HDL cholesterol subclasses are associated with serum uric acid in Japanese men

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ABSTRACT

Objective Uric acid (UA) and high-density lipoprotein (HDL) subclasses are associated with inflammation, coronary heart disease, and metabolic syndrome (MetS). However, the relation between UA and HDL subclasses is not well understood.

Methods Subjects included 848 Japanese men not taking medication for hyperuricemia, hypertension, diabetes mellitus, dyslipidemia, or chronic renal disease; they underwent an annual health examination that included HDL subclass analyses.

Results When subjects were stratified by HDL2-C or HDL3-C levels, UA level decreased as HDL2-C level increased, while UA levels increased as HDL3-C levels increased. In a multiple linear regression analysis, age, waist circumference (WC), diastolic blood pressure (BP), logarithmic transformed triglyceride ln(TG) and HDL3 cholesterol (HDL3-C), were associated with UA level. In a multiple logistic regression analysis for upper tertile of UA (≥ 6.8 mg/dL), WC, diastolic BP, ln(TG), HDL2-C and HDL3-C were associated. Since this analysis indicated that MetS components were determinants of UA level along with HDL-C subclass, possible synergistic effects of HDL-C subclass and MetS components to determine UA level were assessed. A combination of the number of MetS components and stratification of HDL3-C affected UA levels; the mean UA level increased in subjects with increased MetS components and HDL3-C level.

Conclusion HDL-C subclasses were associated with UA level; particularly, a high HDL3-C level was associated with high UA level related to MetS in Japanese men. (HEP. 2018; 45: 563-568.)

Key words HDL2 cholesterol, HDL3 cholesterol, uric acid, metabolic syndrome

INTRODUCTION

Serum uric acid (UA) has been reported to be positively associated with the risk of atherosclerosis¹⁾, cardiovascular disease (CVD)²⁾, hypertension³⁾, and metabolic syndrome (MetS)⁴⁾. Given that UA is associated with several conditions, this raised the possibility that UA might work synergistically with other risk factors during the development of atherosclerosis. In order to prevent the incidence of the above disorders, it is important to investigate the influence of individual risk factors and to understand the inter-relationships of various risk factors with UA. Previous reports suggested a negative relationship between UA and high-density lipoprotein cholesterol (HDL-C) levels^{5, 6)}, however, interactions between UA and HDL-C subclasses are not well understood.

HDL particles are heterogeneous with respect to density, size, composition, and surface charge. These particles can be separated into two main subfractions, HDL2 and HDL3, based on their density after ultracentrifugation⁷⁾, and both have been reported to exhibit inverse associations with the incidence of CVD⁸⁾. We previously demonstrated the importance of analyzing levels of HDL-C subclasses, as well as overall HDL-C, when evaluating

an individual's lifestyle habits⁹⁻¹¹⁾, insulin resistance, and MetS¹²⁾. HDL2-C levels are associated with alcohol consumption, waist circumference (WC), smoking, and exercise. On the other hand, HDL3-C levels are only associated with alcohol consumption¹¹⁾. We reported that the HDL2-C/HDL3-C ratio was associated with MetS components, insulin resistance, and high-molecular-weight adiponectin (HMW-Ad) levels and was thus useful for evaluating MetS in Japanese individuals¹²⁾. We also reported that changes in HDL2-C/HDL3-C were inversely correlated with changes in WC, insulin resistance, and low-density lipoprotein cholesterol (LDL-C), and positively correlated with HMW-Ad and good lifestyle habits¹³⁾. It is apparent that not only HDL-C, but also HDL subclasses and ratios should be investigated with respect to coronary heart disease (CHD) risk, obesity, MetS, and lifestyle habits.

Therefore, it is important to investigate possible connections between serum HDL-C subclasses and UA level to protect against atherosclerotic events and MetS. This study aimed to clarify the relationship between HDL-C subclasses and UA in subjects not receiving treatment for hyperuricemia, hypertension, dyslipidemia, diabetes mellitus, or chronic renal disease.

SUBJECTS AND METHODS

Subjects

A total of 1,282 subjects underwent annual health examinations, including HDL-C subclass analyses, at the Health Evaluation and Promotion Center, Tokai University Hachioji Hospital, between April 2011 and March 2016. After excluding 439 sub-

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jects who were taking medication for hyperuricemia, hypertension, diabetes mellitus, dyslipidemia, or chronic renal disease, 848 subjects were ultimately included in this study. Medical histories were obtained using self-administered questionnaires and interviews conducted by nurses.

