Endocr (2011) 39:182–189
DOI 10.1007/s12020-010-9417-2

ORIGINAL ARTICLE

Total, direct, and indirect serum bilirubin concentrations and metabolic syndrome among the Korean population

Jaeseong Jo · Ji Eun Yun · Heeyeon Lee · Heejin Kimm · Sun Ha Jee

Received: 8 June 2010 / Accepted: 24 October 2010 / Published online: 30 November 2010

Abstract Total bilirubin, not direct or indirect bilirubin, has been reported to associate inversely with metabolic syndrome. Therefore, we aimed to evaluate the association between bilirubin subtypes and metabolic syndrome among the Korean population. This study included 5,231 Koreans (3,008 men, 2,223 women) aged 30–87 years, who visited the Health promotion centers in Seoul from April, 2006 to June, 2007. The associations of direct, indirect, and total bilirubin classified in quartiles with metabolic syndrome were measured by logistic regression analyses in men and women. Odds ratios (95% confidence intervals) of the lowest, 2nd and 3rd quartiles of direct serum bilirubin compared with the highest quartile (reference) were 2.3 (1.6–3.2), 1.8 (1.3–2.4), and 1.8 (1.4–2.4) among men, and 5.5 (2.6–11.5), 3.1 (1.5–6.7), and 1.9 (0.9–4.3) among women, respectively. In a multivariable adjusted model, however, the significance of inverse associations with total and indirect bilirubin became attenuated. The relation was consistent particularly with direct bilirubin in subgroups of metabolic syndrome components such as central obesity, hypertriglyceridemia, hyperglycemia, and low HDL-cholesterol in both men and women. Of the three subtypes of serum bilirubin, the inverse association of metabolic syndrome was significantly apparent and consistent with direct bilirubin.

Keywords Serum bilirubin · Metabolic syndrome

J. Jo · J. E. Yun · H. Lee · H. Kimm · S. H. Jee (✉)
Department of Epidemiology and Health Promotion, Institute for Health Promotion, Graduate School of Public Health, Yonsei University, Seoul, Republic of Korea
e-mail: jsunha@yuhs.ac

Introduction

Serum bilirubin, which is a bile pigment, is known as a major breakdown product of heme catabolism [1]. Bilirubin circulates in the bloodstream in two forms: direct and indirect bilirubin. It has been found to have potent endogenous antioxidant properties in in vitro studies, whether it is direct or indirect, protect lipid membranes, protein albumin, and other proteins, especially consisting of poor antioxidant defense system such as myocardium and nervous system [1, 2]. Bilirubin concentrations were found to have inverse associations with cardiovascular disease (CVD) [3]. Similar associations have also been shown between bilirubin concentrations and coronary heart disease [4], peripheral vascular disease [5], carotid intimal-medial thickening [6], and stroke [7].

Metabolic syndrome is a complex disorder combining central obesity, hyperglycemia, elevated blood pressure, low concentration of high density lipoprotein cholesterol (HDL-C), and hypertriglyceridemia [8]. Metabolic syndrome has been found to be associated with atherosclerosis [9] and an important predictor of CVD [10]. However, the possible importance of serum bilirubin in metabolic syndrome is still largely unknown. Moreover, the studies on the epidemiology of metabolic syndrome in relation to bilirubin were limited to total bilirubin concentrations [11, 12]. In several studies, direct bilirubin has reported to be a better prognostic value than total bilirubin [13–16]. Therefore, it is of interest to evaluate the association of different circulating forms of bilirubin concentrations, such as total, direct, and indirect, with metabolic syndrome and additionally with metabolic syndrome components among the Korean population.

Results

Table 1 shows general characteristics of the study population according to the presence or absence of metabolic syndrome. The percentage of participants aged 30–85 years classified as having metabolic syndrome was 17.2% for men and 9.6% for women, respectively. Comparing with both men and women classified as not having metabolic syndrome, these classified as having metabolic syndrome were older and had higher levels of BMI, waist circumference, systolic blood pressure, diastolic blood pressure, total cholesterol, triglyceride, fasting serum glucose, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and uric acid. However, HDL-C and total serum bilirubin levels were lower in both men and women with metabolic syndrome than those without metabolic syndrome. They were statistically significant (P value < 0.05). In addition, the mean of direct, indirect, and total bilirubin concentrations in both men and women decreased with increasing numbers of metabolic syndrome components (Fig. 1).

