sex hormones play an important role, possibly through their regulation of lipoprotein metabolism. During adulthood, premenopausal women have higher levels of high density lipoprotein (HDL) cholesterol but lower levels of triglycerides and low density lipoprotein (LDL) cholesterol than do men. Some evidence suggests that estrogen and testosterone are important determinants of levels of HDL cholesterol. During adolescence, levels of HDL cholesterol decline by more than 10 mg/dl in boys but not in girls, and large decreases are seen after treatment with testosterone derivatives. Although levels of HDL cholesterol increase after administration of estrogenic hormones, levels are low among women using oral contraceptives containing norgestrel, a progestin with androgenic activity. Because of these associations, it has been thought that the sex differential in CHD may be due to the influence of sex hormones on levels of lipids and lipoproteins.

In contrast to these findings, however, several unexpected associations with endogenous levels of estrogen and testosterone have been reported. For example, in contrast to what might be anticipated on the basis of the lower incidence of CHD among women, Phillips reported that 15 men who survived a myocardial infarction had higher levels of estrogen than men in a control group. In addition, although there are exceptions, most cross-sectional studies of men have reported a positive association between endogenous levels of testosterone and HDL choles-
terol with correlation coefficients ranging as high as 0.65. These studies, however, have included only whites, and most were relatively small.

Some investigators have suggested that the positive association between levels of testosterone and HDL cholesterol may be due to the confounding effects of obesity, alcohol intake, or sex hormone-binding globulin (SHBG). Although age-related decreases in testosterone levels are well established, relatively few investigators have examined other correlates of testosterone. Limited evidence, however, suggests that testosterone levels are associated inversely with relative weight and positively with cigarette smoking. The objective of this study of 4,062 men, 500 of whom are black, is to examine further the association between levels of total testosterone and HDL cholesterol. In addition, the influence of several characteristics (e.g., race, relative weight, alcohol consumption, cigarette smoking, and various medications) on the association between levels of testosterone and HDL cholesterol in these 31- to 45-year-old men will be assessed.

Methods

Population

Subjects were participants in a historical cohort study comparing the health of Vietnam veterans with veterans who served elsewhere. Although the study was designed to examine the general experience of military service in Vietnam, much of the initial concern focused on exposure to Agent Orange and its contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin. Data were obtained during 1) a telephone interview and 2) a medical examination of a random sample of telephone interviewees. Sample selection, interviews, and examinations were performed in 1985 and 1986 and have been described in detail elsewhere.

Briefly, a random sample of 48,500 men who entered the US Army between 1965 and 1971 was generated. To increase the baseline comparability of men who were stationed in Vietnam with other veterans, participation was limited to men who 1) served only one term, 2) had 16 weeks or more of active service, 3) earned a military occupational specialty other than "trainee" or "duty soldier," and 4) had a pay grade at discharge no higher than that of sergeant. A total of 17,867 veterans were considered eligible for the study, with 9,078 (51%) having served in Vietnam.

Of these men, 15,288 (87% of Vietnam veterans and 84% of non-Vietnam veterans) were located and given a structured interview by telephone. Of those interviewed, 6,443 men were randomly selected to participate in medical and psychological examinations conducted at the Lovelace Medical Foundation in Albuquerque, N.M. Of those invited to the examination, 4,462 men (75% of Vietnam veterans and 63% of other veterans) actually participated.

Our analyses are restricted to the 3,562 white men and 500 black men who were between the ages of 31 and 45 years at the time of the medical examination. Men were excluded for one or more of the following reasons: Hispanics (n=200), Asians (n=34), Native Americans (n=49), and men older than 45 years of age (n=5). Also excluded were men who reported that they had not fasted before the examination (n=47), whose weight was not recorded at the medical examination (n=1), or who had been told by a physician that they were diabetic (n=46) or had suffered a heart attack (n=18). In addition, men who did not answer questions concerning frequency of alcohol consumption (n=10) or cigarette smoking (n=2) were deleted from analyses involving these variables.

Risk Factor Information

Telephone interview and medical examination. Information on race was obtained during the telephone interview for more than 99% of the veterans, and Army records were used to find this information for the remaining men. Data on household income and educational achievement were also obtained during the telephone interview. Data on cigarette smoking (current smoking status and mean number of daily cigarettes) and alcohol consumption (average number of days per month during which alcohol was consumed and mean number of daily drinks) were obtained in a standardized interview during the medical examination. Information on the current use of medications, including specific names, was also collected. Use of β-blockers, hydantoins, and androgenic steroids is included in several analyses because of reported associations with levels of HDL cholesterol. Quetelet index, a measure of relative weight, was calculated as weight in kilograms divided by height in meters squared.

