

## Bilirubin and Endothelial Function

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Bilirubin is a fundamental metabolic end product of heme degradation. Despite acting as a cytotoxic metabolite at high concentrations, bilirubin at physiological concentrations has antioxidant effects, such as scavenging reactive oxygen species, leading to a decrease in oxidative stress. Endothelial dysfunction is an early feature of and plays an important role in the development and progression of atherosclerosis, leading to cardiovascular complications. One mechanism of endothelial dysfunction is an increase in oxidative stress, by which the bioavailability of nitric oxide is decreased. Therefore, bilirubin is expected to improve endothelial function, to inhibit the progression of atherosclerosis, and to reduce cardiovascular complications by inactivating oxidative stress through its antioxidant effects. In this review, we will focus on the clinical associations of the antioxidant bilirubin with endothelial function and cardiovascular complications.

**Key words:** Bilirubin, Antioxidant, Oxidative stress, Endothelial function, Gilbert's syndrome

### Introduction

Bilirubin is an end product of heme degradation by heme oxygenases (HOs). At high concentrations, bilirubin acts as a highly cytotoxic metabolite, which can cause brain damage, whereas at low concentrations, it serves as an endogenous antioxidant<sup>1, 2)</sup>. Experimental studies have shown that bilirubin has antioxidant properties, such as scavenging reactive oxygen species (ROS) and inhibiting nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, resulting in a decrease in oxidative stress, which is critically involved in the pathogenesis and development of atherosclerosis<sup>1, 3)</sup>. Therefore, mild hyperbilirubinemia is expected to exert antiatherosclerotic effects through a decrease in oxidative stress. Indeed, the results of population-based studies and epidemiological studies have shown a protective effect of higher physiological levels of serum bilirubin against cardiovascular and metabolic diseases. In addition, the prevalence of ischemic heart disease or diabetic vascular complications has been shown to be

lower in patients with Gilbert's syndrome, an inherited disorder characterized by mild hyperbilirubinemia, than in patients without Gilbert's syndrome.

Endothelial dysfunction is critically involved in the development and progression of atherosclerosis. Endothelial function is influenced by the balance between endothelium-derived vasodilators, especially nitric oxide (NO), and ROS. Endothelial dysfunction is caused by decreased NO bioavailability (increased NO inactivation and/or decreased NO production) due to an excess of ROS. Therefore, bilirubin, an endogenous antioxidant, is expected to improve endothelial dysfunction by decreasing oxidative stress through scavenging ROS. Improvement of endothelial function will prevent the progression of atherosclerosis, leading to a reduction in cardiovascular events. This review focuses on the antioxidant ability of bilirubin and the clinical associations of bilirubin with endothelial function and cardiovascular complications.

### Endothelial Function

The vascular endothelium had been thought to

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be just a structural barrier separating the blood vessel wall and the inside cavity. In the 1980s, experimental studies revealed that the vascular endothelium is not just a structural barrier, but rather it functions as an endocrine organ that secretes vasoactive agents, including vasodilators (e.g., NO, prostacyclin, and endothelium-derived hyperpolarizing factor) and vasoconstrictors (e.g., endothelin-1, angiotensin II, and thromboxane A<sub>2</sub>)<sup>4</sup>. If the vascular endothelium in the whole body could be collected, its total weight is estimated to be equal to that of the liver, and its total area is estimated to be equal to that of six tennis courts. Thus, the vascular endothelium can be regarded as one of the largest endocrine organs in the human body. A healthy endothelium acts as a gatekeeper that maintains vascular function and structure by regulating the balance between vasodilation and vasoconstriction, cell growth inhibition and promotion, anti-thrombosis and prothrombosis, antioxidation and prooxidation, and anti-inflammation and proinflammation. Endothelial dysfunction refers to a condition in which the balance between endothelium-derived vasorelaxing factors and endothelium-derived vasoconstricting factors is disturbed in favor of vasoconstriction, leading to disturbed vascular homeostasis and progression of atherosclerosis.

Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis and plays a critical role in the development and progression of this condition, leading to cardiovascular complications<sup>5, 6</sup>. Thus, endothelial dysfunction has emerged as a therapeutic target for the prevention of atherosclerosis and cardiovascular events in individuals with cardiovascular risk factors, such as hypertension, dyslipidemia, and diabetes mellitus, as well as in individuals with established cardiovascular diseases. Moreover, endothelial function has been shown to be an independent predictor of cardiovascular events<sup>7, 8</sup>. Therefore, assessment of endothelial function in atherosclerotic patients is helpful for cardiovascular risk stratification and for selecting appropriate interventions to improve endothelial dysfunction in clinical settings.

### Endothelial Function Tests

Several methods have been developed and performed for the assessment of endothelial function in humans<sup>9</sup>. Endothelial function tests performed in the coronary artery, including the epicardial endothelial function test and coronary microvascular function test, have been considered as the gold standard techniques for the assessment of endothelial function. These techniques enable direct assessment of endothelial function in the clinically important vascular bed. However, coronary endothelial function tests are lim-



**Fig. 1.** Measurement of flow-mediated vasodilation (FMD) of the brachial artery

Modified from Higashi Y, *et al.* *Int Heart J* 2015; 56: 633-638<sup>10</sup>.

ited by their invasive nature and the need for specific expertise and equipment, leading to difficulty in repeated measurements, especially in asymptomatic subjects. An alternative approach for the assessment of endothelial function involves the peripheral circulation. Recently, flow-mediated vasodilation (FMD) in the brachial artery (**Fig. 1**) and reactive hyperemia-peripheral arterial tonometry (RH-PAT) in the fingertip have been widely used for the assessment of endothelial function as noninvasive methods<sup>10</sup>. Both of these endothelial function tests are based on the same principle of the reactive hyperemia phenomenon. During reactive hyperemia, FMD measures any change in the diameter of the brachial artery induced by vasodilatory substances (mainly NO) released from the endothelium, whereas RH-PAT measures any change in the finger arterial pulse waves. Meta-analyses have shown that not only endothelial function in coronary arteries but also endothelial function in peripheral arteries is a significant predictor of cardiovascular events independent of conventional cardiovascular risk factors with power similar to that of coronary endothelial function tests for predicting cardiovascular events<sup>7, 8</sup>. Although measurement of endothelial function in the peripheral circulation does not enable a direct assessment of endothelial function in the coronary circulation, previous studies have shown that there is a significant correlation between coronary endothelial function and peripheral endothelial function<sup>11-14</sup>. This association may explain, in part, the ability of noninvasive peripheral endothelial function tests to predict future cardiac events.

## NO

NO, a free-radical gas, is one of the vasoactive agents released from the endothelium. NO is mainly produced by endothelial NO synthase (eNOS) in the vasculature using the substrate L-arginine and cofactor tetrahydrobiopterin (BH<sub>4</sub>). NO also has a wide range of antiatherosclerotic effects, such as vasodilation, inhibition of leucocyte adhesion, inhibition of platelet adhesion and aggregation, and suppression of vascular smooth muscle cell proliferation<sup>15, 16</sup>. Therefore, reduced NO bioavailability is an important feature of endothelial dysfunction. Endothelial function is normally maintained by a balance between NO and oxidative stress. One mechanism of endothelial dysfunction is an increase in oxidative stress, by which NO inactivation is increased and NO production is decreased, leading to decreased NO bioavailability and consequent endothelial dysfunction<sup>17</sup>.

## Oxidative Stress

Oxidative stress plays a critical role in the pathogenesis of various diseases and conditions, such as hypertension, diabetes mellitus, myocardial infarction, heart failure, atherosclerosis, and endothelial dysfunction. Oxidative stress refers to a state in which the balance between ROS and the antioxidant defense system is disturbed in favor of oxidation. The susceptibility of vascular cells to oxidative stress is determined by the overall balance between the extent of oxidative stress and the antioxidant defense capability. Under a condition in which excessively generated ROS cannot be sufficiently counteracted by the antioxidant defense system, deleterious effects of ROS, such as lipid peroxidation, DNA fragmentation, and protein or enzyme oxidation, become clinically evident, leading to the development of atherosclerosis. There are several enzyme systems potentially producing ROS in the vasculature, including NADPH oxidases, xanthine oxidase, enzymes of the mitochondrial respiratory chain, and uncoupled eNOS. ROS include superoxide anions (O<sub>2</sub><sup>-</sup>), hydroxyl radicals (OH), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hypochlorous acid (HOCl), NO, and peroxynitrite (ONOO<sup>-</sup>).