After adjusting for age, direct, indirect, and total bilirubin concentrations were significantly correlated with metabolic components, in particular, positively with HDL-C and inversely with other metabolic components, such as systolic blood pressure, diastolic blood pressure, waist circumference, triglyceride, fasting serum glucose, and triglyceride/HDL-C ratio in both men and women (Table 2). The inverse correlation was stronger between direct serum bilirubin and triglyceride than that between indirect bilirubin and triglyceride. BMI, waist circumference, total cholesterol, and TG/HDL-C ratio also showed stronger associations with direct bilirubin. However, systolic and diastolic pressure showed strong correlations with indirect bilirubin, while HDL-C and fasting glucose had apparent correlations with total bilirubin in men and with indirect bilirubin in women.

Tables 3 and 4 list multivariable adjusted odds ratios for metabolic syndrome and metabolic components according to bilirubin subtypes classified into quartiles. Low concentrations of serum bilirubin were associated with increased risk of metabolic syndrome in both men and women. Significant inverse associations were determined between metabolic syndrome and bilirubin subtypes for both genders. Multivariable adjusted odds ratios (95% confidence intervals) of the lowest, 2nd, and 3rd quartiles of direct bilirubin compared with the highest quartile

Table 1 General characteristics of study population according to the presence or absence of metabolic syndrome

	Men (n = 3,008)		Women (n = 2,223)	
	With MS (n = 517) Mean ± SD	Without MS (n = 2,491) Mean ± SD	With MS (n = 213) Mean ± SD	Without MS (n = 2,010) Mean ± SD
Age (year)	45.9 ± 9.0	44.3 ± 8.3	51.8 ± 11.4	43.8 ± 8.9
Body mass index (kg/m^2)	26.8 ± 2.6	23.9 ± 2.5	25.8 ± 2.9	22.2 ± 2.6
Waist circumference (cm)	92.2 ± 6.5	83.8 ± 6.9	85.3 ± 6.1	74.5 ± 7.4
Systolic blood pressure (mmHg)	133.0 ± 12.3	121.7 ± 12.3	131.3 ± 15.3	114.2 ± 13.2
Diastolic blood pressure (mmHg)	85.5 ± 10.8	76.7 ± 10.2	80.5 ± 11.8	70.8 ± 10.1
Total cholesterol (mmol/l)	5.2 ± 1.0	5.0 ± 0.8	5.2 ± 0.9	4.8 ± 0.9
Triglyceride (mmol/l)	2.6 ± 1.5	1.4 ± 0.7	2.0 ± 1.0	0.9 ± 0.4
HDL-cholesterol (mmol/l)	1.1 ± 0.2	1.3 ± 0.3	1.2 ± 0.2	1.5 ± 0.3
Fasting serum glucose (mmol/l)	6.1 ± 1.5	5.3 ± 0.9	6.0 ± 1.8	5.0 ± 0.7
Total bilirubin (μmol/l)	15.7 ± 5.5	16.8 ± 5.9	10.9 ± 4.3	12.8 ± 5.1
Direct bilirubin (μmol/l)	6.7 ± 2.4	7.3 ± 2.7	4.4 ± 2.1	5.6 ± 2.1
Indirect bilirubin (μmol/l)	9.0 ± 3.1	9.5 ± 3.2	6.5 ± 3.1	7.2 ± 3.0
Alanine aminotransferase (U/l)	39.2 ± 27.8	26.4 ± 20.7	22.3 ± 11.4	16.4 ± 12.4
Gamma-glutamyl transferase (U/l)	68.4 ± 65.4	40.0 ± 37.7	27.0 ± 21.9	17.5 ± 15.1
Uric acid (mg/dl)	6.4 ± 1.3	6.0 ± 1.2	4.6 ± 1.0	4.1 ± 0.8
	%	%	%	%
Smoking status				
Ex smoker	33.9	35.3	0.5	1.6
Current smoker	46.4	40.3	1.4	3.8
Alcohol intake				
Yes	92.3	91.0	41.8	56.3

