Serum Total Bilirubin Level and Prevalent Lower-Extremity Peripheral Arterial Disease

National Health and Nutrition Examination Survey (NHANES) 1999 to 2004

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Background—Bilirubin, with recently recognized antioxidant and antiinflammatory activity, has emerged as a candidate for atheroprotection. We hypothesized that higher levels of bilirubin would reduce susceptibility to peripheral arterial disease (PAD).

Methods and Results—We analyzed 7075 adults with data available on the ankle brachial index, serum total bilirubin level, and PAD risk factors in the National Health and Nutrition Examination Survey (1999 to 2004), a nationally representative cross-sectional examination of the United States population. A 0.1 mg/dL increase in bilirubin level was associated with a 6% reduction in the odds of PAD (OR 0.94 [95% CI 0.90 to 0.98]) after adjustment for age, gender, race/ethnicity, smoking status, diabetes, hypertension, hypercholesterolemia, chronic kidney disease, CRP, and homocysteine. This result was not dependent on bilirubin levels above the reference range, liver disease, or alcohol intake. The inverse association of bilirubin with PAD tended to be stronger among men (OR 0.90 [95% CI 0.85 to 0.96]) compared with women (OR 0.97 [95% CI 0.91 to 1.04]; $P_{interaction}=0.05$), and was stronger among active smokers (OR 0.81 [95% CI 0.73 to 0.90]) compared with nonsmokers (OR 0.97 [95% CI 0.93 to 1.02]; $P_{interaction}<0.01$).

Conclusions—Increased serum total bilirubin level is associated with reduced PAD prevalence. This result is consistent with the hypothesis that bilirubin is protective from PAD. (***Arterioscler Thromb Vasc Biol*. 2008;28:166-172.)**

Key Words: bilirubin ■ peripheral vascular disease ■ PVD ■ epidemiology ■ Centers for Disease Control and Prevention ■ CDC ■ National Health and Nutrition Examination Survey ■ NHANES

Bilirubin, once considered simply the metabolic end product of heme degradation, has emerged as a potential endogenous inhibitor of atherosclerosis. Bilirubin is a potent antioxidant under physiological conditions.[1] It acts as an antioxidant whether it is free or albumin bound,[2] unconjugated or conjugated,[3,4] and it inhibits both lipid and protein oxidation.[5] Bilirubin protects cells from a 10 000-fold excess of oxidants through rapid regeneration of bilirubin by biliverdin reductase.[6] Additionally, bilirubin exerts antiinflammatory effects on the vasculature.[7,8] Oxidative stress and inflammation are fundamental to the pathogenesis of atherosclerosis.[9–11] The inverse association of serum total bilirubin with coronary artery disease (CAD) suggests that the antiinflammatory and antioxidant properties of bilirubin might offer protection from atherosclerosis.[12]

Peripheral arterial disease (PAD) is an important manifestation of atherosclerosis, associated with significant morbidity including intermittent claudication, critical limb ischemia, and amputation, and portends a 2- to 6-fold increased cardiovascular mortality risk.[13,14] Although risk factors such as cigarette smoking and diabetes mellitus are known to be important risk factors for PAD, little is known about endogenous protective mechanisms that reduce the risk of PAD.

Given the remarkable antioxidant, cytoprotective, and antiinflammatory properties of bilirubin, and the role of inflammation, oxidative stress, and cellular injury in atherosclerosis, we hypothesized that individuals with higher bilirubin level would be less likely to develop PAD. We therefore examined the association of bilirubin level with prevalent PAD in the National Health and Nutrition Examination Survey 1999 to 2004, a nationally representative cross-sectional examination of the United States civilian population.

Methods

The National Health and Nutrition Examination Surveys (NHANES) is a program designed to assess the health and nutritional status of adults and children in the United States. The study was approved by the National Center for Health Statistics (NCHS) institutional review board and all subjects gave informed consent. The NHANES detailed interview includes demographic, socioeconomic, dietary, and health-related questions. The examination component consists of medical and dental examinations, physiological measurements, and laboratory tests administered by highly trained medical personnel.