Measurements

WC was measured at the level of the umbilicus while the subject was standing and during slight expiration. Blood pressure (BP) was measured on the upper right arm using an automatic BP monitor (TM-2655P; A&D, Tokyo, Japan) while the subject was seated. Blood samples were collected in tubes coated with heparin early in the morning after an overnight fast. UA levels were measured using the uricase-*N*-(3-sulfopropyl)-3-methoxy-5-methylaniline method with a Wako L-Type UA M kit (Wako Pure Chemicals, Osaka, Japan). Hyperuricemia was defined as plasma UA levels > 7.0 mg/dL¹⁴. Fasting plasma glucose (FPG) was measured using the hexokinase glucose 6-phosphate dehydrogenase method with a Wako L-type Glu 2 kit (Wako Pure Chemicals, Osaka, Japan). Fasting immunoreactive insulin (FIRI) levels were measured using a fluorescence enzyme immunoassay (ST AIA-PACK IRI; Toso, Tokyo, Japan). Serum high-sensitivity C-reactive protein (hsCRP) levels were measured using latex agglutination turbidimetry. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using JSCC transferable method with L-Type AST.J2 and L-Type ALT.J2 (Wako Pure Chemicals, Osaka, Japan). LDL-C was calculated using the Friedewald formula¹⁵. HDL-C and triglyceride (TG) levels were measured using visible spectrophotometry (Determiner L HDL-C, and Determiner L TG II, respectively; Kyowa Medex, Tokyo, Japan). HDL2-C and HDL3-C levels were determined via ultracentrifugation. Briefly, after plasma was centrifuged using an L-60 centrifuge (Beckman Coulter, Brea, USA) at $22,300 \times g$ for 4 h at a plasma density of 1.063 kg/L and a solvent density of 1.125 kg/L, adjusted by adding solid KBr, 40% volume from the top was aspirated, yielding HDL (a) and HDL3 (b) fractions. The cholesterol concentration of each fraction was measured, and HDL2-C was calculated as follows: $[(a) - (b) \times 1.54] \times 0.6^{16}$.

Verbal consent for the analytical use of anonymized health records was obtained from all subjects. The study protocol was approved by the institutional ethics committee of the Tokai University School of Medicine.

Definitions of MetS

A diagnosis of MetS requires any three of the following five factors: central obesity, as determined by WC (≥ 85 cm for men, ≥ 90 cm for women); increased FPG levels (> 5.5 mmol/L; 100 mg/dL); increased TG levels (≥ 1.7 mmol/L; 150 mg/dL); low HDL-C levels (< 1.0 mmol/L; 40 mg/dL in males and < 1.3 mmol/L; 50 mg/dL in females), or an elevated BP [systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg]^{17, 18}.

Statistical analysis

Due to skewed distributions of TG and hsCRP, logarithmic transformation was applied. Scheffe's multiple comparisons test was used to compare mean values across more than two groups. The relationships between HDL-C subclasses and UA were

investigated using Pearson's correlation coefficient. A multiple linear regression analysis was performed to identify significant determinants of UA. Age, BMI, WC, systolic BP, diastolic BP, FPG, FIRI, LDL-C, HDL2-C, HDL3-C, logarithmic transformed TG ($\ln(TG)$), $\ln(hsCRP)$, AST, ALT, exercise habit, amount of alcohol consumption, and current smoking status were used as independent variables. A multiple logistic regression analysis to calculate odds ratios (ORs) for the hyperuricemia (UA > 7.0 mg/dL) was performed. The same variables listed for the multiple linear regression analyses were used for this analysis. Subjects were classified as smoker or non-smoker. Additionally, those who exercised for ≥ 30 min/day more than twice per week were classified as habitual exercisers. Alcohol consumption was surveyed by asking how many units of sake were consumed in a day, where 1 unit (180 mL) was taken to be the equivalent to 25 g of alcohol. A stepwise procedure was used to select variables for the multiple linear and logistic regression analyses. Statistical analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). All *P*-values were two-tailed, and a *P*-value < 0.05 was considered statistically significant.

