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4. Association of Low Serum Concentration of Bilirubin
with Increased Risk of Coronary Artery Disease

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11. SUPPLEMENTARY NOTES

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factors for angiographically documented coronary artery disease (CAD). The studies
involved a "training" set of 619 men for whom complete data on all risk factors
considered were available, and a "test" set of 258 men for whom some risk factor
data were not available. In both study groups, the liver enzymes were not related
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related to the presence of CAD, both univariately and multivariately after adjust-
ment for the established risk factors of age, total cholesterol, high-density
lipoprotein cholesterol, smoking history, and systolic blood pressure. A 50% de-
crease in total bilirubin was associated with a 47% increase in the odds of being in
a more severe CAD category. Our data suggest that serum bilirubin is an inverse and
independent risk factor for CAD, with an association equivalent in degree to that of
systolic blood pressure.

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Is Serum Bilirubin a Risk Factor for Coronary Artery Disease?

Coronary artery disease (CAD), a major cause of morbidity and death in North America, has several known risk factors, e.g., history of cigarette smoking, obesity, age, diabetes mellitus, systolic blood pressure, and increased serum lipids. As far as lipids are concerned, animal studies have long shown a liaison between hypercholesterolemia and the presence of an inflammatory reaction that seems to characterize vascular smooth muscle cell proliferation in atherosclerosis. This has engendered an intense interest in the role of lipid oxidation in atherogenesis (1). As early as 1980, Brown and Goldstein showed that, during the above-mentioned chronic inflammatory process, macrophages have a very limited ability to ingest native low-density lipoprotein (LDL), but they bind chemically modified LDL by a high-affinity receptor also called a scavenger receptor (2). In fact, there is a family of related scavenger receptors, of which one is a high-affinity receptor for oxidized LDL (3). Because of their chemical composition, lipoproteins, particularly LDL, are highly susceptible to oxidations in vitro and in vivo. Subsequently, several lines of evidence in humans and animals have lent credence to the proposal that oxidized LDL is taken up by intimal macrophages, which contribute to formation of lipid-rich foam cells (3). These and related data have consolidated the suggestion that oxidized LDL is a risk factor for the development of atherosclerosis.

In the current issue of *Clinical Chemistry*, Schwertner et al. present an unexpected finding that serum bilirubin is an inverse and independent risk factor for CAD (4). They examined serum bilirubin and various liver-function enzymes as possible risk factors for angiographically confirmed CAD in 619 men with complete data for all risk factors considered and in 258 men with incomplete data for risk factors. All subjects were US Air Force pilots and navigators. From statistical analyses of the data according to various hypotheses, the authors deduced that a 50% decrease in bilirubin was associated with a 47% increase in the probability of being in a more severe CAD category.

The finding of an inverse relationship between serum bilirubin and the risk of CAD is novel simply because it has never been so suggested. That novelty is even more striking against the background that bilirubin has, since more than a century ago, been used as a marker for many hepatobiliary disorders but not for any heart conditions. If further substantiated, bilirubin may be a welcome addition to the barrage of tests for CAD risk. It will also necessitate a revision in current concepts about bilirubin.

The paper by Schwertner et al. (4) raises a number of interesting questions. For example, because total bilirubin (TBIL) in serum comprises multiple subfractions (5)—unconjugated bilirubin (Bu), mono- and di-sugar bilirubin conjugates, and delta bilirubin (Bd or BP)—one wonders if one or more bilirubin fractions were involved in this putative relationship with CAD. To explore this issue, we will apparently first have to separately quantify bilirubin fractions at concentrations well below the "normal" value for serum TBIL (e.g., 17–22 µmol/L). This is not an impossible challenge. Monoclonal antibodies targeted against serum Bu and Bd have recently been prepared (Wu et al., unpublished).