## Oxidative Stress and Endothelial Dysfunction

O<sub>2</sub><sup>-</sup> is produced by the one-electron reduction of molecular oxygen. Although O<sub>2</sub><sup>-</sup> is not a highly potent oxidant, it is critically involved in the progression of endothelial dysfunction. O<sub>2</sub><sup>-</sup> reacts directly with NO with high affinity, resulting not only in a decrease in NO bioavailability through degradation and inactivation of NO, but also in an increase in ONOO<sup>-</sup> formation. ONOO<sup>-</sup> is a highly potent oxidant that can oxidize the critical cofactor BH<sub>4</sub> to the

biologically inactive trihydrobiopterin. In the absence of a sufficient intracellular concentration of BH<sub>4</sub>, eNOS, a main source of endothelial NO production, is converted from an NO-producing enzyme to an enzyme generating O<sub>2</sub><sup>-</sup>. This phenomenon has been referred to as eNOS uncoupling<sup>18</sup>. Therefore, when there is an increase in the production of O<sub>2</sub><sup>-</sup>, oxidative stress is further enhanced through a vicious cycle of increased O<sub>2</sub><sup>-</sup>, increased formation of ONOO<sup>-</sup> as a result of the reaction of O<sub>2</sub><sup>-</sup> with NO, oxidation of BH<sub>4</sub> by ONOO<sup>-</sup>, and eNOS uncoupling, leading to a further increase in O<sub>2</sub><sup>-</sup> production, a further decrease in NO bioavailability, and a consequent exacerbation of endothelial dysfunction.

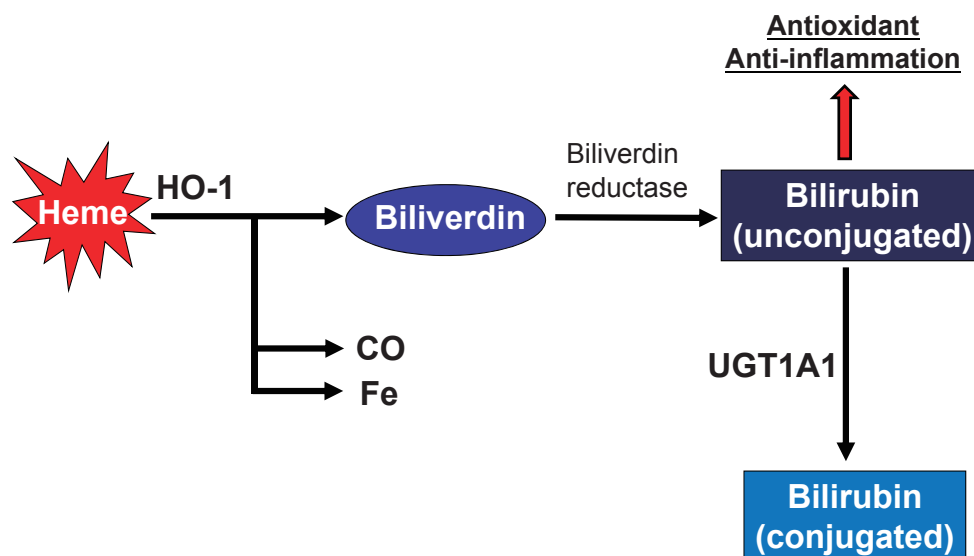
## Bilirubin

Bilirubin is a metabolic end product of heme catabolism. Heme is released when heme-containing proteins, such as cytochrome P450 and hemoglobin, are degraded. Serum bilirubin levels are thought to be usually derived from the breakdown of red blood cells. Heme is metabolized to carbon monoxide, iron, and biliverdin by HO, especially HO-1 (Fig. 2)<sup>19</sup>. Biliverdin, a green water-soluble compound, is rapidly reduced to poorly water-soluble unconjugated bilirubin by bilirubin reductase and is released into the blood. Albumin-bound unconjugated bilirubin travels to other organs or is transported to the liver, where hydrophobic unconjugated bilirubin is conjugated with glucuronic acid by the UDP-glucuronosyltransferase 1A1 (UGT1A1) enzyme before being excreted from the liver into the bile.

