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Review

Gilbert syndrome, UGT1A1*28 allele, and cardiovascular disease risk: Possible protective effects and therapeutic applications of bilirubin

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Abstract

Serum bilirubin has been shown to be inversely related to cardiovascular disease (CVD) in both retrospective and prospective studies. Meta-analysis of existing studies has also confirmed that serum bilirubin concentrations are inversely related to CVD. Less information is known about the protective effects of slightly elevated serum bilirubin concentrations. In this review, we will focus primarily on the association of serum bilirubin and CVD and the possible protective roles of bilirubin, heme oxygenase (HO), and bilirubin UDP-glucuronosyltransferase (UGT1A1). HO and biliverdin reductase control the formation of bilirubin, whereas UGT1A1 controls bilirubin conjugation and clearance. Because of the health and therapeutic implications of slightly elevated serum bilirubin concentrations, we will discuss the recent prospective studies on cardiovascular risk in individuals with Gilbert syndrome (GS) as well as those with the UGT1A1*28 allele. Such individuals have decreased hepatic bilirubin UDP-glucuronosyltransferase activity, decreased bilirubin clearance, and increased serum bilirubin concentrations. Lastly, we will discuss some of the therapeutic approaches that could be used to increase serum bilirubin concentrations to prevent CVD and other oxidative and inflammatory diseases.
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Keywords: Serum bilirubin; Heme oxygenase; Gilbert syndrome; UGT1A1*28 allele; Cardiovascular disease risk; Coronary heart disease; Oxidative stress

Contents

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1. Heme-bilirubin-carbon monoxide metabolic pathway

In recent years, a considerable amount of attention has been focused on the role of heme oxygenase-1 (HO-1) and the heme-bilirubin-carbon monoxide pathway in the development of atherosclerosis [1–9]. As shown in Fig. 1, heme oxygenase (HO) removes heme which has pro-oxidative and pro-atherosclerotic properties, and it produces biliverdin and carbon monoxide (CO) which appear to have anti-atherosclerotic properties [1–9]. HO therefore plays an important role not only in heme metabolism but also in the production of biliverdin, bilirubin, and carbon monoxide.

Bilirubin concentrations in serum are controlled by three keys enzymes: HO, biliverdin reductase, and bilirubin uridine diphosphate-glucuronosyltransferase (UGT1A1). HO and bilirubin reductase govern the formation of bilirubin whereas UGT1A1 regulates the clearance of bilirubin by the liver and bile. In addition, there is some evidence to suggest that bilirubin reductase has the ability to regenerate bilirubin from biliverdin by the biliverdin reductase antioxidant cycle [10].

Most of the emphasis of previous review articles has been on the possible protective role of HO-1 in *in vitro* studies and in animal models [1–9]. In this review, we will focus primarily on human studies and on the association of serum bilirubin and CVD. We also will discuss the possible protective role of slightly elevated serum bilirubin concentrations such as found in individuals with GS and the UGT1A1*28 allele. In addition, we will discuss the possible role that bilirubin, the HO pathway, and the UGT1A1 genes could have on the prevention of CVD as well as other oxidative and inflammatory diseases and vascular complications.

2. Protective properties of bilirubin

There has been a considerable amount of research on the factors that cause CVD; however, much less research has been performed on substances that might prevent CVD and other oxidative and inflammatory related diseases. One substance that has been shown to be protective is high density lipoprotein cholesterol (HDL-cholesterol). Bilirubin may be another protective factor in that it has both antioxidant [11,12] and anti-inflammatory properties [13,14]. The antioxidant and anti-atherogenic properties of bilirubin are thought to result from its ability to inhibit the oxidation of LDL and other lipids [15,16], scavenge oxygen radicals [11], and counteract oxidative stress [17,18]. The anti-atherosclerotic properties of biliverdin and bilirubin have been found in several in vitro studies [11,15,16] as well as in animal studies [19–21]. Bilirubin also has been shown to be directly related to the serum total antioxidant capacity in humans [18,22] and to be a more effective protector of human ventricular monocytes than either vitamin C or vitamin E [16]. In addition, bilirubin appears to have anti-proliferative properties [19,20,23].

3. Protective effects of HO-1

Several studies have shown that HO induction and up-regulation decreases experimental atherosclerosis in the Watanabe heritable hyperlipidemic rabbits and in LDL-receptor knockout mice whereas HO-1 inhibition increases atherosclerosis [19,20]. HO-1 induction and up-regulation have also been shown to decrease intimal thickening in experimental restenosis [23] and to protect against experimental ischemia and reperfusion injury [24]. HO-1 inhibition and down-regulation, on the other hand, has been shown to increase experimental atherosclerosis [19,20] and to increase intimal thickening [23]. The proposed mechanisms by which HO-1 exerts its cytoprotective effects are not known, however, bilirubin and biliverdin treatment have been shown to prevent restenosis in rats with balloon-induced injury [21]. The protective effects of HO-1 might also result from the formation of carbon monoxide (CO) which has vasodilatory and antiproliferative properties [23,24], and from the degradation of heme which is capable of oxidizing low density lipoproteins [25]. Because of their low antioxidant content, the small dense low density lipoprotein fractions are known to be particularly susceptible to oxidation [26,27].

Recent studies on the pharmacologic up-regulation of HO-1 with cobalt protoporhyrin IX in mice with non-autoimmune arthritis also have shown that up-regulation of HO-1 produces both protective anti-oxidative and anti-inflammatory responses in this mouse model [28]. Likewise, inhibition of HO-1 by in vivo injection of anti-HO-1 siRNA was found to be effective in suppressing the protective effects of HO-1 [28]. It remains to be established if the protective effects of HO-1 are due to the production of biliverdin, bilirubin, and CO, the removal of heme, or to a combination of these or other factors. Serum bilirubin concentrations were not analyzed in most of the studies involving HO-1 up-regulation or down-regulation. Such information is needed in order to determine if the protective effects are due to bilirubin or some other factors. Likewise, CO or some surrogate of CO like carbonyl-hemoglobin could provide more detailed information on the causative factors involved in atherosclerosis. Additional clinical and experimental studies which support a protective role of HO-1 in atherosclerosis and related diseases have been described in more detail in the recent reviews of Vítek and Schwertner, Stocker and Perella, and Morita [1–3].

