Cardiovascular Events as a Function of Serum Bilirubin Levels in a Large Statin-Treated Cohort

Running title: Horsfall et al.; Cardiovascular events as a function of bilirubin

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Abstract:

Background—Serum bilirubin is an endogenous antioxidant that is routinely measured prior to statin prescription primarily to assess liver function, but the association with cardiovascular disease (CVD) in this population has not been explored.

Method and Results—We identified patients from a United Kingdom primary care database (The Health Improvement Network) with measurements of serum total bilirubin levels recorded three months prior to first statin treatment between January 1st 2000 and December 31st 2010 and with no history of liver disease or CVD. In total 130,052 patients met the inclusion criteria and after a median follow-up of 43 months, there were 7,850 CVD events. In men, the incidence of CVD in the lowest decile category of bilirubin (1-6 μmol/L [0.06-0.35 mg/dL]) was 215 per 10,000 person years (PYs) compared with 163 per 10,000 PYs in the highest decile (19-40 μmol/L [1.1-2.3 mg/dL]). Similar differences were seen for women. After accounting for conventional CVD risk factors, the associations with bilirubin were non-linear (L-shaped) and the models predicted that, compared to patients with a bilirubin level of 10 μmol/L (0.6 mg/dL), those with a similar CVD risk profile but a bilirubin level of 5 μmol/L (0.3 mg/dL) had a 18% (95%CI; 9-27%) higher risk of any CVD event, a 34% (95%CI; 13-56%) higher risk of myocardial infarction, and a 33% (95%CI; 21-46%) higher risk of death from any cause.

Conclusions—Serum bilirubin level measured prior to statin prescription in the UK to assess liver function is an independent risk factor for CVD and death in both men and women.

Key words: antioxidants; cardiovascular disease risk factors; cardiovascular events; epidemiology; follow-up studies
Serum unconjugated bilirubin is the end product of natural heme degradation via the heme oxygenase enzyme pathway. The hepatic enzyme UDP-glucuronosyltransferase 1-1 (UGT1A1) converts bilirubin to a soluble (conjugated) form suitable for renal and biliary elimination.\(^1\) In healthy European populations, common genetic variation of the \(UGTA1\) promoter region explains around 45-50% of the variability in serum bilirubin levels with homozygosity for the low activity allele UGT1A1*28 the main cause of Gilbert’s syndrome (mild familial unconjugated hyperbilirubinemia) in Europeans.\(^2-4\) A range of socio-demographic and lifestyle variables have also been associated with moderately higher bilirubin levels including younger age, male sex, lower body mass index (BMI) and non-smoking status.\(^3,5\)

Though once regarded as a metabolic waste product useful for assessing liver function, bilirubin has demonstrated potent antioxidant and anti-inflammatory properties \textit{in vitro} and \textit{in vivo}.\(^6-10\) Amongst other known serum antioxidants, bilirubin seems to be particularly effective at suppressing the oxidation of blood lipids including low-density lipoprotein (LDL).\(^6,8,11\) In addition, direct application of bilirubin to vascular endothelial tissue appears to improve markers of oxidative stress and cellular dysfunction.\(^12,13\) These observations suggest a role in protecting against cardiovascular disease (CVD) and, indeed, a number of small prospective studies have reported much lower rates of CVD for men with comparatively higher bilirubin levels after accounting for other risk factors.\(^14\) Less is known on the association in women. Furthermore, cohort studies have shown that homozygosity for the UGT1A1*28 allele is associated with a much lower risk of CVD events.\(^15,16\) UGT1A1*28 is not associated with conventional CVD risk factors\(^12,13\) but has been linked with a significantly smaller brachial artery diameter and increased cold pressor test reactivity in young people suggesting a beneficial influence on the size and structure of arteries.\(^3\) Thus serum bilirubin level may be an independent marker for
environmental and genetically determined CVD risk, however the small sample size of previous studies mean it is difficult to understand the clinical implications for women and the wider population.

The aim of our study was to prospectively assess whether serum bilirubin level measured routinely prior to statin prescription, primarily to assess liver function, was also related to future CVD events and death from any cause in a large primary care patient population.