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Ankle Brachial Blood Pressure Examination Protocol

Beginning in 1999, participants 40 years of age and older were asked to participate in the ankle brachial index (ABI) examination. Those who weighed over 400 pounds or had bilateral amputations were not eligible. Participants lay supine during the examination. Systolic pressure was measured on the right arm (brachial artery) and at both ankles (posterior tibial arteries) using an 8-MHz Doppler probe. If the right arm could not be used, the left arm was used. Each limb pressure was measured twice in participants aged 40 to 59 years and once in participants 60 years and older. The ankle brachial blood pressure index (ABI) was automatically calculated (Parks Mini-Laboratory IV, Model 3100) by dividing the systolic blood pressure at the ankle by the brachial artery systolic blood pressure. PAD was defined as either leg ABI being $\leq$0.90. If neither leg ABI was $\leq$0.90 and either leg ABI was $>$1.40, that subject was excluded from this analysis because of noncompressible vessels.

Laboratory Methods

Serum total bilirubin, albumin, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glucose were determined by automated biochemical profiling (Beckman Synchron LX20); fractionation of total bilirubin was not performed. The LX20 uses a timed end point Diazo method to measure the total bilirubin level. The analytical range for this assay is 0.1 to 30 mg/dL, and the reference range is 0.2 to 1.3 mg/dL. C-reactive protein was quantified by latex-enhanced nephelometry. Total cholesterol and HDL cholesterol were measured by automated enzymatic assay (Hitachi 704 Analyzer serviced by Roche Diagnostics). Total homocysteine in plasma was measured by the Abbott Homocysteine assay.

Covariates

Self-reported race was defined as non-Hispanic white, non-Hispanic black, Mexican-American, or other. A diagnosis of hypertension was assigned if the subject reported a physician diagnosis of hypertension, if the subject reported taking prescription medications for hypertension, or if the systolic blood pressure was $\geq$140 mm Hg or the diastolic blood pressure was $\geq$90 mm Hg. A diagnosis of hypercholesterolemia was assigned if the subject reported a physician diagnosis of hypercholesterolemia, if the subject reported taking prescription medications for hypercholesterolemia, or if the total cholesterol level was $\geq$62.1 mmol/L (240 mg/dL). A diagnosis of diabetes mellitus was assigned if the subject reported a physician diagnosis of diabetes, if the subject reported taking prescription medications (either insulin or oral agents) for diabetes, if nonfasting plasma glucose was $\geq$11.1 mmol/L (200 mg/dL), or if fasting plasma glucose was $\geq$7.0 mmol/L (126 mg/dL). Subjects were characterized as active smokers if the subject answered yes to "do you now smoke cigarettes", as former smokers if they were not active smokers and they answered yes to "have you smoked at least 100 cigarettes in your life", or as never smokers if they denied smoking at least 100 cigarettes. Alcohol intake was categorized as $<$1 drink per day, 1 to 4 drinks per day, or $\geq$5 drinks per day. The presence of active liver disease was determined by the subject's answer to the questions "Has a doctor or other health professional ever told you that you have liver disease?" and "Do you still have a liver condition?" We used the Modification of Diet in Renal Disease (MDRD) Study equation for estimating glomerular filtration rate (GFR) from serum creatinine (GFR mL/min/1.73 m^2)=$175\times(\text{Scr})^{-1.154}\times(\text{Age})^{-0.203}\times$(0.742 if female)$\times$(1.210 if African American). Subjects whose estimated GFR was $<$60 mL/min/1.73 m^2 were classified as having chronic kidney disease.[15,16] Further laboratory and examination details are available at http://www.cdc.gov/nchs/about/major/nhanes/datalink.htm.

Derivation of the Sample

Of the 7571 subjects that had ABI data available, 113 were excluded for noncompressible vessels. Of the remaining subjects, 7075 had complete information on the main covariates of interest and therefore constitute the sample used in this analysis. Of these subjects, 6924 had alcohol intake data available.