4. Serum bilirubin and CVD risk

A possible role of bilirubin and HO in atherosclerotic vascular disease was first suggested in 1994 in studies showing an inverse relationship of serum total bilirubin concentrations and risk of coronary artery disease (CAD) [29]. In those studies, low serum total bilirubin concentrations were found to be related to an increased risk of coronary artery disease (CAD) whereas higher serum bilirubin concentrations were related to a decreased risk of CAD. The strength of the association

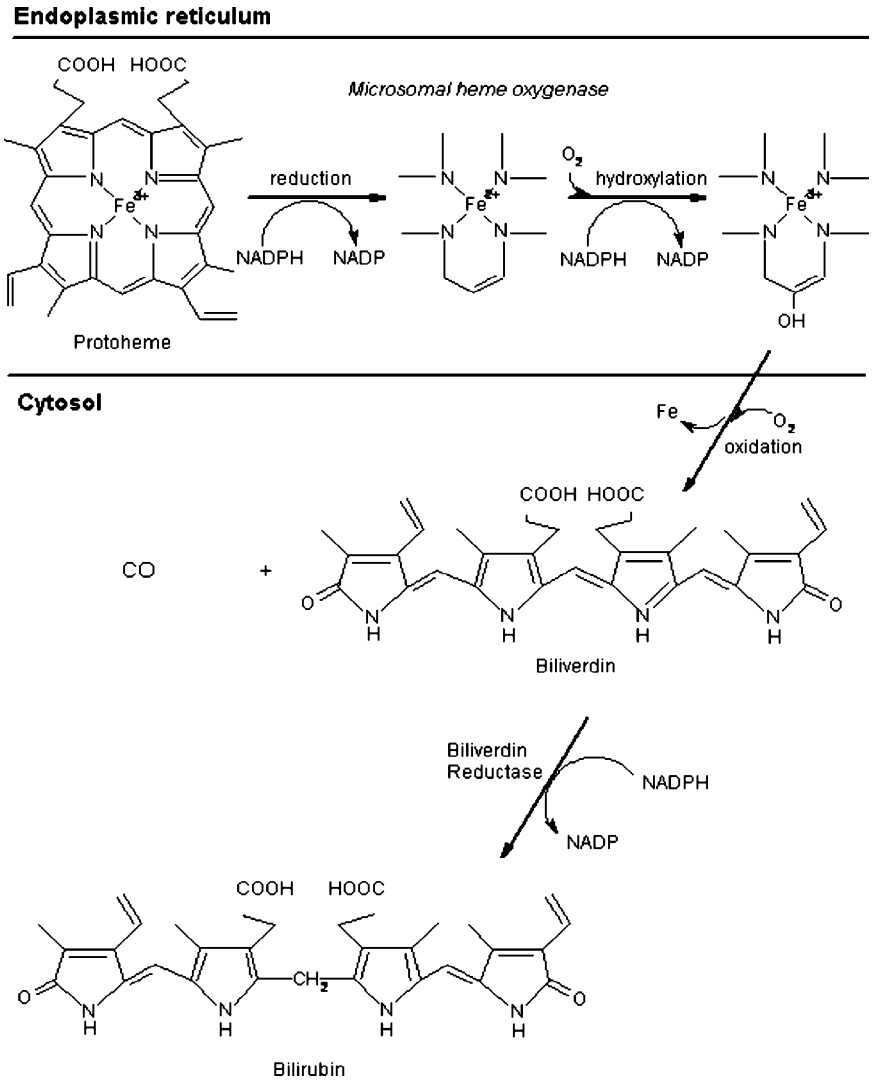

Fig. 1. Heme-bilirubin catabolic pathway.

between serum bilirubin and CAD was found to be similar to that of smoking, elevated systolic blood pressure, and HDL-cholesterol [17,29,30]. The results also indicated that a 50% decrease in serum bilirubin was associated with a 47% increase in the odds of having a more severe disease [29]. Hopkins et al. [30] confirmed that serum bilirubin concentrations were inversely related to the severity of CAD in both men and women. They also found that individuals with serum bilirubin concentrations in the top quintile had an 80% reduction in CAD risk compared to those in the lowest quintile. Similar inverse associations have now been shown between serum bilirubin concentrations and CVD, peripheral vascular disease (PVD), carotid intimal-medial thickening, and stroke ([29–47], Table 1).

The inverse association between serum bilirubin concentrations and CVD has been found in seven prospective studies ([48–53], Table 1). Such findings are important in that they suggest that bilirubin or some other factor or factors associated with bilirubin prevents future CVD. In addition, such studies provide more direct support that bilirubin may have a causal role in CVD prevention. Breimer et al. performed a prospective study on 7685 middle-aged British men and found a U-shaped relationship [48]. The cause of the U-shaped relations is not known, however, it could be due to underlying liver disease since the investigators did not properly adjust for possible liver disease. In recent prospective studies, Vítek et al. found a significantly lower 3-year incidence of coronary heart disease (CHD) in individuals with GS than that predicted based on multifactorial analysis of standard risk factors for CHD [18]. Similar inverse relationships between serum bilirubin and early and late onset CHD were found by Hunt et al. [49].

An inverse relationship between serum bilirubin and risk of myocardial infarction, coronary disease death, and PVD risk was recently found in a Framingham Offspring Study involving 4276 men and women [50]. In this study, Djousse et al. evaluated the association between serum bilirubin and myocardial infarction (MI), coronary death, and any cardio-