**Method**

**Data source**

The Health Improvement Network (THIN) primary care database includes anonymized records entered using the VISION software including more than ten million patients from the United Kingdom (UK) (http://csdmruk.cegedim.com). All diagnoses, interventions, symptoms, and referrals to secondary care are electronically recorded as Read codes, which is a hierarchical coding system used in UK primary care. Clinical measurements and laboratory tests are also included as well as data on all drug prescriptions. Postcode level variables are available including the Townsend Index, which is a composite measure of social deprivation derived using figures on unemployment, car ownership, home ownership and household overcrowding in the local area. A date is provided that relates to the year in which the general practice achieved a standardized mortality rate similar to the general population and is termed the acceptable mortality recording date (AMR date). This can be used to restrict analyses to time periods after which primary care clinicians were using their computer systems adequately. The demographics and consultation behaviour in THIN data are broadly representative of the general practice population and clinical diagnoses recorded by general practitioners (primary care clinicians) have recently been shown to be comparable to other reliable sources. The THIN scheme of
providing anonymized data to researchers was approved by the NHS South-East Multi-centre Research Ethics Committee in 2002 and the University College London Scientific Review Committee approved the present study.

**Study population**

All patients with a total bilirubin level, alkaline phosphatase (ALP) as a measure of biliary function and at least one transaminase (alanine aminotransferase [ALT] or aspartate aminotransferase [AST]) as a measure of liver function recorded in the three months before their first statin prescription between January 1st 2000 and December 31st 2010 were included in the study. This population was selected because patients prescribed statins have important CVD risk factors recorded prior to prescription and also have a broadly similar risk profile (i.e. at least a 20% 10-year risk of CVD). There is evidence that bilirubin levels may increase in response to disease processes, possibly due to stimulation of heme oxygenase,\(^{21}\) and therefore, to reduce the risk of any dilution effect, patients a history of CVD at the time of first prescription were excluded. Likewise patients with a history of diseases that can affect bilirubin levels including liver disease, alcoholism, and hemolytic anaemia were excluded. Patients were followed up from first statin prescription to the event of interest, death, date they left the practice or the end of the study period.

**Exposure**

Serum total bilirubin levels in standard international units of \(\mu\text{mol/L}\) (divide by 17.1 to convert to mg/dL) measured before statin prescription was the exposure variable. Bilirubin levels have been shown to be fairly stable within healthy individuals with only around 5% variation between tests on the same person on two different days.\(^{4}\) The upper level of normal for bilirubin levels is often 17 or 21 \(\mu\text{mol/L}\). However, applying this cut-off this would have excluded a large numbers
of healthy individuals with the Gilbert’s genotype, which is part of the normal genetically-determined variation in bilirubin level.\textsuperscript{2,15} Therefore we considered “normal” bilirubin levels to range between 1 and 40 μmol/L with the upper level approximating three standard deviations from the mean levels reported for healthy people with the Gilbert’s genotype.\textsuperscript{2,15}

**Outcomes**

The main outcome was CVD event (diagnosis or cause of death) including MI, ischemic heart disease, angina, cerebrovascular accident or surgical procedures related to these events. Lists of Read codes for identifying CVD events were developed using previously reported guidelines.\textsuperscript{22} Within CVD, we examined coronary heart disease (CHD) including MI, ischemic heart disease or angina. We also examined MI and death from any cause as outcomes that are probably less susceptible to any misclassification bias. Death from any cause was determined by the presence of a date of death in the patient records.

**Explanatory variables**

Conventional CVD risk factors typically recorded prior to statin prescription were selected as potential confounders in the primary analyses. These included age at first prescription, sex, self-reported smoking (current, ex, never-smoker) and drinking status (current, ex, never-drinker), body mass index (BMI), blood pressure (systolic and diastolic), high-density lipoprotein (HDL), LDL, ethnicity, and the Townsend index of social deprivation. Where multiple measurements were recorded the one closest to the date of the first statin prescription was used. Smoking and drinking status are self-reported by patients and electronically recorded by primary care clinicians or practice nurses using structured data areas in the VISION system with options for “never”, “ex” or “current”. Smoking levels recorded in THIN have been shown to be very similar to other UK data sources.\textsuperscript{23} Patients reporting to be never smokers or drinkers at the time
of first statin prescription but were recorded as current or ex previously were reclassified as ex drinkers or smokers. According to the 2001 census, over 92% of the UK is of white European descent and ethnicity is generally poorly recorded in UK primary care. To maintain statistical power ethnicity was assumed to be European if not recorded.