Data collected during the telephone interviews were used to assess the similarity between the 4,462 men who participated in the medical examination and the 10,826 men who did not, and only minor differences were found. For example, 11% of the 10,826 men interviewed by telephone only were black, whereas 12% of the 4,462 men who participated in the medical examination were black. Furthermore, participation in the medical examination was not associated with marital status, household income, cigarette smoking status, Quetelet index (calculated from self-reported weight and height), or alcohol consumption. Men who participated in the medical examination, however, tended to be slightly more educated than those interviewed only by telephone.

Laboratory determinations. Because animal studies have suggested that exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin may lead to hyperlipidemia and decreased levels of testosterone, these characteristics were measured as part of the medical examination. Venipuncture was performed after an overnight fast. Although levels of testosterone exhibit a strong diurnal cycle, the effects of this variation on the study results should be minimal because all blood samples were obtained between 8 and 10 AM, near the time of peak testosterone levels.
Levels of total cholesterol were assayed by an enzymatic method, and HDL cholesterol was measured after precipitating the LDL and very low density lipoprotein cholesterol fractions with dextran sulfate. The laboratory used an Ektachem 700 (Eastman Kodak, Rochester, N.Y.), and in addition to internal controls, the Centers for Disease Control standardized and monitored the laboratory.26 Levels of triglycerides were determined by an enzymatic method, and levels of total testosterone were measured with a standard, double-antibody radioimmunoassay system (Leeco Diagnostics, Inc., Southfield, Mich.).27 All laboratory determinations were monitored by using bench and “blind” repeat quality control procedures. The coefficient of variation for the various laboratory measurements ranged from 2.6% to 3.7% for HDL cholesterol and from 4.5% to 9.7% for testosterone.26

**Statistical Methods**

Using t tests, we assessed differences in mean levels of HDL cholesterol, testosterone, and other characteristics between black and white men; differences in categorical variables were assessed using χ² tests. In addition to regression analyses, we examined graphically the association between levels of testosterone and age using smoothed medians28 because of 1) the small numbers of black men at various ages and 2) uncertainty concerning the shape of the association between age and testosterone. We examined race-specific associations between levels of testosterone and other characteristics using stratification and age-adjusted correlation coefficients; both Pearson and Spearman correlations yielded similar results. F tests were used to examine linear trends in levels of testosterone across categorical variables having more than two levels.

We then examined whether the observed association between levels of testosterone and HDL cholesterol was due to confounding by extraneous variables. The association between levels of testosterone and HDL cholesterol was examined within strata of several covariates (e.g., age and cigarette smoking). With HDL cholesterol as the dependent variable in regression analyses, we also examined changes in the magnitude of the coefficient for testosterone after adjusting for additional covariates.29 In all analyses, the use of logarithmically transformed values for testosterone and HDL cholesterol yielded results similar to those obtained by using the original values.

**Results**

**Descriptive Characteristics and Black/White Comparisons**

Levels of selected characteristics are compared between white and black men in Table 1. The mean age among both groups was 38 years, and similar proportions of black men and white men were Vietnam veterans. Levels of total cholesterol, Quetelet index, and alcohol consumption were also similar for both groups. Among blacks, however, mean levels of triglycerides were 26% (31 mg/dl) lower, while mean HDL cholesterol levels were 18% (8 mg/dl) higher than among whites. We also found smaller but statistically significant differences in testosterone levels, with mean levels 5% (33 ng/dl) higher among black men than among whites. A larger proportion of black men smoked cigarettes than did whites, but among the smokers, white men reported smoking more cigarettes...
per day. Fewer than 1% of the examined men reported using β-blockers, hydantoins, or anabolic steroids.

Although levels of total testosterone were positively skewed (Figure 1), the difference in median levels between the two groups (684 ng/dl in blacks versus 656 ng/dl in whites) was comparable to the 33 ng/dl difference in mean levels. Despite these differences in average levels, similar proportions of black and white men had low levels of testosterone: the fifth percentiles were 341 and 349 ng/dl, and the 10th percentiles were 405 and 415 ng/dl, respectively.

Despite the relatively young ages of the subjects, levels of testosterone were inversely associated with age: correlation coefficients were −0.17 among whites and −0.29 among blacks. An examination of median testosterone levels by age (Figure 2) suggested that the difference between black and white men decreased with age. As estimated by regression analyses that included race × age terms, the higher mean testosterone levels among blacks decreased from 97 ng/dl (among 31- to 35-year-olds) to 7 ng/dl (among 41- to 45-year-olds), a significant \( p<0.01 \) interaction. In contrast to these associations, HDL cholesterol levels were not linearly associated with age \( (r=−0.01 \text{ [whites]} \text{ and } −0.04 \text{ [blacks]}), \text{ and age did not modify the black/white difference, } p=0.37 \).