RESULTS

Table 1 lists the subjects' characteristics. The mean HDL-C, HDL2-C, HDL3-C, and UA levels for the total subjects were 59.0 mg/dL, 36.6 mg/dL, 21.5 mg/dL, and 6.3 mg/dL, respectively, and the prevalence of hyperuricemia was 29.5%. The characteristics of the study subjects according to the tertile value of UA level (T1: < 5.8 mg/dL, T2: 5.8 to < 6.8 mg/dL, T3: ≥ 6.8 mg/dL) are also shown in **Table 1**. The upper tertile of UA was often accompanied by the worst metabolic profile (BMI, WC, BP, FIRI, TG, HDL-C, AST, ALT and number of MetS components). HDL2-C level of the middle and upper tertiles of UA were significantly lower than the lower tertile. HDL3-C level of the upper tertile of UA were significantly higher than the lower tertile. Although no significant differences were observed, LDL-C levels gradually increased as UA levels increased.

Pearson's correlation coefficient was used to assess the univariate associations between HDL-C subclasses and UA. UA levels exhibited a positive correlation with HDL3-C ($r = 0.110$, confidence interval (CI): 0.043 – 0.176, $P = 0.0013$), but showed negative correlations with HDL-C and HDL2-C ($r = -0.131$, CI: -0.196 – -0.064, $P = 0.0001$ and $r = -0.164$, CI: -0.229 – -0.098, $P < 0.0001$, for each).

To test the relationship between HDL subclasses and UA, mean UA levels were compared when subjects were stratified by tertile for either HDL2-C (**Fig. 1** (a)) or HDL3-C levels (**Fig. 1** (b)). UA levels decreased as HDL2-C increased. UA levels in the middle and upper tertiles of HDL2-C were significantly higher than the lower tertile. On the contrary, UA levels increased as HDL3-C increased. UA levels in the upper tertile of HDL3-C were significantly higher than the lower tertile.

Next, determinants of UA level were analyzed by multiple linear regression analysis (**Table 2**). Among the following variables, age, BMI, WC, systolic BP, diastolic BP, FPG, FIRI, LDL-C, HDL2-C, HDL3-C, $\ln(TG)$, $\ln(hsCRP)$, AST, ALT, exercise

Table 1 Characteristics of study subjects

Variables	Total n=848	T1 (<5.8 mg/dL) n=282	T2 (5.8 – <6.8 mg/dL) n=266	T3 (≥ 6.8 mg/dL) n=300
Age (years)	53.2 ± 12.3	54.8 ± 12.9	52.8 ± 12.4	52.0 ± 11.6*
BMI (kg/m ²)	23.9 ± 3.3	23.0 ± 3.1	23.9 ± 3.1**	24.8 ± 3.6**.,##
Waist circumference (cm)	84.5 ± 9.2	81.9 ± 8.8	84.4 ± 8.3**	87.1 ± 9.5**.,##
Systolic BP (mmHg)	122.5 ± 17.3	119.7 ± 16.9	123.0 ± 16.4	124.6 ± 18.1**
Diastolic BP (mmHg)	78.0 ± 13.0	75.4 ± 13.0	78.0 ± 11.9	80.4 ± 13.3**
FPG (mg/dL)	103.2 ± 19.5	103.0 ± 18.2	102.2 ± 13.4	104.1 ± 24.5
FIRI (μU/mL)	6.50 ± 4.93	5.49 ± 3.91	6.46 ± 5.12	7.49 ± 5.42**.,#
TG (mg/dL)	128.9 ± 99.5	106.6 ± 64.7	128.9 ± 86.4*	149.7 ± 128.9**.,#
HDL-C (mg/dL)	59.0 ± 14.6	61.3 ± 14.7	58.4 ± 14.2	57.2 ± 14.5**
HDL2-C (mg/dL)	36.6 ± 12.3	39.0 ± 12.8	36.4 ± 11.8*	34.5 ± 11.9**
HDL3-C (mg/dL)	21.5 ± 3.5	21.1 ± 3.4	21.4 ± 3.3	21.9 ± 3.7*
LDL-C (mg/dL)	122.9 ± 31.8	119.9 ± 29.1	123.3 ± 31.6	125.5 ± 34.1
UA (mg/dL)	6.3 ± 1.2	5.0 ± 0.6	6.2 ± 0.3**	7.6 ± 0.7**.,##
hsCRP (mg/dL)	0.118 ± 0.300	0.109 ± 0.279	0.129 ± 0.400	0.116 ± 0.203
AST (IU/L)	24.0 ± 12.3	23.2 ± 15.4	22.9 ± 7.5	25.7 ± 12.2 [#]
ALT (IU/L)	27.5 ± 21.7	24.4 ± 22.2	27.2 ± 19.3	30.7 ± 22.9**
Numbers of MetS	1.3 ± 1.2	1.0 ± 1.1	1.3 ± 1.1	1.7 ± 1.2**.,##