HDL high density lipoprotein

All of the above differences were statistically significant (P value < 0.05) by Student's t test or χ^2 test

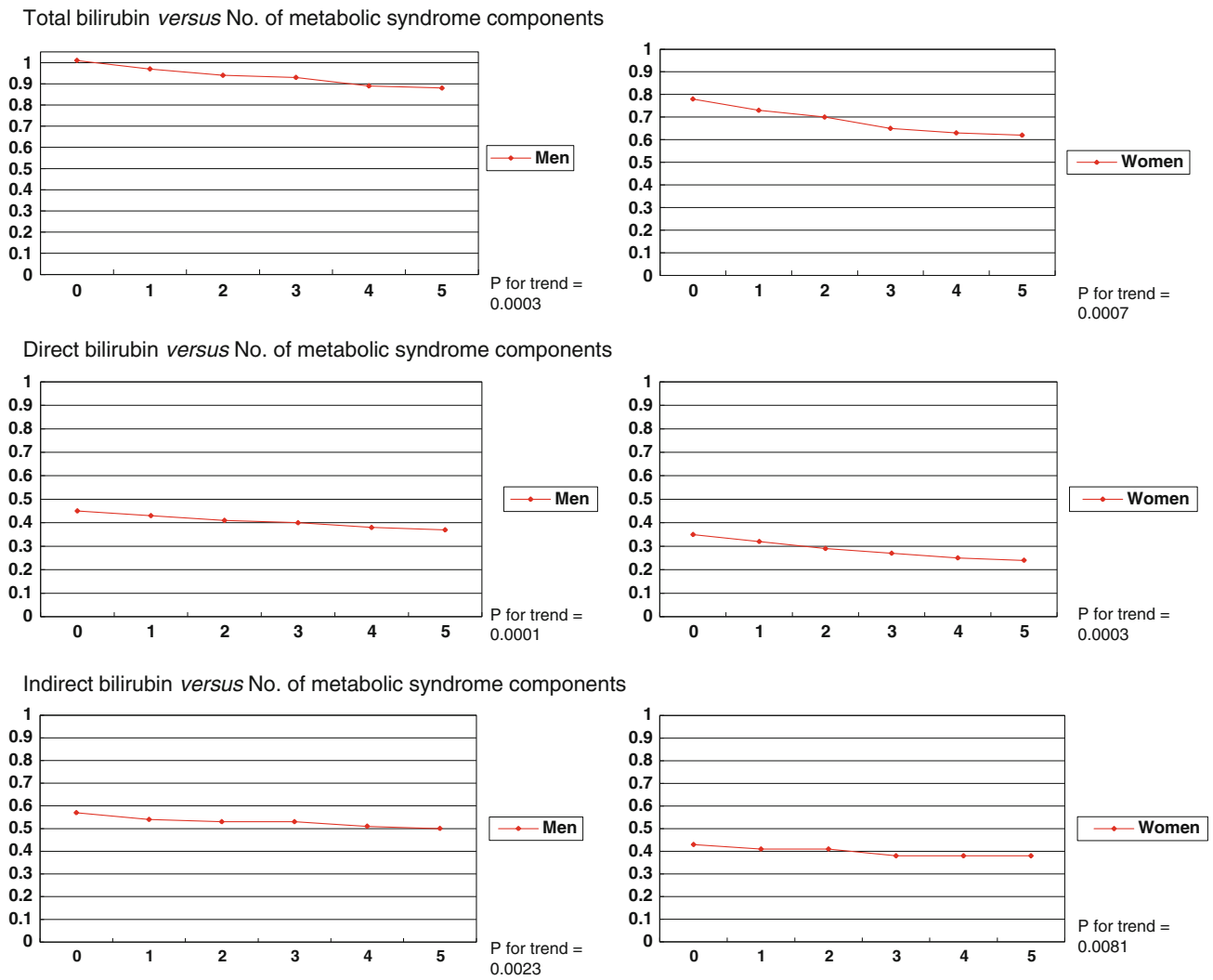

Fig. 1 Total, direct, and indirect bilirubin concentrations according to number of metabolic components