**Association Between Levels of Testosterone and High Density Lipoprotein Cholesterol**

Mean levels of the lipids and lipoproteins according to testosterone quintile and correlation coefficients are shown in Table 2. Levels of total testosterone showed little association with total cholesterol but were associated inversely with levels of triglycerides \( (r=−0.18 \text{ to } −0.19) \) and positively with levels of HDL cholesterol \( (r=0.22 \text{ to } 0.26) \). Men in the highest testosterone quintile had mean levels of HDL cholesterol that were 7 mg/dl (whites) to 12 mg/dl (blacks) higher than those observed among men in
the lowest quintile. Adjustment for age did not affect the magnitudes of the examined associations.

Adjustment for Other Characteristics

Table 3 shows mean age-adjusted levels of HDL cholesterol and testosterone within categories of several characteristics. Although there were small differences between blacks and whites in the magnitudes of the observed associations, levels of HDL cholesterol were, as expected, related inversely to both Quetelet index and cigarette smoking and positively to alcohol consumption. (As compared with white men, the association between smoking and levels of HDL cholesterol in blacks was more strongly confounded by alcohol consumption.) Levels of testosterone were most strongly associated with Quetelet index, with the heaviest men (>28 kg/m²) having the largest effect, however, was seen after controlling for cigarette smoking, with correlation coefficients of 0.27 among both white and black men. The largest effect, however, was seen after controlling for Quetelet index: partial correlation coefficients were reduced to 0.13 (whites) and 0.15 (blacks). Similar results were seen in the stratum-specific analyses, with a correlation coefficient of only 0.09 among white men who had a Quetelet index below 24.5 kg/m².

We then examined the magnitude of the association between levels of HDL cholesterol and testosterone after controlling for several characteristics by stratification and partial correlations (Table 4). Adjustment for most characteristics, however, had little influence on the strength of the association. Among white men, for example, correlations within each age group were identical to the unadjusted association (r=0.22) between testosterone and HDL cholesterol.

The association between levels of HDL cholesterol and testosterone, however, increased slightly after controlling for cigarette smoking, with correlation coefficients of 0.27 among both white and black men. The largest effect, however, was seen after controlling for Quetelet index: partial correlation coefficients were reduced to 0.13 (whites) and 0.15 (blacks). Similar results were seen in the stratum-specific analyses, with a correlation coefficient of only 0.09 among white men who had a Quetelet index below 24.5 kg/m².

Coefficients from several regression models examining the cross-sectional association between levels of HDL cholesterol and several characteristics are shown in Table 5. Before adjustment for any covariate (model 1), a 100 ng/dl increase in testosterone was associated with a 1.2 mg/dl increase in levels of HDL.

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*p values have been age adjusted.

Discussions

Results of prospective studies have shown that baseline levels of testosterone and estrogen are not strongly predictive of subsequent CHD, and it has been suggested that the associations between levels of sex hormones and risk factors be studied further. In agreement with the results of previous studies, our cross-sectional findings indicate that levels of total testosterone and HDL cholesterol are positively associated (r=0.22 to 0.26). Furthermore, we observed that mean levels of HDL cholesterol and testosterone were higher among black men than among whites and that both were related similarly to obesity (inversely) and alcohol consumption (positively). We found different relations, however, with age and cigarette smoking. Age was inversely associated with testosterone levels (estimated yearly decrements of 15 ng/dl for whites and 25 ng/dl for blacks) but not with levels of HDL cholesterol. Cigarette smokers had lower mean levels of HDL cholesterol but higher levels of testosterone than did nonsmokers. Analyses indicated that the positive association between levels of HDL cholesterol and testosterone was, at least in part, due to their inverse associations with obesity.

Levels of total testosterone were measured in the current study, and only a small proportion of total testosterone exists unbound to protein, with most...
women and 2) HDL cholesterol levels decrease in the context of testosterone resulting from a testosterone-stimulated plausible biologic mechanism that may explain the inverse association, with decreased levels of HDL cholesterol, this relation is somewhat surprising due to their joint association with another factor. Investigators have found that several indexes of obesity are inversely related to levels of both HDL cholesterol and testosterone, and we found that controlling for Quetelet index reduced the strength of the association between levels of testosterone and HDL cholesterol by about 30%.