Degradation of heme and excretion of end product bilirubin have been considered to be straightforward detoxification. Excess levels of bilirubin are potentially toxic. Newborns with severe neonatal jaundice or patients with Crigler–Najjar syndrome, an inherited disorder characterized by the absence of UGT1A1 activity, show very high levels of unconjugated bilirubin, which can cause severe brain damage. High levels of bilirubin act as a cytotoxic metabolite. However, bilirubin at a physiological level has been shown to be a potent endogenous antioxidant.

## Bilirubin as an Antioxidant

In 1987, Stocker *et al.* for the first time demonstrated that bilirubin scavenged peroxy radicals more efficiently than did the powerful antioxidant  $\alpha$ -tocopherol *in vitro*<sup>1</sup>. They also demonstrated that bilirubin, when bound to human albumin at concentrations present in normal human plasma, prevents albumin-bound fatty acid from undergoing peroxy radical-induced oxidation *in vitro*<sup>2</sup>. These pioneering studies introduced the concept that unconjugated bili-



**Fig. 2.** Heme degradation pathway

HO-1: heme oxygenase-1; CO: carbon monoxide; UGT1A1: UDP-glucuronosyltransferase 1A1. Modified from Higashi Y, *et al.* Trends Cardiovasc Med 2014; 24: 165-169<sup>17)</sup>.

rubin, which had been regarded as a potentially cytotoxic waste product of heme catabolism, has a beneficial role as an endogenous antioxidant directly scavenging ROS. When acting as a ROS scavenger, bilirubin and albumin-bound unconjugated bilirubin are oxidized to the nontoxic metabolic precursor biliverdin and are then recycled back to bilirubin by biliverdin reductase<sup>2, 20, 21)</sup>. It has been shown in *in vitro* studies that albumin-bound unconjugated bilirubin is oxidized by  $O_2^-$ , OH, and  $HO_2$  and that unconjugated bilirubin protects albumin from oxidation by  $O_2^-$ , OH,  $HO_2$ , and  $ONOO^-$ , indicating that unconjugated bilirubin can serve as an antioxidant through directly scavenging ROS<sup>2, 21, 22)</sup>. In addition, bilirubin has been shown to inhibit the activity of NADPH oxidase, the major source of ROS in the vasculature, *in vitro* and *in vivo*, suggesting that the production of ROS is decreased by bilirubin<sup>3, 23)</sup>. Moreover, bilirubin has been shown to interact with other antioxidants to synergistically inhibit lipid peroxidation<sup>24, 25)</sup>. Taken together, these experimental findings indicate that bilirubin may function as an antioxidant through scavenging ROS, inhibiting NADPH oxidase activity, and synergistically interacting with other antioxidants to inhibit lipid oxidation.

### Bilirubin and Cardiovascular Complications

Clinical studies have shown significant associations between serum bilirubin concentrations and the risk of cardiovascular diseases, including coronary artery disease, stroke, and peripheral artery disease

(PAD)<sup>26-28)</sup>. Schwertner *et al.* reported a negative association between serum bilirubin concentrations and the presence of coronary artery disease in 877 male subjects<sup>26)</sup>. Kimm *et al.* reported that the risk of ischemic stroke was significantly lower in individuals with high levels of bilirubin than in individuals with low levels of bilirubin in 78,724 male subjects<sup>29)</sup>. Perlstein *et al.* showed that the prevalence of PAD decreased in relation to an increase in the bilirubin level and that an increase in the bilirubin level of 0.1 mg/dL was associated with a 6% reduction in the odds of PAD, defined as an ankle-brachial index of less than 0.9 in 7,075 adults<sup>28)</sup>. Cohort studies have also shown that low bilirubin levels are associated with an elevated risk of cardiovascular diseases<sup>30, 31)</sup>. In addition, a meta-analysis of 11 studies showed a strong inverse relationship between serum bilirubin levels and severity of atherosclerosis in men<sup>32)</sup>.

