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Direct bilirubin is associated with low-density lipoprotein subfractions and particle size in overweight and centrally obese women

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KEYWORDS
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Abstract *Background and aims:* Bilirubin has antioxidant and anti-inflammatory properties; serum bilirubin levels have been known to be inversely associated with cardiovascular disease. However, the effects of different bilirubin subtypes on cardiometabolic traits are unknown. In this study, we aimed to determine whether direct bilirubin is more strongly correlated with small, dense low-density lipoprotein (sdLDL) compared to other bilirubin subtypes. We also investigated which LDL subfractions exhibited the highest correlation with direct bilirubin.
Methods and results: A total of 288 overweight and centrally obese women were included in this study. The Pearson correlation and Steiger's Z test were used to compare the correlation coefficients between bilirubin subtypes and lipoproteins. Multiple linear regression analyses were used to evaluate the independent association between direct bilirubin and mean LDL particle size. Only direct bilirubin levels were significantly associated with the sdLDL subfraction and mean LDL particle size. Mean LDL particle size exhibited a significantly stronger correlation with direct bilirubin than sdLDL, percent sdLDL, and the sdLDL:large LDL ratio. Regression analysis showed that direct bilirubin was significantly associated with mean LDL particle size, according to both the stepwise method ($\beta = 11.445$, P value $= 0.002$) and the enter method ($\beta = 11.655$, P value $= 0.002$).
Conclusions: Direct bilirubin is more strongly correlated with the sdLDL subfraction compared with total and indirect bilirubin, and is independently associated with mean LDL particle size in overweight and centrally obese women.

Introduction

Bilirubin is a degradation product of heme proteins that has long been considered a waste product that is harmful to the central nervous system; it is also an indicator of abnormal liver function [1,2]. Elevated bilirubin could be caused by diseases, resulting in unconjugated hyperbilirubinemia or conjugated hyperbilirubinemia [3].

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Conditions that enhance bilirubin production (e.g. hemolysis, dyserythopoiesis) and reduce the rate of biliary secretion (e.g. biliary obstruction, intrahepatic cholestasis) lead to increased levels of indirect and direct bilirubin [4].

Serum bilirubin levels are affected not only by intrinsic factors, such as gender and certain enzyme levels [UDP-glucuronosyltransferase and biliverdin reductase (BVR)], but also by external factors such as stress, fasting, and certain medications [5]. However, many studies have found that modestly elevated serum bilirubin levels are inversely associated with metabolic syndrome [6], diabetes [7], and cardiovascular disease [8]. Heme is catalyzed into biliverdin by heme oxygenase, and biliverdin is converted into bilirubin by BVR [9]. In this continuous biliverdin/bilirubin redox cycle, bilirubin acts a potent antioxidant and has anti-inflammatory activity [10]. Bilirubin circulates in the bloodstream as both indirect and direct bilirubin forms, and total bilirubin is the sum of these moieties. Several studies have suggested that both indirect and direct bilirubin have antioxidant properties [11]. However, the differences between bilirubin subtypes with respect to metabolic traits and antioxidant capacities are unknown.

Low-density lipoprotein (LDL) particle size is a crucial factor in cardiovascular disease [12]. Specifically, ischemic heart disease (IHD) is largely attributed to small, dense LDL (sdLDL) particles, while the association between large LDL particles and IHD is inconsistent [13,14]. Consistent with this observation, sdLDL particles are more easily oxidized and atherogenic than are either normal or large LDL particles [15]. Oxidized LDL leads to the release of proinflammatory cytokines by endothelial cells [16]. Several studies have shown that bilirubin impedes the oxidation of LDL [17] and decreases the formation of sdLDL [18]. For example, Tapan et al. [18] suggested that the amount of sdLDL is reduced in subjects with Gilbert's syndrome, and Kang et al. [8] found that lower bilirubin levels are associated with an increased abundance of calcified plaques.

Direct bilirubin has been investigated in epidemiologic studies as a biomarker, and is more closely associated with metabolic syndrome [19], ischemic stroke [20], and gestational diabetes mellitus than is either total bilirubin or indirect bilirubin [21]. In addition, a previous study suggested that direct bilirubin is a more available form of bilirubin than is indirect bilirubin [22]. However, few studies have investigated the relationship between levels of direct bilirubin, LDL subfraction, and LDL particle size, which are important contributors to cardiovascular disease.

The aims of our study were to determine whether direct bilirubin exhibits a stronger correlation with the sdLDL subfraction compared with other bilirubin types and to determine which LDL subfraction is most closely correlated with direct bilirubin.

Methods

Study population

A total of 288 overweight and centrally obese women who voluntarily visited the obesity clinic at Severance Hospital in Seoul, Korea, from October 2010 to August 2016, were included in the study. This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Gangnam Severance Hospital. Apparently healthy overweight and centrally obese subjects, aged 18–65 years without underlying medical conditions, were included in this study. Subjects with a body mass index (BMI) greater than or equal to 23 kg/m^2 and/or the presence of central obesity (waist circumference $\geq$ 80 cm) were included in this study [23]. Patients with a history of malignancy or of renal, thyroid, chronic liver (e.g. cirrhosis, hepatitis B or hepatitis C) or cardiovascular disease were excluded. We also excluded patients with participants with abnormal liver enzyme level of aspartate aminotransferase (AST) or alanine aminotransferase (ALT) $\geq$ 2.5 times above the upper limit.

Anthropometric and clinical data

Past and current histories of all medical conditions and health-related behaviors were collected from the patients' medical records. Physical measurements were obtained by trained medical staff. Body weights and heights were measured to the nearest 0.1 kg and 0.1 cm, respectively. BMI was calculated as body weight divided by height squared. Waist circumference was measured midway between the costal margin and the iliac crest with patients in the standing position. Systolic blood pressure (SBP) and diastolic blood pressure were measured twice by sphygmomanometry, and the average of two measurements was recorded. Smoking status was defined as positive if the subject was a current smoker. Positive alcohol intake was defined as the consumption of more than 20 g alcohol greater than once per week. Menopause was defined as amenorrhea for 12 consecutive months.

Biochemical measurements

All blood samples were collected from the antecubital vein after a 12-h overnight fast. Fasting plasma glucose, total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, AST, and ALT levels were measured by enzymatic methods using a chemistry analyzer (Hitachi 7600-110; Tokyo, Japan). Fasting insulin was measured by electrochemiluminescence immunoassay using an Elecsys 2010 (Roche, Indianapolis, IN, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: fasting plasma glucose (mg/dL) $\times$ fasting insulin (μIU/mL)/405. Serum total and direct bilirubin levels were measured using 3,5-dichlorophenyl diazomium tetrafluoroborate on a Beckman coulter analyzer (Beckman coulter Inc., Fullerton, CA, USA).

Lipoprotein profiles

LDL subfractions were scanned using a Quantimetrix Lipoprint™ system (Quantimetrix, Redondo Beach, CA, USA),

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