Table 2 Age-adjusted correlation coefficients between bilirubin and metabolic components in men and women

	Men			Women		
	Direct B	Total B	Indirect B	Direct B	Total B	Indirect B
Systolic blood pressure	0.062**	−0.010	−0.056*	0.034	−0.074**	−0.127**
Diastolic blood pressure	0.023	−0.021	−0.046*	0.039	−0.082**	−0.143**
Body mass index	−0.073**	−0.032*	0.002	−0.089**	−0.082**	−0.072**
Waist circumference	−0.098**	−0.046*	−0.002	−0.125**	−0.079**	−0.027
Total cholesterol	−0.181**	−0.004	0.115**	−0.171**	−0.004	0.112**
Triglyceride	−0.205**	−0.116**	−0.032	−0.197**	−0.146**	−0.071**
HDL-C	0.133**	0.154**	0.137**	0.070**	0.129**	0.134**
TG/HDL-C ratio	−0.202**	−0.128**	−0.052*	−0.174**	−0.145**	−0.086**
Fasting serum glucose	−0.040*	−0.052*	−0.050	−0.044*	−0.085**	−0.089**

* P value < 0.05, ** P value < 0.01

B bilirubin, *TG* triglyceride, *HDL-C* high density lipoprotein cholesterol

Table 3 Multivariable adjusted odds ratios and 95% confidence intervals for metabolic syndrome according to total, direct, and indirect serum bilirubin classified into quartiles in both men and women

Quartiles	Direct B		Total B		Indirect B	
	Model 1[a]	Model 2[b]	Model 1[a]	Model 2[b]	Model 1[a]	Model 2[b]
Men						
Quartile 4	1.0	1.0	1.0	1.0	1.0	1.0
Quartile 3	1.8 (1.4–2.3)	1.8 (1.4–2.4)	1.3 (1.0–1.6)	1.3 (1.0–1.7)	1.3 (1.0–1.7)	1.2 (0.9–1.5)
Quartile 2	1.7 (1.3–2.2)	1.8 (1.3–2.4)	1.3 (1.0–1.7)	1.2 (0.9–2.6)	1.1 (0.9–1.4)	1.0 (0.8–1.3)
Quartile 1	1.7 (1.3–2.3)	2.3 (1.6–3.2)	1.5 (1.1–2.1)	1.4 (1.0–2.0)	0.9 (0.6–1.3)	0.7 (0.4–1.0)
Women						
Quartile 4	1.0	1.0	1.0	1.0	1.0	1.0
Quartile 3	1.8 (0.9–3.8)	1.8 (0.9–4.3)	1.4 (0.8–2.7)	1.2 (0.5–2.7)	1.9 (1.1–3.2)	1.7 (0.7–4.0)
Quartile 2	2.8 (1.4–5.5)	3.1 (1.5–6.7)	2.0 (1.1–3.7)	1.9 (0.9–4.1)	1.9 (1.2–3.0)	1.9 (0.9–4.0)
Quartile 1	3.5 (1.8–6.8)	5.5 (2.6–11.5)	3.0 (1.6–3.7)	2.7 (1.3–5.6)	2.2 (1.4–3.6)	2.1 (1.0–4.5)

B bilirubin

[a] Model 1: adjusted for age

[b] Model 2: adjusted for age, alanine aminotransferase, gamma-glutamyl transferase, uric acid, smoking status, and alcohol consumption

Table 4 Multivariable adjusted odds ratios and 95% confidence intervals for metabolic syndrome according to total, direct, and indirect serum bilirubin concentrations classified into quartiles in subgroups of metabolic syndrome components