Gilbert's syndrome is an inherited disorder characterized by mild unconjugated nonhemolytic hyperbilirubinemia caused by decreased UGT1A1 activity, which is reduced to approximately 30% of normal. The incidence of Gilbert's syndrome has been reported to be 5–10% in the general population<sup>33, 34)</sup>. Clinical studies have shown that the prevalence of cardiovascular complications is significantly lower in subjects with Gilbert's syndrome than in those without. Vitek *et al.* reported that the prevalence of ischemic heart disease was 2% in patients with Gilbert's syndrome, which is much lower than the prevalence of 12% in the general population<sup>35)</sup>. In patients with diabetes mellitus, the

**Table 1.** Clinical Studies Investigating the Association between Bilirubin and Endothelial Function

Study	Study subjects	No. of subjects	Measurement for endothelial function	Difference in endothelial function between the groups/ Correlation between endothelial function and bilirubin	Independent predictor for endothelial function
Yoshino, 2013 <sup>37)</sup>	Patients without coronary artery disease	141	%change in CBF by ACh/% change in epicardial coronary artery diameter by ACh (coronary angiography)	Yes	Yes
Yoshino, 2011 <sup>38)</sup>	Obese patients without coronary artery disease	36	FMD in the epicardial coronary artery by papaverine (coronary angiography)	Yes	N/A
Erdogan, 2006 <sup>39)</sup>	Healthy subjects	Low bilirubin group; 47 High bilirubin group; 44	FMD of the brachial artery	Yes	Yes
Maruhashi, 2012 <sup>40)</sup>	Healthy subjects with and without Gilbert's syndrome	Control; 108 Gilbert's syndrome; 108	FMD of the brachial artery	Yes	Yes
Yeh, 2009 <sup>41)</sup>	Healthy subjects and patients with diabetes mellitus	Healthy controls; 52 Type 1 diabetes; 37 Type 2 diabetes; 213	FMD of the brachial artery/ Vascular reactivity of the skin microcirculation by ACh infusion	Yes (negative correlation)	No

CBF: coronary blood flow; ACh: acetylcholine.

prevalence of vascular complications, including retinopathy, macroalbuminuria, and coronary artery disease, has been shown to be significantly lower in patients with Gilbert's syndrome than in those without, with significantly lower oxidative stress marker urinary excretion of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in patients with Gilbert's syndrome than in those without<sup>36)</sup>. These findings suggest that bilirubin has beneficial effects on the development of atherosclerosis, probably through its antioxidant ability.

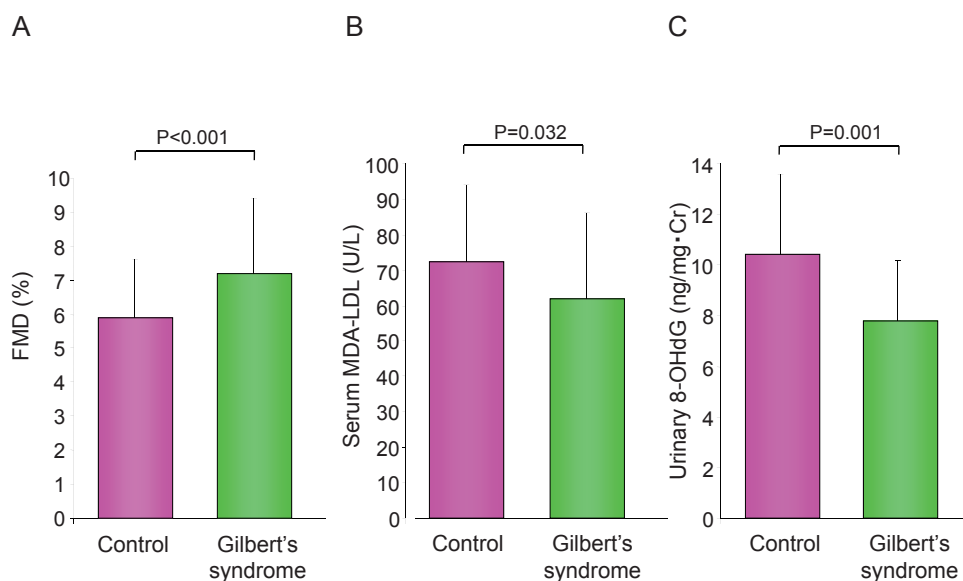
### Bilirubin and Endothelial Function

Considering the antioxidant effects of bilirubin, it is postulated that endothelial function is maintained or augmented in individuals with high serum bilirubin concentrations through a decrease in oxidative stress and consequent restoration of NO bioavailability. The associations of serum bilirubin concentrations with endothelial function in coronary arteries and endothelial function in peripheral arteries have been

investigated in clinical settings (Table 1)<sup>37-41)</sup>. To our knowledge, there is no study investigating the association between bilirubin levels and peripheral endothelial function using RH-PAT in the fingertips.