Statistical Analysis

NHANES uses a complex, multistage, probability-sampling design to select participants representative of the civilian, noninstitutionalized U.S. population. All analyses accounted for the complex sampling method. A 6-year mobile examination center (MEC) weight variable was created by assigning 2/3 of the 4 year weight for 1999 to 2002 if the person was sampled in 1999 to 2002 or assigning 1/3 of the 2 year weight for 2003 to 2004 if the person was sampled in 2003 to 2004.

Analyses were performed with SAS version 9.1 (SAS Institute Inc) callable SUDAAN version 9.01 (Research Triangle Institute). Hypothesis testing was 2-tailed, with a probability value of $<$0.05 considered significant. Subject characteristics are reported as the weighted mean and standard error (SE) or the weighted percentile and SE, unless indicated otherwise. Odds ratios and 95% confidence intervals (CI) were estimated by logistic regression. Based on recognized risk factors for lower extremity PAD and previous analyses in the NHANES, age, gender (female versus male), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other), hypertension (yes versus no), diabetes mellitus (yes versus no), hypercholesterolemia (yes versus no), chronic kidney disease (yes versus no), smoking status (never, former, active), homocysteine level ($<$10 μmol, 10 to 15 μmol, $>$15 μmol),[17] and CRP level ($<$1 mg/L, 1 to 3 mg/L, $>$3 mg/L)[18] were adjusted for in the multivariable model.[16,19]

We examined the likelihood of PAD associated with a 0.1 mg/dL increase and by a 1 standard deviation (0.28 mg/dL) increase in bilirubin level. Based on observations regarding the relationship between bilirubin level and coronary disease,[20,21] a priori we examined whether the relationship between bilirubin level and PAD varied by gender in stratified analyses and by adding a bilirubin*gender interaction term in the full multivariable model. We also examined a priori whether smoking status influenced the association of bilirubin level with PAD, as has been suggested previously.[22] For analyses examining the interaction of smoking status with bilirubin level, subjects were characterized either as nonsmokers or active smokers.

Results

Of the 7075 analyzed subjects, 599 subjects had an ABI $\leq$0.90. The weighted mean prevalence of PAD was 5.8% (0.3). Subject characteristics are summarized in Table 1. The mean serum total bilirubin was 0.70 (0.01) mg/dL, the median was 0.70 (interquartile range 0.5 to 0.8) mg/dL, and the mode was 0.60 mg/dL. The distribution of bilirubin levels can be seen in the Figure.

Serum total bilirubin level was associated with multiple subject characteristics (Table 2). Female gender, non-Hispanic black race/ethnicity, active smoking status, and higher CRP category were associated with lower bilirubin levels, whereas higher homocysteine category was associated with higher bilirubin levels.

PAD was also associated with multiple subject characteristics (Table 1). Increasing age, non-Hispanic black race/ethnicity, hypertension, diabetes, hypercholesterolemia, smoking, chronic kidney disease, higher CRP level, and higher homocysteine level were all associated with an increased likelihood of PAD, in agreement with previous analyses of the NHANES cohort.[16,19]

Association of Serum Total Bilirubin Level With PAD

Subjects with PAD had a significantly lower mean total bilirubin level than subjects without PAD (0.65 [95% CI 0.62 to 0.67] versus 0.71 [95% CI 0.69 to 0.72] mg/dL). PAD