As a plausible mechanism for their observations, Schwertner et al. (4) suggested that bilirubin, which behaves as an antioxidant (see below), may prevent oxidation of LDL and hence reduce accumulation of cholesterol plaque (3). The idea that bilirubin is an antioxidant is not new. Several groups of workers (e.g., 6–8) had advanced this concept since the 1950s. The key finding was that Bu either in chloroform or in multilamellar liposomes protects phospholipids against damage from in situ-generated peroxyl radicals (8). However, most of these studies were not performed in the presence of living cells or in vivo. Albumin-bound Bu (especially Bd) was first demonstrated in 1991 to protect cultured hepatocytes and rat livers from oxyradical damage (9, 10). Also in 1991, the same bilirubins were shown to be substantially more effective protectors of human ventricular myocytes than several known antioxidants such as vitamin C and a vitamin E analog called Trolox (11). However, the fact that bilirubin is a cytoprotective antioxidant does not necessarily mean that it must protect LDL from oxidation. This is a critical point that must await further experimental testing. For example: (a) Is there concrete physicochemical evidence of bilirubin's being consumed in vitro and in vivo so as to preserve LDL from oxidative damage? (b) If so, is the effect reflected by angiography and at least some other risk factors of CAD? Eventually, it will be important to ascertain what is the relative impact of bilirubin in the total endogenous anti-LDL activity of the body, in the context of the other known native antioxidants, some of which are effective in preserving LDL integrity (3). There may even be other, less obvious, metabolic linkages between bilirubin and cholesterol/bile acids involved here.

Most of the serum Bu is strongly adsorbed, whereas all of the bilirubin in Bd is irreversibly bonded to albumin, the most abundant protein in serum and a qualitatively important antioxidant (5, 11). Against this

background, it seems logical to ask: Does albumin contribute to the body's ability to prevent LDL oxidation and therefore play a role in protecting LDL? Although in vivo evidence is wanting, our group has shown that albumin profoundly modulates the antioxidant activity of bilirubin in human cardiomyocytes (11) and erythrocytes (10). Eventually, this question must be answered more directly by studying the physicochemical interaction between purified LDL and bilirubin fraction(s) in the presence or absence of albumin. Overall, Schwertner et al. (4) have provoked our thinking about serum bilirubin in the important context of CAD. Bilirubin may be more closely linked to the heart than we have been taught.

References

1. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modification of low-density lipoprotein that increases its atherogenicity. N Engl J Med 1989;320:915-25.
2. Brown MS, Basu SK, Falck JR, Ho YK, Goldstein JL. The scavenger cell pathway for lipoprotein degradation. Specificity of binding site that mediates the uptake of negatively-charged LDL by macrophages. J Supramol Struct 1980;13:67-81.
3. Esterbauer H, Gebicki J, Puhl H, Jurgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radical Biol Med 1992;13:341-90.
4. Schwertner HA, Jackson WG, Tolan G. Association of low serum bilirubin with increased risk of coronary artery disease. Clin Chem 1994;40:18-23.
5. Wu T-W. Bilirubin analysis—the state of the art and future prospects [Review]. Clin Biochem 1984;17:221-9.
6. Bernhard K, Ritzel G, Steiner KU. Uber einer biologische Bedeutung der Gallenfarbstoffe. Bilirubin and Biliverdin als Antioxydantien fur des Vitamin A und die essentiellen Fettsaure. Helv Chim Acta 1954;37:306-13.
7. Blyuger AF, Dudnik LB, Maiore AY, Mieze IE. Role of bilirubin as a natural antioxidant in regulation of the intensity of lipid peroxidation in acute virus hepatitis. Bull Exp Biol Med 1985;99:166-8.
8. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043-6.
9. Wu T-W, Au J-X, Arbour F, Carey D, Yazawa N, Hashimoto N, et al. Serum biliprotein is a cytoprotective agent of potential physiologic significance [Abstract]. Hepatology 1991;19:881.
10. Wu T-W, Carey D, Wu J, Sugiyama H. The cytoprotective effects of bilirubin and biliverdin on rat hepatocytes and human erythrocytes and the impact of albumin. Biochem Cell Biol 1991;69:828-34.
11. Wu T-W, Wu J, Li R-K, Mickle DAG, Carey D. Albumin-bound bilirubins protect human ventricular myocytes against oxyradical damage. Biochem Cell Biol 1991;69:683-8.

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Association of Low Serum Concentration of Bilirubin with Increased Risk of Coronary Artery Disease

Harvey A. Schwertner,[1] William G. Jackson,[2] and Gil Tolan[2]

We examined serum bilirubin and various liver-function enzymes as possible risk factors for angiographically documented coronary artery disease (CAD). The studies involved a "training" set of 619 men for whom complete data on all risk factors considered were available, and a "test" set of 258 men for whom some risk factor data were not available. In both study groups, the liver enzymes were not related to CAD; however, ln[total bilirubin] was inversely and statistically significantly related to the presence of CAD, both univariately and multivariately after adjustment for the established risk factors of age, total cholesterol, high-density lipoprotein cholesterol, smoking history, and systolic blood pressure. A 50% decrease in total bilirubin was associated with a 47% increase in the odds of being in a more severe CAD category. Our data suggest that serum bilirubin is an inverse and independent risk factor for CAD, with an association equivalent in degree to that of systolic blood pressure.