Bilirubin purportedly has systematic antioxidant/anti-inflammatory properties and may therefore protect against a range of diseases. As such there are certain patient characteristics that could play a role on the causal pathway such as a history of diabetes, certain drug prescriptions and a family history of CVD. These were included in a supportive analysis in combination with the risk factors in the primary analysis. Evidence of moderately raised ALP (>128 IU/L in men and >98 IU/L in women), ALT (>50 IU/L in men and >38 IU/L in women) or AST (>40 IU/L in men and >34 IU/L in women) was also included in this supportive analysis to account for any sub-clinical/undiagnosed hepatobiliary disease. We also accounted for antihypertensive or corticosteroid prescriptions in the six months before first statin prescription, which have been associated with higher and lower bilirubin levels respectively.

**Statistical analyses**

Descriptive statistics included mean values ± standard deviation for continuous variables and percentages for categorical variables. Multivariable Poisson regression was used to estimate the incidence rate ratios (IRRs) of CVD, CHD, MI and death after the addition of conventional cardiovascular risk factors (age, sex, blood pressure, LDL, HDL, BMI, ethnicity, alcohol, smoking, social deprivation) and year of statin prescription. Continuous covariates were entered as such into any models. Restricted cubic spline interpolation was used to investigate non-linear relationships between bilirubin levels and clinical events. This method can flexibly capture dose-response relationships without the loss of information and potential biases associated with
Bilirubin level was transformed into restricted cubic splines with four knots (points at which the relationship with events is allowed to vary). The four knots were selected to using percentiles 5%, 35%, 65%, and 95% (Harrell’s recommended percentiles), which corresponds to 5 μmol/L, 9 μmol/L, 12 μmol/L, and 20 μmol/L respectively. The Akaike information criterion (AIC) was used to identify the model explaining the most variation using the least degrees of freedom. The cubic spline model was selected over the linear model (with log-transformed bilirubin) if the AIC was improved by four. The regression coefficients from restricted cubic spline models are difficult to interpret and so to visualize the results adjusted IRRs for each 1 μmol/L increase in bilirubin assuming average values for all other risk factors.

general practices were included as random effects in all models to account for extra Poissonian variability (overdispersion). All p-values were two-tailed and a value of less than 0.05 was considered statistically significant. All analyses were done using Stata version 12.

Supportive analyses

It is possible that pre-treatment serum bilirubin levels are also a marker for UGT1A-mediated statin metabolism and any observed association in this statin-treated population could also reflect differential drug efficacy or toxicity. As a supportive analysis, we restricted the cohort to a subgroup with LDL recorded both before and within one year of statin prescription and adjusted the associations for LDL reduction. We also examined the relationship between pre-treatment bilirubin levels and the incidence of any statin intolerance and more specifically statin-induced myopathy in the six months following first prescription. Myopathy was defined using Read codes for myopathy or muscle weakness in combination with an entry of statin-related drug intolerance within the structured data area of the VISION system for recording adverse events.
Results

The cohort consisted of 69,209 men and 60,843 women. At the end of the study period (31st December 2010), 16% of the cohort had transferred to a different general practice or were registered with practices that left the THIN scheme before the study period ended. The average age of cohort entry was 60 years for men and 64 years for women (Table 1). Median serum total bilirubin levels were 11 μmol/L (Q1-to-Q3: 8-15) in men and 9 μmol/L (Q1-to-Q3: 7-12) in women. Except for age and blood pressure, factors associated with a higher risk of CVD were associated with moderately lower bilirubin levels (Table 1).

