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Association of lower total bilirubin level with statin usage: The United States National Health and Nutrition Examination Survey 1999-2008

Running title: Statins and total bilirubin level

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Abstract

Objective: A low circulating level of bilirubin is associated with increased cardiovascular risk. As statins can stimulate heme oxygenase-1 (HO-1), which increases bilirubin production, we investigated whether statins in routine use increase total bilirubin levels in subjects at high cardiovascular risk.

Methods**:** Data from 3290 subjects with self-reported history of hypercholesterolemia, diabetes, or cardiovascular diseases in the United States National Health and Nutrition Examination Survey (NHANES) 1999-2008 were analyzed.

Results: **S**ubjects taking statins (n=1156) had lower total bilirubin levels than those not taking any lipid-lowering medication (n=2134) after adjusting for age, sex, race/ethnicity, and survey period (adjusted mean=0.699 vs 0.729 mg/dl respectively, P=0.001). The association remained significant after adjusting for more covariates (P=0.002), but was attenuated after further adjusting for glycosylated hemoglobin, insulin resistance index, and low-density lipoprotein (LDL) cholesterol (P=0.043). The use of lovastatin, rosuvastatin, and cerivastatin was associated with lower total bilirubin levels in the full adjustment model (P<0.05).

Conclusion: The use of statins was associated unexpectedly with lower total bilirubin levels. This could be explained at least partly by the effect of statins on glycemia and LDL cholesterol. Our results do not suggest that the anti-oxidant and anti-inflammatory effects of statins are due to HO-1 induction and increased serum bilirubin levels.

Keywords: bilirubin; cardiovascular risk; heme oxygenase; NHANES; statins

1. Introduction

Bilirubin has antioxidant and anti-inflammatory effects [1]. It is the metabolic product of heme catabolism and circulates in the blood in either a direct (conjugated) or an indirect (unconjugated) form. Heme oxygenase (HO) is the rate-limiting enzyme in the degradation of heme to biliverdin, carbon monoxide, and ferritin. Biliverdin is then converted to bilirubin by the enzyme biliverdin reductase [2]. There are three isoforms of HO, namely, HO-1, HO-2, and HO-3 [3]. HO-1 is an inducible enzyme whereas HO-2 and HO-3 are constitutively expressed. Bilirubin can protect lipid from oxidation, and the deletion of HO-2 results in greater lipid than protein oxidation [4]. Bilirubin is a protective biomarker for cardiovascular risk. Previous studies have demonstrated an association of lower circulating total bilirubin levels with higher risk of the metabolic syndrome [5], coronary artery disease [6], peripheral arterial disease (PAD) [7], stroke [8], and other cardiovascular diseases (CVDs) [1]. A low circulating level of total bilirubin is also associated with increased body mass index (BMI), blood pressure, and insulin resistance [1,5].

HO-1 has been suggested as a promising therapeutic target in vascular diseases [9,10]. The increase in HO-1 expression, induced by either pharmacological methods or gene transfer, improves vascular dysfunction in animal models of various CVDs including atherosclerosis, thrombosis, myocardial infarction, and hypertension [10]. Moreover, HO-1 also plays a role in vascular repair by increasing circulating endothelial progenitor cells [11]. Previous studies have demonstrated that statins can activate HO-1, an observation that has been implicated as contributing to their anti-inflammatory and anti-proliferative effects [12-14]. Furthermore, the anti-oxidant effect of HO-1 induction by statins has been attributed to the reaction products of HO-1, biliverdin, and ferritin [14]. Therefore, statin treatment in human subjects with high cardiovascular risk may increase HO-1 activity and hence bilirubin

production. Therefore, we investigated whether statins in routine use could affect total bilirubin levels using data from the US National Health and Nutrition Examination Survey (NHANES). This could help to explain whether the anti-oxidative and anti-inflammatory effects of statins may be mediated through induction of HO-1 and an increase in serum bilirubin levels.

2. Methods

2.1. Study subjects

NHANES was conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention to monitor the health and nutritional status of the civilian, non-institutionalized US population [15]. Since 1999, NHANES has become a continuous cross-sectional survey program and data are released for every two-year cycle. The detailed measurement procedures and protocols are described in its website [15]. All participants gave informed consent and the study received approval from the Centers for Disease Control and Prevention Institutional Review Board. In NHANES 1999-2008, after excluding pregnant women, there were 23,539 subjects aged ≥20 years who were both interviewed and examined in the mobile examination center.