Bilirubin quartiles	Men				Women			
	Q4	Q3	Q2	Q1	Q4	Q3	Q2	Q1
Direct bilirubin								
Central obesity[a]	1.0	1.3 (1.0–1.6)	1.3 (1.1–1.7)	1.2 (0.9–1.6)	1.0	1.4 (0.9–2.0)	1.7 (1.2–2.5)	2.4 (1.7–3.5)
High blood pressure[b]	1.0	1.0 (0.8–1.2)	1.1 (0.9–1.4)	1.0 (0.8–1.3)	1.0	0.9 (0.6–1.4)	1.4 (0.9–2.0)	1.2 (0.8–1.8)
Hypertriglyceridemia[c]	1.0	1.7 (1.4–2.2)	2.3 (1.8–3.0)	3.7 (2.7–4.9)	1.0	2.5 (0.9–7.2)	4.0 (1.5–10.8)	14.2 (5.5–36.8)
Hyperglycemia[d]	1.0	1.6 (1.2–2.1)	1.2 (0.8–1.6)	1.4 (0.9–2.1)	1.0	1.8 (0.7–4.7)	1.7 (0.6–4.3)	3.0 (1.2–7.4)
Low HDL-C[e]	1.0	1.5 (1.1–1.9)	1.7 (1.3–2.3)	2.9 (2.1–3.9)	1.0	1.2 (0.8–1.7)	1.6 (1.1–2.2)	2.5 (1.8–3.5)
Total bilirubin								
Central obesity[a]	1.0	1.3 (1.0–1.6)	1.1 (0.9–1.4)	1.2 (0.8–1.4)	1.0	1.6 (1.0–2.4)	1.6 (1.0–2.4)	2.1 (1.4–3.2)
High blood pressure[b]	1.0	0.9 (0.8–1.1)	1.1 (0.9–1.3)	1.0 (0.7–1.3)	1.0	1.1 (0.7–1.8)	1.2 (0.8–1.9)	1.4 (0.9–2.1)
Hypertriglyceridemia[c]	1.0	1.2 (1.0–1.5)	1.5 (1.2–1.9)	1.9 (1.4–2.6)	1.0	1.5 (0.6–4.0)	2.4 (0.9–5.9)	5.0 (2.1–12.1)
Hyperglycemia[d]	1.0	1.2 (0.9–1.6)	1.1 (0.8–1.6)	1.2 (0.8–1.8)	1.0	1.3 (0.4–4.3)	2.2 (0.7–6.5)	2.3 (0.8–6.8)
Low HDL-C[e]	1.0	1.6 (1.2–2.0)	1.4 (1.0–1.8)	2.2 (1.6–3.1)	1.0	1.0 (0.7–1.5)	1.2 (0.8–1.7)	1.6 (1.1–2.4)
Indirect bilirubin								
Central obesity[a]	1.0	1.1 (0.9–1.4)	1.1 (0.9–1.4)	0.9 (0.6–1.3)	1.0	1.4 (0.8–2.3)	1.9 (1.3–3.0)	1.5 (1.0–2.3)
High blood pressure[b]	1.0	0.9 (0.7–1.1)	1.0 (0.8–1.2)	0.8 (0.6–1.1)	1.0	1.1 (0.7–1.9)	1.2 (0.7–1.8)	1.1 (0.7–1.8)
Hypertriglyceridemia[c]	1.0	1.1 (0.9–1.4)	1.1 (0.9–1.4)	1.0 (0.7–1.4)	1.0	3.8 (1.4–10.7)	3.1 (1.2–8.2)	4.4 (1.7–11.6)
Hyperglycemia[d]	1.0	1.2 (0.9–1.6)	1.0 (0.7–1.3)	1.2 (0.8–1.8)	1.0	1.2 (0.3–4.1)	2.0 (0.7–5.9)	2.1 (0.7–6.4)
Low HDL-C[e]	1.0	1.4 (1.1–1.8)	1.3 (1.0–1.6)	1.4 (1.0–1.9)	1.0	1.3 (0.7–1.7)	1.0 (0.7–1.5)	1.3 (0.9–1.9)

