Serum Glutathione in Adolescent Males Predicts Parental Coronary Heart Disease

John A. Morrison, PhD; Donald W. Jacobsen, PhD; Dennis L. Sprecher, MD; Killian Robinson, MD; Philip Khoury, MS; Stephen R. Daniels, MD, PhD

Background—Traditional risk factors account for only half of the morbidity and mortality from coronary heart disease (CHD). There is substantial evidence that oxidative injury plays a major role in the atherosclerotic process. Thus, antioxidants may protect against development of atherosclerosis. Glutathione, an intracellular tripeptide with antioxidant properties, may be protective.

Methods and Results—This case-control study compared total serum glutathione (tGSH) in 81 adolescent male offspring of parents with premature CHD (ie, before 56 years of age) and 78 control male offspring of parents without known or suspected CHD. Case offspring had significantly lower tGSH than control offspring. In multiple logistic regression with parental CHD status as the dependent variable, age entered as a covariate, and other CHD risk factors competing to enter the model as significant independent predictor variables, LDL cholesterol (odds ratio [OR], 2.15 [units=1.5 SD]; 95% CI, 1.21 to 3.82), tGSH (OR, 0.40; 95% CI, 0.22 to 0.71), HDL cholesterol (OR, 0.42; 95% CI, 0.22 to 0.78), and total serum homocysteine (OR, 2.6; 95% CI, 1.35 to 5.02) entered the model as significant predictors of parental CHD status.

Conclusions—Low tGSH in adolescent boys is a significant independent predictor of parental CHD, in addition to elevated LDL cholesterol, low HDL cholesterol, and elevated total serum homocysteine concentrations. (Circulation. 1999;100:2244-2247.)

Key Words: cholesterol ■ coronary disease ■ antioxidants

The atherosclerotic process begins in childhood and adolescence.1,2 Traditional risk factors for this process, eg, lipid abnormalities, cigarette smoking, and blood pressure elevation, account for only half of the morbidity and mortality from coronary heart disease (CHD).3 Newer factors, such as hyperhomocysteinemia, have recently been identified as risk factors for vascular disease, including coronary artery disease.4 Furthermore, parameters associated with oxidation are being tested for association with coronary artery disease. There is substantial evidence that oxidative injury plays an important role in the atherosclerotic process.5 Antioxidants may protect against development of atherosclerosis. Hence, red blood cell glutathione, an intracellular tripeptide with antioxidant properties, may be protective.6 It has been reported that the level of oxidized glutathione in serum may serve as an index of myocardial oxidative stress during and after cardiac reperfusion.7–9 Few data are available to evaluate whether serum glutathione (GSH) is protective against oxidative vascular damage and development of coronary artery disease; however, ≧2 reports indicate that glutathione levels are lower in adult patients with CHD.5,10 Given the familial nature of CHD and its risk factors, we conducted a case-control study of total serum glutathione (tGSH) in which adolescent sons of patients with documented premature CHD were compared with boys without such a family history. The association of tGSH was assessed in the context of established risk factors, including total serum homocysteine (tHcy).

Methods
The subjects for this case-control study were drawn from a 3-year cohort study of changes in sex steroid hormones, body composition, and lipids during sexual maturation conducted from 1984 to 1987.11 This cohort included boys with and without family histories of premature CHD. During the course of the cohort study, blood samples were separated into serum and stored at −20°C. The protocols for the original cohort study and this investigation were approved by the Institutional Review boards at the University of Cincinnati College of Medicine and the Children’s Hospital Medical Center (Cincinnati, Ohio). All subjects were 10 to 17 years of age. Cases had a parent with premature CHD, defined as a documented myocardial infarction or CABG surgery, at or before 55 years of age. Cardiac surgeons at the 3 major hospitals in Cincinnati performing CABG procedures provided lists of all patients with CABG surgeries who were ≦55 years of age in the previous 12 months. These patients were contacted to determine whether they had a son 10 to 15 years of age to participate in a study of changes in cholesterol levels...
during adolescence. In addition, cardiologists at the same hospitals provided lists of consecutive patients with confirmed premature myocardial infarction. Control subjects were drawn from a study of pubertal development and changes in cholesterol profiles in boys conducted in public and parochial schools in urban, urban residential, and suburban areas of Cincinnati. The schools were selected to obtain students with a wide range of income levels in both major race groups and to parallel referrals of adults to cardiologists and cardiac surgeons. Students whose parents reported no known CHD at the time of the study, 1984 to 1987, were used as control subjects.

Baseline demographic data were collected by interview with the participant’s parents, including subject’s date of birth; race; biological relationship of mother and father; and family history of CHD, stroke, hypertension, and hyperlipidemia. Adoptive, foster, and stepchildren were excluded.

