Serum Bilirubin and Risk of Ischemic Heart Disease in Middle-Aged British Men
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The possibility that low concentrations of serum bilirubin may be associated with increased risk of ischemic heart disease has been examined in a prospective study of 7685 middle-aged British men. During 11.5 years there were 737 major ischemic heart disease (IHD) events. A U-shaped relationship was observed between serum bilirubin and risk of IHD. Low bilirubin was associated with several cardiovascular risk factors, in particular smoking, low concentrations of high-density lipoprotein cholesterol, low forced expiratory volume in 1 s, and low serum albumin. The U-shaped relationship persisted even after adjusting for several risk factors. Compared with men in the lowest fifth of the distribution (bilirubin < 7 μmol/L), those in the middle range (8–9 μmol/L) showed a 30% reduction in relative risk [RR = 0.68 (95% confidence intervals 0.51–0.89)] in IHD, whereas men in the top fifth (> 12 μmol/L) showed similar risk to the lowest fifth [RR = 0.99 (95% confidence intervals 0.73–1.34)], which persisted after exclusion of men with bilirubin > 17 μmol/L. The significance of this U-shaped relationship is unclear, but it could be interpreted as support for the role of endogenous antioxidants in the etiology of IHD.

Indexing Terms: antioxidants/cholesterol/risk factors

The major risk factors for ischemic heart disease (IHD) are well documented and include smoking, blood cholesterol, and hypertension (1). There is substantial variation in IHD risk between countries (2), within populations (3), and between socioeconomic groups (4). Major IHD risk factors do not predict subsequent myocardial infarction accurately (5) and do not fully explain declining secular trends in IHD (6) or social class differences (7). The search for risk factors for IHD that might explain these variations has been stimulated by evidence that free radicals are involved in the pathogenesis of atheroma (8) and that antioxidants, both dietary and endogenous, may be important protective factors (9). The antioxidant properties of bilirubin have been recognized for many years (10–12). Recently, Schwertner et al. (13) reported a strong inverse association between serum bilirubin and severity of coronary artery stenosis in 877 asymptomatic US Air Force pilots undergoing routine assessments of fitness for flight. These observations were tentatively interpreted as implicating higher concentrations of serum bilirubin in the prevention of low-density lipoprotein (LDL) oxidation, thereby reducing the risk of IHD. Further work has demonstrated low concentrations of serum bilirubin in patients with peripheral vascular disease (14). These observations suggest that low serum bilirubin may be a risk factor for IHD. To test this hypothesis, we have examined data from a large prospective study of the etiology of cardiovascular diseases, The British Regional Heart Study (BRHS).

Subjects and Methods

The BRHS is a prospective study of cardiovascular disease involving 7735 men, ages 40–59 years, selected from the age–sex registers of one group general practice in each of 24 towns in England, Wales, and Scotland. The criteria for selecting each town, the general practice, and the subjects, as well as the methods of data collection, have been reported (3). Research nurses administered to each man a standard questionnaire that included questions on smoking habits, alcohol intake, and medical history. Several physical measurements were made, and blood samples (nonfasting) were taken for measurement of biochemical and hematological variables. Details of the measurement of serum lipid concentrations have been described (15). The London School of Hygiene sphygmomanometer was used to measure blood pressure twice in succession with the subjects seated and the arm supported on a cushion. The mean of the two readings was used in the analysis, and all blood pressure readings were adjusted for observer variation within each town (16). The men were classified according to their current smoking status: those who had never smoked, ex-cigarette smokers, and current smokers. Those who had only smoked pipes and (or) cigars were grouped as “never smoked.” Ex-cigarette smokers who were currently pipe and (or) cigar smokers were classified as ex-cigarette smokers. Alcohol consumption was recorded from answers to questions on frequency, quantity, and type, similar to those used in the 1978 General Household Survey (17). Men were classified into five groups on the basis of their estimated weekly intake: none, occasional, light, moderate, and heavy (18). Heavy drinkers were defined as those regularly drinking more than six drinks daily. The longest-held occupation of each man was recorded and then coded in accordance with the Registrar General's occupational classification. Body mass index, calculated as weight/height², was used as an index of relative weight. Forced expiratory volume in 1 s (FEV1) was measured by using a Vitalograph spirometer (Model J49-B2; Vitalograph...
Medical Instrumentation, Buckingham, UK) with the subject seated. The FEV1 values were height-standardized to 1.73 m, the average height of the men in this study. The men were asked to indicate their usual pattern of physical activity, and a score based on frequency and type of leisure activity (19) was devised for each man. The men were grouped into six broad categories on the basis of the total score of their activity levels: inactive, occasional, light, moderate, moderately vigorous, and vigorous. Active men were those whose physical activity was moderate or greater.