Median cohort follow-up was 43 months (Q1-to-Q3: 24-64 months) during which there were there were 7,850 CVD events. The incidence rate for CVD, CHD, MI and death tended to decrease with increasing bilirubin decile categories for both genders (Table 2). After accounting for conventional risk factors, the relationships with bilirubin were non-linear with the four-knot restricted cubic spline models a better fit to the data than linear models for all the events examined. The introduction of further knots to the data did not improve model fit. Bilirubin level was significantly related to all outcomes examined (p<0.0001). The models estimated an L-shaped relationship with steep negative associations up to around 10-15 μmol/L (Figure 1). Data used to generate these graphs are also reported for the median bilirubin level within decile categories (Table 3). Using the median level of 10 μmol/L as the reference value, the models predicted that, patients with a similar CVD risk profile but a bilirubin level of 5 μmol/L had a 18% (95%CI; 9-27%) higher risk of any CVD event, a 15% (95%CI; 5-26%) higher risk of CHD, a 34% (95%CI; 13-56%) higher risk of MI, and a 33% (95%CI; 21-46%) higher risk of death (Table 3).

There were no significant interactions between bilirubin and sex. However, bilirubin
distributions are different for men and women and a stratified analysis was undertaken for CVD events with four-knot cubic spline models fitted within sex. Bilirubin was significantly associated with CVD in men (p<0.0001) and women (p<0.0001) and the relationship was broadly similar with level below 10 μmol/L associated with excess risk. The addition of covariates potentially on the causal pathway did not materially alter the associations. In the subgroup of patients with LDL recorded both before and within one year of statin prescription, there was a weak positive association with baseline bilirubin and LDL reduction with each 1 μmol/L increase in serum bilirubin associated with a 0.002 mg/dl greater average decrease in LDL (p<0.0001). The relationship with CVD events and death in this subgroup was similar to the whole cohort and the addition of LDL reduction to the model made no material difference to the associations. There was no significant association between pre-treatment bilirubin level and recorded adverse events to statins in the six months following prescription.

**Discussion**

This is the first study to show that serum bilirubin level measured in the context of assessing liver function prior to statin prescription may provide information on disease risk in addition to conventional CVD risk factors. We found that in people with a median serum bilirubin level of 10 μmol/L compared to those with level of 5 μmol/L and similar cardiovascular risk factors, there was a 18% (95%CI; 9-27%) higher risk of having a CVD event, 15% (95%CI; 5-26%) higher risk of CHD event, a 34% (95%CI; 13-56%) higher risk of MI, and a 33% (95%CI; 21-46%) higher risk of death from any cause. The results are directly applicable to primary care and clinicians should be particularly concerned about the one in five patients at risk of CVD with bilirubin levels below 6 μmol/L.
Smaller studies on bilirubin and CVD events have reported similar magnitudes of association as those seen in the present study. A meta-analysis of 11 studies conducted in men and published up to 2001 reported a 6.5% decrease in atherosclerotic disease rates per 1 μmol/L increase in bilirubin level. The Framingham Offspring Cohort study (n=1,780) reported a statistically significant 10% reduction in CVD events per 1.7 μmol/L (0.1 mg/dL) increase in bilirubin, a 13% reduction in CHD, and a 13% reduction in MI over a 24-year follow-up after adjusting for conventional risk factors. These findings are similar in magnitude to our results of around 3-5% decrease per 1 μmol/L increase at bilirubin levels below 10-15μmol/L. The slightly weaker associations in THIN data may be due to adjustments for additional risk factors such as social deprivation or possibly the presence of some reverse causation where bilirubin was measured closer to the event date than in other cohorts and any stimulation of heme oxygenase in response to advancing yet undiagnosed disease may dilute the observed associations.

