Cardiovascular Disease and Cardiovascular Risk Factors

Serum bilirubin and the risk of hypertension

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Abstract

Background: Experimental studies suggest oxidative stress could lead to the development of hypertension. Serum bilirubin is a major contributor to the antioxidant capacity in blood plasma and has been identified as an independent cardiovascular risk factor in cohort studies. However, data on the relationship between bilirubin and blood pressure are scarce and inconclusive.

Methods: We analysed data from the National Health and Nutrition Examination Surveys (NHANES) 1999–2012 (N = 31,069). Fifty multiple imputed data sets were generated and analysed to avoid selection/confounding bias due to excluding individuals/variables with missing values. A minimal sufficient adjustment set of variables (MSAS) needed to estimate the unconfounded effect of bilirubin on blood pressure and hypertension (systolic/diastolic blood pressure ≥140/90 mmHg or using antihypertensive medication) was identified using the back-door criterion and included in all regression models.

Results: After adjustment for the MSAS variables, systolic blood pressure decreased progressively up to −2.5 mmHg (p < 0.001) and the prevalence of hypertension was up to 25% lower (P < 0.001) in those with bilirubin ≥1.0 mg/dl—the highest two deciles—compared with those with 0.1–0.4 mg/dl—the lowest decile. Sensitivity analyses showed these results were unlikely to be explained by residual confounding or selection bias.

Conclusions: High serum bilirubin may decrease the risk of hypertension by inactivating and inhibiting the synthesis of reactive oxygen species in vascular cells. Strategies to boost the bioavailability of circulating and tissue bilirubin or to mimic bilirubin’s antioxidant properties could have a significant impact on prevention and control of hypertension as well as coronary heart disease.

Key words: Bilirubin, blood pressure, direct acyclic graphs, antioxidants, reactive oxygen species, multiple imputation, sensitivity analysis, NHANES
Introduction

Although elevated blood pressure (BP) is a major cause of cardiovascular diseases in all populations and the leading risk factor for global disease burden, our knowledge on risk factors for the development of hypertension is still limited. Serum bilirubin is a powerful antioxidant and has been shown to decrease the risk of cardiovascular outcomes in prospective cohort studies. Experimental studies in animal models suggest that bilirubin may reduce BP by decreasing vascular oxidative stress, and a few epidemiological studies point to an association between bilirubin and BP. However, the results of these studies are uncertain because they were based on cross-sectional data, were limited to narrow age ranges and patient groups, had limited sample size or did not account for confounding and selection biases. Moreover, the role of oxidative stress in the incidence of hypertension has been questioned, due in part to contradictory findings from epidemiological and clinical studies assessing the benefits of supplementing diets with antioxidants such as vitamins C and E. In view of the potential clinical and public health significance of this association, in this study we examined the role of serum bilirubin as a possible risk factor for hypertension in a representative sample of the US adult population.

Methods

This study is based on data from the 1999 to 2012 National Health and Nutrition Examination Surveys (NHANES). NHANES samples are chosen using a stratified, multistage probabilistic cluster design of the US population. Participants ≥20 years old were eligible for this study, excluding pregnant women (n = 1351), individuals without BP data (n = 3622) and Native and Asian Americans (n = 1932).

NHANES data were collected through interviews and a physical examination administered by highly trained personnel. Three to four BP measurements were taken following standard procedures and the mean of all values, excluding the first one in those with more than one measurement, was used in our analysis. Individuals with an average systolic BP ≥ 140 mmHg and/or average diastolic BP ≥ 90 mmHg and/or taking prescribed antihypertensive drugs were considered as hypertensives. Serum total bilirubin levels were measured using Hitachi and Beckman analysers and reported values were adjusted to make them comparable when needed.

Statistical analysis: estimating the effect of bilirubin

In spite of the high quality of NHANES data, 44% of the individuals in our analysis had at least one missing value for at least one of our study variables. Excluding those individuals from the analysis would have reduced statistical power and increased the likelihood of selection bias, whereas excluding those variables would have increased the likelihood of residual confounding. In consequence, we used multivariate imputation by chained equations (MICE) to fill out missing values, and generated and analysed 50 imputed data sets. Also the underlying BP, our outcome variable, could not be measured in 70% of the 38% (of total participants) with hypertension, because they were taking antihypertensive drugs. Excluding these individuals, treating observed BP as underlying BP values, and including treatment as a covariate in the analysis could have resulted in bias. To address this problem we considered the measured BP as a right-censored variable and imputed BP values among treated individuals using interval regression (see Supplementary for details on our imputation approach).