Serum Bilirubin

The men attended the examination center between 0830 and 1830. Blood samples (nonfasting) were collected into evacuated tubes for measurement of biochemical and hematological variables. All samples reached the Department of Haematology, Queen Elizabeth Hospital (Birmingham, UK) by the next morning, and estimations were completed by 1200 of that day. Bilirubin was measured in serum with a Technicon SMA 12/60 AutoAnalyzer (Bayer, Basingstoke, Hants, UK). Estimates of bilirubin were not available for 46 of the men. Examination times were not available for four men.

A previous report from the BRHS has shown marked diurnal variation in bilirubin concentrations (20). There was little difference in mean values up to 1600, but after this, the concentrations decreased steadily and significantly through the afternoon, being 9.1 and 7.2 mmol/L in men screened before and after 1600, respectively. This difference was not caused by the differences in various other risk factors such as smoking in men screened before and after 1600. The explanation for this decrease is uncertain, but may be the result of food intake (20), fasting having been shown to increase bilirubin concentration (21). Because of the concern regarding the marked diurnal variation and the uncertainty of the nature of the decrease in bilirubin after 1600, we analyzed the data separately for the men screened before and after 1600. The major analyses reported here involved the two-thirds of men screened before 1600, with reference made to the findings in the other third screened after 1600.

Preexisting Disease

The men were asked to recall a doctor’s diagnosis of angina, myocardial infarction, diabetes, and several other disorders listed on the questionnaire. The WHO (Rose) chest pain questionnaire was administered to all men at the initial examination (22), and a three-orthogonal lead electrocardiogram was recorded with the subject recumbent and having been at rest at least 30 min before the recording. Men with evidence of IHD were defined as those with a diagnosis of angina or heart attack made by a doctor, a response on WHO (Rose) questionnaire indicating angina or possible myocardial infarction, or electrocardiographic evidence of definite or possible myocardial ischemia or myocardial infarction. In all, 1914 men (25%) had preexisting evidence of IHD.

Follow-up

All men were followed up for all-cause mortality and for cardiovascular morbidity for 11.5 years (23). Information on death was collected through the established “tagging” procedures provided by the National Health Service registers in Southport (England and Wales) and Edinburgh (Scotland). A nonfatal myocardial infarction was diagnosed according to WHO criteria, which included any report of myocardial infarction accompanied by at least two of the following: a history of severe chest pain, electrocardiographic evidence of myocardial infarction, and cardiac enzyme changes associated with myocardial infarction. Fatal events were defined as death from IHD (International Classification of Disease Ninth Revision Codes 410–414) as the underlying code.

Statistical Methods

Cox’s proportional hazards model (24) was used to obtain relative risks adjusted for the risk factors. Age, body mass index, systolic blood pressure, blood cholesterol, FEV1, high-density lipoprotein (HDL)-cholesterol, and blood glucose were fitted as continuous variables. Physical activity, smoking, social class, alcohol, preexisting IHDs, diabetes, and antihypertensive treatment were fitted as categorical variables. Subjects with missing values for covariates in the various adjustments made with the use of Cox’s model were excluded from that particular analysis. The distribution of bilirubin was skewed, and log transformation was used. In Table 1, the χ² test for linear trend was used to assess the significance of the trends in proportions for the categorical risk factors (%) across the bilirubin groups, and linear regression was used to assess the trend for the continuous risk factors (means). Multiple regression was used to assess the relationship between bilirubin and the risk factors, adjusting for each of the other variables. To assess the U-shaped relationship between serum bilirubin and risk of ischemic heart, we entered bilirubin (log) both as a linear and quadratic term in its original continuous form in the model; the analysis indicates a U-shaped relation if the quadratic term is significant.