[a] Central obesity: waist circumference $\geq$ 90 (men), 80 (women) cm; [b] high blood pressure: systolic blood pressure $\geq$ 130 mmHg or diastolic blood pressure $\geq$ 85 mmHg; [c] hypertriglyceridemia: triglyceride $\geq$ 150 mg/dl; [d] hyperglycemia: fasting blood sugar $\geq$ 100 mg/dl; and [e] low high density lipoprotein cholesterol (HDL-C): HDL-C $<$ 40 (men), 50 (women) mg/dl

All ORs were adjusted for age, alanine aminotransferase, gamma-glutamyl transferase, uric acid, smoking status, and alcohol consumption

(reference) were 2.3 (1.6–3.2), 1.8 (1.3–2.4), and 1.8 (1.4–2.4) among men, and 5.5 (2.6–11.5), 3.1 (1.5–6.7), and 1.9 (0.9–4.3) among women. In this multivariable adjusted model, the significance of inverse associations with total and indirect bilirubin became attenuated; however, this inverse association remained significant with direct bilirubin in both men and women. As shown in Table 4, these associations between bilirubin and metabolic syndrome were remained in most of the metabolic syndrome components subgroups, despite no significant

increase of ORs in high blood pressure groups. Increased ORs were detected in central obesity group among women and low HDL-C group among men in the relationship with total and indirect serum bilirubin. On the other side, direct bilirubin was consistently associated with central obesity, hypertriglyceridemia, hyperglycemia, and low HDL-C groups in both men and women.

Discussion

This study showed the association between serum bilirubin concentrations and metabolic syndrome among the Korean population. Low concentrations of bilirubin subtypes, such as direct, indirect, and total, were associated with increased risk of metabolic syndrome. Particularly, such association was significant, apparent, and consistent with direct bilirubin. This cross-sectional study could be one of the newest studies that evaluated the association between bilirubin subtypes and metabolic syndrome among the Korean population.

Metabolic syndrome is known as an important predictor of CVD [10]. Numerous studies have determined the associations between total serum bilirubin and CVD. In the relationship of total bilirubin to CVD, inverse associations were found in retrospective studies while U-shaped associations were observed in prospective studies [13–15]. Several studies have been published on the relationship between total serum bilirubin and metabolic syndrome. In these studies, inverse associations between total bilirubin and metabolic syndrome were determined; however, the importance of bilirubin, as an antioxidant, in metabolic syndrome needs further investigation. At the same time, most studies on the epidemiology of metabolic syndrome in relation to bilirubin were limited to total bilirubin concentrations [11, 12]. There was only one study showing the association between serum bilirubin subtypes and metabolic syndrome [17].

In this study, total bilirubin as well as direct and indirect bilirubin were analyzed in relation to metabolic syndrome in Korean men and women. This study indicated the apparent appearance of direct bilirubin in the association with metabolic syndrome. From a recent study, the highest quartile of serum direct bilirubin had a 40–70% reduced risk of metabolic syndrome compared to the lowest quartile in both men and women. In addition, women of the top quartile had a 35% reduction of the metabolic syndrome in serum total bilirubin and 20% reduction in serum indirect bilirubin [17]. In another study about outcomes from patients with ischemic stroke, elevated levels of direct bilirubin in patients had an acute severe stroke when compared to those with lower levels of direct bilirubin, which implies the role of bilirubin as an antioxidant in acute phase. Interestingly, no significant relationship between total bilirubin concentration and initial stroke severity, or first diagnosed stroke severity, was found among patients with ischemic stroke. Although serum bilirubin, whether it is total or direct, acts as an indicator of oxidative stress, it is unclear why direct bilirubin showed a significant association with initial stroke severity, whereas total bilirubin did not [16]. Some studies indicated that direct bilirubin might act on the target organ and molecule because direct bilirubin is weakly bound to albumin, thus easily separated from albumin and be in active form compared to other bilirubin subtypes [18]. Still, a variety of epidemiological studies among individuals with general medical conditions including hepatic disease have suggested that direct bilirubin concentrations may be of better prognostic values than total bilirubin concentrations [16–21]. Further investigation should be performed for determination of the diagnostic value of serum bilirubin, particularly direct bilirubin, in relation to diseases such as CVD and metabolic syndrome.