Prospective studies of individuals homozygous UGT1A1*28, in combination with cell and animal studies demonstrating cytoprotective properties, provide persuasive support for a causal relationship between bilirubin and CVD risk. The Framingham Offspring cohort study (average age 36 years at baseline) demonstrated that the 11% of participants homozygous for the UGT1A1*28 allele (mean bilirubin level 19 μmol/L) had 64% lower rates of CVD (95%CI; 26-82%; p=0.005) and 70% lower rates of CHD (95%CI; 26-88%; p=0.009) over a 24-year follow-up period. Similar associations were reported for the cohort of East-Asian hemodialysis patients (n=661) where homozygosity for UGT1A1*28 was associated with 88% lower rates of CVD events (95%CI; 57-96%; p=0.001) and 75% lower rates of all-cause mortality (95%CI; 21-98%; p=0.019) over a 12-year period. This study was also able to demonstrate that UGT1A1*28 heterozygotes had a 36% lower rates of CVD (95%CI; 11-55%;
p=0.008) compared to patients with no copies of UGT1A1*28 even though heterozygotes tend only to have bilirubin levels 10-30% (1-3 \( \mu \text{mol/L} \)) higher on average. Conversely, two large cohort studies in Danish populations found no association between a variant in linkage disequilibrium with the main \( UGT1A1 \) locus associated with bilirubin levels and the risk of ischemic heart disease.\(^{28}\) Retrospective case-control studies have produced conflicting results.\(^{28}\) One of the largest such studies reported odds ratios similar to the risk ratios of the prospective studies and also identified some significant associations with other genes associated with bilirubin levels.\(^{29}\) All other case-control studies did not report significant associations with \( UGT1A1 \) variation.\(^{28}\) Bias is an issue for genetic association studies of conditions with high lethality like CVD\(^{30, 31}\) and associations would be biased toward the null hypothesis if the UGT1A1*28 allele improves the probability of surviving an acute CVD event or is associated with greater drug efficacy. Indeed, a recent clinical study reported a 60% increase in serum bilirubin levels during MI with levels remaining high for around 21 hours.\(^{32}\) This increase was strongly correlated with the level of serum heme oxygenase and patients whose bilirubin level increased more dramatically during MI had significantly better collateral flow into the ischemic myocardium.\(^{32}\) Although this was a small study, it does raise the possibility of survival bias in case-control studies of alleles influencing bilirubin levels and CVD. Further replication using large cohorts with a young recruitment age, extended follow-up, careful recording of CVD related drug prescriptions, and inclusion of variants influencing bilirubin levels in addition to UGT1A1*28\(^{2, 33}\) is required to clarify the relationship between alleles causing hyperbilirubinemia and CVD risk.

Randomized controlled trials to examine the effect of agents causing moderate increases in serum bilirubin on the risk of CVD could also help to understand whether a causal relationship
exists. Niacin (vitamin B3), for instance, can increase bilirubin levels by stimulating heme oxygenase activity and is sometimes used as a provocation test for Gilbert’s syndrome; a 50mg intravenous injection is associated with a 23 µmol/L average increase in people with Gilbert’s syndrome and a 10 µmol/L average increase in patients without the condition.34 A meta-analysis of randomized controlled trials reported that one to three grams of niacin per day can reduce CVD events by around 25%.35 Part of the mechanism appears to be increases in HDL although an increase in bilirubin was recently shown to explain some of the beneficial effects at least in animal models and human vascular endothelial tissue.36 It would be interesting to explore whether baseline bilirubin levels and UGT1A1 genotype modify the treatment effect. For example, if the dose-response relationships suggested by the cubic spline models are true effects, we would expect patients with bilirubin levels below 10 µmol/L to derive greater benefit from niacin/statin combination treatment compared to those with higher levels. Drugs that stimulate heme oxygenase, such as niacin, will produce carbon monoxide and ferritin in equal measure to bilirubin meaning it will be difficult to conclude with certainty that bilirubin is the causal agent.

Direct intravenous administration of bilirubin or its soluble precursor biliverdin have been shown to benefit rats exposed to endotoxins,37 but use in humans would require further preclinical development. Other possible candidates for investigation are drugs that specifically inhibit or down regulate UGT1A1 activity but strategies to increase bilirubin levels in this way should be balanced against a potential increased risk of gallstones38 or adverse drug reactions.39

To our knowledge, this is the largest longitudinal analysis of the association between bilirubin level and CVD events, and one of the first with sufficient statistical power to demonstrate that the relationships are similar for men and women. During 2010, 89% of new statin prescriptions recorded in THIN included a bilirubin measure in the three months before
prescription suggesting our cohort is broadly representative of patients newly prescribed statins in primary care across the UK. The mean and standard deviation of serum bilirubin levels in this cohort were near identical to those of healthy British men and women reported by others.\(^{40}\) We found no strong evidence that differences in drug efficacy (LDL reduction) or toxicity explained the relationship between pre-treatment bilirubin and CVD events. There was a slightly greater LDL reduction amongst patients with higher bilirubin levels, which may reflect differences in glucuronidation capacity or other characteristics such as treatment adherence. Overall it seems the associations are mainly mediated by the protective properties of bilirubin and/or the influence of unmeasured environmental CVD risk factors that are also associated with lower bilirubin level.