As serum triglycerides and low-density lipoprotein (LDL) cholesterol were measured only in subjects who were examined in the morning session and had fasted for 8-24 hours [15], our analysis was limited to 9891 subjects. Among these subjects, information on prescription medications for lowering blood lipids was available in 9835 subjects with 3824 of them having a self-reported history of hypercholesterolemia, diabetes, or CVDs. After further excluding subjects with abnormal liver function (defined as a serum aspartate aminotransferase [AST] or alanine aminotransferase [ALT] >100 U/l, a serum

gamma-glutamyltransferase [GGT] >100 U/l, or total bilirubin level >3 mg/dl) [16] or a self-reported history of liver diseases, there were 3397 subjects. A total of 107 subjects taking lipid-lowering medications other than statins, such as fibrates, bile acid sequestrants, and nicotinic acid were also excluded from the analysis. Therefore, 3290 subjects were included in this analysis.

2.2. Variables of interest

The usage of lipid lowering medications in the past month was assessed by questionnaires. Subjects who reported the use of atorvastatin, simvastatin, pravastatin, lovastatin, rosuvastatin, fluvastatin, or cerivastatin were defined as receiving statin treatment, regardless of whether the drugs were taken as one combination pill or as two different pills. Subjects with unspecified lipid-lowering medications were excluded from the analysis (n=17). Information on race/ethnicity, history of hypercholesterolemia, diabetes, CVDs (heart attack, congestive heart failure, coronary heart disease, angina, or stroke) and liver diseases were obtained from self-reported questionnaires. Pregnancy was determined in women by a self-reported questionnaire and a urine pregnancy test. BMI was calculated as weight in kilograms divided by the square of height in meters. Hypertension was defined as blood pressure ≥140/90 mmHg or taking anti-hypertensive medications. Smokers were defined as subjects who had smoked ≥100 cigarettes in their lives. Current and former smokers were then classified based on the question, “Do you now smoke cigarettes?”. Alcohol drinking was defined as consumption of any type of alcoholic beverage at least once a week in the past one year.

2.3. Laboratory measurement

The laboratory analytical methods used in NHANES have been described in detail elsewhere

[15]. Briefly, serum creatinine, alkaline phosphatase (ALP), ALT, AST, GGT, and total bilirubin levels were measured as the routine laboratory blood parameters using an Hitachi Model 704 multichannel analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN) in 1999-2000, a Beckman Synchron LX20 system in 2003-2007 and a Beckman UniCel® DxC800 Synchron (Beckman Coulter Inc, Fullerton, CA) in 2008. In 2001-2002, these levels were measured using either an Hitachi Model 704 multichannel analyzer or a Beckman Synchron LX20. The reported values have been adjusted by regression equations to allow comparison across the two methods [15]. Correction for serum creatinine data in 1999-2000 and 2005-2006 according to the NHANES protocol [15] was performed before calculating the glomerular filtration rate (GFR). GFR was estimated from the modified prediction equation from the Modification of Diet in Renal Disease study [17]. Total bilirubin levels, but not direct or indirect bilirubin levels, were measured in NHANES 1999-2008.

Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured with an Hitachi Model 704 multichannel analyzer in 1999-2004, a Roche Hitachi 717 (Roche Diagnostics, Indianapolis, IN) in 2005, both Roche Hitachi 717 and 912 (Roche Diagnostics, Indianapolis, IN) in 2006, and a Roche Modular P chemistry analyzer (Roche Diagnostics, Indianapolis, IN) in 2007-2008. LDL cholesterol was calculated using the Friedewald equation. Fasting glucose was measured by a Cobas Mira Chemistry System (Roche Diagnostic Systems, Indianapolis, IN) in 1999-2004, a Roche/Hitachi 911 (Roche Diagnostics, Indianapolis, IN) in 2005-2006, and a Roche Modular P Chemistry Analyzer in 2007-2008. Fasting insulin was measured using a Pharmacia Insulin RIA kit (Pharmacia Diagnostics AB, Uppsala, Sweden) in 1999-2002, a Tosoh AIA-PACK IRI (a two-site immunoenzymometric assay) in 2003-2004, and an ELISA kit (Mercodia AB, Uppsala, Sweden) in 2005-2008. Glycosylated hemoglobin (HbA1c) was determined by a Primus