In agreement with these results, Dai et al observed that controlling for relative weight reduced the longitudinal association between changes in testosterone and HDL cholesterol. SHBG may be involved in these joint associations with obesity, and an inverse association between levels of testosterone and HDL cholesterol has been reported after controlling for SHBG. An increased peripheral conversion of testosterone to estradiol in obese men may also be important.

Although we found a positive association between levels of testosterone and HDL cholesterol even after controlling for Quetelet index, this index is a very indirect measure of obesity. If adiposity was more accurately assessed (e.g., by measurement of skinfold thicknesses), the association between testosterone and HDL cholesterol may have been further reduced. In addition, body fat distribution is correlated with levels of both HDL cholesterol and SHBG, and it may also play a role in the association between levels of testosterone and HDL cholesterol. Other mechanisms, however, may also be involved. For example, testosterone may lead to a generalized increase in microsomal enzyme activity, resulting in increased HDL production by the liver.

Previous reports have shown that testosterone levels in middle-aged men are associated negatively with age and positively with cigarette smoking. Although we found similar associations, testosterone levels appear to decrease with age beginning with men in their early 30s, a younger age than is generally realized. The positive correlation that we observed between alcohol consumption and levels of testosterone does not agree, however, with findings of a previous study and needs confirmation. To our knowledge, differences in testosterone levels between black and white men have not been previously described: we found that mean levels were approximately 5% higher among black men. This black/white difference, however, is 1) smaller than the corresponding 18% difference in mean levels of HDL cholesterol and 2) not constant across age groups. As observed by other investigators, only a small proportion of the variability in levels of testosterone can be accounted for by the examined characteristics, and other factors, such as physical activity and diet, may be important.

The importance of testosterone levels in the etiology of CHD is uncertain. Although some case–control studies indicate that testosterone levels among myocardial infarction survivors do not differ significantly from levels among controls, there are several reports of lower testosterone levels among men with CHD and with arteriographically confirmed stenotic disease. Breier et al have suggested that...
the decreased levels of HDL cholesterol among men with CHD may be secondary to the association between testosterone and lipoprotein lipase activity. Interestingly, some results suggest that levels of total testosterone are as strongly associated with CHD as are levels of free testosterone.42,46

Because levels of sex hormones may be altered by a myocardial infarction, the results of prospective studies may be more valid. Results of a nested case-control study, in which sex hormone levels were measured in plasma samples obtained as many as 12 years before the clinical event, suggest that high levels of testosterone may be somewhat protective against CHD mortality.32 The results, however, were not statistically significant. Two other similarly designed studies28,31 provide no evidence that testosterone levels are related to CHD risk, but relatively few cases were studied. Deterioration of sex hormones during long-term storage of blood samples31 may reduce the magnitude of the association between levels of testosterone and CHD in nested case-control studies.

Our results are based on men who entered the US Army between 1965 and 1971, and extrapolations to the general population should be made with caution. Furthermore, as a result of the multistep procedure leading to inclusion of men in the current analyses, which included tracing the veterans and obtaining their agreement to take part in both the telephone interview and medical examination, final participation rates ranged from 53% (non-Vietnam veterans) to 66% (Vietnam veterans). Several of the findings, however, suggest that the observed associations may be generalizable. Mean levels of testosterone among men in our study were comparable with those previously reported among similarly aged men19,32 and slightly higher than those reported among 45- to 59-year-old men.17 Other findings in this study, such as differences between black and white men in levels of HDL cholesterol and the relation of HDL cholesterol levels to relative weight, cigarette smoking, and alcohol consumption, also agree with the results of other investigations.35

Although a previously reported cross-sectional association between levels of testosterone and CHD was reduced after controlling for levels of triglycerides,17 others have found that the association is independent of HDL cholesterol levels.40 Our findings concerning the interrelations among levels of testosterone, HDL cholesterol, cigarette smoking, and obesity emphasize the importance of multivariable analyses in studies that examine the relation of testosterone levels to CHD.

Acknowledgments

We thank the leaders of veterans service organizations who provided important input and support for the study and the veterans whose cooperation made this study possible.

References


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*All analyses are restricted to the 4,051 men for whom values for all variables were available.
†Values are regression coefficients and, in parentheses, the absolute value of the t statistic; a t value of 2.6 is statistically significant at the 0.01 level; a value of 3.3 is statistically significant at the 0.001 level.
Levels of Testosterone and HDL Cholesterol

Freedman et al


Key Words • high density lipoprotein cholesterol • testosterone • blacks
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