The purported pleotropic effects of statins have led to speculation on a protective mechanism distinct from LDL reduction. Animal studies have reported that statin administration increases heme oxygenase expression in heart and lung tissue together with increases in serum bilirubin level.\(^{41,42}\) Increases in heme oxygenase levels in response to statins have also been identified in human endothelial tissues\(^{43}\) and studies in humans show a modest increase in serum bilirubin, independent of changes in liver enzymes, of around 10-20% following statin treatment.\(^{44,45}\) If heme oxygenase stimulation does explain some the mechanism of statin efficacy, via bilirubin or other by-products, and this stimulation is more pronounced in patients with a higher bilirubin level measured prior to prescription, this could also partially explain the negative relationships with CVD described in the present study. However, if this was the case, we might expect our results to appear stronger relative to similar epidemiological studies in non-statin treated cohorts whereas they were in fact slightly weaker. In addition, a recent paper found bilirubin increases following statin prescription were independent of \textit{UGT1A1} variants in
Chinese patients with hypercholesterolemia.\textsuperscript{44} We could not account for over the counter (OTC) prescriptions of low-dose statins and it is possible that some of the cohort were not treatment naïve at the time of CVD risk assessment. If people with low bilirubin were more likely to be on OTC statin treatment prior to baseline, the associations could have been diluted. Read codes have been shown to be accurate compared with other reliable sources\textsuperscript{20} but there is still the potential for misclassification bias. The associations with bilirubin did appear more pronounced for the “harder” outcomes (MI and death), which might reflect some misclassification of CVD and CHD with respect to bilirubin level. For example, a raised bilirubin level might prompt further general investigations by the clinician and could lead to a CVD diagnosis. Also, patients with higher bilirubin levels may have higher levels of education, not fully accounted for by adjusting for social deprivation, and be more likely to be informed on the symptoms of CVD. It is also worth noting that the patients included in the THIN cohort were those already deemed at risk of CVD by the clinician and appropriate for statin prescription, and although others have reported that bilirubin can improve the performance of CVD risk scores applied to the general population\textsuperscript{46}, we could not examine this in the present study.

Serum bilirubin is a cheap and routine test that captures risk information over and above that of conventional CVD risk factors recorded prior to statin prescription. Further research is warranted to examine whether the inclusion of bilirubin can improve the performance of CVD risk scores applied to the general population and to determine whether there exists a causal relationship between serum bilirubin level and the development of CVD.

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**Conflict of Interest Disclosures:** None.
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<td>1182 (14)</td>
<td>808 (12)</td>
<td>11774 (19)</td>
</tr>
<tr>
<td>Non-European</td>
<td>408 (15)</td>
<td>646 (15)</td>
<td>820 (14)</td>
<td>878 (12)</td>
<td>906 (12)</td>
<td>799 (12)</td>
<td>645 (11)</td>
<td>483 (10)</td>
<td>766 (9)</td>
<td>613 (9)</td>
<td>6964 (11)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; SD, standard deviation
* Categorical variables are reported as No. (%) and continuous are reported as mean (SD)
Table 2. Incidence rates of coronary heart disease events, stroke and death from any cause stratified by sex and decile categories of total serum bilirubin levels measured prior to statin prescription.