We used directed acyclic graphs (DAG) and Pearl’s back-door criterion, as implemented in DAGitty, to select a minimal sufficient adjustment set (MSAS) of variables that would allow the identification of an unconfounded effect of bilirubin on BP. The DAG (Figure 1) was built by identifying all known factors...
affecting bilirubin or BP, and then including all common causes of any pair of variables already in the DAG. Variables in the MSAS blocked all non-causal but not the causal pathway between bilirubin and BP, and included age, gender, race, abdominal obesity, alcohol use, education (≥ high school) and serum creatinine, albumin and uric acid.

Multiple linear and logistic regression were used to estimate the effect of bilirubin on current systolic and diastolic BP and on the prevalence of hypertension, respectively. NHANES sampling weights were not used in our analysis, because we aimed to estimate conditional instead of marginal effects. All variables in the MSAS and only those variables were included in our full models. An age-squared term and a gender-by-age interaction term were included in our regression models to account for the nonlinearity in the age-BP relationship and for the age-dependent effect of gender on BP and hypertension risk. Also, a term for an abdominal obesity-by-age interaction was included and retained if it was statistically significant. This approach to select model covariates and functional forms avoided excessive data mining, bias towards small P-values, and confidence intervals (CIs) with true coverage less than their nominal rates.

We modelled bilirubin as: a continuous variable with increments of 0.2 mg/dl (slightly over one-tenth of the normal range); as a categorical variables with seven levels, defined by its observed deciles among non-hypertensive individuals; and as a dichotomous variable defined as bilirubin ≥ 0.7 mg/dl, the median value among non-hypertensive individuals. Abdominal obesity was defined using gender and race specific cut points from Herrera et al.

We assessed the fit of our models in each imputed data set, because varying predicted probabilities across imputed datasets make average model goodness-of-fit tests and residual analyses non-interpretable. For logistic models we
report the maximum and the minimum value of the Hosmer and Lemeshow test\textsuperscript{58} in each imputed data set. Also, we assessed the robustness of our results from logistic models by re-estimating the effect of bilirubin after excluding the top 10% of observations with high influence, as defined by Pregibon’s leverage statistic.\textsuperscript{59} Similarly, for linear models we re-estimated the effect of bilirubin after excluding observations having a Cook’s distance\textsuperscript{60} greater than four divided by the total number of observations.\textsuperscript{61}

**Sensitivity analysis: assessing the robustness of study assumptions**

We conducted sensitivity analyses to evaluate the robustness of our results to different assumptions and potential biases. A particularly strong assumption was that an unconfounded effect of bilirubin on BP could be estimated by adjusting only for those variables in the MSAS.\textsuperscript{37} To test this assumption we conducted a calibrated simulation-based sensitivity analysis based on propensity score matching,\textsuperscript{62} as proposed by Ichino et al.\textsuperscript{63} and implemented by Nannicini.\textsuperscript{64} Briefly, we assessed the reasonableness of this assumption by comparing our estimate of the effect of the bilirubin with estimates that were further adjusted for simulated potential confounders (SPC) drawn from the distribution of observed variables (see Supplementary for details).\textsuperscript{63,64}

Another important assumption was that kidney function, as measured through serum creatinine levels, modulates BP level.\textsuperscript{65} However, there is evidence that high BP induces early renal damage and results in increased serum creatinine.\textsuperscript{66} If that is the case, then adjusting for serum creatinine can result in an inconsistent estimate of the effect of bilirubin on BP.\textsuperscript{67} In addressing this issue, we argued that creatinine acts as a confounder in some but not all individuals in our sample and compared the estimates of effects from models with and without creatinine as a covariate. The unbiased causal effect of bilirubin on BP should lie between these two estimates.