Table 1. Subject Characteristics

	All Subjects n=7075 Percent (95% CI)	Subjects Without PAD n=6476 Percent (95% CI)	Subjects With PAD n=599 Percent (95% CI)
Age group, years			
40–49	36.6 (34.7–38.6)	38.3 (36.3–40.4)	9.4 (6.1–14.0)
50–59	28.3 (26.8–29.9)	29.1 (27.4–30.9)	16.5 (12.4–21.5)
60–69	17.9 (16.8–19.1)	17.5 (16.3–18.7)	24.2 (20.1–28.8)
70 or over	17.2 (16.0–18.4)	15.1 (14.1–16.2)	50.0 (45.0–55.0)
Female sex	51.6 (50.5–52.7)	51.2 (49.9–52.4)	58.3 (53.0–63.5)
Race/ethnicity			
Non-Hispanic White	78.7 (75.2–81.8)	78.6 (75.1–81.7)	79.4 (74.9–83.3)
Non-Hispanic Black	8.9 (7.3–10.9)	8.6 (7.1–10.5)	13.6 (10.2–17.8)
Mexican American	4.5 (3.2–6.3)	4.6 (3.3–6.4)	3.1 (1.7–5.6)
Other	7.9 (5.9–10.5)	8.1 (6.1–10.7)	4.0 (2.1–7.3)
Hypertension	47.3 (45.0–49.5)	45.7 (43.4–48.0)	73.7 (68.4–78.3)
Diabetes mellitus	11.5 (10.9–12.8)	11.1 (10.1–12.1)	24.6 (19.7–30.3)
Hypercholesterolemia	46.7 (45.5–48.9)	46.5 (44.7–48.3)	58.5 (53.6–63.2)
Smoking status			
Never	45.6 (42.7–46.5)	45.2 (43.2–47.3)	33.8 (29.1–39.9)
Former	33.7 (28.7–32.4)	30.1 (28.3–32.0)	37.0 (31.5–42.8)
Active	20.7 (23.1–26.8)	24.6 (22.8–26.6)	29.2 (25.8–32.9)
Chronic kidney disease	11.7 (10.6–12.9)	10.3 (9.2–11.6)	34.0 (29.2–39.2)
C-reactive protein, mg/L			
<1	25.0 (23.5–26.7)	25.8 (24.2–27.5)	12.7 (9.5–16.8)
1–3	34.4 (33.1–35.7)	34.4 (33.0–35.8)	33.4 (29.1–37.9)
>3	40.6 (38.8–42.4)	39.8 (37.9–41.7)	53.9 (49.9–57.9)
Homocysteine, μmol/L			
<10	69.4 (67.3–71.5)	70.7 (68.5–72.8)	49.3 (44.2–54.4)
10–15	25.3 (23.6–27.0)	24.6 (22.9–26.4)	36.2 (36.2–41.6)
>15	5.3 (4.6–6.1)	4.8 (4.1–5.6)	14.5 (11.1–18.8)
Peripheral arterial disease	5.8 (5.2–6.5)	n/a	n/a

prevalence was lower among subjects with higher bilirubin levels ($P_{trend}<0.001$; Figure). In unadjusted logistic regression analysis, a 0.1 mg/dL increase in bilirubin was associated with a 9% reduced odds of PAD (OR 0.91 [95% CI 0.88 to 0.95]): the standardized estimate was an OR of 0.77 (95% CI 0.69 to 0.86). In a multivariable model adjusting for age, gender, race/ethnicity, diabetes, hypertension, hypercholesterolemia, smoking status, chronic kidney disease, homocysteine, and CRP, a 0.1 mg/dL increase in total bilirubin was associated with a 6% reduced odds of PAD (OR 0.94 [95%CI 0.90 to 0.98]): the standardized estimate was an OR of 0.83 (95% CI 0.73 to 0.95). Excluding the 3% of subjects with bilirubin level greater than the upper-limit of the reference range (1.3 mg/dL) did not change this result (OR 0.93 [95% CI 0.88 to 0.99]). Additional adjustment for HDL cholesterol also did not change the association of bilirubin with PAD (not shown). Also, adjustment for total cholesterol:HDL cholesterol ratio in place of hypercholesterolemia did not change the results (OR 0.94 [95% CI 0.89 to 0.98]).