Pubertal stage was assessed by trained male examiners following the modification of Tanner staging described by Biro et al. Height, weight, and skinfold thicknesses at the triceps, subscapular, and suprailiac sites were made according to standard protocols to assess body composition.13 Body mass index (weight in kilograms divided by height in meters squared) was used as a measure of ponderosity, and the ratio of the subscapular plus suprailiac skin folds to triceps skin folds was used as a measure of central adiposity. Cigarette smoking in the subjects was determined by interview. Blood pressure was measured as described previously14 with a standard sphygmomanometer after the subject had been sitting quietly for 5 minutes. Blood specimens were drawn in the morning from participants after a 12-hour fast. Specimens for cases and control subjects were handled identically. After 60 minutes at room temperature, blood specimens were centrifuged at 4°C. Serum was removed and stored in cryovials at −20°C.

tGSH, tHcy, and total serum cysteine were determined by use of the method of Jacobsen et al.14 Briefly, 100 μL serum was reduced with sodium borohydride to liberate low-molecular-weight thiols. The free thiols were then derivatized with monobromobimane. After precipitation of protein with perchloric acid and neutralization of the supernatant, the thiol-bimane conjugates were separated by high-performance liquid chromatography on a reverse-phase column and detected fluorometrically. This method measures both reduced and oxidized species of glutathione, homocysteine, and cysteine. A matched comparison of tGSH and tHcy values in a subsample of participants 18 months apart revealed no deterioration of either component. Measurements were made in random order without identification of case or control status. Measurements of plasma total cholesterol, triglyceride, and HDL cholesterol, with calculation of LDL cholesterol, were performed at the time of the original cohort study in an NHLBI-CDC standardized lipid laboratory on a Hitachi 705 with enzymatic procedures for cholesterol and triglyceride measurement, triglyceride blanking, and the modified Lipid Research Clinic procedure (heparin-2 molar MnCl2) to isolate the HDL cholesterol as described previously.11

### Statistical Methods

Data were double entered, and the resulting data sets were compared and checked for completeness and accuracy of entry. Analyses were performed with SAS statistical software.14 Distributions of variables were examined, and variance-stabilizing transformations were performed when appropriate. Student’s t tests were performed to compare the mean ages for the case and control groups. The distributions of maturation stages for the case and control groups were compared using χ² analysis. Student’s t tests were also performed to compare mean differences between offspring and control subjects for body composition, lipids, systolic and diastolic blood pressures, tHcy, and tGSH. A stepwise logistic regression analysis was performed to determine which factors were independently associated with a positive family history of early CHD. Variables included as independent risk factor candidates for the model are shown in Table 1. Because the case and control groups were constructed to have similar ages, age was included in the final logistic regression model as a covariate. Odds ratios and 95% CIs were calculated by use of set

### Results

Serum samples for 81 cases and age-matched control subjects were identified for analysis. Because the parents of 3 control students drawn for this analysis reported possible CHD, these samples were excluded. Thus, the analysis sample included 81 cases and 78 control subjects.

Summary statistics for the cases and control subjects are presented in Table 1. The mean age of the fathers of cases was 45.0 years; that of fathers of control subjects, 42.5 years. The mean age of mothers of cases was 42.5 years, and that of mothers of control subjects was 40.7 years. Most of the CHD events in case parents were in fathers, and the mean age at the time of the event (myocardial infarction or coronary bypass graft surgery) was 39.7 years. Socioeconomic status as measured by level of education achieved by the fathers and mothers did not differ by group. Cases and control subjects did not differ in mean age, height, weight, body mass index, or distribution of pubertal stages (data not shown), but offspring of parents with premature CHD had greater central adiposity. Cases also had significantly higher concentrations of total and LDL cholesterol, triglycerides, and tHcy and lower concentrations of HDL cholesterol. Cases had significantly lower concentrations of tGSH (P=0.0002) compared with control subjects. In contrast, total serum cysteine was similar in cases and control subjects. The prevalence of

### Table 1. Mean and SD of Age, Body Composition, Blood Pressure, Lipids, and Serum Thiols in Adolescent Male Offspring of Parents With (Cases) and Without (Control Subjects) Premature CHD