Indexing Terms: *antioxidants/risk factors/cholesterol/lipoproteins/ liver-function enzymes*

Lipids and lipoproteins are important risk factors for coronary artery disease (CAD); however, they do not account for disease in 30–40% of the population with CAD.[3] To further improve our ability to predict CAD, we examined several nonlipid factors as possible risk factors. In preliminary studies, we found that serum bilirubin concentrations were decreased in individuals with CAD, whereas some of the liver-function enzyme activities were increased. Because decreased bilirubin concentrations and increased enzyme activities had not been previously associated with CAD, we sought to determine whether such associations existed in larger patient populations.

It is not known whether bilirubin has a role in preventing CAD; however, it could be involved in CAD in several ways. Bilirubin is a naturally occurring antioxidant (*1*, *2*) and, as such, could have a role in protecting lipids and lipoproteins against oxidation. Given that oxidized lipids and lipoproteins are known to be atherogenic (*3*, *4*), low bilirubin concentrations could be associated with increases in oxidized lipids and lipoproteins.

A low concentration of bilirubin might also prevent solubilization of cholesterol and its clearance through the bile, thereby increasing serum cholesterol concentrations (*5*, *6*).

To further determine whether bilirubin is associated with CAD, we examined its concentrations in a larger study of individuals who had undergone coronary angiography. In this study, we compared bilirubin and the liver-function enzymes with several established risk factors: age, total cholesterol, high-density lipoprotein (HDL) cholesterol, smoking history, systolic blood pressure, triglycerides, fasting blood sugar, and the ratio of total to HDL cholesterol. In addition, we sought to determine whether bilirubin and the liver enzymes are independent risk factors, i.e., if they add information about coronary risk beyond that of the established risk factors.

Materials and Methods

Subjects

Subjects were asymptomatic male United States Air Force (USAF) pilots and navigators who underwent coronary angiography to determine their fitness for flying duty. Most subjects were initially identified by subtle serial changes on their routine resting 12-lead electrocardiogram (ECG) obtained during their biennial flight physical. An internist at the USAF Central ECG Library compared abnormalities on the recent ECG with a baseline tracing obtained before the subjects reached age 28. Aviators with serial changes, such as nonspecific ST-T wave changes, underwent a local symptom-limited treadmill test. Those aviators with repolarization or rhythm abnormalities on local stress testing were referred to the USAF Aeromedical Consultation Service. In addition, aviators over age 30 who were referred for any reason also underwent cardiovascular screening tests.

Exercise Test Procedures

The cardiovascular screen included an ECG at rest, at least 16 h of ambulatory ECG monitoring, a symptom-limited treadmill test, cardiac fluoroscopy (beginning 1 October 1982), and a thorough history, physical examination, and extensive blood tests.

The symptom-limited treadmill tests were performed after an overnight fast. Serum potassium concentrations were within the normal range. The treadmill tests were abnormal if an ST segment depression of 1.0 mm or more occurred 80 ms after the J point. Patients determined to be at risk for coronary artery disease had a repeat symptom-limited treadmill test with injection of ^{201}Tl 1 min before peak exercise; these subjects underwent planar thallium scanning with an Anger-type

camera immediately after exercising and 4 h later. Elective coronary arteriography was performed for abnormal repolarization, decreased thallium uptake, cardiac calcification demonstrated by fluoroscopy, tachycardia (three or more consecutive ectopic beats), acquired left bundle branch block, or valvular abnormalities.

Coronary Angiography

Each angiogram was read jointly by at least two cardiologists. Coronary artery lesions were magnified, traced, and measured with calipers to determine the percentage of diameter narrowing of the artery. A total of 877 USAF aircrew members who underwent coronary angiography between 1 August 1978 and 8 May 1990 are included in this study.

For 258 of the 877 subjects in this study, information on one or another of the risk factors was awaiting entry into the computer database. Because procedures for selection of variables require complete data, we divided the patients into a training group of 619 for whom data were complete and saved the other 258 to test the most complete model possible based on the risk factors selected in the analysis of the 619.