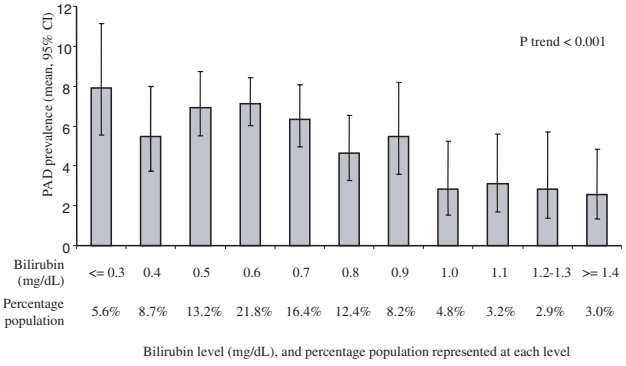

Figure. The prevalence of PAD by serum total bilirubin level, and the percentage of the population at each bilirubin level. To convert bilirubin values from mg/dL to μmol/L, multiply by 17.1.

Possible Influence of Liver Disease and Alcohol Intake

We repeated the multivariable analysis excluding subjects who reported a physician diagnosis of active liver disease (n remaining=6953). The multivariable-adjusted odds ratio for PAD associated with a 0.1 mg/dL increase in bilirubin was unchanged (0.93 [95% CI 0.89 to 0.98]). Further excluding subjects with laboratory evidence of possible liver disease (any of serum bilirubin >2.0 mg/dL, serum albumin ≤35

Table 2. Mean (95% Confidence Intervals) Bilirubin Level Described by Subject Characteristics

	Bilirubin (mg/dL), Mean (95% CI)
Age group, years	
40–49	0.71 (0.69–0.72)
50–59	0.70 (0.68–0.72)
60–69	0.70 (0.67–0.72)
70 or over	0.71 (0.69–0.73)
Gender	
Male	0.79 (0.77–0.81)
Female	0.63 (0.61–0.64)
Race/ethnicity	
Non-Hispanic White	0.72 (0.70–0.73)
Non-Hispanic Black	0.65 (0.62–0.68)
Mexican American	0.70 (0.67–0.73)
Other	0.65 (0.60–0.71)
Hypertension	
No	0.71 (0.69–0.72)
Yes	0.70 (0.69–0.72)
Diabetes mellitus	
No	0.71 (0.69–0.72)
Yes	0.68 (0.65–0.71)
Hypercholesterolemia	
No	0.71 (0.70–0.73)
Yes	0.70 (0.68–0.71)
Smoking status	
Never	0.71 (0.69–0.72)
Former	0.74 (0.72–0.76)
Active	0.64 (0.62–0.66)
Chronic kidney disease	
No	0.70 (0.69–0.72)
Yes	0.71 (0.67–0.74)
C-reactive protein, mg/L	
<1	0.79 (0.76–0.81)
1–3	0.72 (0.70–0.74)
>3	0.64 (0.63–0.65)
Homocysteine, μmol/L	
<10	0.69 (0.68–0.70)
10–15	0.74 (0.71–0.76)
>15	0.76 (0.71–0.81)

To convert bilirubin from mg/dL to μmol/L, multiply by 17.1.

gm/L [3.5 mg/dL], or aspartate aminotransferase or alanine aminotransferase >2× gender-specific upper-limit [n remaining=6679]), the association of bilirubin with PAD was unchanged (OR 0.92 [95% CI 0.87 to 0.98]).

Alcohol might influence the relationship between bilirubin and PAD, as moderate alcohol intake may confer protection from PAD,[23] and alcohol intake could plausibly increase serum bilirubin levels through adverse effects on liver function or induction of heme oxygenase (HO).[24] In subjects with alcohol intake data available (n=6924), the multivariable-adjusted odds ratio for PAD associated with a 0.1 mg/dL increase in bilirubin was 0.93 (95% CI 0.89 to 0.98), similar to the whole cohort. Alcohol intake was categorized as <1 drink per day, 1 to 4 drinks per day, and ≥5 drinks per day, representing 41.6%, 51.7%, and 6.8% of the subjects, respectively. Mean (95% CI) bilirubin levels were 0.68 (0.67 to 0.70), 0.75 (0.73 to 0.77), and 0.76 (0.70 to 0.82) mg/dL among subjects within the 3 categories of alcohol intake. PAD prevalence estimates in each alcohol intake category were 6.5% (95% CI 5.7 to 7.4), 4.1% (95% CI 3.2 to 5.2), and 4.8% (95% CI 3.2 to 7.1), respectively. The addition of alcohol intake to the multivariable model did not change the association of bilirubin with PAD (OR 0.93 [95% CI 0.89 to 0.98]).