It is well documented that survival is increased among individuals with higher levels of serum bilirubin,\textsuperscript{68–70} and is decreased among individuals with higher BP.\textsuperscript{71} Therefore, due to their cross-sectional nature, the NHANES samples are biased towards including individuals with lower BP and higher bilirubin than the original population that generated those samples. This creates a potential for selection bias because our analysis was limited to survivors and survival was a consequence of both the exposure and the outcome (i.e. survival is a collider).\textsuperscript{72,73} Also, the exclusion of individuals without BP data from the analysis could have resulted in selection bias if having a BP measure depended on both the actual value of BP and the level of serum bilirubin. To assess these sources of bias we assumed that the selection mechanisms that resulted in the sample included in our analysis would operate in the same way if NHANES participants were followed prospectively. In both cases we mimicked the selection mechanism by simulating a collider variable that was an additive function of the effects of bilirubin and BP on inclusion in the study. Then we estimated the effect of bilirubin \( \geq 0.7 \text{ mg/dL} \) on hypertension excluding individuals with high values of the simulated collider, and compared these estimates with the one obtained from the analysis with all individuals (see Supplementary for details).

All the analyses were conducted using Stata 13.0 (StataCorp 2013, College Station, TX).

**Results**

Our analysis included 31,069 individuals, with an average age of 50 years, 50% men, 38% hypertensive, and a median serum bilirubin of 0.7 mg/dl (range 0.1 to 13.1; Table 1). Individuals with serum bilirubin \( \geq 0.7 \text{ mg/dL} \) had higher serum creatinine and uric acid, but had slightly lower systolic BP and prevalence of hypertension, and were considerably less likely to be African Americans, to have abdominal obesity, to smoke or to drink alcohol regularly.

Crude and multivariate adjusted models showed that systolic BP was lower among individuals with higher serum bilirubin (Table 2). After adjusting for variables in the MSAS, systolic BP decreased progressively with increasing levels of bilirubin, up to \(-3.4 \text{ mmHg} \) in those with the highest (\( \geq 1.2 \text{ mg/dl} \)) as compared with those with the lowest (0.1–0.4 mg/dl) levels of bilirubin (\( P < 0.001 \)). In fact, systolic BP decreased by 0.41 mmHg for each increase of 0.20 mg/dl of bilirubin (\( P < 0.001 \); Table 2). Also, systolic BP was 1.67 mm Hg (\( P < 0.001 \)) lower among individuals with bilirubin \( \geq 0.7 \text{ mg/dL} \). In contrast, bilirubin-associated changes in diastolic BP were inconsistent, small and statistically non-significant in all models (Table 3).

The prevalence of hypertension decreased progressively with increasing levels of serum bilirubin (Table 4). Indeed, after accounting for variables in the MSAS, the prevalence of hypertension was 25% lower in individuals with the highest (\( \geq 1.2 \text{ mg/dl} \)) as compared with those with the lowest (0.1–0.4 mg/dL) levels of bilirubin (\( P < 0.001 \)), and decreased by 14% among those with bilirubin \( \geq 0.7 \text{ mg/dL} \) (\( P < 0.001 \)) and 5% per each 0.20 mg/dl increase in bilirubin (\( P < 0.001 \)). A bilirubin level above the median resulted in similar decreases in hypertension prevalence in men and women (\( P \)-value for interaction: 0.144).
Discussion
We found that serum bilirubin was inversely associated with systolic BP and with the prevalence of hypertension. Specifically, after adjusting for relevant confounders, systolic BP was 1.67 mm Hg lower and hypertension was 14% less likely among individuals with bilirubin \( \geq 0.7 \) mg/dl. These inverse associations were also observed when bilirubin was analysed as a continuous and as an interval variable. A significant relationship between bilirubin and diastolic BP was not observed.

Serum bilirubin has previously been shown to be an independent cardiovascular risk factor in prospective cohort studies.\(^5\)–\(^8\) There are few reports on the association between bilirubin and BP, but most seem to support our findings. In a study among young adults from the Bogalusa study, Madhavan et al.\(^12\) found that bilirubin was negatively and weakly correlated with systolic but not with diastolic BP, after adjustment for age, body mass index, smoking and alcohol intake.\(^12\) Also, in crude comparisons in a small group of non-smokers with primary dyslipidaemia, serum bilirubin was significantly lower in those who were hypertensive, regardless of antihypertensive treatment.\(^13\) Moreover, Chin et al. conducted a cohort study among 1208 Korean outpatients recruited from a health promotion clinic over a 10-year period, and found that the relative risk of hypertension was 0.71 (\(P = 0.048\)) in patients with total bilirubin \( \geq 1.1 \) mg/dl as compared with those with lower levels, after adjustment for other risk factors.\(^14\) However, this was a small study with only 43 new cases in the exposed group and staggered non-planned follow-up evaluations, and the decrease in risk was statistically significant only in women and smokers. In contrast, bilirubin was not associated with BP in a small study (\(N = 868\)) among related Amish.\(^74\) This finding is likely explained by selection bias due to the exclusion of individuals with high levels of both BP (systolic/diastolic BP \( \geq 140/90 \) mm Hg) or taking antihypertensive medication.