Table 1
Bilirubin and atherosclerosis, human studies

Study characteristics

Type	Population	Outcome	Reference
Bilirubin and atherosclerosis Retrospective studies	Prevalence study on men with angiographically proven CHD ($n = 877$)	50% decrease in serum bilirubin associated with increased odds for CHD by 47%	[29]
	Prevalence study of PVD ($n = 31$) and serum bilirubin	Cases had significantly lower serum bilirubin levels as compared to population mean	[31]
	Case-control study patients with early CHD ($n = 161$) compared to controls ($n = 155$)	75% reduction in CHD risk associated with increase of 17 μM in serum bilirubin level	[30]
	Prevalence study in men who underwent coronary angiography ($n = 171$)	Inverse relationship between serum bilirubin and coronary artery stenoses	[32]
	Case-control study on the association between serum bilirubin levels and both CHD and PVD ($n = 111$)	Lowest serum bilirubin levels detected in PVD as compared to CHD and controls (6.0 vs. 8.0 vs. 12.0 μM, respectively)	[33]
	Prevalence study in men and women with early CHD ($n = 328$)	7% increase of CHD prevalence for each 1 μM decrease in serum bilirubin	[34]
	Prevalence analysis of the relationship between serum bilirubin levels and carotid intimo-medial thickness (IMT) ($n = 1741$, 330 cases)	Significantly lower serum bilirubin levels in subjects with carotid plaques	[35]
	Prevalence study of serum bilirubin levels and both CHD and PVD in men and women ($n = 456$)	Inverse relationship between serum bilirubin levels and PVD, the same, but non-significant trend demonstrated also for CHD	[36]
	Prevalence study comparing prevalence of CHD in hyperbilirubinemic Gilbert syndrome subjects ($n = 50$) and aged-matched general population	Significantly lower prevalence of CHD in GS subjects (2% vs. 12.1%, $p < 0.05$)	[18]
	Analysis of serum bilirubin levels between male CHD patients ($n = 161$) and healthy controls ($n = 322$)	Inverse relationship between serum bilirubin levels and CHD risk in males, however, this relationship not observed in females	[37]
	Analysis of serum bilirubin levels between CHD ($n = 544$) and non-CHD patients ($n = 359$)	As in previous study, inverse relationship between serum bilirubin levels and CHD demonstrated only in males	[38]
	Analysis of association between echocardiographic signs of coronary atherosclerosis and serum bilirubin levels in healthy subjects ($n = 160$)	Inverse relationship between serum bilirubin levels and coronary flow reserve impairment and coronary microvascular dysfunction	[39]
	Analysis of relationship between carotid IMT and serum bilirubin levels in healthy men ($n = 111$)	Inverse relationship between carotid IMT and serum bilirubin levels	[40]
	Prevalence study in both men and women with angiographically proven CHD ($n = 312$), compared to healthy controls ($n = 50$)	Significantly lower serum bilirubin levels in cases sompared to controls (9.4 ± 6.7 vs. 13.9 ± 5.5, $p < 0.01$)	[41]
	Analysis of endothelial dysfunction and carotid IMT in healthy subjects ($n = 91$)	Inverse relationship between serum bilirubin levels and both endothelial dysfunction and carotid IMT	[42]
	Analysis of serum bilirubin levels between CHD patients ($n = 262$) and healthy controls ($n = 50$)	Substantially lower serum bilirubin levels in CHD patients	[43]
	Analysis of PVD in US adults ($n = 7{,}074$)	1 mg/dL increase in serum bilirubin associated with 48% reduced risk of PAD (OR 0.52; 0.33–0.83). In categorized analysis, subjects with serum bilirubin >1 mg/dL (consistent with GS diagnosis) had reduced risk of PAD by 51% (OR 0.49; 0.32–0.76).	[44]
	Analysis of stroke history in US adults ($n = 13{,}223$)	1 mg/dL increase in serum bilirubin associated with 63% reduced risk of stroke (OR 0.37; 0.21–0.63). Adjustment for liver disease further strenghtened this relationship (OR 0.30; 0.18–0.49). In categorized analysis, subjects with serum bilirubin >1 mg/dL (consistent with GS diagnosis) had reduced risk of stroke by 48%.	[45]

	Prevalence study of vascular complications in diabetics ($n = 96$) with and without GS ($n = 426$)	Lower prevalence of CHD, cerebrovascular diseases, arterial hypertension and diabetic retinopathy in diabetic patients with GS	[46]
	Retrospective study on subjects with dyslipidemia without overt vascular disease ($n = 376$)	Inverse relationship between serum bilirubin levels and CHD risk	[47]
Bilirubin and atherosclerosis Prospective studies	Prospective study on middle-aged British men ($n = 7,685$)	U-shaped relationship between serum bilirubin levels and CHD, however, proper adjustment for underlying liver disease was not performed	[48]
	Prospective study of men and women with familial CHD ($n = 1,240$)	Significant inverse relationship between serum bilirubin levels and both early- and late-onset CHD	[49]
	Framingham Offspring Study (both men and women) on the relationship between serum bilirubin and risk of CHD ($n = 4,276$)	Inverse relationship between serum bilirubin levels and risk of myocardial infarction, coronary disease death and cardiovascular disease risk	[50]
	10 year follow-up on cardivascular mortality in relation to serum bilirubin levels in men ($n = 5,460$) and women ($n = 4,843$)	Lower, but non-significant cardiovascular mortality associated with higher serum bilirubin levels	[51]
	Analysis of 3-year incidence of CHD in GS subjects ($n = 50$)	Significantly lower 3-year incidence of CHD in hyperbilirubinemic GS subjects	[18]
	Framingham Offspring Study as described above, focus on interrelation between serum bilirubin and albumin levels to risk of CHD	Highest CHD risk demonstrated in low bilirubin/low albumin group, risk the most pronounced in males	[52]
	Comparison of patients who developed CHD at 5-year follow-up ($n = 216$) to matched controls ($n = 434$)	Significantly lower serum bilirubin in cases as compared to controls, hyperbilirubinemic subjects which lacked protection were not adjusted for underlying liver disease	[53]
Bilirubin and atherosclerosis Meta-analysis	Meta-analysis of all studies published up to 2002 on the relationship between serum bilirubin levels and atherosclerosis	Inverse relationship between serum bilirubin and both CHD and PVD, 10 μM serum bilirubin cut-off level discriminating cardiovascular risk. Confounding effect of underlying liver disease demonstrated.	[54] (in this paper following studies were meta-analyzed: [18,29–32,35,43–45,48,75[a]]
UGT1A1*28 and atherosclerosis	Retrospective, case-control study on association between UGT1A1*28 allele and risk for myocardial infarction in men and women (185 cases, 255 controls)	Association between UGT1A1*28 allele homozygocity and risk for myocardial infarction detected. However, serum bilirubin levels in UGT1A1*28 homozygotes were substantially lower than expected, probably due to high proportion of normobilirubinemic homozygotes.	[56]
	Retrospective, case-control study on association between UGT1A1*28 allele and risk for myocardial infarction in men ($n = 680$)	Association between UGT1A1*28 allele homozygocity and risk of myocardial infarction detected. However, serum bilirubin levels and other confounding factors were not determined.	[59]

Table 1(*Continued*).