Variables are given as means ± standard deviations. Subjects were stratified into four groups according to UA level. T1; lower tertile, T2; middle tertile, T3; upper tertile of UA in each group. BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; FIRI, fasting immunoreactive insulin; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; HDL2-C, high-density lipoprotein 2 cholesterol; HDL3-C, high-density lipoprotein 3 cholesterol; LDL-C, low-density lipoprotein cholesterol calculated by Friedewald formula; UA, uric acid; hsCRP, high sensitivity C-reactive protein; AST, aspartate transaminase; ALT, alanine transferase

**p<0.01, *p<0.05 (T1 vs T2, T1 vs T3), ##p<0.01, #p<0.05 (T2 vs T3) by Scheffe's multiple comparison test.

Fig. 1 Bar graph of mean UA values with 95% confidence intervals after stratifying subjects according to HDL2-C (a) and HDL3-C (b) levels.

*P< 0.05, **P< 0.01 by Scheffe's multiple comparison test.

UA, uric acid; HDL2-C, high-density lipoprotein 2 cholesterol; HDL3-C, high-density lipoprotein 3 cholesterol

habit, amount of alcohol consumption, and current smoking status, six variables (age, WC, diastolic BP, ln(TG), ln(hsCRP), and HDL3-C) were selected by a stepwise procedure. Standardized regression coefficients in the multiple regression analysis were higher for WC, ln(TG), and HDL3-C than for diastolic BP, ln(hsCRP) and age. Thus, WC, ln(TG), and HDL3-C were mainly and positively associated with UA levels.

Determinants of hyperuricemia (UA > 7.0 mg/dL) were analyzed by multiple logistic regression analysis (**Table 3**). Five

variables (WC, diastolic BP, ln(TG), HDL2-C, and HDL3-C) were selected by a stepwise procedure. The OR for ln(TG) was the highest and HDL3-C was second among the selected variables, indicating that ln(TG) and HDL3-C was dominantly associated with hyperuricemia.

According to the multiple linear regression for UA and multiple logistic regression analysis for the hyperuricemia, selected variables were components of MetS (WC, diastolic BP and ln(TG)). Accordingly, the association of UA and HDL3-C levels

Table 2 Multiple liner regression analysis for UA

	Multiple liner regression			
	RC	SRC	<i>t</i>	<i>P</i>
Age	-0.0096	-0.09605	-2.89	0.0039
WC	0.02101	0.15608	4.25	<0.0001
Diastolic BP	0.01096	0.11506	3.36	0.0008
Ln(TG)	0.37412	0.16391	4.52	<0.0001
Ln(hsCRP)	0.07482	0.07027	2.04	0.0417
HDL3-C	0.05336	0.15235	4.60	<0.0001

Variable selection was made by a stepwise procedure. RC, regression coefficient; SRC, standardized regression coefficient; WC, waist circumference; BP, blood pressure; Ln(TG), logarithmic transformed triglyceride; Ln(hsCRP), logarithmic transformed high sensitivity C-reactive protein; HDL3-C, high-density lipoprotein 3 cholesterol

Table 3 Multiple logistic regression analysis for upper tertile of UA

	Multiple logistic regression				
	RC	SE	OR	95% CI	<i>P</i>
WC	0.0337	0.00969	1.034	1.015–1.054	0.0005
Diastolic BP	0.0194	0.00637	1.020	1.007–1.032	0.0024
Ln(TG)	0.3626	0.1678	1.437	1.034–1.997	0.0307
HDL2-C	-0.0176	0.00820	0.983	0.967–0.998	0.0319
HDL3-C	0.0854	0.0238	1.089	1.040–1.141	0.0003

Variable selection was made by a stepwise procedure. RC, regression coefficient; SE, standard error; OR, odds ratio; CL, confidence interval; WC, waist circumference; BP, blood pressure; Ln(TG), logarithmic transformed triglyceride; HDL2-C, high-density lipoprotein 2 cholesterol; HDL3-C, high-density lipoprotein 3 cholesterol

Fig. 2 (a) Bar graph of mean UA values with 95% confidence intervals after stratifying subjects according to number of MetS components. (b) Bar graph of mean UA values after stratifying subjects according to number of MetS components and HDL3-C levels.