CLC330 or a Primus CLC 385 (Primus Corporation, Kansas City, MO) in 1999-2004, a Tosoh A1c 2.2 Plus Glycohemoglobin Analyzer (Tosoh Medics Inc, San Francisco, CA) in 2005-2006, and an A1c G7 HPLC Glycohemoglobin Analyzer (Tosoh Medics Inc, San Francisco, CA) in 2007-2008. Data on fasting glucose, insulin, and HBA1c were adjusted using regression equations so that measurements across survey periods could be combined [15]. Insulin resistance was assessed by the homeostasis model assessment of insulin resistance index (HOMA-IR) [18]. C-reactive protein (CRP) was measured by a Dade Behring Nephelometer II Analyzer System (Dade Behring Diagnostics Inc, Somerville, NJ).

2.4. Statistical analysis

Data analysis was performed using the complex sample function of SPSS version 18.0 (SPSS Inc, Chicago, IL). Data are expressed as mean or percent (SE). Variables with skewed distributions were log-transformed before analysis and are expressed as a geometric mean (95% CI). Fasting sampling weights were used in all analyses to adjust for non-response bias and the oversampling of blacks, Mexican Americans, and the elderly among the fasting sub-sample in NHANES [15]. Multiple linear regression was used to adjust the association for covariates. Variables were used as covariates in the regression model if they were significantly different between subjects with and without statin treatment. For variables that were highly correlated, only one was entered into the regression analysis. To take account for the variations which may be due to changes in measurement sites and methods, survey year was included in the regression model as a categorical independent variable. The *P* for interaction was estimated by including each multiplicative interaction term in the linear regression models after adjusting for the main effects of all covariates.

3. Results

In this study, there were 2134 (percent [SE] = 66.0 [1.1] %) subjects without taking any lipid-lowing medication and 1156 (percent [SE] = 34.0 [1.1] %) subjects taking statins. Among 1156 subjects taking statin, 1022 (percent [SE] = 87.2 [1.2] %) subjects took statins as the only lipid-lowering medication. Table 1 shows the clinical characteristics of these subjects. Compared to subjects not on any lipid-lowing medication, those taking statins were more likely to be men and non-Hispanic white. They had higher mean age, BMI, HbA1c, fasting glucose, fasting insulin, HOMA-IR, systolic blood pressure, ALT and GGT, but lower total cholesterol, LDL cholesterol, GFR, and CRP level. The percentage of former smoking, and prevalence of coronary heart disease, angina, heart attack, any CVD, diabetes, hypercholesterolemia and hypertension were all significantly higher in subjects taking statins than those without taking any lipid-lowering medication. Similar results were obtained when subjects not on any lipid-lowing medication were compared to subjects taking statins as the only lipid-lowering medication, except that the difference in the prevalence of any CVD, GFR, and CRP level did not reach statistical significance (Table 1).

As shown in Table 2, although subjects taking statins had a higher unadjusted level of total bilirubin than those not taking any lipid-lowering medication, this trend was reversed after adjustment for age, sex, race/ethnicity, and survey period (model 1, P=0.001). In this adjustment model, the adjusted mean (SE) of total bilirubin level was 0.729 (0.008) mg/dl in subjects not taking any lipid-lowering medication and 0.699 (0.008) mg/dl in subjects taking statins (P=0.001). Further adjustment for BMI, smoking, hypertension, history of any CVD, GFR, CRP, ALT, and GGT did not attenuate the association (model 2, P=0.002). However, the association was attenuated after further adjusting for HbA1c, HOMA-IR, and LDL cholesterol (models 3 and 4, P=0.020 and 0.043, respectively). There was no significant interaction between statin usage and any of the covariates (P for interaction >0.05). A

similar trend was obtained after exclusion of 134 subjects taking other types of lipid-lowering drugs, in which the association was not significant in the full adjustment model (Table 2).

We then investigated whether different types of statins may differ in their effect on total bilirubin level. One subject, who took more than one type of statin (i.e. atorvastatin and cerivastatin), was excluded from the analysis. As shown in Table 3, the use of lovastatin, rosuvastatin, and cerivastatin were associated with lower total bilirubin levels in the full adjustment model (P=0.001, 0.028, and 0.004 respectively), in which the effect size of cerivastatin tended to be the greatest (regression coefficient [SE] = -0.098 [0.033] mg/dl). When subjects taking lipid-lowering medications other than statins were excluded, similar results were obtained, in which the use of simvastatin was also associated with lower total bilirubin level with a borderline significance (regression coefficient [SE] = -0.044 [0.022] mg/dl, P=0.046 in full adjustment model). In a separate analysis, replacement of LDL cholesterol with total cholesterol in the regression models as shown in Tables 2 and 3 did not affect the results significantly (data not shown).