### Coronary Endothelial Function and Bilirubin

Yoshino *et al.* investigated the associations of serum bilirubin concentrations with endothelial function in the epicardial coronary artery (conduit artery) and coronary microvasculature (resistance arteries) using intracoronary acetylcholine (ACh) infusion in 141 patients without coronary artery disease<sup>37)</sup>. Coronary endothelial function in conduit and resistance arteries correlated positively with serum bilirubin concentrations. Moreover, multivariate analyses showed that higher serum bilirubin concentrations were significantly associated with augmented coronary endothelial function in both the conduit artery and the microvasculature, independent of conventional cardiovascular risk factors, indicating that bilirubin has a



**Fig. 3.** Flow-mediated vasodilation (FMD) of the brachial artery (A), serum malondialdehyde-modified low-density lipoprotein (MDA-LDL) (B), and urinary excretion of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in control subjects and patients with Gilbert's syndrome

Modified from Maruhashi T, *et al.* *Circulation* 2012; 126: 598-603<sup>40</sup>.

protective effect on endothelial function in coronary arteries.

### Peripheral Endothelial Function and Bilirubin

High serum bilirubin concentrations have been shown to be associated with augmented peripheral endothelial function in young individuals<sup>39, 40</sup>. Erdogan *et al.* evaluated FMD in young healthy subjects, including 44 subjects with total serum bilirubin concentrations of more than 1.0 mg/dL and 47 subjects with total serum bilirubin concentrations of less than 0.5 mg/dL<sup>39</sup>. They showed that FMD was significantly higher in subjects with higher bilirubin concentrations than in those with lower bilirubin concentrations ( $11.6 \pm 4.4\%$  versus  $7.2 \pm 4.7\%$ ,  $P < 0.0001$ ). We measured FMD in the brachial artery and oxidative stress markers, including malondialdehyde-modified low-density lipoprotein (MDA-LDL) and urinary excretion of 8-OHdG, in young healthy male subjects with or without Gilbert's syndrome (108 patients with Gilbert's syndrome and 108 age-matched control subjects)<sup>40</sup>. Serum bilirubin concentrations were higher in patients with Gilbert's syndrome than in the control subjects ( $1.72 \pm 0.68$  mg/dL versus  $0.55 \pm 0.16$  mg/dL). FMD was significantly greater in patients with Gilbert's syndrome than in the control subjects ( $7.2 \pm 2.2\%$  versus  $5.9 \pm 1.7\%$ ,  $P < 0.001$ ) (Fig. 3A), whereas oxidative stress markers were significantly lower in patients with Gilbert's syndrome than in the

control subjects (MDA-LDL,  $61.8 \pm 24.5$  U/L versus  $72.5 \pm 21.8$  U/L,  $P = 0.032$ ; urinary excretion of 8-OHdG,  $7.8 \pm 2.4$  ng/mg·Cr versus  $10.4 \pm 3.2$  ng/mg·Cr,  $P = 0.001$ ) (Fig. 3B, Fig. 3C). In addition, there were negative correlations between FMD and oxidative stress markers, a positive correlation between serum bilirubin concentrations and FMD, and negative correlations between serum bilirubin concentrations and oxidative stress markers. These findings indicate that endothelial function is enhanced by mild hyperbilirubinemia through its antioxidant effect in patients with Gilbert's syndrome. However, Yeh *et al.* reported that there was no significant association between serum bilirubin concentrations and endothelial function assessed by FMD in the brachial artery or skin endothelium-dependent vasodilation using laser Doppler flowmetry in 302 individuals, of whom 37 patients had type 1 diabetes mellitus and 213 had type 2 diabetes mellitus<sup>41</sup>. Although there are conflicting results regarding the association between bilirubin and endothelial function, possibly due to the different study populations, clinical studies have shown that mild hyperbilirubinemia is associated with enhanced endothelial function through a decrease in oxidative stress in healthy subjects and have also indicated the possibility that bilirubin is a therapeutic target for the prevention of endothelial dysfunction and cardiovascular complications.