Using a cross-sectional sample could have resulted in survival bias because both low bilirubin and high BP are positively associated with increased mortality.\(^68\),\(^75\) However, our sensitivity analysis showed that if survival bias had occurred, the true effect of bilirubin on BP should be slightly stronger than what we have estimated in our study (Supplementary Table 3 and Figure 7). This is consistent with the fact that mortality increases with higher BP but decreases with higher bilirubin levels. Similarly, our assessment of potential selection bias due to the exclusion of individuals without BP data showed that our estimate of the effect of bilirubin was likely biased towards the null, but that the bias was very small (Supplementary Table 4 and Figure 8). This is a reflection of a non-significant effect.

Table 1. Characteristics of the study population by levels of serum bilirubin (means and (95% confidence intervals))

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Low bilirubin (&lt;0.7 mg/dl)</th>
<th>High bilirubin (( \geq 0.7 ) mg/dl)</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.0 (49.7, 50.3)</td>
<td>51.1 (50.9, 51.4)</td>
<td>50.6 (50.4, 50.8)</td>
</tr>
<tr>
<td>Serum creatinine (( \mu g/ml ))</td>
<td>8.6 (8.5, 8.7)</td>
<td>9.5 (9.4, 9.5)</td>
<td>9.1 (9.0, 9.1)</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>4.2 (4.2, 4.2)</td>
<td>4.3 (4.3, 4.3)</td>
<td>4.3 (4.3, 4.3)</td>
</tr>
<tr>
<td>Serum uric acid (mg/dl)</td>
<td>5.2 (5.2, 5.2)</td>
<td>5.7 (5.7, 5.7)</td>
<td>5.5 (5.5, 5.5)</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>132.3 (131.8, 132.7)</td>
<td>131.2 (130.8, 131.6)</td>
<td>131.7 (131.4, 132.0)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75.4 (75.1, 75.7)</td>
<td>75.4 (75.1, 75.6)</td>
<td>75.4 (75.2, 75.6)</td>
</tr>
<tr>
<td>Male gender</td>
<td>36.6 (35.8, 37.4)</td>
<td>62.2 (61.4, 62.9)</td>
<td>50.3 (49.7, 50.9)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican American</td>
<td>21.0 (20.3, 21.7)</td>
<td>18.5 (17.9, 19.1)</td>
<td>19.6 (19.2, 20.1)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>8.1 (7.7, 8.6)</td>
<td>7.2 (6.8, 7.6)</td>
<td>7.7 (7.4, 7.9)</td>
</tr>
<tr>
<td>White</td>
<td>45.4 (44.5, 46.2)</td>
<td>55.3 (54.5, 56.0)</td>
<td>50.7 (50.1, 51.2)</td>
</tr>
<tr>
<td>African American</td>
<td>25.5 (24.8, 26.2)</td>
<td>19.0 (18.4, 19.6)</td>
<td>22.0 (21.6, 22.5)</td>
</tr>
<tr>
<td>Education ≥high school</td>
<td>66.1 (65.3, 66.8)</td>
<td>72.6 (71.9, 73.3)</td>
<td>69.6 (69.0, 70.1)</td>
</tr>
<tr>
<td>Abdominal obesity(^a)</td>
<td>52.7 (51.9, 53.6)</td>
<td>46.3 (45.6, 47.2)</td>
<td>49.3 (48.8, 49.9)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>25.9 (25.2, 26.7)</td>
<td>19.1 (18.5, 19.7)</td>
<td>22.3 (21.8, 22.7)</td>
</tr>
<tr>
<td>Alcohol drinkers</td>
<td>72.5 (71.6, 73.3)</td>
<td>77.4 (76.7, 78.2)</td>
<td>75.1 (74.5, 75.7)</td>
</tr>
<tr>
<td>Hypertensive(^b)</td>
<td>38.6 (37.8, 39.4)</td>
<td>37.2 (36.4, 37.9)</td>
<td>37.8 (37.3, 38.4)</td>
</tr>
</tbody>
</table>

\(^a\)Waist circumference (cm) in females/males \( \geq 96.8/102.1 \) in Whites, \( \geq 95.5/95.0 \) in African Americans and \( \geq 93.5/99.2 \) in Hispanics and Mexican Americans.