Laboratory Tests

Phosphotungstate–magnesium reagents, prepared in the laboratory, were used for HDL-cholesterol analyses until July 1987. Total cholesterol and HDL-cholesterol were determined enzymatically with BMC Autoflo reagents (Boehringer Mannheim Diagnostics, Indianapolis, IN). After July 1987, dextran sulfate, M_r 50 000 (Ciba Corning, Oberlin, OH), was used for HDL-cholesterol analyses. Total and HDL-cholesterol also were determined with Ciba Corning reagents. Triglycerides were determined enzymatically without correction for free glycerol (Abbott Laboratories Diagnostics Division, Irving, TX, and Ciba Corning). Hyperlipemic samples were rarely present, but when encountered, they were centrifuged (Beckman Airfuge; Beckman Instruments, Palo Alto, CA) to remove chylomicrons before the HDL-cholesterol analysis. Total bilirubin was analyzed with diazotized sulfanilic acid reagent with blank correction (Malloy and Evelyn method; Abbott Laboratories and Ciba Corning).

Day-to-day coefficients of variation (CVs) were 2.5% for assay of cholesterol (5.2 mmol/L), 4.6% for HDL-cholesterol (1.3 mmol/L), 5.0% for triglycerides (1.48 mmol/L), and 5.6% for total bilirubin (1.03 mmol/L). CVs for liver enzymes were in the 4–10% range, depending on the type of enzyme and its activity. Throughout the study, cholesterol was calibrated against the Abell–Kendall method with cholesterol standards from the National Institute of Standards and Technology (Gaithersburg, MD). The laboratory participated in both intra- and interlaboratory quality-control programs sponsored by the College of American Pathologists.

Statistical Methods

Parallel-lines logistic regression models with ordinal responses were fitted by using SAS PROC LOGISTIC

(7). Coronary disease was divided into three groupings according to the maximum coronary stenosis at angiography: <10% (no gradeable disease), >10% but <50% (mild disease), and ≥50% (severe disease). All of the independent variables, including systolic blood pressure and fasting serum glucose, were treated as continuous variables. Cigarette smoking was measured as the reported average number of cigarettes smoked per day, provided the subject had not quit smoking >1 year before. For the set of 619 men, results for independent variables with coefficient of skewness >2.0 [total bilirubin, triglycerides, aspartate aminotransferase (AST), and alanine aminotransferase (ALT)] were transformed logarithmically in all the regressions. In the analysis of the 619 patients with complete data, variables were entered stepwise based on the likelihood ratio test (8). From the variables selected, a submodel including those variables for which the remaining 258 patients had complete data was tested.

Selection of variables was also examined on the training set of 619 by linear regression, with maximum percentage of obstruction as a continuous dependent variable, by using SAS PROC RSQUARE. In addition, the Mantel–Haenszel odds ratio for association between CAD (severe vs mild or none) and total bilirubin (<10 vs ≥10 μmol/L), adjusted for other categorized covariates, was estimated by using SAS PROC FREQ.

Results

Patient Groups and Angiographic Data

Summary statistics for the two groups are given in Table 1. In the training group of 619 patients, 111 (18%) had severe CAD, 87 (14%) had mild disease, and 421 (68%) were free of CAD. In the test group of 258 subjects, 35 (14%) had severe CAD, 47 (18%) had mild disease, and 176 (68%) had no CAD. Table 2 shows summary statistics by severity of CAD for the training group.

Logistic Regression Models

The results of the univariate and multivariate logistic regression modeling on the training set of 619 subjects are shown in Table 3. All of the univariate variables were significant at or near $P = 0.05$ except for ln[AST], alkaline phosphatase, and lactate dehydrogenase. Stepwise logistic regression selected a model that included age, total cholesterol, HDL-cholesterol, cigarettes per day, systolic blood pressure, and ln[total bilirubin]. The adjusted regression coefficient for ln[total bilirubin] was −0.560 (SE 0.242), giving 95% confidence limits of −1.034 to −0.0857. This means that in comparing no CAD vs mild + severe CAD, or when comparing no + mild CAD vs severe CAD, the associated odds ratio for a 50% decrease in total bilirubin was given by [−0.560 ln(0.5)] = 1.47, with 95% confidence limits of 1.06 to 2.05. Thus, for the training set, a 50% decrease in total bilirubin was associated with a 47% increase in the odds of being in a more-severe CAD category.

Table 4 shows test results from fitting one submodel to the test set of 258 subjects. The subset of independent

Table 1. Prevalence of angiographically determined CAD plus clinical and laboratory characteristics of asymptomatic study subjects.