Study characteristics			
Type	Population	Outcome	Reference
	Study on the association of UGT1A1*28 allele, serum bilirubin and risk for CHD and cardivascular diseases performed in Framingham Offspring cohort ($n = 1{,}780$) followed up for 24 years	UGT1A1*28 allele homozygocity associated with 1/3 the risk for both CHD and cardiovascular diseases as compared to wild type genotype	[57]
Bilirubin and risk factors for atherosclerosis	Retrospective study on association of cardiovascular risk factors and serum bilirubin levels in Hong Kong Chinese ($n = 1{,}508$)	Inverse relationship between serum bilirubin levels and glycated hemoglobin, triglycerides and VLDL cholesterol	[60]
	Cross-sectional study in children and young adults focused on assesment of cardiovascular risk factors and serum bilirubin levels ($n = 4{,}156$)	Inverse relationship between serum bilirubin levels and adiposity, triglycerides, LDL and VLDL cholesterol, insulin, glucose, systolic blood pressure and parental histoty of heart attack or hypertension	[61]
	Retrospective study on relationship between serum bilirubin levels and arterial hypertension in dyslipidemic men and women ($n = 491$)	Significantly lower serum bilirubin levels detected in untreated hypertensive patients as compared to normotensive subjects	[62]
	Study on the association of serum bilirubin levels and advanced glycation-end products (AGEs) in GS ($n = 23$) and control subjects ($n = 21$)	Inverse relationship between serum levels of bilirubin and both pentosidine and carboxy-methyllysine	[63]
	Study on aortic function in healthy hypobilirubinemic ($n = 42$) subjects and healthy subjects with hyperbilirubinemia ($n = 40$)	Impairment of aortic elastic properties in subjects with lower serum bilirubin levels	[64]
	Study comparing the effect of serum bilirubin levels on total antixidant capacity in GS ($n = 50$), CHD ($n = 38$) and control subjects ($n = 38$)	Positive relationship between serum bilirubin levels and total serum antioxidant capacity	[18]
	Analysis of total antioxidant capacity according to serum bilirubin levels in healthy men and women ($n = 224$)	Positive relationship between serum bilirubin levels and total serum antioxidant capacity	[22][a]
	Study comparing the effect of serum bilirubin levels on urinary excretion of biopyrrins in GS ($n = 33$) and control subjects ($n = 25$)	Inverse relationship between serum bilirubin levels and urinary excretion of biopyrrins, a known predictor of CHD	[65]
	Analysis of hsCRP levels according to serum bilirubin levels in healthy men and women ($n = 288$)	Subjects in the lowest bilirubin quartile had the highest hsCRP levels as compared to higher bilirubin quartiles	[22][a]
	Study on diabetic patients with ($n = 96$) and without GS ($n = 426$)	Lower serum levels of HbA_{1c}, LDL and total cholesterol, triglycerides, hsCRP, lower urinary excretion of 8-hydroxy-2′-deoxyguanosine (a marker of oxidative stress), and higher levels of HDL cholesterol in diabetics with GS	[46]

[a] Abstract.

vascular event (any clinical form of CHD, congestive heart failure, intermittent claudication, or stroke). A higher total serum bilirubin concentration was found to be associated with a lower risk of MI, CHD, and any CVD event in men; however, the bilirubin-CHD relationships were only suggestive in women. The authors concluded that the limited number of MI and CHD cases and the relatively young age of the women at baseline (39.5 years) might have accounted for the differences in men and women. In separate studies, Temme et al. [51] found lower, but non-significant, cardiovascular mortality to be associated with a higher serum bilirubin concentration and Djousse et al. [52] found an inverse relationship between serum bilirubin and albumin and MI. In other studies, lower serum bilirubin concentrations were found in individuals with CHD compared to controls, however, the hyperbilirubinemia did not protect against CHD possibly due to the lack of adjustment for underlying liver disease [53].

5. Meta-analysis of studies on serum bilirubin and CVD risk

A meta-analysis study of eleven studies has shown that serum bilirubin concentrations are inversely related to the severity of atherosclerosis in men ($r = -0.31$, $p < 0.0001$) ([54], Table 1). Women were not included in the study because of the limited number of studies involving women. Non-parametric, regression, and stratified analyses all showed an inverse and dose-dependent relationship between serum bilirubin concentrations and different types and severities of CVD. Based on these studies, each 1.0 μmol/L increase in serum bilirubin was found to be associated with a 6.5% decrease in CVD [54]. The studies also found that a serum bilirubin concentration of 10.0 μmol/L was the cut-point for discrimination of cardiovascular risk.

6. Gilbert syndrome and CVD risk

While it is clear that low serum bilirubin concentrations are associated with an increased risk of CVD, less information is available on the role of moderately elevated serum bilirubin concentrations such as found in individuals with GS. Such information is important because approximately 5–10% of the population worldwide has GS [55]. Such elevations in serum bilirubin would have significant implications for CVD prevention in this rather large population. Secondly, if moderately elevated levels of serum bilirubin are found to reduce the risk of CVD, then bilirubin synthesis, clearance, and transport mechanisms might serve as potential therapeutic target sites for new drug development initiatives.

Most studies of serum bilirubin and atherosclerosis have been performed on individuals with normal serum bilirubin concentrations (≤1.0 mg/dL, i.e. ≤17.1 μmol/L). Several studies, however, have included individuals with slightly elevated bilirubin concentrations. For example, we found a protective effect for angiographically documented CAD in individuals with slightly elevated serum bilirubin concentrations [29]. Similarly, Ishizaka et al. found protective effects between slightly elevated serum bilirubin and carotid atherosclerosis [35].

The first study on CHD risk in individuals with GS was performed by Vítek et al. [18]. As mentioned above, individuals with GS were found to have mild elevations in fasting serum unconjugated bilirubin concentrations and a marked reduction in CHD risk. Individuals for the study were selected on the basis of chronic unconjugated hyperbilirubinemia in the absence of any hemolytic or altered hepatic function. Approximately, 2% of the individuals with GS were found to have CHD compared to 12.1% in the general population and the significantly low incidence rate was found also in a 3-year follow-up of these subjects. The individuals with GS also had higher serum bilirubin and HDL-cholesterol concentrations and higher total antioxidant capacities than did the controls. In addition, elevated bilirubin levels were found to be equal to HDL cholesterol in protecting individuals from CHD. The results provide important new evidence that mildly elevated levels of bilirubin (33 ± 14 μmol/L) are associated with a lower prevalence of CHD and that mildly elevated levels of bilirubin appear to protect against the development of future heart disease.

7. UGT1A1*28 allele and CVD risk

Since individuals with GS were found to have a lower risk of CHD, studies were performed to determine if individuals with the homozygous form of the UGT1A1*28 allele (genotype $(TA)_7/(TA)_7$) might also have a lower risk of CVD. This polymorphism occurs in 10–16% of the population and is known to have a significant role in regulating bilirubin glucuronidation, bilirubin clearance, and serum bilirubin concentrations [55]. Bosma et al. [56] examined the risk of MI in individuals with the UGT1A1*28 allele, however, they did not find a protective effect in individuals homozygous for the UGT1A1*28 allele even though such individuals had significantly elevated serum bilirubin concentrations [55]. While there might not be an association between UGT1A1*28 and this specific endpoint (MI), the association might have been missed because of the relatively advanced population age at entry into the study, the lack of adjustment for liver disease, the use of non-standardized blood collection procedures, lack of sub-stratification of individual genotypes according to serum bilirubin levels, survival bias, low number of cases and controls, or population substructure influences [56–58]. In their study, low serum bilirubin concentrations also were not found to be associated with an increased risk of MI and no information was presented on the concentrations of other risk factors such as cholesterol, LDL-cholesterol, HDL-cholesterol, or blood pressure or on their association with risk of MI. The same criticism might also apply to a recent Spanish study where no correlation between genotype and phenotype was found [59].