4. Discussion

Statins can lower LDL cholesterol by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and are used widely to reduce CVD risk [19]. Besides their lipid-lowering effect, statins also possess other properties such as the inhibition of inflammation and oxidation, and the attenuation of endothelial dysfunction and vascular remodeling [19]. The anti-oxidant and anti-inflammatory effects of statins exist even at routine clinical dosage, as demonstrated by the reduction of systemic levels of oxidative stress markers such as protein-bound nitrotyrosine [20] and inflammatory markers such as CRP [21,22]. As statins can stimulate HO-1 and increase the production of bilirubin

[12-14,23], we expected that statin usage would be associated with a higher total bilirubin level, which may explain part of their anti-oxidant and anti-inflammatory effects. However, in this study, statin usage was unexpectedly associated with a lower total bilirubin level among subjects at high cardiovascular risk (i.e. with a self-reported history of diabetes, hypercholesterolemia, or CVDs) in NHANES 1999-2008. Therefore, the anti-oxidant and anti-inflammatory effects of statins reflect the activation of antioxidant and anti-inflammatory pathways that are independent of HO-1 induction.

Previous cell and animal studies have focused on the effect of statins on HO-1 expression and activity. Lee et al. first reported that simvastatin can activate HO-1 in human and rat aortic vascular smooth muscle cells, but not in endothelial cells or macrophages [12]. Ali et al. then demonstrated that HO-1 plays a central role in the anti-oxidant properties of atorvastatin in human vascular endothelial cells through the generation of biliverdin and ferritin [14]. In mice, the plasma total bilirubin level increased after three weeks of treatment with rosuvastatin or atorvastatin, without affecting other liver enzyme activities (ALT, AST, ALP, and GGT) [23].

The long-term effect of statins on bilirubin levels in humans is not known. In a randomized trial, the use of pravastatin in patients with advanced hepatocellular carcinoma was associated with a lower serum total bilirubin level after one year compared to the control group [24]. However, the sample size of this study was small and the effect of pravastatin on bilirubin levels could have been confounded by the presence of chronic liver disease. The present study is therefore the first report showing that statin treatment is associated with a lower total bilirubin level in subjects at high cardiovascular risk.

A low circulating level of bilirubin is associated with increased cardiovascular risk [1]. For example, each 0.1 mg/dl increase in total bilirubin level is associated with 6% and 9% decrease in the odds of PAD and stoke, respectively [7,8]. Therefore, the reduced bilirubin level in subjects treated with statins could predispose them to higher cardiovascular risk. As statin can stimulate HO-1 activity in cell and animal studies [12-14,23], the mechanism underlying the association of statin usage with a lower, rather than a higher, total bilirubin level in the present study is not clear. However, HO-1 can have pro-oxidative effect, in addition to its well-studied anti-oxidative effect [25]. Moreover, the effect of statins on *in vivo* HO-1 expression is tissue-specific [13] and thus the circulating level of bilirubin may not reflect the local tissue-specific effect of statins on HO-1 induction. The decrease in total bilirubin levels in subjects with statin treatment may probably be due to the inhibition of HO-1 activity in some tissues, or increased degradation or excretion of bilirubin by statins. Further studies are needed to investigate these speculative mechanisms.

Statins vary in their ability to lower LDL cholesterol and raise HDL cholesterol [26,27]. For example, in a recent meta-analysis, rosuvastatin was found to lower LDL cholesterol and apolipoprotein B more effectively than atorvastatin and simvastatin at the same dose [26]. The effect of statins on *in vivo* HO-1 expression is also statin-specific, with lovastatin having a larger effect than simvastatin, atorvastatin, and rosuvastatin in the liver [13]. These differential effects of statins on blood lipids and HO-1 expression may contribute to the differences in the association of different types of statins with total bilirubin level in this study. Among different types of statins, cerivastatin is associated with the largest decrease in total bilirubin level (Table 3).