\(^b\)Systolic/diastolic blood pressure \( \geq 140/90 \) mm Hg or taking antihypertensive medication.
of bilirubin levels on missing the BP measurement. Although bilirubin and BP were measured at the same time, reverse causality bias was unlikely, as there is no evidence of mechanisms by which BP could drive bilirubin levels. In contrast, an effect of bilirubin on BP is supported by biological mechanisms as well as evidence from experimental studies in animal models\textsubscript{10,11} and observational genetic studies in humans.\textsubscript{76,77}

Our sensitivity analysis for residual confounding showed that even if an unobserved confounding factor had outcome effects (odds ratios from 0.57 to 2.86) and exposure effects (odds ratios from 0.41 to 12.24) as large as those of the 29 observed covariates used to generate SPC, it would not cause a large change in the estimated effect of bilirubin on BP (Supplementary Table 3). Although seven of the 29 SPC induced bias towards a null effect, the biases were small, and none rendered the effect of bilirubin statistically non-significant. Nevertheless, we cannot rule out the existence of confounders with distributions different from the distribution of variables in our calibration set, or residual confounding due to measurement errors.

Adjusting for serum creatinine level, a variable that seems to be both a consequence and a cause of the outcome,\textsuperscript{42,65} could have biased our estimate of the effect of bilirubin on BP.\textsuperscript{66} However, creatinine-adjusted and un-adjusted models resulted in virtually identical odds ratios of hypertension for bilirubin $\geq 0.7$ mg/dl: $0.86$ (0.81, 0.92) and $0.87$ (0.82, 0.93), respectively. On the other hand, our logistic models fitted the data well, as indicated by $P$-values for goodness of fit tests in each imputed data set ranging from 0.43 to 0.95 and from 0.52 to 0.96 when bilirubin was analysed as a continuous and as a dichotomous variable, respectively. Also, excluding the observations with strong influence from the analysis resulted in insubstantial changes in the estimates of effect of bilirubin, as

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**Table 2.** Average difference in systolic blood pressure (mm Hg) by level of serum total bilirubin

<table>
<thead>
<tr>
<th>Models</th>
<th>Bilirubin (mg/dl)</th>
<th>Change in systolic BP</th>
<th>95% confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>0.1–0.4</td>
<td>0.00</td>
<td>–0.94, 0.88</td>
<td>0.945</td>
</tr>
<tr>
<td></td>
<td>0.5–0.5</td>
<td>–0.03</td>
<td>–0.97, 0.88</td>
<td>0.945</td>
</tr>
<tr>
<td></td>
<td>0.6–0.6</td>
<td>0.34</td>
<td>–0.77, 1.18</td>
<td>0.420</td>
</tr>
<tr>
<td></td>
<td>0.7–0.7</td>
<td>0.11</td>
<td>–0.84, 0.88</td>
<td>0.813</td>
</tr>
<tr>
<td></td>
<td>0.8–0.8</td>
<td>–0.22</td>
<td>–0.85, 0.88</td>
<td>0.642</td>
</tr>
<tr>
<td></td>
<td>0.9–1.1</td>
<td>–0.58</td>
<td>–0.95, 0.88</td>
<td>0.200</td>
</tr>
<tr>
<td></td>
<td>1.2–1.31</td>
<td>–1.92</td>
<td>–0.96, 0.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Per 0.20 mg/dl</td>
<td>–0.41</td>
<td>–0.96, 0.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>≥0.7 mg/dl</td>
<td>–0.69</td>
<td>–1.94, 0.88</td>
<td>0.003</td>
</tr>
</tbody>
</table>