Results

In the 7685 men with complete data on bilirubin and time of examination, the mean (geometric) concentration of bilirubin was 8.3 (range 1–86 μmol/L). During the follow-up period of 11.5 years, 737 major IHD events (fatal and nonfatal) occurred in the 7685 men. To assess the relationship between bilirubin and risk of major IHD, we divided the entire cohort of men by absolute concentrations in an attempt to approximate fifths of the overall distribution. The relationship was examined separately in men screened before and after 1600. The IHD event rate/1000 person-years was similar in both groups (8.9 vs 9.0/1000 person years). Table 2 shows the major IHD event rate/1000 person-years and age-adjusted relative risk by fifths of bilirubin concentrations separately by
Table 1. Serum bilirubin and coronary risk factors in the 4916 men measured before 1600 tabulated in quintiles (1, lowest; 5, highest).

<table>
<thead>
<tr>
<th>Bilirubin quintile</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Trend*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>50.6</td>
<td>50.6</td>
<td>50.4</td>
<td>50.1</td>
<td>50.0</td>
<td>**</td>
</tr>
<tr>
<td>BMI, kg m⁻²</td>
<td>25.3</td>
<td>25.3</td>
<td>25.5</td>
<td>25.6</td>
<td>25.6</td>
<td>NS</td>
</tr>
<tr>
<td>% smokers</td>
<td>52.9</td>
<td>51.2</td>
<td>43.1</td>
<td>35.4</td>
<td>30.2</td>
<td>***</td>
</tr>
<tr>
<td>% active</td>
<td>34.4</td>
<td>36.5</td>
<td>33.3</td>
<td>37.7</td>
<td>41.5</td>
<td>***</td>
</tr>
<tr>
<td>% manual</td>
<td>63.8</td>
<td>61.0</td>
<td>60.9</td>
<td>55.4</td>
<td>53.8</td>
<td>***</td>
</tr>
<tr>
<td>% nondrinkers</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>% heavy drinkers</td>
<td>12.2</td>
<td>11.2</td>
<td>11.3</td>
<td>9.2</td>
<td>10.9</td>
<td>NS</td>
</tr>
<tr>
<td>% preexisting IHD (any)</td>
<td>23.0</td>
<td>24.4</td>
<td>27.6</td>
<td>24.4</td>
<td>24.9</td>
<td>NS</td>
</tr>
<tr>
<td>% MI</td>
<td>5.5</td>
<td>5.7</td>
<td>5.9</td>
<td>4.9</td>
<td>5.5</td>
<td>NS</td>
</tr>
<tr>
<td>% diabetes</td>
<td>2.3</td>
<td>1.5</td>
<td>1.7</td>
<td>1.2</td>
<td>1.3</td>
<td>NS</td>
</tr>
<tr>
<td>% antihypertensive drugs</td>
<td>3.6</td>
<td>3.9</td>
<td>4.5</td>
<td>5.9</td>
<td>6.5</td>
<td>*</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.28</td>
<td>6.38</td>
<td>6.34</td>
<td>6.38</td>
<td>6.17</td>
<td>**</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.12</td>
<td>1.14</td>
<td>1.14</td>
<td>1.15</td>
<td>1.19</td>
<td>***</td>
</tr>
<tr>
<td>FEV1, mL</td>
<td>322.9</td>
<td>328.1</td>
<td>334.8</td>
<td>337.6</td>
<td>343.5</td>
<td>***</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>43.7</td>
<td>44.3</td>
<td>44.5</td>
<td>44.7</td>
<td>45.0</td>
<td>***</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>145.0</td>
<td>144.7</td>
<td>144.8</td>
<td>143.9</td>
<td>144.9</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.52</td>
<td>5.47</td>
<td>5.52</td>
<td>5.47</td>
<td>5.47</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Significant at * P < 0.05; ** P < 0.01; *** P < 0.001; NS, not significant.

Bilirubin and Coronary Risk Factors

The relationship between bilirubin and factors previously shown to be associated with major coronary heart disease events or mortality was examined. Table 1 shows the distribution of coronary risk factors by the five quintiles. Smoking was inversely and strongly associated with bilirubin concentrations. Significant inverse associations were also seen with age and social class. Physical activity and use of antihypertensive drugs were significantly positively associated with bilirubin concentrations. Prevalence of diabetes tended to decrease with increasing bilirubin, but the trend was not significant. The mean cholesterol concentration was significantly lower in men in the top bilirubin quintile. A significant positive association was observed between bilirubin and HDL-cholesterol, FEV1, and albumin. No association was seen with body mass index, preexisting IHD, systolic blood pressure, or blood glucose.