**Table 2.** Associations between Treatment with Atazanavir, Bilirubin, and Endothelial Function

Study	Study subjects	No. of subjects	Dose of atazanavir	Treatment period	Total bilirubin, mg/dL	Method	Significance
Dube, 2008 <sup>42)</sup>	Healthy subjects	9	400 mg daily	4 weeks	Before; 0.6 After; 1.2	% change in leg blood flow measured using a modified thermodilution technique by methacholine	No significant change in leg blood flow before and after the treatment
Dekker, 2011 <sup>43)</sup>	Type 2 diabetes mellitus	15	300 mg twice daily	3 days	Before; 0.4 After; 3.8	% change in forearm blood flow measured using venous occlusion strain gauge plethysmography by ACh	Significant difference between placebo and treatment
Hileman, 2012 <sup>44)</sup>	HIV-infected adults	Control; 62 Atazanavir; 36		28.5 months (median)	Control (median); 0.4 Atazanavir (median); 1.8	FMD of the brachial artery	No difference

HIV: human immunodeficiency virus; ACh: acetylcholine; FMD: flow-mediated vasodilation

### Human Immunodeficiency virus Protease Inhibitor and Endothelial Function

Atazanavir, a human immunodeficiency virus 1 (HIV-1) protease inhibitor, has an inhibitory effect on UGT1A1 activity, leading to an increase in the unconjugated bilirubin level and consequent mild hyperbilirubinemia in subjects undergoing treatment with atazanavir. Therefore, treatment with atazanavir is postulated to have a beneficial effect on endothelial function through an increase in the antioxidant capacity in relation to mild hyperbilirubinemia. The association between peripheral endothelial function and serum bilirubin concentrations in individuals undergoing treatment with atazanavir has been investigated in a few studies (Table 2)<sup>42-44)</sup>. Two randomized studies showed conflicting results. Three-day atazanavir treatment (300 mg twice daily) significantly improved endothelial function assessed by venous occlusion plethysmography in response to ACh infusion with increased serum bilirubin concentrations from 0.4 to 3.8 mg/dL and increased plasma antioxidant capacity in patients with type 2 diabetes mellitus<sup>43)</sup>. In contrast, four-week atazanavir treatment (400 mg daily) did not improve endothelial function assessed by percent change in leg blood flow using a thermodilution technique in response to methacholine infusion despite an increase in serum bilirubin concentrations from 0.6 to 1.2 mg/dL in healthy subjects<sup>42)</sup>. The reasons for these conflicting results are unclear, but some explanations, including differences in the study popu-

lations, doses of atazanavir, average serum bilirubin concentrations induced by atazanavir treatment, treatment periods, and methods of assessment of endothelial function, have been postulated. A cross-sectional study showed no association between endothelial function assessed by FMD in the brachial artery and atazanavir treatment (median treatment period: 28.5 months) despite the higher bilirubin levels in patients undergoing treatment with atazanavir than in those not undergoing treatment with atazanavir (1.8 mg/dL versus 0.4 mg/dL) in HIV-infected patients on stable antiretroviral therapy<sup>44)</sup>. In addition, two randomized studies in which HIV-infected patients were randomly assigned to continue current protease inhibitor treatment or switch to atazanavir treatment showed no significant difference in FMD in the brachial artery between the two groups after 24-week treatment, although no data were presented on serum bilirubin concentrations and its association with FMD<sup>45, 46)</sup>. Further studies are needed to determine whether atazanavir-induced hyperbilirubinemia is associated with the augmentation of endothelial function.

### Conclusion

Bilirubin has been shown to be a potent endogenous antioxidant. Clinical studies have shown associations of bilirubin at a high physiological concentration with enhanced endothelial function, lower levels of oxidative stress markers, and a lower risk of cardiovas-

cular complications, indicating the possibility that bilirubin is a therapeutic target for the prevention of endothelial dysfunction and cardiovascular events. An increase in bilirubin might be achieved by the inhibition of UGT1A1 activity or induction of HO-1 through statin treatment or suppression of Bach-1, a transcriptional repressor of HO-1<sup>17, 43, 47, 48</sup>). Further research is needed for the development of new therapeutic strategies to safely and effectively increase bilirubin levels for the enhancement of endothelial function and consequent reduction in cardiovascular complications.

### Conflicts of Interests

None.

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