The most comprehensive study to date has been the recent study of Lin et al. [57]. In that study, the UGT1A1*28 allele status, serum bilirubin concentrations, and CVD was followed for 24 years in 1,780 individuals participating in the Framingham Offspring Study [57]. Individuals with liver disease were excluded from the study. The CVD category consisted of men and women with CHD, stroke, transient ischemic attack, intermittent claudication, congestive heart failure, or CHD death and the CHD category consisted of fatal and nonfatal MI, angina pectoris, or coronary insufficiency. Individuals with the genotype $(TA)_7/(TA)_7$ had approximately one third the risk of CVD and CHD as the carriers of the wild type allele and were found to have significantly higher serum bilirubin concentrations (mean ± S.D. 1.14 ± 0.44 mg/dL) than those with genotypes $(TA)_6/(TA)_6$ and $(TA)_6/(TA)_7$ (mean ± S.D. 0.69 ± 0.27 mg/dL, $p < 0.01$). The association of the *UGT1A1* polymorphism with MI, however, was of borderline significance. Bilirubin was found to be highly protective in that each 0.1 mg/dL increase in serum bilirubin was found to be decrease CVD, CHD, and MI risks by 10%, 13%, and 13%, respectively, when the genotype was not included in the Cox regression model. When the *UGT1A1* polymorphism and bilirubin levels were both included only the bilirubin effect remained in the model. This means that serum bilirubin is probably more closely associated with CVD than the $(TA)_7/(TA)_7$ genotype most likely due to its incomplete penetrance.

The study is important in that it is the first well-designed study to show that individuals with the genotype $(TA)_7/(TA)_7$ have both a higher serum bilirubin concentration and a lower risk of developing future CVD and CHD than individuals with the wild ($(TA)_6/(TA)_6$) and heterozygous genotypes ($(TA)_6/(TA)_7$). The study is also important because the protective effects of bilirubin were found in both men and women. In addition, approximately 11% of the study population had the genotype $(TA)_7/(TA)_7$, 43% had the genotype $(TA)_6/(TA)_7$, and 46% had the genotype $(TA)_6/(TA)_6$. In the study, smoking status did not have an effect on the association between the polymorphism and any of the CVD events. It would be very interesting to see the results for each genotype after sub-clustering the subjects with serum bilirubin concentrations above 1.0 mg/mL. If mild hyperbilirubinemia is a protective factor, this analysis should reveal a further decrease of CVD risk in this subset of "true" GS individuals.

8. Protective role of HO, UGT1A1*28 allele, and bilirubin in other diseases and medical conditions

Even though CVD is a very complicated disease involving many risk factors, HO-1, UGT1A1*28, and bilirubin appear to have a protective effect in the prevention of CVD. HO-1, UGT1A1*28 allele, and bilirubin might also confer protection in other oxidative stress and inflammatory-related diseases such as in certain cancers, rheumatological and neuropsychiatric diseases, diabetes, as well as in other medical conditions. Most of the studies to date have been on the association of HO-1, UGT1A1*28, and bilirubin in chronic diseases such as CVD. Less is known about their protective effects in acute medical conditions such as sepsis and oxidative stress after surgery. There is some evidence in animal models that bilirubin and biliverdin prevent vascular complications associated with coronary artery bypass surgery and percutaneous transluminal angioplasty such as vein graft surgery failure and restenosis [22]. Further studies also need to examine the association between the HO-1, UGT1A1 polymorphism, serum bilirubin concentrations, and CVD mortality and all-cause mortality.

9. Bilirubin and other risk factors for CVD disease

Serum bilirubin has been shown to be independently related to many of the existing risk factors as well as to other compounds ([22,46,60–65], Table 1). Serum bilirubin has been shown to be directly related to HDL-cholesterol [17,29,30] and total serum antioxidant capacities [18,22] concentrations and to be inversely related to LDL- and VLDL-cholesterol, triglycerides, hsCRP, systolic blood pressure, parental history of heart attack or hypertension, insulin, and glucose [61]. In addition low serum bilirubin has been shown to be related to impairment of aortic elastic properties [64], increased urinary excretion of biopyrrins, a known predictor of CHD [65], pentosidine and carboxy-methylysine belonging to the group of advanced glycation end products (AGE's) [63], and glycated hemoglobin [60].

10. Serum bilirubin as a predictor of CVD

Serum bilirubin and UGT1A1*28 analyses may improve our ability to diagnose individuals at risk for CVD and possibly other diseases. Since serum bilirubin concentrations appear to be related to those of HDL-C and both bilirubin and HDL-cholesterol are decreased with CAD, various cholesterol, LDL-cholesterol, HDL-cholesterol and bilirubin ratios were tested as possible predictors of CAD. Stepwise logistic regression analysis performed in a retrospective study of 644 middle aged males who had undergone coronary angiography indicated that serum bilirubin was more closely associated with CAD than HDL-cholesterol and of the laboratory risk factors was second only to total cholesterol and LDL-cholesterol as a risk predictor [66]. Further evaluations of risk factor combinations indicated that the cholesterol/(HDL-C + bilirubin) and LDL-cholesterol/(HDL-C + bilirubin) ratios were more accurate in identifying severe CAD than either cholesterol/HDL-C or LDL-C/HDL-C ratios alone.

Studies were also performed to determine if information on serum bilirubin improves the predictive ability of the Framingham risk factors (cholesterol, HDL-cholesterol, systolic blood pressure, smoking, and age). The results indicated

that the addition of serum bilirubin improved the prediction of CAD over that achieved with the Framingham formula alone [66]. If confirmed in other studies, algorithms of serum bilirubin and other risk factor combinations may improve our ability to predict existing and future CAD. It should be mentioned that studies comparing the diagnostic sensitivity and specificity of serum bilirubin have not been determined in women. In addition, studies using the Framingham Offspring study group have shown that the relationship of serum bilirubin and CHD is only suggestive in women [50]. Lin et al. [57], however, found elevated serum bilirubin levels to be protective in both men and women.

These data are perfectly in line with very recent study by Japanese authors showing that both male and female diabetic patients with GS have much lower prevalence of vascular complications as well as reduced markers of oxidative stress and inflammation [46].