Table 3. The Multivariable-Adjusted Association of Bilirubin Level With PAD, Stratified by Sex and Smoking Status

	n	Odds Ratio	95% CI
Men			
Nonsmokers	2583	0.93	0.88–0.99
Active smokers	1017	0.82	0.71–0.94
Women			
Nonsmokers	2862	1.01	0.94–1.07
Active smokers	613	0.82	0.68–0.98

Variation by Gender and Smoking

Mean bilirubin levels were lower in men with PAD (0.69 mg/dL [95% CI 0.66 to 0.72]) compared with men without PAD (0.79 mg/dL [95% CI 0.77 to 0.81]), while mean bilirubin levels were not different in women with PAD (0.61 mg/dL [95% CI 0.58 to 0.64]) compared with women without PAD (0.63 mg/dL [95% CI 0.61 to 0.64]). The inverse association of bilirubin with PAD tended to be stronger among men (OR 0.90 [95% CI 0.85 to 0.96]) than among women (OR 0.97 [95% CI 0.91 to 1.04]); this difference was on the cutoff of statistical significance ($P_{interaction}=0.05$). Additional adjustment for HDL cholesterol did not change this result, nor did additional adjustment for the use of hormonal therapy in women (not shown).

Mean bilirubin levels tended to be lower in nonsmokers with PAD (0.67 mg/dL [95% CI 0.64 to 0.70]) compared with nonsmokers without PAD (0.72 mg/dL [95% CI 0.70 to 0.73]). Mean bilirubin levels were lower in active smokers with PAD (0.58 mg/dL [95% CI 0.55 to 0.61]) compared with active smokers without PAD (0.67 mg/dL [95% CI 0.65 to 0.70]). The inverse association of bilirubin with PAD was stronger among active smokers (OR 0.81 [95% CI 0.73 to 0.90]) than among non-smokers (OR 0.97 [95% CI 0.93 to 1.01]) ($P_{interaction}=0.006$).

Given these results, we examined the multivariable-adjusted odds for PAD in analyses stratified by both gender and smoking status (Table 3). The inverse association of bilirubin with PAD was strongest among active smokers, regardless of gender. Among nonsmokers, bilirubin was associated with PAD in men only.

Discussion

In a large nationally representative cohort, we found an independent association between increasing concentration of

serum total bilirubin and decreasing prevalence of PAD. We did not find evidence that this association is dependent on bilirubin levels beyond the reference range, on the presence of liver disease, or on alcohol intake. These data, together with evidence from experimental atherosclerosis, are consistent with the hypothesis that bilirubin is an endogenous protectant mechanism against PAD.

Inflammation and oxidative stress are essential to the pathogenesis of atherosclerosis.[9–11] Bilirubin is a antioxidant under physiological conditions and suppresses inflammation in the vasculature.[1,7] Additionally, bilirubin functions as a cytoprotectant.[6] These properties appear to allow bilirubin to inhibit multiple steps in atherogenesis. Bilirubin inhibits inflammatory cytokine-induced endothelial cell expression of vascular cell adhesion molecule (VCAM)-1,[7] an initial step in atherosclerosis.[9] Bilirubin also inhibits monocyte transmigration,[25] prevents the formation of oxidized LDL,[26] inhibits endothelial inflammation and dysfunction,[8] inhibits vascular smooth muscle proliferation,[27] and prevents thrombus formation.[28] Human studies demonstrate that low bilirubin is associated with impaired endothelial function and increased carotid intima media thickness, 2 predictors of atherosclerosis, in healthy individuals free of cardiovascular risk factors.[29,30] These data provide a biological basis for the inverse association of bilirubin with PAD, a manifestation of systemic atherosclerosis.