In the present study, the association of total bilirubin level with the use of statin was

attenuated after further adjustment for HbA1c, HOMA-IR, and LDL cholesterol. Therefore, the statin-associated decrease in total bilirubin level could be explained at least partly by the effect of statins on glycemia and LDL cholesterol. As other lipid-lowering medications may have the possibility to affect HO-1 activity and bilirubin production, subjects taking other concomitant types of lipid-lowering medications such as fibrates, bile acid sequestrants, and nicotinic acid were excluded in a separate analysis and similar results were still observed in this study.

Despite the adverse effect on total bilirubin level, statins can lower LDL cholesterol and reduce CVD risk [21,22,28,29]. These beneficial effects of statins overweigh their small but negative effects on total bilirubin level. In fact, there is a residual cardiovascular risk in subjects treated with statins, which could be contributed by the low HDL cholesterol [29]. Whether the reduced total bilirubin level in subjects treated with statins could contribute to this residual risk remains unknown and needs to be further investigated.

Our study has some limitations. There is a lack of data on the circulating level of HO-1. HO-1 levels are elevated in subjects with chronic diseases such as type 2 diabetes [30], probably as a protective mechanism against oxidative stress and inflammation. Another limitation is the small sample size in NHANES, which limits the power to perform sub-group analysis. However, our study has the advantage of using data from NHANES with good sampling design, quality control, and nationally representative estimates. As different types of statins show differential effects in the association with total bilirubin levels (Table 3), the mixed analysis in all statin group (Table 2) might have influenced the result. A major limitation of this study is that subjects treated with statins have a high cardiovascular risk profile. Due to the cross-sectional nature of the NHANES survey, it is difficult to establish

the causal and temporal relationship between statin therapy and total bilirubin levels. Therefore, future studies of bilirubin levels before and after statin treatment will be of crucial importance in order to investigate the nature of such relationships.

5. Conclusion

We found an unexpected association of statin usage with lower total bilirubin level in subjects at high cardiovascular risk in NHANES 1999-2008. Our results do not suggest that statins in routine use exert anti-oxidative and anti-inflammatory effects by inducing HO-1 and increasing serum bilirubin levels.

Conflicts of Interests

None

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Table 1. Clinical characteristics among subjects in NHANES 1999-2008.

Characteristics	No lipid-lowering medication	Taking statins	
		All subjects	Subjects with statins as the only lipid-lowering medication†
n	2134	1156	1022
Age, y	53.0 (0.5)	62.2 (0.5)‖	62.4 (0.5)‖
Women, %	54.6 (1.5)	48.2 (1.6)‖	49.3 (1.7)‖
BMI, kg/m^2	29.3 (0.2)	29.7 (0.2)§	29.7 (0.2)§
Race/ethnicity, %			
Non-Hispanic white	73.3 (1.7)	81.2 (1.7)§	80.6 (1.9)‡
Non-Hispanic black	11.2 (1.1)	7.5 (1.0)‡	8.1 (1.1)
Mexican American	6.1 (0.8)	3.3 (0.5)‖	3.2 (0.5)§
Others	9.5 (1.1)	8.1 (1.3)	8.0 (1.3)
Smoking, %			
Never	47.9 (1.4)	43.1 (1.6)	44.1 (1.7)
Former	30.9 (1.1)	41.9 (1.4)‡	41.7 (1.6)‡
Current	21.1 (1.2)	15.1 (1.4)	14.2 (1.4)
Alcohol drinking, %	30.3 (1.4)	29.4 (2.1)	28.5 (2.3)
History of CVD, %			
Congestive heart failure	5.0 (0.6)	7.3 (0.9)	6.9 (0.9)
Coronary heart	4.5 (0.5)	19.7 (1.2)‖	16.5 (1.3)‖