As shown in this study, bilirubin concentrations had significant associations with metabolic syndrome within most of the metabolic syndrome components subgroups including central obesity, hypertriglyceridemia, hyperglycemia and low HDL-C for both genders, particularly with direct bilirubin. Our current findings support the view that serum bilirubin concentrations within the normal range might be in association with serum lipid profile such as triglyceride and HDL-C as risk factors for CVD under non-pathological conditions [22]. Additionally, high triglyceride and low HDL-C in association with low concentrations of bilirubin implies the possible relationship between bilirubin concentrations and insulin resistance. However, some investigators observed no association between total bilirubin concentrations, triglyceride and HDL-C [23].

Serum bilirubin has antioxidant, anti-inflammatory properties and anti-atherogenic properties through inhibition of low-density lipoprotein oxidation [1, 24–26]. With an experiment in vivo, ascorbic acid, a documented physiological antioxidant, helped reduce the secretion of bilirubin metabolites and suppress the endotoxin-stimulated hepatic concentration of heme oxygenase (HO) mRNA [18]. In addition, bilirubin, as well as induction of HO-1 by hemin, prevented this stress-invoked response, suggesting that HO activity and/or bilirubin serve a protective function against injury-mediated proliferation of intimal cells [27]. Human studies also have led to similar conclusions that bilirubin production is involved in antioxidant defense mechanisms and that higher bilirubin concentrations are associated with a lower incidence of oxygen radical mediated injury [28, 29]. In the presence of metabolic syndrome, an increase in oxidative damage

decreased superoxide dismutase activity and increased lipid peroxidation malondialdehyde levels. These are evidenced by decreased antioxidant protection such as bilirubin [30].

The limitation of our study was that it is a cross-sectional study. This cross-sectional study precludes the determination of causality. Selection bias may exist as most participants included in the study are volunteers who may have had healthier lifestyle. In addition, bilirubin concentration was measured only once. However, the advantage of this study is that this relatively large-sample was studied to evaluate the inverse association between bilirubin concentrations and metabolic syndrome. To clarify the results, apparently healthy population without high possibility of having Gilbert's syndrome (total bilirubin concentrations > 34.2 μmol/l) [12] and any other diseases affecting metabolic syndrome were included in this study. Gilbert syndrome is a genetic disorder of bilirubin metabolism which can result in production of elevated levels of indirect bilirubin in the bloodstream without any serious consequences [31]. In Korea, participants with Gilbert's syndrome showed to have low prevalence and incidence of coronary heart diseases and higher levels of high density lipoprotein and antioxidant levels [32]. In this study, we could not find any differences in the risk of metabolic syndrome when we included the participants with Gilbert's syndrome. Serum bilirubin concentration was shown to have significant associations with metabolic syndrome. This study used the Korean population, whereas the aforementioned studies of the association of serum bilirubin to the metabolic syndrome were limited in Caucasians, hardly Asians.

In conclusion, serum bilirubin, whether it was total or direct or indirect, were inversely associated with metabolic syndrome in Korean men and women. In bilirubin subtypes, direct bilirubin concentration was apparent and consistent in the association with metabolic syndrome, even in the metabolic components subgroups, particularly, central obesity, hypertriglyceridemia, hyperglycemia and low HDL-C groups. Bilirubin is easily measured in many clinical laboratories and costless. Although low levels of serum bilirubin, particularly direct bilirubin, explain only a small part of clinical susceptibility of metabolic syndrome in the population, measurements of serum bilirubin could be helpful for prevention of serious diseases such as metabolic syndrome and CVD. Still, the prognostic value of direct bilirubin concentration is unclear and determination of the significance of direct bilirubin, as an antioxidant, also needs further studies. Further studies are also needed for determination of the association between serum bilirubin and insulin resistance.