Thus, overall, subjects with low serum bilirubin are characterized by older age, more cigarette smoking, lower social class, more diabetes, higher serum cholesterol, lower FEV1, lower HDL-cholesterol, and lower serum albumin.

To determine whether the factors shown to be associated with bilirubin in the univariate analysis, i.e., age, smoking, social class, physical activity, use of antihypertensive treatment, FEV1, HDL-cholesterol, and albumin, were independently associated with bilirubin, we examined the relationship, adjusting for each of these other factors. Smoking, use of antihypertensive treatment, FEV1, HDL-cholesterol, and albumin remained significantly associated with bilirubin. No significant association was seen with age, physical activity, and social class after adjustment. Similar

Table 2. Effect of time of measurement on bilirubin and major IHD event rate/1000 person-years and age-adjusted relative risk.

<table>
<thead>
<tr>
<th>Bilirubin, μmol/L</th>
<th>No. of men</th>
<th>No. of cases</th>
<th>Rate/1000 person-years</th>
<th>Age-adjusted relative risk</th>
<th>No. of men</th>
<th>No. of cases</th>
<th>Rate/1000 person-years</th>
<th>Age-adjusted relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;7</td>
<td>788</td>
<td>90</td>
<td>11.2</td>
<td>1.00</td>
<td>1150</td>
<td>112</td>
<td>9.2</td>
<td>1.00</td>
</tr>
<tr>
<td>7</td>
<td>715</td>
<td>77</td>
<td>10.2</td>
<td>0.91 (0.68–1.23)</td>
<td>459</td>
<td>54</td>
<td>11.1</td>
<td>1.23 (0.89–1.68)</td>
</tr>
<tr>
<td>8</td>
<td>1371</td>
<td>114</td>
<td>7.8</td>
<td>0.71 (0.53–0.93)</td>
<td>577</td>
<td>50</td>
<td>7.9</td>
<td>0.87 (0.82–1.22)</td>
</tr>
<tr>
<td>10</td>
<td>905</td>
<td>79</td>
<td>8.1</td>
<td>0.75 (0.56–1.00)</td>
<td>260</td>
<td>17</td>
<td>6.0</td>
<td>0.64 (0.39–1.08)</td>
</tr>
<tr>
<td>12</td>
<td>1137</td>
<td>110</td>
<td>9.0</td>
<td>0.85 (0.64–1.13)</td>
<td>323</td>
<td>34</td>
<td>9.9</td>
<td>1.12 (0.76–1.63)</td>
</tr>
<tr>
<td>Total</td>
<td>4916</td>
<td>470</td>
<td>8.9c</td>
<td></td>
<td>2769</td>
<td>267</td>
<td>9.0c</td>
<td></td>
</tr>
</tbody>
</table>

* 95% confidence intervals in parentheses.

b Mean.
patterns of relationship between bilirubin and the risk factors were observed in the men screened after 1600.

Adjustment for Confounding Factors

To assess the effects of confounding factors and because the biological factors may serve as mediating factors, we adjusted first for preexisting disease and lifestyle factors, i.e., age, smoking, physical activity, social class, alcohol intake, body mass index, preexisting IHD, history of diabetes, and use of antihypertensive treatment (Table 3). Adjustment for these factors made little difference to the U-shaped relationship seen. Further adjustment for the biological factors, i.e., systolic blood pressure, cholesterol, HDL-cholesterol, FEV1, blood glucose, and serum albumin, increased the risk in the top fifth (Table 3). As compared with men in the third group, men in the top and bottom fifths of the distribution both showed significantly higher risk ($P = 0.007$ and $P = 0.006$, respectively). A test for the U-shaped relationship fitting a linear and quadratic effect of bilirubin showed a significant indication of a U-shaped relationship (quadratic term; $P = 0.005$).

In men screened after 1600, a similar but less consistent U-shaped curve was seen. The relative risks (and 95% confidence intervals) for the five groups were 1.0, 1.2 (0.8–1.6), 0.9 (0.7–1.3), 0.7 (0.4–1.2), and 1.2 (0.8–1.8), but a formal test for the U-shaped relationship fitting a quadratic term was not statistically significant.

Current Smoking and Preexisting IHD

Because of the strong association between smoking and bilirubin, we also examined the relationship separately in current and noncurrent cigarette smokers. Analysis was confined to the men screened before 1600. A U-shaped relationship was seen in both groups. The U-shaped relationship was seen in men both with and without preexisting IHD (data not shown).