Characteristic	Mean (and range)	
	619 subjects with complete data	258 subjects with incomplete data
Max. stenosis, %		
0	421 (68)[a]	176 (68)[a]
10–49	87 (14)[a]	47 (18)[a]
50–100	111 (18)[a]	35 (14)[a]
Age, years	41.8 (21–61)	43.7 (23–65)
Cholesterol, mmol/L	5.55 (3.03–12.23)	5.61 (2.92–9.49)
HDL-chol, mmol/L	1.18 (0.52–2.48)	1.08 (0.36–2.04)
Chol/HDL-chol	4.99 (2.1–14.9)	5.59 (2.3–11.8)
Triglyc, mmol/L	1.63 (0.20–10.98)	1.81 (0.44–20.88)
Fast. gluc., mmol/L	5.64 (4.00–8.49)	5.59 (4.27–7.83)
Cigarettes/day[b]	7.1 (0–60)	6.7 (0–50, n = 96)[c]
Never smoked or quit >1 year ago	430 (69.5)[a]	68 (70.8)[a]
Current smoker or quit ≤1 year ago	189 (30.5)[a]	28 (29.2)[a]
Systolic BP, mmHg	126.9 (94–182)	125.6 (98–160, n = 52)
Total bilirubin, μmol/L	14.1 (3.4–55.7)	13.5 (5.0–39.3)
ALT, U/L	26.5 (4–159)	25.9 (7–128, n = 254)
AST, U/L	22.3 (6–94)	23.4 (6–64, n = 255)
AP, U/L	50.4 (15–111)	59.3 (26–137, n = 254)
LDH, U/L	155.7 (65–251)	145.5 (83–247, n = 250)

[a] CAD prevalence and smoking status given as no. (and %).

[b] Cigarettes per day = 0, if subject never smoked or quit smoking >1 year before; or, average reported cigarettes smoked per day, if subject was a current smoker or reported quitting ≤1 year ago.

[c] n is listed when some subjects are missing data.

Chol, cholesterol; triglyc, triglycerides; BP, blood pressure; AP, alkaline phosphatase; LDH, lactate dehydrogenase; fast. gluc., fasting glucose.

Table 2. Clinical and laboratory characteristics of 619 asymptomatic study subjects with complete data, by degree of CAD.

	Maximum stenosis, %		
	<10	10–49	50–100
n	421	87	111
Age, years	40.0 ± 6.2[a]	45.7 ± 5.4	45.5 ± 5.5
Cholesterol, mmol/L	5.33 ± 1.00	5.88 ± 0.83	6.12 ± 1.14
HDL-chol, mmol/L	1.21 ± 0.31	1.16 ± 0.25	1.11 ± 0.25
Chol/HDL-chol	4.72 ± 1.63	5.30 ± 1.28	5.76 ± 1.59
Triglyc, mmol/L	1.56 ± 1.11	1.69 ± 0.89	1.84 ± 0.84
Fast. gluc., mmol/L	5.60 ± .56	5.64 ± 0.50	5.79 ± 0.62
Cigarettes/day	5.8 ± 11.0	8.5 ± 13.9	10.9 ± 14.0
Systolic BP, mmHg	125.7 ± 13.2	127.2 ± 13.6	131.1 ± 13.5
Total bilirubin, μmol/L	14.8 ± 7.2	13.1 ± 4.9	12.5 ± 5.9
ALT, U/L	25.8 ± 16.2	26.2 ± 13.3	29.3 ± 19.3
AST, U/L	22.0 ± 9.3	21.5 ± 7.4	24.3 ± 13.8
AP, U/L	50.2 ± 14.9	51.8 ± 14.0	50.4 ± 11.7
LDH, U/L	155.8 ± 26.9	154.7 ± 27.2	156.1 ± 27.5

[a] Mean ± SD.

Abbreviations as in Table 1.

variables for which complete data were available is included. In the multivariate model, the four variables age, total cholesterol, HDL-cholesterol, and ln[total bilirubin] were forced into the model, and ln[triglycerides], [total cholesterol]/[HDL-cholesterol], and fasting glucose were tested for inclusion. Once again, ln[total bilirubin] was found to be statistically significant and inversely associated with prevalence of CAD.

Figure 1 shows a univariate representation of the relationship between CAD prevalence and total bilirubin for all 877 subjects. CAD prevalence is clearly increasing as total bilirubin decreases.