Materials and methods

Study subjects

This study included 9,995 Koreans (men: 5,824, women: 4,171) aged 30–87 years who visited the Health promotion centers in Seoul for routine health examinations from April, 2006 to June, 2007. Detailed purpose and methods of the data collection were described in our previous study [32]. Of the 9,995 participants, 3,725 participants aged < 30 years were excluded as having missing data for serum bilirubin subtypes, such as total, direct, and indirect bilirubin. Another 611 participants with a past history of cardiovascular diseases and liver diseases written in the self-questionnaire were excluded. Furthermore, participants with total bilirubin concentrations > 34.2 μmol/l (2 mg/dl) were excluded due to high possibility of Gilbert's syndrome [12]. Additional 428 participants with missing data for metabolic syndrome risk factors, such as systolic blood pressure, diastolic blood pressure, total cholesterol, waist circumference, triglyceride and fasting serum glucose, and for alcohol consumption were excluded. Therefore, the study population was finalized as 5,231 Koreans (men: 3,008, women: 2,223). Recruitment of volunteers only took place after obtaining written informed consents. The institutional Review Board of Human Research of Yonsei University approved the study.

Data collection

During a standardized examination at the Health promotion centers, participants were asked if they have ever smoked or if they exercised regularly via a standardized health questionnaire. Their demographic characteristics such as age, gender, and family and past history of clinical diseases, cigarette smoking status (never smoker, ex smoker, and current smoker), and alcohol consumption status (never-drinker and ever-drinker) were also collected.

For the clinical chemistry assay, serum was separated from peripheral venous blood samples that were obtained from each participant after 12 h of fasting. Serum bilirubin concentrations and metabolic syndrome components, such as fasting blood glucose, total cholesterol, triglyceride, and HDL-C were measured using the Hitachi-7600 analyzer (Hitachi Ltd., Tokyo, Japan). Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). A waist circumference was measured midway between the lower rib and iliac crest.

Definition of metabolic syndrome

Metabolic syndrome was defined by the Third report of the National Cholesterol Education Program Expert Panel on

Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (NCEP/ATP III) [33]. High waist circumferences were defined as ≥90 cm for men and ≥80 cm for women according to Asia-Pacific criteria [34]. Participants with at least three of the following five criteria were considered to have metabolic syndrome:

(1) Central obesity: waist circumference ≥ 90 cm (men), 80 cm (women).
(2) High blood pressure: systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg.
(3) Hyperglycemia: fasting blood sugar ≥ 110 mg/dl.
(4) Hypertriglyceridemia: triglyceride ≥ 150 mg/dl.
(5) Low high density lipoprotein cholesterol (HDL-C): HDL-C < 40 mg/dl (men), 50 mg/dl (women).

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Differences between groups with and without metabolic syndrome were assessed by Student's t test for continuous variables or by χ^2 test for categorical variables in men and women, respectively. Pearson correlation analyses were performed to express the correlation between direct, indirect, or total bilirubin concentration and metabolic syndrome components. Serum bilirubin concentrations were classified into quartiles [direct bilirubin: $0 < Q1 < 5.00$ μmol/l, $5.00 \leq Q2 < 6.50$ μmol/l, $6.50 \leq Q3 < 8.55$ μmol/l, and $8.55 \leq Q4$; indirect bilirubin: $0 < Q1 < 5.13$ μmol/l, $5.13 \leq Q2 < 7.35$ μmol/l, $7.35 \leq Q3 < 10.26$ μmol/l, and $10.26 \leq Q4$; and total bilirubin: $0 < Q1 < 10.26$ μmol/l, $10.26 \leq Q2 < 13.68$ μmol/l, $13.68 \leq Q3 < 18.81$ μmol/l, and $18.81 \leq Q4$]. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated, and analyses were performed separately for men and women. Logistic regression analyses were conducted to display the association between metabolic syndrome and different circulating forms of bilirubin concentrations (direct, indirect, and total) by quartiles. SAS statistical software, version 9.0 (SAS Institute Inc, Cary, NC), was used in all analyses. A statistical significance was considered as $P < 0.05$ when needed.

Acknowledgments This study was supported by a grant (10526) from the Seoul City R&BD program and by a grant from the National R&D Program for Cancer Control, Ministry for Health, Welfare and Family affairs, Republic of Korea (0920330).

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