High Bilirubin and Gilbert Syndrome

Gilbert syndrome is characterized by hyperbilirubinemia in the absence of functional liver disease. To assess whether the increased risk of IHD seen in the top fifth of the bilirubin distribution compared with men in the third and fourth quintiles could be skewed by men with very high concentrations of bilirubin (which may reflect liver disease), we separated the men in the top 5% of the distribution ($>17 \mu$mol/L; $n = 291$ men; 29 IHD cases). These men with bilirubin $>17 \mu$mol/L were then further divided between those with (γ-glutamyltransferase $>24$ or aspartate aminotransferase $>28$; top fifth of the distribution: $n = 116$ men; 18 IHD cases) and those without raised liver enzyme (n = 175 men; 11 cases), possibly representing Gilbert syndrome (3.6% of the men screened before 1600). Even when all men with high concentrations of bilirubin ($\geq17 \mu$mol/L) were removed, those in the top fifth still showed a risk similar to those in the lowest fifth (relative risk = 0.94). Men with possible Gilbert syndrome showed a risk (relative risk = 0.90) similar to men in the top quintile. However, men with increased concentrations of bilirubin and liver enzymes showed markedly higher risk, although the numbers were too small to achieve statistical significance (relative risk = 1.82, 95% confidence interval 0.8–4.0).

Discussion

Our observations demonstrate that serum bilirubin (nonfasting) appears to show diurnal variability and that low bilirubin concentration is also strongly associated with several cardiovascular risk factors. The association with smoking is particularly important and may confound the relationships reported between serum bilirubin and severity of coronary artery disease and peripheral vascular disease (13, 14).

However, our analyses demonstrate that both low and high concentrations of serum bilirubin are associated with an increased risk of IHD, forming a U-shaped relationship. This relationship is observed in subjects measured before and after 1600. Moreover, the relationship is well within the “reference” range of bilirubin and is still apparent when subjects with possible occult liver disease or Gilbert syndrome are excluded from the analysis. Men with increased concentrations of serum bilirubin and liver enzymes appeared to be at a much increased risk of IHD, although the numbers were small. There is no evidence that people with Gilbert syndrome are at lower risk of IHD. Because the bilirubin–IHD relationship may be subject to confounding by any of the variables associated with both serum bilirubin and IHD, adjustment was carried out by means of multivariate analyses and by stratified analysis for the smoking habit. After adjustment for lifestyle factors, biological factors, and preexisting disease, the relationship remained U-shaped. Residual confounding caused by imprecise measurement of variables such as smoking history might possibly explain the persisting relationship. This is unlikely, however, because, for this to be true, one would have to postulate that smoking history was assessed to different degrees of accuracy depending on the concentration of bilirubin. An alternative explanation of our findings is that they simply reflect the play of chance despite the statistical significance observed.
If the relationship is not due to confounding or to chance but is a real phenomenon, it is necessary to explain how both low and high serum bilirubin might influence risk of disease. Schwertner et al. (13) suggested that the relationship of increased IHD risk with low bilirubin was a reflection of its "consumption" in endogenous antioxidant activity. The roles and relative importance of endogenous antioxidants such as bilirubin, uric acid (25), and lipoprotein(a) (26) and dietary antioxidants are not well understood. Possibly, an interaction takes place between endogenous and exogenous systems such that in people with deficient dietary intake of antioxidants, compensatory increases in endogenous production of antioxidants occur. Inverse relationships between the intake of dietary antioxidants and risk of cardiovascular diseases have been reported (27). Therefore, in people with poor diets, i.e., low antioxidants (a factor not measured in the BRHS), bilirubin might be a compensatory mechanism and thus act as a marker for dietary antioxidant deficiency.

Bilirubin is only one of a series of endogenous antioxidants; possibly, these compounds operate in complex ways modulated by each other, by exogenous antioxidant intake, and by the amount of oxidant stress experiences. Oxidant stress is thought to cause damage in atherosclerosis both by initiating lesions and in aiding their progression to clinical events (28–30). Thus bilirubin may be operating as a factor both in the early phase of development of atherosclerosis and in the period closer to the onset of clinical events.

In conclusion, serum bilirubin shows a U-shaped relationship with IHD events that is not explained by confounding by major cardiovascular risk factors. The significance of this relationship is unclear but might support a role for endogenous antioxidants in the etiology of IHD.

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References