<table>
<thead>
<tr>
<th>Bilirubin decile (μmol/L)</th>
<th>1-6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12-13</th>
<th>14-15</th>
<th>16-18</th>
<th>19-40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHD Events (PYs (10,000))</td>
<td>467</td>
<td>350</td>
<td>392</td>
<td>433</td>
<td>435</td>
<td>424</td>
<td>667</td>
<td>467</td>
<td>413</td>
<td>416</td>
</tr>
<tr>
<td>CVD IR (95%CI)</td>
<td>215</td>
<td>224</td>
<td>195</td>
<td>194</td>
<td>189</td>
<td>193</td>
<td>173</td>
<td>171</td>
<td>167</td>
<td>163</td>
</tr>
<tr>
<td>CHD Events (PYs (10,000))</td>
<td>338</td>
<td>253</td>
<td>295</td>
<td>302</td>
<td>308</td>
<td>311</td>
<td>455</td>
<td>332</td>
<td>279</td>
<td>305</td>
</tr>
<tr>
<td>CVD IR (95%CI)</td>
<td>153</td>
<td>160</td>
<td>145</td>
<td>133</td>
<td>132</td>
<td>140</td>
<td>116</td>
<td>120</td>
<td>112</td>
<td>118</td>
</tr>
<tr>
<td>MI Events (PYs (10,000))</td>
<td>52</td>
<td>47</td>
<td>44</td>
<td>34</td>
<td>40</td>
<td>39</td>
<td>32</td>
<td>32</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>CVD IR (95%CI)</td>
<td>52</td>
<td>47</td>
<td>44</td>
<td>34</td>
<td>40</td>
<td>39</td>
<td>32</td>
<td>32</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>MI Events (PYs (10,000))</td>
<td>319</td>
<td>201</td>
<td>254</td>
<td>266</td>
<td>243</td>
<td>197</td>
<td>363</td>
<td>276</td>
<td>217</td>
<td>246</td>
</tr>
<tr>
<td>CVD IR (95%CI)</td>
<td>139</td>
<td>122</td>
<td>120</td>
<td>113</td>
<td>101</td>
<td>85</td>
<td>90</td>
<td>97</td>
<td>84</td>
<td>92</td>
</tr>
<tr>
<td>Death Events (PYs (10,000))</td>
<td>100</td>
<td>158</td>
<td>214</td>
<td>243</td>
<td>242</td>
<td>233</td>
<td>197</td>
<td>138</td>
<td>266</td>
<td>192</td>
</tr>
<tr>
<td>CVD IR (95%CI)</td>
<td>107</td>
<td>105</td>
<td>101</td>
<td>95</td>
<td>86</td>
<td>90</td>
<td>85</td>
<td>76</td>
<td>81</td>
<td>75</td>
</tr>
<tr>
<td>MI Events (PYs (10,000))</td>
<td>33</td>
<td>31</td>
<td>30</td>
<td>26</td>
<td>23</td>
<td>21</td>
<td>21</td>
<td>22</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>CVD IR (95%CI)</td>
<td>33</td>
<td>31</td>
<td>30</td>
<td>26</td>
<td>23</td>
<td>21</td>
<td>21</td>
<td>22</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>Death Events (PYs (10,000))</td>
<td>151</td>
<td>193</td>
<td>275</td>
<td>289</td>
<td>306</td>
<td>272</td>
<td>229</td>
<td>192</td>
<td>366</td>
<td>281</td>
</tr>
<tr>
<td>CVD IR (95%CI)</td>
<td>157</td>
<td>124</td>
<td>126</td>
<td>110</td>
<td>106</td>
<td>103</td>
<td>96</td>
<td>104</td>
<td>109</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>(134-184)</td>
<td>(108-143)</td>
<td>(112-142)</td>
<td>(98-123)</td>
<td>(95-119)</td>
<td>(91-116)</td>
<td>(84-109)</td>
<td>(90-120)</td>
<td>(99-121)</td>
<td>(95-121)</td>
</tr>
</tbody>
</table>

Abbreviations: CHD, coronary heart disease; CVD, cardiovascular disease; IR, incidence rate; MI, myocardial infarction; PYs, person years.
Table 3. Predicted incidence rate ratios using restricted cubic splines for CHD, stroke and death across serum bilirubin deciles in people prescribed statins and after accounting for conventional cardiovascular risk factors.