disease			
Angina	4.6 (0.6)	12.8 (1.1)‖	11.1 (1.1)‖
Heart attack	6.1 (0.6)	15.7 (1.1)‖	14.1 (1.2)§
Stroke	6.6 (0.7)	9.1 (0.9)	9.3 (1.0)
Any of the above	17.4 (1.2)	31.8 (1.6)§	28.7 (1.9)
HbA1c, %*	5.61 (5.55-5.67)	5.89 (5.82-5.97)‖	5.88 (5.80-5.97)‖
Fasting glucose, mmol/l*	6.02 (5.95-6.10)	6.32 (6.22-6.42)§	6.34 (6.23-6.45)‖
Fasting insulin, mU/l*	8.3 (8.0-8.7)	9.6 (9.1-10.1)‖	9.4 (8.8-9.9)‖
HOMA-IR*	2.23 (2.12-2.34)	2.69 (2.53-2.86)‖	2.64 (2.47-2.82)‖
Diabetes, %	18.2 (1.1)	23.6 (1.6)§	23.4 (1.7)‡
Total cholesterol, mmol/l	5.75 (0.03)	4.83 (0.04)‖	4.86 (0.05)‖
HDL cholesterol, mmol/l	1.35 (0.01)	1.34 (0.01)	1.35 (0.01)
LDL cholesterol, mmol/l	3.56 (0.03)	2.65 (0.03)‖	2.69 (0.04)‖
Triglycerides, mmol/l*	1.60 (1.55-1.65)	1.60 (1.54-1.67)	1.60 (1.54-1.67)
Hypercholesterolemia, %	79.7 (1.3)	91.4 (1.0)‖	91.4 (1.1)‖
SBP, mmHg	125.9 (0.5)	128.1 (0.8)‡	128.5 (0.7)
DBP, mmHg	72.1 (0.4)	68.3 (0.6)	68.6 (0.6)
Hypertension, %	39.4 (1.4)	65.9 (1.8)‖	65.9 (1.8)‖
GFR, ml/min/1.73m^2	83.8 (0.7)	75.0 (0.9)‡	75.3 (0.9)

CRP, mg/dl*	0.25 (0.23-0.27)	0.21 (0.19-0.24)‡	0.22 (0.20-0.25)
ALP, U/l*	68.0 (66.7-69.3)	68.3 (66.8-69.8)	68.7 (67.2-70.4)
ALT, U/l*	22.3 (21.8-22.8)	22.8 (22.3-23.4)‡	22.6 (22.0-23.2)‡
AST, U/l*	22.6 (22.2-22.9)	23.8 (23.4-24.2)	23.5 (23.1-24.0)
GGT, U/l*	22.8 (22.1-23.5)	24.2 (23.3-25.1)‡	24.3 (23.4-25.3)§

Data are expressed as mean or percent (SE), or geometric mean (95% CI) unless otherwise noted.

*Data were log-transformed before analysis.

†Defined as subjects not taking other types of lipid-lowering medications (such as fibrates, bile acid sequestrants, and nicotinic acid) concomitantly.

‡$P<0.05$, §$P<0.01$, and ‖$P<0.001$ for comparison between subjects without any medication and those taking statins after adjusting for age, sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican Americans, and others), and survey period (1999-2000, 2001-2002, 2003-2004, 2005-2006, and 2007-2008), where appropriate.

SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2. Association of total bilirubin level with the use of statins in NHANES 1999-2008.

	Unadjusted model	Model 1	Model 2	Model 3	Model 4
All subjects					
Total bilirubin level, mg/dl					
No medication (n=2134)	0.738 (0.009)	0.729 (0.008)	0.718 (0.009)	0.721 (0.009)	0.724 (0.010)
Statins (n=1156)	0.762 (0.008)	0.699 (0.008)	0.688 (0.009)	0.698 (0.010)	0.698 (0.012)
Regression coefficient (SE), mg/dl*	0.024 (0.010)	-0.030 (0.009)	-0.029 (0.009)	-0.023 (0.010)	-0.026 (0.013)
P	0.017	0.001	0.002	0.020	0.043
Subjects with statins as the only lipid-lowering medication					
Total bilirubin level, mg/dl					
No medication (n=2134)	0.74 (0.01)	0.730 (0.008)	0.718 (0.009)	0.721 (0.009)	0.724 (0.009)
Statins (n=1022)	0.76 (0.01)	0.703 (0.010)	0.688 (0.011)	0.697 (0.011)	0.700 (0.014)
Regression coefficient (SE), mg/dl*	0.019 (0.010)	-0.027 (0.010)	-0.030 (0.010)	-0.024 (0.011)	-0.024 (0.013)
P	0.068	0.010	0.006	0.027	0.072

Total bilirubin level is expressed as mean (SE).

Model 1: Adjusted for age, sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican Americans, and others), and survey period

(1999-2000, 2001-2002, 2003-2004, 2005-2006, and 2007-2008).