**Table 3.** Average difference in diastolic blood pressure (mm Hg) by level of serum total bilirubin

<table>
<thead>
<tr>
<th>Models</th>
<th>Bilirubin (mg/dl)</th>
<th>Change in diastolic BP</th>
<th>95% confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>0.1–0.4</td>
<td>0.00</td>
<td>–0.46, 0.78</td>
<td>0.616</td>
</tr>
<tr>
<td></td>
<td>0.5–0.5</td>
<td>0.16</td>
<td>–0.51, 0.66</td>
<td>0.807</td>
</tr>
<tr>
<td></td>
<td>0.6–0.6</td>
<td>0.07</td>
<td>–0.31, 0.90</td>
<td>0.339</td>
</tr>
<tr>
<td></td>
<td>0.7–0.7</td>
<td>0.29</td>
<td>–0.14, 1.14</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td>0.8–0.8</td>
<td>0.50</td>
<td>–0.18, 1.05</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>0.9–1.1</td>
<td>0.44</td>
<td>–0.42, 0.91</td>
<td>0.475</td>
</tr>
<tr>
<td></td>
<td>1.2–1.31</td>
<td>0.24</td>
<td>–0.10, 0.10</td>
<td>0.944</td>
</tr>
<tr>
<td></td>
<td>Per 0.20 mg/dl</td>
<td>0.00</td>
<td>–0.39, 0.76</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>≥0.7 mg/dl</td>
<td>0.29</td>
<td>–0.03, 0.61</td>
<td>0.071</td>
</tr>
</tbody>
</table>

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\textsuperscript{a}Adjusted by age and gender.

\textsuperscript{b}Adjusted by age, gender, race, alcohol use, education, abdominal obesity, serum creatinine, serum albumin and serum uric acid.

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\textsuperscript{a}Adjusted by age and gender.

\textsuperscript{b}Adjusted by age, gender, race, alcohol use, education, abdominal obesity, serum creatinine, serum albumin and serum uric acid.
fully to those from the analysis of the full sample (odds ratio: 0.87, P < 0.001) materially identical. In turn peroxynitrite oxidizes tetrahydrobiopterin, a critical cofactor for the eNOS, resulting in ‘uncoupling’ of eNOS and production of ROS rather than NO.\textsuperscript{84,85} In addition, free radicals catalyze the transformation of arachidonic acid to F\textsubscript{2}-isoprostanes, including 8-epiprostaglandin F\textsubscript{2}:x, a potent vasoconstrictor and mitogen.\textsuperscript{86} Therefore, an increase in ROS could decrease the bioavailability of vascular NO, impair the homeostatic vasodilating mechanisms aimed to regulate arterial BP\textsuperscript{87,88} and contribute to the development of hypertension.\textsuperscript{89–91}

Many studies have shown that bilirubin is a powerful antioxidant both \textit{in vitro}\textsuperscript{92,93} and \textit{in vivo}.\textsuperscript{2–4} Bilirubin contributes to 23% of the total antioxidant activity of the five major radical scavenging antioxidants in plasma (albumin, urate, ascorbate, \textit{z}-tocopherol and bilirubin) in 5-days-old term babies.\textsuperscript{94} Moreover, it has been postulated that the main role of bilirubin is inhibiting NADPH oxidase, the enzyme mainly responsible for vascular ROS production.\textsuperscript{95–98} Also, high bilirubin is associated with higher brachial artery flow-mediated vasodilation, an indicator of endothelial function potentially related to the development of hypertension.\textsuperscript{89–91,99}

There are currently no proven interventions to induce safe and persistent increases in bilirubin levels that may lead to important changes in BP. Limited data suggest that smoking cessation,\textsuperscript{100} vigorous exercise\textsuperscript{101} and weight loss through diet\textsuperscript{102} could lead to significant short-term increases in serum bilirubin. Bilirubin levels could also be increased by drugs that increase the expression of heme oxygenase-1 (HO-1)\textsuperscript{98,103,104} and drugs that inhibit UGT. Unfortunately, it is uncertain whether chronic induction of HO-1 can have detrimental effects \textit{in vivo},\textsuperscript{103} and UGT inhibitors could also increase the plasma half-life of other drugs that are metabolized through conjugation with glucuronic acid.\textsuperscript{105}

In view of our findings and those from previous studies, it is reasonable to infer that serum bilirubin has a likely casual effect on BP. However, bilirubin’s effects on systolic BP and hypertension were relatively weak, in spite of its high antioxidant capacity. This suggests that strategies to boost the bioavailability of bilirubin or to mimic its antioxidant properties would have a limited impact on prevention and treatment of hypertension and may partly explain the failure of large controlled trials of antioxidant supplements for prevention and management of cardiovascular diseases.\textsuperscript{106,107}

### Supplementary Data

Supplementary data are available at IJE online.
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Conflict of interest: None declared.

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