Concerned that the observed association might be due to outliers in the data, we performed logistic regressions on mutually exclusive subsets of subjects, balanced with respect to CAD prevalence and to levels of the covariates. The standardized estimates of regression parameters from these analyses are shown in Table 5. Consistency across the subsets was sufficient to indicate that the inverse association between CAD and total bilirubin was pervasive in our data set. Not only do the signs of the regression coefficients for ln[total bilirubin] tend to point in the same direction, but also, because the independent variables are standardized to means of 0 and standard deviations of 1, the strength of the association relative to the other risk variables can be assessed. In our data set, the association between CAD and total bilirubin was about the same as between CAD and either smoking (as measured by cigarettes per day) or systolic blood pressure.

Concerned that increased total bilirubin might indicate a liver disease, which somehow protected against CAD, we compared subjects in the "normal" subsets, who had all liver-function tests in the healthy ranges, with "abnormal" subjects, who had at least one abnormal liver-function test result. The healthy ranges were: 2–18 μmol/L for total bilirubin, 1–175 U/L for lactate dehydrogenase, 1–42 U/L for ALT, 1–35 U/L for AST, and 1–90 U/L for alkaline phosphatase. The adjusted P values for ln[total bilirubin] in the four subsets from multivariate tests on models such as those in Tables 3 and 4 were somewhat inconsistent: normal subset of the training set (n = 342) P = 0.744; abnormal subset of the training set (n = 277) P <0.001; normal subset of the test set (n = 162) P = 0.0091; abnormal subset of the test set (n = 96) P = 0.0574.

Other Modeling Approaches

Concerned that our arbitrary trifurcation of CAD into three categories might be too broad, we reanalyzed the training set of 619 subjects, using linear regression. The data were the same as for logistic regression except that maximum percentage of coronary stenosis was in its original continuous form, as read from angiography. We used SAS PROC RSQUARE to pick the model that minimized Mallows' Cp statistic. The only result that differed from stepwise logistic regression was that ln[triglycerides] was included at the final step, after ln[total bilirubin]. The increase in r^2 with the inclusion of ln[total bilirubin] was from 0.199 to 0.206; i.e., for purposes of predicting the maximum percentage of stenosis in individuals, the total unexplained variability in linear regression models is not much reduced.

Table 3. Summary of stepwise logistic regression modeling on 619 subjects with complete data, and parameter estimates for computing risk of mild or severe CAD.[a]

Variable	Univariate		Multivariate		
	χ^2 (1 df)	P	χ^2 (1 df)	P	Parameter ± SE
Intercept 1					−10.24 ± 1.47
Intercept 2					−11.22 ± 1.48
Age	101.6	<0.001	72.3	<0.001	0.134 ± 0.017
Cholesterol	62.9	<0.001	39.8	<0.001	0.586 ± 0.095
Cigarettes/day	15.6	<0.001	10.3	0.0013	0.0239 ± 0.0074
Systolic BP	12.2	<0.001	7.2	0.007	0.0189 ± 0.0072
HDL-chol	11.0	<0.001	4.1	0.044	−0.679 ± 0.341
ln[Tot Bil]	16.3	<0.001	5.3	0.021	−0.560 ± 0.242 [b]
ln[Triglyc]	17.6	<0.001	2.0	0.154	
Chol/HDL-chol	40.1	<0.001	3.0	0.085	
Fast. gluc.	8.3	0.004	0.03	0.860	
ln[ALT]	3.4	0.066	1.7	0.190	
ln[AST]	1.4	0.234	1.0	0.312	
AP	0.3	0.581	0.07	0.797	
LDH	0.007	0.932	2.1	0.150	

[a] Risk = 1/[1 + exp (−Intercept − B_1 age − B_2 chol − ... − B_6 ln[Tot Bil])], where intercept = intercept 1 to compute risk that maximum stenosis is ≥10% and = intercept 2 to compute risk that maximum stenosis is ≥50%; B_x respective parameter estimates.

[b] Entries above the line are likelihood ratio χ^2 values for deletion with 1 df after these six variables were entered into the model; below are likelihood ratio χ^2 values for inclusion with 1 df. Likelihood ratio χ^2 values were computed with the SAS PROC LOGISTIC, and variables were entered stepwise.

Tot Bil, total bilirubin; other abbreviations as in Table 1.