Model 2: Further adjusted for BMI, smoking (current, former, and never), hypertension, history of any CVD (no and yes), GFR, CRP (log-transformed), ALT (log-transformed), and GGT (log-transformed).

Model 3: Further adjusted for HbA1c (log-transformed) and HOMA-IR (log-transformed).

Model 4: Further adjusted for LDL cholesterol.

*Subjects not taking any lipid-lowering medication as the referent group.

Table 3. Association of total bilirubin level with the use of different types of statins in NHANES 1999-2008.

	n	% (SE)	Model 1			Model 2		
			Adjusted total bilirubin level, mg/dl	Regression coefficient (SE), mg/dl	*P*	Adjusted total bilirubin level, mg/dl	Regression coefficient (SE), mg/dl	*P*
All subjects								
No lipid-lowering medication	2134	66.1 (1.1)	0.729 (0.008)	referent	-	0.724 (0.010)	referent	-
Atorvastatin	482	14.6 (0.8)	0.717 (0.013)	-0.012 (0.012)	0.322	0.714 (0.016)	-0.010 (0.015)	0.518
Simvastatin	391	11.0 (0.7)	**0.694 (0.015)**	**-0.035 (0.016)**	**0.033**	0.689 (0.019)	-0.034 (0.020)	0.087
Pravastatin	105	3.0 (0.4)	0.718 (0.022)	-0.011 (0.024)	0.645	0.718 (0.023)	-0.006 (0.025)	0.819
Lovastatin	94	2.5 (0.3)	**0.624 (0.024)**	**-0.105 (0.025)**	**<0.001**	**0.632 (0.026)**	**-0.092 (0.027)**	**0.001**
Rosuvastatin	43	1.5 (0.3)	**0.617 (0.036)**	**-0.112 (0.036)**	**0.002**	**0.652 (0.032)**	**-0.072 (0.032)**	**0.028**
Fluvastatin	29	0.9 (0.2)	0.719 (0.047)	-0.010 (0.047)	0.836	0.732 (0.045)	0.008 (0.046)	0.857
Cerivastatin	12	0.5 (0.2)*	**0.658 (0.033)**	**-0.071 (0.034)**	**0.042**	**0.625 (0.031)**	**-0.098 (0.033)**	**0.004**
Overall trend	-	-	-	-	0.001	-	-	0.005
Subjects with statins as the only lipid-lowering medication								

No lipid-lowering medication	2134	69.1 (1.0)	0.730 (0.009)	referent	-	0.724 (0.010)	referent	-
Atorvastatin	444	14.1 (0.8)	0.726 (0.015)	-0.004 (0.013)	0.766	0.720 (0.018)	-0.004 (0.016)	0.823
Simvastatin	322	9.1 (0.6)	**0.685 (0.018)**	**-0.044 (0.019)**	**0.024**	**0.680 (0.021)**	**-0.044 (0.022)**	**0.046**
Pravastatin	94	2.7 (0.3)	0.728 (0.023)	-0.002 (0.025)	0.950	0.725 (0.023)	0.001 (0.026)	0.958
Lovastatin	88	2.5 (0.3)	**0.628 (0.024)**	**-0.102 (0.025)**	**<0.001**	**0.636 (0.026)**	**-0.088 (0.027)**	**0.002**
Rosuvastatin	37	1.3 (0.3)	**0.619 (0.032)**	**-0.111 (0.032)**	**0.001**	**0.645 (0.032)**	**-0.079 (0.031)**	**0.014**
Fluvastatin	25	0.7 (0.2)	0.761 (0.045)	0.032 (0.046)	0.491	0.766 (0.052)	0.042 (0.052)	0.427
Cerivastatin	11	0.5 (0.2)*	0.661 (0.035)	-0.069 (0.036)	0.061	**0.628 (0.031)**	**-0.096 (0.033)**	**0.005**
Overall trend	-		-	-	0.001	-	-	0.005

Total bilirubin level is expressed as mean (SE).

Model 1: Adjusted for age, sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican Americans, and others), and survey period (1999-2000, 2001-2002, 2003-2004, 2005-2006, and 2007-2008).

Model 2: Further adjusted for BMI, smoking (current, former, and never), hypertension, history of any CVD (no and yes), GFR, CRP (log-transformed), ALT (log-transformed), GGT (log-transformed), HbA1c (log-transformed), HOMA-IR (log-transformed), and LDL cholesterol.

*Estimate was unreliable due to coefficient of variation >0.30.