11. Therapeutic approaches for HO-1 induction/up-regulation or UGT1A1 inhibition/down-regulation

The low prevalence of CVD in individuals with GS [18] and in individuals with the UGT1A1*28 [57] suggests a possible protective role for HO-1, bilirubin, and CO. A rather large number of drugs are known to induce HO-1 and they include heme, metalloporphyrins, inflammatory cytokines, prostaglandins, ultraviolet light, heat shock, bacterial lipopolysaccharides, phorbol esters, and thiol scavengers [2,6]. In addition, aspirin [67] and statins [68,69], which are widely prescribed for CVD prevention and treatment are known to induce HO-1. Studies, however, have not been performed to determine if the beneficial effects of aspirin and statins are due to their effects on HO-1 leading to increased production of CO and increased serum bilirubin concentrations, or due to their effects on inflammation. Similar HO-1 inducing effects have been described for probucol, rapamycin, ethanol, nitric oxide, CO, dopamine, diclofenac, COX-2 inhibitors, PPAR γ activators, resveratrol, curcumin, and polyphenolic chalcones (rosolic acid) [1–9].

Recent experimental studies of rat models have shown that bilirubin and biliverdin are effective in preventing restenosis in balloon-induced injury [21]. Other interesting studies have shown that protoporphyrin IX induction of HO-1 resulted in decreases in IL-1β, IL-6, and TNFα levels, joint swelling, cartilage degradation, and proliferation of inflammatory tissue in joints of mice with non-immune arthritis [28]. A recent study performed by Brydun et al. [70] also indicated that patients with coronary atherosclerosis have a reduced expression of HO-1. If confirmed in other studies, this finding provides critical support for the role of HO-1 in coronary atherosclerosis.

Substances which inhibit bilirubin UDP-glucuronosyl transferase or which prevent bilirubin oxidation might be effective in increasing serum bilirubin concentrations [71]. Inhibition of UDP-glucuronosyl transferase activity, however, would need to be evaluated for safety effects since such inhibition may adversely impact the conjugation and clearance of drugs, toxins, and carcinogens. Several studies have reported on the effectiveness of gene therapy with HO-1 in inhibiting inflammation and oxidative stress in animals [7]. Plant derived phycobilins might be also be effective and might be a readily available source of compounds with properties similar to bilirubin [71].

Even though the above-mentioned drugs might be able to increase serum bilirubin concentrations, hepatoxicity needs to be ruled out as a possible cause for the increases in serum bilirubin concentrations. A number of studies have shown that abnormal liver function tests are associated with an increased risk of CVD [1]. Abnormal liver function tests are also known predictors of atherosclerotic diseases [72–74]. As a result, liver function tests should always be included in the atherosclerosis risk assessment of an individual and when evaluating new drugs for their ability to increase serum bilirubin concentrations.

12. Summary

The recent retrospective and prospective studies indicate that high normal and moderately elevated serum bilirubin concentrations protect against CVD. Prospective studies also show that individuals with GS and the UGT1A1*28 allele who have moderate elevations in serum bilirubin concentrations have a lower risk of CHD and CVD. A protective role of elevated bilirubin levels is less clear for women. It remains to be seen if bilirubin is responsible for the protection or if it is acting in concert with other protective factors such as HO-1 and CO. Further prospective studies need to be performed to determine if elevated serum bilirubin concentrations are associated with decreased CVD mortality. There is, however, some evidence that individuals with moderately elevated serum bilirubin levels have a lower CVD mortality than those with low serum bilirubin concentrations [57,75].

Most of the human studies have involved the association of serum bilirubin and risk of CVD. Human studies on the association between bilirubin and other diseases have been limited only on certain forms of cancer and to certain rheumatological and neuropsychiatric diseases (for review see Ref. [1]). In addition, most of the human studies have examined the role of bilirubin in chronic diseases [1]. Very few studies have been performed to determine if bilirubin or HO-1 induction might alleviate some of the acute complications associated with coronary artery bypass and other surgical procedures. Bilirubin has been found to protect against sepsis and several other acute medical conditions in neonates [1]. Drugs, diet, and exercise also might be effective in increasing serum bilirubin concentrations. While some studies have been performed on drugs which induce HO-1, there have not been any studies on the effects of the drugs on CVD prevention in animal models. Even though aspirin, statins, and probucol all

appear to induce HO-1, more detailed studies will need to be performed to determine if such HO-1 up-regulation increases serum bilirubin concentrations and prevents CVD. Such studies offer great promise and could have an impact on the health of individuals with low serum bilirubin concentrations and in individuals at risk for CVD and possibly other diseases.