Table 4. Results from fitting selected logistic regression models on 258 subjects in the test set.[a]

Variable	Univariate		Multivariate	
	χ^2 (1 df)	P	χ^2 (1 df)	P
Age	20.5	<0.001	11.4	<0.001
Cholesterol	30.8	<0.001	25.8	<0.001
HDL-chol	24.8	<0.001	20.7	<0.001
ln[Tot Bil]	9.5	0.002	12.3	<0.001 [b]
ln[Triglyc]	30.7	<0.001	1.8	0.182
Chol/HDL-chol	41.9	<0.001	0.5	0.485
Fast. gluc.	2.1	0.147	0.2	0.641

[a] Risk factors analyzed were those with values for all 258 subjects. Likelihood ratio χ^2 values were computed by using SAS PROC LOGISTIC with age, total cholesterol, HDL-C, and ln[Tot Bil] forced into the multivariate model.

[b] Entries above the line are likelihood ratio χ^2 for deletion with 1 df; below are χ^2 values for inclusion with 1 df.

Abbreviations as in Table 3.

Fig. 1. Prevalence of coronary artery disease, according to concentration of total bilirubin in 877 patients: <10 μmol/L (n = 219), 10–11.9 (199), 12–15.9 (229), ≥16 (230).

Degree of CAD:
☐ None
■ Mild
■ Severe

Still concerned that our unexpected inverse relationship was influenced by a few outliers, we carried out a discrete analysis of the 619 subjects. CAD was divided into only two groupings: severe CAD vs mild + no CAD; total bilirubin was bifurcated into values <10 and ≥10 μmol/L; age was split into <35, 35–44, and ≥45 years; total cholesterol was divided into <5.2, 5.2–5.7, and >5.7 mmol/L; HDL-cholesterol was either <1.2 or ≥1.2 mmol/L; cigarette smoking was broken up into cigarettes per day = 0 or >0; and systolic blood pressure was ≤140 vs >140 mmHg. Having arranged the tables so that an odds ratio >1.0 indicated an inverse a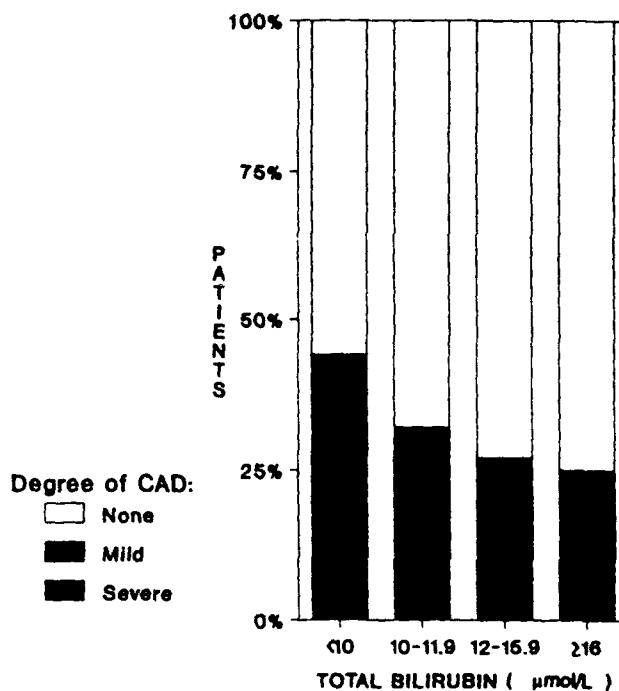ssociation between CAD and total bilirubin, we used SAS PROC FREQ to compute a Mantel–Haenszel odds ratio of 1.94, averaged over all combinations of the other variables. The Mantel–Haenszel test for an odds ratio different from 1 yielded a χ^2 of 7.97 (1 df; P = 0.005). The test-based 95% confidence limits were 1.22–3.06.

Table 5. Logistic regression coefficients for risk of mild or severe CAD from 10 mutually exclusive subsets of the 619 subjects with complete data.

No. subjects with max % stenosis of			Logistic regression coefficients (risk factors standardized)					
<10	10–49	≥50	Age	Cholesterol	HDL	Cigarettes/day	Systolic BP	ln[Tot Bili]
42	8	11	0.77	0.30	−0.08	−0.13	0.36	−0.65
43	8	11	1.30	0.50	0.17	−0.05	0.46	−0.57
42	9	11	1.40	0.72	−0.24	−0.18	−0.18	−0.58
42	9	11	0.82	0.43	−0.56	0.37	0.23	0.06
42	9	11	0.61	1.01	−0.64	0.09	0.08	−0.14
42	9	11	0.29	0.80	−0.26	0.28	0.62	−0.56
42	9	11	0.40	0.79	−0.40	0.61	0.70	−0.39
42	9	11	1.18	0.89	0.05	0.55	0.30	0.06
42	9	11	0.97	1.06	0.77	1.29	0.20	−0.19
42	8	12	2.26	0.73	−0.04	0.05	0.14	−0.14
	Mean		1.00	0.72	−0.12	0.31	0.29	−0.31
	SD		0.58	0.25	0.41	0.42	0.26	0.27
	t (9 df)		5.5	9.3	−1.0	2.3	3.5	−3.6
	P		<0.001	<0.001	0.362	0.044	0.006	0.006