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=81)</th>
<th>Control Subjects (n=78)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>13.2 1.9</td>
<td>13.4 1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Body composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>158.0 14.1</td>
<td>158.9 13.6</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>51.5 16.3</td>
<td>49.9 15.2</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>20.2 3.9</td>
<td>19.3 3.5</td>
<td>NS</td>
</tr>
<tr>
<td>Central adiposity*</td>
<td>2.14 0.6</td>
<td>1.83 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>111.3 13.4</td>
<td>108.5 11.4</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic</td>
<td>66.7 11.8</td>
<td>67.9 8.6</td>
<td>NS</td>
</tr>
<tr>
<td>Lipids, mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>174.2 30.0</td>
<td>160.9 28.8</td>
<td>0.005</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>78.5 37.3</td>
<td>63.4 30.1</td>
<td>0.006</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>51.6 10.5</td>
<td>56.1 12.4</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>110.6 27.7</td>
<td>95.1 23.5</td>
<td>0.0002</td>
</tr>
<tr>
<td>Thiols, μmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tHcy</td>
<td>9.26 2.5</td>
<td>8.08 2.4</td>
<td>0.003</td>
</tr>
<tr>
<td>tGSH</td>
<td>3.20 2.2</td>
<td>4.35 2.1</td>
<td>0.0009</td>
</tr>
<tr>
<td>Total cysteine</td>
<td>262.2 37.1</td>
<td>247.9 53.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Ratio of subscapular and suprailiac skin folds to triceps skin folds.
reported cigarette smoking was low, and the number of cigarettes reported smoked per day varied: 5 cases reported smoking (1, 2, 10, 12, and 20 cigarettes a day), and 3 control subjects reported smoking (2, 5, and 10 cigarettes a day). The reported length of time of smoking ranged from <1 to 48 months.

Results of the logistic regression analysis are presented in Table 2. This analysis showed that LDL cholesterol, HDL cholesterol, tHcy, and tGSH concentrations were all significant independent predictors of parental CHD status for these boys. Homocysteine was positively and significantly associated with parental CHD. GSH concentration was inversely associated with parental CHD. Lower concentrations of tGSH were associated with the presence of parental CHD. HDL cholesterol was also inversely associated with parental CHD. LDL cholesterol was directly associated with parental CHD. There were no additional significant predictors of parental CHD.

**Discussion**

The finding that lower tGSH in adolescents predicts parental CHD, even after traditional risk factors such as LDL and HDL cholesterol and recently reported risk factors such as tHcy are accounted for, is new and could identify glutathione as a risk factor for the development of atherosclerosis. Substantial evidence suggests that oxidative injury plays a major role in atherogenesis and that antioxidants may protect patients from the development of atherosclerosis. Intraacellular glutathione, which is normally present at millimole per liter concentrations, has a protective role against oxidative damage. Oxidative stress may be important in the development of coronary artery disease because the heart, in contrast to the liver or lung, is relatively poorly defended against oxidative stress. The level of oxidized glutathione in serum serves as an index of myocardial oxidative stress during and after reperfusion.

Data on homocysteine and risk of cardiovascular disease in children and adolescents are sparse. Tonstad et al evaluated tHcy levels in Norwegian children 8 to 12 years of age and found an association with family history of cardiovascular disease. They did not, however, evaluate tGSH, nor did they find a relationship between lipid levels and family history of CHD. In a subsequent study, Tonstad et al assessed carotid intimal-medial thickening and the presence of plaque by B-mode ultrasonography and their relationship to risk factors in 10- to 19-year-old children of both sexes with familial hypercholesterolemia, ascertained by the presence of specific LDL receptor mutations, and control subjects. Mean and maximum intimal-medial thicknesses were determined in the common carotid artery and carotid bulb. In multivariate analyses, tHcy was associated with all 4 measurements controlling for pubertal stage. The present finding that higher levels of tHcy in adolescents predict parental CHD status is in agreement with the work of Tonstad et al.

In the present study, all forms of GSH, including reduced, oxidized, and protein-bound GSH (another oxidized form of GSH), were measured. The levels of tGSH observed in this study are in agreement with other reported values for tGSH. tGSH is a significant independent predictor of parental CHD in the participants of the present study by multivariate analyses. These findings support the hypothesis that tGSH is a protective factor against the development of atherosclerosis.

Given the mean age of parents of control subjects, it is possible that some of these parents may have subsequently developed CHD. On average, however, the age of the fathers of control subjects was 3 years greater than the average age of the CHD event in parents of cases (42.5 versus 39.7 years). We do not have data on the subsequent CHD experience of the parents of control subjects. If some parents of control subjects have developed CHD, this would result in misclassification. Assuming that the relationship between tGSH and parental CHD is strong in boys, it would be parents of boys with lower tGSH who would have been misclassified in the control group. However, if this misclassification is present, the effect would be to make the control group more like the case group and thus make it more difficult to find a relationship between family history of CHD and the presence of risk factors. This means that our findings are conservative and that the relationships may, in fact, be stronger than observed in the present study. Confirmation of these results in other populations is important.

Identification of high tHcy and low tGSH as potential risk factors in children and adolescents for the development of CHD may be clinically important. Current dietary intervention focuses on weight control and lowering the intake of fat, saturated fat, and cholesterol to achieve more favorable lipid and lipoprotein concentrations. Levels of tHcy can also be lowered by changes in diet, including increased dietary folate or supplementation with folic acid. Less is known about dietary influences on tGSH. Although Hagen et al showed increased plasma glutathione after oral administration of glutathione in rats, Flagg et al found only weak (and negative) correlations between dietary glutathione and...
plasma glutathione in humans. Further research is necessary to evaluate the determinants of and the ability to modify GSH.

Acknowledgments
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References
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