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References

[1] Vítek L, Schwertner HA. The heme catabolic pathway and its protective effects on oxidative stress-mediated diseases. Adv Clin Chem 2007;43:1–57.
[2] Stocker R, Perrella MA. Heme oxygenase-1: a novel drug target for atherosclerotic diseases? Circulation 2006;114:2178–89.
[3] Morita T. Heme oxygenase and atherosclerosis. Arterioscler Thromb Vasc Biol 2005;25:1786–95.
[4] Siow RCM, Sato H, Mann GE. Heme oxygenase-carbon monoxide signaling pathway in atherosclerosis: anti-atherogenic actions of bilirubin and carbon monoxide? Cardiovasc Res 1999;41:385–94.
[5] Elbirt KK, Bonkovsky HL. Heme oxygenase: Recent advances in understanding its regulation and role. Proc Assoc Am Physicians 1999;111:438–47.
[6] Bach FH. Heme oxygenase-1: A therapeutic amplification funnel. FASEB J 2005;19:1216–9.
[7] Abraham NG, Asija A, Drummond G, Peterson S. Heme oxygenase-1 gene therapy: Recent advances and therapeutic applications. Curr Gene Therapy 2007;7:89–108.
[8] Perrella MA, Yet SF. Role of heme oxygenase-1 in cardiovascular function. Curr Pharm Des 2003;9:2479–87.
[9] Scott JR, Chin BY, Bilban MH, Otterbein LE. Restoring HOmeostasis: is heme oxygenase-1 ready for the clinic? Trends Pharmacol Sci 2007;28:200–5.
[10] Sedlak TW, Synder SH. Bilirubin benefits: cellular protection by a biliverdin reductase antioxidant cycle. Pediatrics 2004;113:1776–82.
[11] Stocker R, Yamamoto Y, McDonagh A, Glazer A. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043–6.
[12] Stocker R, Keaney Jr JF. Role of oxidative modifications in atherosclerosis. Physiol Rev 2004;84:1381–478.
[13] Willis D, Moore AR, Frederik R, Willoughby DA. Heme oxygenase: A novel target for the modulation of the inflammatory response. Nat Med 1996;2:87–90.
[14] Nakagami T, Toyomura K, Kinoshita T, Morisawa S. A beneficial role of bile pigments as an endogenous tissue protector: Anticomplement effects of biliverdin and conjugated bilirubin. Biochim Biophys Acta 1993;1158:189–93.
[15] Neuzil J, Stocker R. Free and albumin-bound bilirubin are efficient co-antioxidants for α-tocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation. J Biol Chem 1994;269:16712–9.
[16] Wu TW, Fung KP, Wu J, Yang CC, Weisel RD. Antioxidation of human low density lipoprotein by unconjugated and conjugated bilirubins. Biochem Pharmacol 1996;51:859–62.
[17] Schwertner HA. Association of smoking and low serum bilirubin antioxidant concentrations. Atherosclerosis 1998;136:383–7.
[18] Vítek L, Jirsa Jr M, Brodanová M, et al. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. Atherosclerosis 2002;160:449–56.
[19] Ishikawa K, Sugawara D, Goto J, et al. Heme oxygenase-1 inhibits atherogenesis in Watanabe heritable hyperlipidemic rabbits. Circulation 2001;104:1831–6.
[20] Ishikawa K, Sugawara D, Wang X, et al. Heme oxygenase-1 inhibits atherosclerotic lesion formation in LDL-receptor knockout mice. Circ Res 2001;88:506–12.
[21] Ollinger R, Yamashita K, Bilban M, et al. Bilirubin and biliverdin treatment of atherosclerotic diseases. Cell Cycle 2007;6:39–43.
[22] Vítek L, Malíková I, Kvasnička J, Benáková H, Novotný L. Relationship between serum bilirubin and markers of inflammation and oxidative stress (abstract). J Gastroenterol Hepatol 2007;22(Suppl. 2):A235.
[23] Duckers HJ, Boehm M, True AL, et al. Heme oxygenase-1 protects against vascular constriction and proliferation. Nat Med 2001;7:693–8.
[24] Yet SF, Tian R, Layne MD, et al. Cardiac-specific expression of heme oxygenase-1 protects against ischemia and reperfusion injury in transgenic mice. Circ Res 2001;89:168–73.
[25] Camejo G, Halberg C, Manschich-Lundin A, et al. Hemin binding and oxidation of lipoproteins in serum: Mechanism and effect on the interaction of LDL with human macrophages. J Lipid Res 1998;39:755–66.
[26] Tribble D, van den Berg JM, Motchnik PA, et al. Oxidative susceptibility of low density lipoprotein subfractions is related to their ubiquinol-10 and α-tocopherol content. Proc Natl Acad Sci USA 1994;91:1183–7.
[27] Tribble DL, Rizzo M, Chait A, et al. Enhanced oxidative susceptibility and reduced antioxidant content of metabolic precursors of small dense low-density lipoproteins. Am J Med 2001;110:103–10.
[28] Benallaoua M, Francois M, Batteux F, et al. Pharmacologic induction of heme oxygenase 1 reduces inflammatory arthritis in mice. Arthritis Rheum 2007;56:2585–94.
[29] Schwertner HA, Jackson WG, Tolan G. Association of low serum concentrations of bilirubin with increased risk of coronary artery disease. Clin Chem 1994;40:18–23.
[30] Hopkins PN, Wu LL, Hunt SC, et al. Higher serum bilirubin is associated with decreased risk for early familial coronary artery disease. Arterioscler Thromb Vasc Biol 1996;16:250–5.
[31] Breimer LH, Spyropolous KA, Winder AF, Mikhailidis DP, Hamilton G. Is bilirubin protective against coronary artery disease? Clin Chem 1994;40:1987–8.
[32] Levinson SS. Relationship between bilirubin, apolipoprotein B, and coronary artery disease. Ann Clin Lab Sci 1997;27:185–92.
[33] Cerne D, Ledinski G, Kager G, et al. Comparison of laboratory parameters as risk factors for the presence and the extent of coronary or carotid atherosclerosis: the significance of apolipoprotein B to apolipoprotein all ratio. Clin Chem Lab Med 2000;38:529–38.
[34] Hunt SC, Kronenberg F, Eckfeldt JH, et al. Association of plasma bilirubin with coronary heart disease and segregation of bilirubin as a major gene trait: the NHLBI family heart study. Atherosclerosis 2001;154:747–54.
[35] Ishizaka N, Ishizaka Y, Takahashi E, Yamakado M, Hashimoto H. High serum bilirubin level is inversely associated with the presence of carotid plaque. Stroke 2001;32:581–3.
[36] Krijgsman B, Papadakis JA, Ganotakis ES, Mikhailidis DP, Hamilton G. The effect of peripheral vascular disease on the serum levels of natural anti-oxidants: bilirubin and albumin. Int Angiol 2002;21:44–52.
[37] Kronenberg F, Coon H, Gutin A, et al. A genome scan for loci influencing anti-atherogenic serum bilirubin levels. Eur J Hum Genet 2002;10:539–46.
[38] Endler G, Hamwi A, Sunder-Plassmann R, et al. Is low serum bilirubin an independent risk factor for coronary artery disease in men but not in women? Clin Chem 2003;49:1201–4.
[39] Gullu H, Erdogan D, Tok D, et al. High serum bilirubin concentrations preserve coronary flow reserve and coronary microvascular functions. Arterioscler Throm Vasc Biol 2005;25:2289–94.
[40] Vítek L, Novotný L, Šperl M, Holaj R, Spáčil J. The inverse association of elevated serum bilirubin levels with subclinical carotid atherosclerosis. Cerebrovasc Dis 2006;21:408–14.