<table>
<thead>
<tr>
<th>Bilirubin decile (μmol/L)</th>
<th>1-5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>14-16</th>
<th>17-40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (μmol/L)</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>No. patients</td>
<td>10,464</td>
<td>9,151</td>
<td>11,798</td>
<td>13,397</td>
<td>13,350</td>
<td>12,733</td>
<td>11,000</td>
<td>17,354</td>
<td>15,538</td>
<td>15,267</td>
</tr>
<tr>
<td>CVD Model 1</td>
<td>1.18-1.35</td>
<td>1.15-1.26</td>
<td>1.11-1.18</td>
<td>1.07-1.11</td>
<td>1.03-1.05</td>
<td>(Reference)</td>
<td>0.95-0.98</td>
<td>0.91-0.96</td>
<td>0.84-0.93</td>
<td>0.83-0.93</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.09-1.27</td>
<td>1.08-1.20</td>
<td>1.06-1.14</td>
<td>1.04-1.09</td>
<td>1.02-1.04</td>
<td>(Reference)</td>
<td>0.96-0.99</td>
<td>0.92-0.98</td>
<td>0.86-0.97</td>
<td>0.85-0.97</td>
</tr>
<tr>
<td>CHD Model 1</td>
<td>1.15-1.36</td>
<td>1.13-1.27</td>
<td>1.11-1.19</td>
<td>1.07-1.13</td>
<td>1.04-1.06</td>
<td>(Reference)</td>
<td>0.94-0.97</td>
<td>0.88-0.95</td>
<td>0.80-0.91</td>
<td>0.79-0.92</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.05-1.26</td>
<td>1.06-1.20</td>
<td>1.06-1.15</td>
<td>1.05-1.10</td>
<td>1.02-1.05</td>
<td>(Reference)</td>
<td>0.94-0.98</td>
<td>0.89-0.96</td>
<td>0.82-0.94</td>
<td>0.82-0.96</td>
</tr>
<tr>
<td>MI Model 1</td>
<td>1.28-1.72</td>
<td>1.23-1.50</td>
<td>1.17-1.33</td>
<td>1.10-1.20</td>
<td>1.04-1.09</td>
<td>(Reference)</td>
<td>0.91-0.98</td>
<td>0.84-0.97</td>
<td>0.73-0.93</td>
<td>0.68-0.91</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.14-1.56</td>
<td>1.12-1.41</td>
<td>1.09-1.27</td>
<td>1.06-1.16</td>
<td>1.02-1.08</td>
<td>(Reference)</td>
<td>0.93-1.00</td>
<td>0.86-1.00</td>
<td>0.78-1.01</td>
<td>0.76-1.04</td>
</tr>
<tr>
<td>Death Model 1</td>
<td>1.41-1.65</td>
<td>1.30-1.44</td>
<td>1.20-1.28</td>
<td>1.11-1.16</td>
<td>1.04-1.07</td>
<td>(Reference)</td>
<td>0.95-0.98</td>
<td>0.91-0.98</td>
<td>0.84-0.95</td>
<td>0.80-0.93</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.21-1.46</td>
<td>1.16-1.32</td>
<td>1.10-1.20</td>
<td>1.05-1.11</td>
<td>1.01-1.05</td>
<td>(Reference)</td>
<td>0.96-1.01</td>
<td>0.94-1.02</td>
<td>0.89-1.03</td>
<td>0.86-1.03</td>
</tr>
</tbody>
</table>

**Abbreviations:** CHD, coronary heart disease; CVD, cardiovascular disease; MI, myocardial infarction.

Four knot spline transformation of bilirubin levels at percentiles corresponding to 5, 9, 12, and 20 μmol/L. Multivariable Poisson models were used with the median bilirubin level of 10 μmol/L used as the reference level and IRs are estimated for median values within the bilirubin decile category. Model 1 adjusted for age and sex; Model 2 adjusted for age, sex, ethnicity, body mass index, blood pressure, smoking status, drinking status, HDL, LDL, social deprivation, and year of statin prescription. Bilirubin level was significantly associated with all events in Model 2 (p<0.0001).
Figure Legend:

**Figure 1.** Estimated relationship between serum bilirubin level, coronary heart disease, stroke, and death with the median level of 10 μmol/L used as the reference value. Smoothed incidence rate ratio plot using a four knot spline transformation at 5, 9, 12, and 20 μmol/L. Median serum bilirubin level is the reference value. Models were adjusted for age, sex, ethnicity, BMI, blood pressure, smoking status, drinking status, HDL, LDL, social deprivation, and year of statin prescription. Red lines indicate 95% CIs. Abbreviations: CHD, coronary heart disease; CVD, cardiovascular disease; MI, myocardial infarction; PYs, person years.
Cardiovascular Events as a Function of Serum Bilirubin Levels in a Large Statin-Treated Cohort
Laura J. Horsfall, Irwin Nazareth and Irene Petersen

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