It is important to consider bilirubin not in isolation but rather in the context of it being a product of heme oxygenase (HO), the rate-limiting enzyme in heme catabolism that generates biliverdin, the bilirubin precursor. HO is one of several enzymes that are induced by inflammation or oxidative stress and that limit vascular damage.[10] Multiple lines of evidence support an atheroprotective role for HO-1, the isoform of HO expressed in the blood vessel wall.[31,32] HO-1 is induced by multiple cardiovascular risk factors including smoking and hypercholesterolemia. Its induction decreases and its inhibition increases experimental atherosclerosis.[33,34] Several investigators have found that the vascular-protective effects of HO can be reproduced by the administration of bilirubin or biliverdin, suggesting that the production of bilirubin is a major component of the vascular protection offered by HO.[8,25,28]

Our result is similar to the inverse relationship reported between bilirubin level and CAD.[12,35] Two studies in this area are particularly noteworthy. The first is a report of the remarkably low prevalence of CAD in patients with Gilbert syndrome, a hereditary unconjugated hyperbilirubinemia secondary to congenital deficiency of uridine diphosphate-glucuronosyltransferase-1 (UGT1).[36,37] The second is an analysis of the prospective relationship between genetic variation in UGT1 and risk for cardiovascular disease (CVD).[38] The variation in UGT1 associated with higher bilirubin levels was associated with one-third the risk of CVD. After adjustment for bilirubin level, the association between UGT1 variation and CVD risk all but disappeared.

The association of bilirubin level with PAD tended to be stronger in men than in women. Some but not all studies have found that the relationship between bilirubin and CAD varies by gender.[20,21] The biology underlying a possible gender-based difference in the association of bilirubin with PAD is not clear and requires investigation. We did not find that differences in HDL cholesterol accounted for the differential association of bilirubin with PAD in men and women, a possibility suggested by Hunt and colleagues.[21] To the extent that bilirubin level is related to oxidative stress,[39,40] our result is similar to finding that F_2 isoprostane level is more weakly associated with coronary calcification in women than in men.[41]

We found the inverse association of bilirubin level with PAD among active smokers to be striking. Smoking is known to be associated with lower bilirubin levels.[39] We speculate that the potential protection offered by an endogenous antioxidant such as bilirubin might be greatest among individuals exposed to a source of oxidant stress such as cigarette smoke.

Strengths of the present study include the national representative nature of the NHANES cohort, that the ABI method has excellent performance characteristics for the diagnosis of PAD,[13] and that the comprehensive nature of the NHANES allowed adjustment for important PAD risk factors. Despite these strengths, several limitations warrant consideration. The ABI was determined from the blood pressure in a single arm and subjects ≥60 years of age had the blood pressure measured only once, potential sources of misclassification: a cohort within which the blood pressure was measured more than once in each limb could provide a more precise estimate of the association of bilirubin with PAD. Plasma bilirubin levels exhibit significant within subject variability[42]; the NHANES performs only a single blood draw, perhaps further limiting the precision of our bilirubin effect estimate. Also, the antioxidant and antiinflammatory properties of bilirubin may vary depending whether it is conjugated or unconjugated,[4] and the NHANES only measured total and not fractionated bilirubin level. Although this cross-sectional study demonstrates an association of increased serum bilirubin with reduced prevalence of PAD, prospective studies are needed to determine the temporal nature of the association, and causation cannot be established. Reverse causation is a possible explanation for our findings, although prospective studies have demonstrated an inverse association of bilirubin level with cardiovascular risk.[38] The finding that the association of bilirubin level with risk for ischemic heart disease may in fact be U-shaped highlights the need for prospective studies of bilirubin level and risk for PAD.[43,44]

In summary, increased serum total bilirubin level is associated with a reduced likelihood of PAD. This association is not dependent on elevated bilirubin levels, liver disease, or alcohol intake, is stronger in men compared with women, and is stronger in smokers compared with nonsmokers. Further work, including prospective epidemiological studies, is needed to better illuminate the role of bilirubin in determining susceptibility to PAD. Bilirubin, with strong antiinflammatory and antioxidant properties, may be an important endogenous protectant from PAD.

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Disclosures

None.

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