[41] Yilmaz N, Cicek HK, Celik A, Davutoglu V. Diagnostic value of bilirubin concentrations compared with novel and traditional biomarkers in atherosclerosis with coronary artery disease. Saudi Med J 2006;27:1262–4.
[42] Erdogan D, Gullu H, Yildirim E, et al. Low serum bilirubin levels are independently and inversely related to impaired flow-mediated vasodilation and increased carotid intima-media thickness in both men and women. Atherosclerosis 2006;184:431–7.
[43] Yilmaz N, Cicek HK, Celik A, et al. Diagnostic value of homocysteine, C-reactive protein and bilirubin for coronary artery disease. East Mediterr Health J 2007;13:522–35.
[44] Perlstein TS, Pande R, Beckman JA, Creager MA. Serum total bilirubin level and prevalent lower-extremity peripheral arterial disease: National Health and Nutrition Examination Survey (NHANES) 1999 to 2004. Arteriocler Thromb Vasc Biol 2008;28:166–72.
[45] Perlstein TS, Pande R, Beckman JA, Creager MA. Serum total bilirubin level is inversely associated with likelihood of nonfatal stroke: National Health and Nutrition Examination Survey, 1999–2004 (abstract). Vasc Med 2007;12:146.
[46] Inoguchi T, Sasaki S, Kobayashi K, Takayanagi R, Yamada T. Relationship between Gilbert syndrome and prevalence of vascular complications in patients with diabetes. J Am Med Assoc 2007;298:1398–400.
[47] Ganotakis ES, Vrentzos GE, Gazi IF, et al. Fibrinogen, lipoprotein (a), albumin and bilirubin (F-L-A-B) levels and cardiovascular risk calculated using the Framingham equation. In Vivo 2007;21:685–94.
[48] Breimer LH, Wannamethee G, Ebrahim S, Shaper AG. Serum bilirubin and risk of ischemic heart disease in middle-aged British men. Clin Chem 1995;41:1504–8.
[49] Hunt SC, Wu LL, Hopkins PN, Williams RR. Evidence for a major gene elevating serum bilirubin concentration in Utah pedigrees. Arterioscler Thromb Vasc Biol 1996;16:912–7.
[50] Djousse L, Levy D, Cupples LA, et al. Total serum bilirubin and risk of cardiovascular disease in the Framingham offspring study. Am J Cardiol 2001;87:1196–200.
[51] Temme EH, Zhang J, Schouten EG, Kesteloot H. Serum bilirubin and 10-year mortality risk in a Belgian population. Cancer Causes Control 2001;12:887–94.
[52] Djousse L, Rothman KJ, Cupples LA, Levy D, Ellison RC. Effect of serum albumin and bilirubin on the risk of myocardial infarction (the Framingham Offspring Study). Am J Cardiol 2003;91:485–8.
[53] Troughton JA, Woodside JV, Young IS, et al. Bilirubin and coronary heart disease risk in the Prospective Epidemiological Study of Myocardial Infarction (PRIME). Eur J Cardiovasc Prev Rehabil 2007;14:79–84.
[54] Novotný L, Vítek L. Inverse relationship between serum bilirubin and atherosclerosis in men. A meta-analysis of published studies. Exp Biol Med 2003;228:568–71.
[55] Bosma PJ. Inherited disorders of bilirubin metabolism. J Hepatol 2003;38:101–7.
[56] Bosma PJ, van der Meer IM, Bakker CT, et al. UGT1A1*28 allele and coronary heart disease: The Rotterdam Study. Clin Chem 2003;49:1180–1.
[57] Lin JP, O'Donnell CJ, Schwaiger JP, et al. Association between the UGT1A1*28 allele, bilirubin levels, and coronary heart disease in the Framingham Heart Study. Circulation 2006;114:1476–81.
[58] Schwertner HA. Bilirubin concentration, UGT1A1*28 polymorphism, and coronary artery disease. Clin Chem 2003;49:1039–40.
[59] Gajdos V, Petit FM, Perret C, et al. Further evidence that the UGT1A1*28 allele is not associated with coronary artery heart disease: The ECTIM Study. Clin Chem 2006;52:2313–4.
[60] Ko GT, Chan JC, Woo J, et al. Serum bilirubin and cardiovascular risk factors in a Chinese population. J Cardiovascular Risk 1996;3:459–63.
[61] Madhavan M, Wattingney WA, Srinivasan SR, Berenson GS. Serum bilirubin distribution and its relation to cardiovascular risk in children and young adults. Atherosclerosis 1997;131:107–13.
[62] Papadakis JA, Ganotakis ES, Jagroop IA, Mikhailidis DP, Winder AF. Effect of hypertension and its treatment on lipid, lipoprotein(a), fibrinogen, and bilirubin levels in patients referred for dyslipidemia. Am J Hypertens 1999;12:673–81.
[63] Kalousová M, Novotný L, Zima T, Braun M, Vítek L. Decreased levels of advanced glycation end-product in patients with Gilbert syndrome. Cell Mol Biol 2005;51:387–92.
[64] Caliskan M, Erdogan D, Gullu H, Tok D, Bilgi M, Muderrisoglu H. Low serum bilirubin concentrations are associated with impaired aortic elastic properties, but not impaired left ventricular diastolic function. Int J Clin Pract 2006 [Epub ahead of print].
[65] Vítek L, Kráslová I, Muchová L, Novotný L, Yamaguchi T. Urinary excretion of oxidative metabolites of bilirubin in subjects with Gilbert syndrome. J Gastroenterol Hepatol 2007;22:841–5.
[66] Schwertner HA, Fischer Jr JR. Comparison of various lipid, lipoprotein, and bilirubin combinations as risk factors for predicting coronary artery disease. Atherosclerosis 2000;150:381–7.
[67] Grosser N, Hemmerlee A, Berndt G, et al. The antioxidant defense proteinheme oxygenase 1 is a novel target for statins in endothelial cells. Free Radic Biol Med 2004;37:2064–71.
[68] Lee TS, Chang CC, Zhu YY, Shyy JY. Simvastatin induces heme oxygenase-1: A novel mechanism for vessel protection. Circulation 2004;110:1296–302.
[69] Muchova L, Wong RJ, Hsu M, et al. Statin treatment increases formation of carbon monoxide and bilirubin in mice: a novel mechanism of in vivo antioxidant protection. Can J Physiol Pharmacol 2007;85:800–10.
[70] Brydun A, Watari Y, Yamamoto Y, et al. Reduced expression of heme oxygenase-1 in patients with coronary atherosclerosis. Hypertens Res 2007;30:341–8.
[71] McCarty MF. Iatrogenic Gilbert syndrome—A strategy for reducing vascular and cancer risk by increasing plasma unconjugated bilirubin. Med Hypotheses 2007;69:1367–70.
[72] Schindhelm RK, Diamant M, Dekker JM, et al. Alanine aminotransferase as a marker of non-alcoholic fatty liver disease in relation to type 2 diabetes mellitus and cardiovascular disease. Diabetes Metab Res Rev 2006;22:437–43.
[73] Schindhelm RK, Dekker JM, Nijpels G, et al. Alanine aminotransferase predicts coronary heart disease events: A 10-year follow-up of the Hoorn Study. Atherosclerosis 2007;191:391–6.
[74] Ioannou GN, Weiss NS, Boyko EJ, Mozaffarian D, Lee SP. Elevated serum alanine aminotransferase activity and calculated risk of coronary heart disease in the United States. Hepatology 2006;43:1145–51.
[75] Wei M, Schwertner HA, Zeng Q, Blair SN. Fasting serum bilirubin concentrations and the risk of subsequent coronary heart disease death in men (abstract). Hepatology 2000;32:314A.