Ten subsets were balanced on CAD prevalence and level of risk factors by first sorting the 619 by CAD severity (none, mild, severe) as the "outside" sort and on the first principal component of the six standardized risk factors as the "inside" sort. Every 10th subject in the sorted list was then assigned to the same subset. All risk factors were restandardized to mean = 0 and SD = 1 within each subset before logistic regressions.

Discussion

In this study, we discovered an unexpected inverse association between serum total bilirubin and CAD. The association was statistically significant in both univariate analyses and multivariate analyses after adjustment for recognized risk factors. Furthermore, the inverse association was found to hold across different subsets of the data and across different types of statistical analyses. The strength of the association with CAD was similar to that of smoking or of systolic blood pressure. To our knowledge, this is the first report showing an association between low bilirubin concentrations and a specific disease. Generally, low serum bilirubin concentrations have been associated with good health, and only high concentrations have had any diagnostic significance (5, 6).

Serum bilirubin is derived primarily from the degradation of hemoglobin, although some originates from the erythroid elements in the reticuloendothelial cells of bone marrow, spleen, and liver (5, 6). In serum, bilirubin occurs mostly in the free form, although some occurs in the conjugated form and some is bound to serum albumin (2, 5, 6). Each of these fractions will need to be analyzed to determine which is most associated with CAD. We also think that the accuracy and precision of methods used to determine serum bilirubin will need to be improved if it, or its subfractions, are to be predictive for CAD.

This study did not include women or older men. It did not include young adult men with diabetes, obesity, or other chronic diseases. It remains to be seen whether serum bilirubin is inversely associated with CAD in these groups. This was a cross-sectional prevalence study. A prospective study is needed to determine whether serum bilirubin helps predict future coronary heart disease. The selection process used in this study was also different from those used in other angiographic studies. For instance, the prevalence of disease in our study group was <18%, whereas the prevalence in other angiographic studies is generally >70%.

We do not know if increased serum bilirubin prevents CAD, if CAD decreases serum bilirubin, or if serum bilirubin is an index of some factor in the pathogenesis of CAD. Bilirubin is, however, an effective antioxidant (1, 2), possibly protecting lipids and lipoproteins against oxidation (3) and against plaque formation in humans (4). We also do not know why bilirubin concentrations are lower in individuals with CAD than in those without CAD. Perhaps this reflects an increase in oxidant activity or increases in iron stores (9).

It remains to be seen whether abstinence from alcohol is related to low bilirubin concentrations. However, no association between increased consumption of alcohol and increased concentrations of bilirubin has been found (10). Bilirubin is also effective in the solubilization of cholesterol and in aiding in its clearance through the bile (5, 6). Further studies are needed to confirm the association between bilirubin and CAD in independent populations and to elucidate the various pathogenic mechanisms involved.

References

1. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043–6.
2. Stocker R, Glazer AN, Ames BN. Antioxidant activity of albumin-bound bilirubin. Proc Natl Acad Sci USA 1987;84:5918–22.
3. Stringer MD, Gorog PG, Freeman A, Kakkar VV. Lipid peroxides and atherosclerosis. Br Med J 1989;298:281–4.

4. Yla-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, Butler S, et al. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. J Clin Invest 1989;84:1086–95.

5. Ockner RK. Laboratory tests in liver disease. In: Wyngaarden JB, Smith LH, eds. Cecil textbook of medicine, 16th ed. Philadelphia: WB Saunders, 1982:775–8.

6. Routh JI. Liver function. In: Tietz NW, ed. Fundamentals of clinical chemistry. Philadelphia: WB Saunders, 1976:1026–62.

7. Statistical Analysis System Institute, Inc. SAS/Stat user's guide; version 6, 4th ed., Vol. 2. Cary, NC: SAS Institute, 1989.

8. Hosmer DW, Lemeshow S. Applied logistic regression. New York: John Wiley, 1989:13–8, 106–14.

9. Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation 1992;86:803–11.

10. Shaper AG, Pocock SJ, Ashby D, Walker M, Whitehead TP. Biochemical and haematological response to alcohol intake. Ann Clin Biochem 1985;22:50–61.