Numbers on the bars represent mean UA values (mg/dL) (upper) and number of subjects (lower) of each group.

UA, uric acid; MetS, metabolic syndrome; HDL2-C, high-density lipoprotein 2 cholesterol; HDL3-C, high-density lipoprotein 3 cholesterol

in relation to MetS was investigated (**Fig. 2**). In agreement with previous reports^{19,20}, mean UA levels gradually increased as the number of MetS components (0, 1, 2, and ≥ 3) in the four groups increased (**Fig. 2 (a)**). Mean HDL2-C levels gradually decreased as the number of MetS components (0, 1, 2, and ≥ 3) in the four groups increased (**Fig. 2 (b)**). On the other hand, it was not clear whether HDL3-C levels were associated with the number of MetS components, since the mean HDL3-C levels stratified by number of MetS components were imbalanced (**Fig. 2 (c)**). However, when the combined effects of HDL3-C and number of MetS

components to UA levels were investigated, mean UA levels generally increased with increasing number of MetS components and HDL3-C levels (**Fig. 2 (d)**).

DISCUSSION

The present study showed that HDL-C subclasses were associated with UA; in particular, high HDL3-C levels were associated with high UA levels in relationship to MetS in Japanese men. To the best of my knowledge, this is the first report to describe the association of HDL3-C and UA levels relative to MetS compo-

nents.

In agreement with previous study which showed higher UA was strong predictor of small, dense HDL particles in middle-aged European subjects²¹, HDL3-C was associated with UA level in the current study. Small, dense HDL particles by gradient gel electrophoresis may be compatible, at least in part, with HDL3 determined by ultracentrifugation. As UA level positively associated with makers of inflammation, the authors speculated that oxidation stress related to the small HDL particles could result in compensatory elevation of UA²¹. The reason why marker of inflammation (ln(hsCRP)) was not correlated with hyperuricemia in the current study could be partly because of the differences in the clinical characteristics of study subjects; those in the previous study had a higher frequency of CHD risk which is often accompanied by inflammation, while subjects in the current study were healthier. On the other hand, mean serum UA level was much higher in the current study (6.3 versus 4.8 mg/dL), partly due to the selection of the current study subjects where women were not studied.

Previous study indicated that serum UA was negatively related to large HDL in Chinese subjects, since multiple linear regression analysis indicated that large HDL was selected⁶. This result was in agreement with the current study, since HDL2-C was selected in multiple logistic regression analysis (**Table 3**). In addition, subjects with high UA level in the current study were accompanied with high CVD risk factors including dyslipidemia and MetS. However, I reported a controversial result showing that serum UA was positively related to small HDL-C (HDL3-C), since multiple linear regression analysis and multiple logistic regression analysis indicated that HDL3-C was determinant for UA (**Table 2** and **3**). It is reported that when subjects were stratified by quintile value of serum UA level, large and intermediate HDL-C decreased, while the trend of small HDL-C was imbalanced⁶.

Visceral obesity and insulin resistance contribute to postprandial hypertriglyceridemia and low HDL-C, both of which are components of MetS. These conditions ultimately stimulate hepatic TG output²². It is widely accepted that serum UA closely related to MetS⁴. Moreover, we previously reported that HDL-C and HDL2-C showed very strong positive correlation, and HDL2-C levels were decreased by obesity¹¹. Therefore, it is reasonable to see the subjects with highest UA concomitant with higher BMI, WC, TG showed lower HDL-C and HDL2-C.

Since both UA and HDL appeared to be associated with CVD, inflammation, and MetS, they may share similar characteristics. UA has antioxidant effects in physiological environments or in association with antioxidants²³. *In vitro*, UA has an antioxidant effect on native LDL as well as a prooxidant effect on mildly oxidized LDL²⁴. However, despite the potential antioxidant effect of UA, numerous studies have revealed positive associations between serum UA concentration and various disorders, most of which are components of MetS. HDL protects LDL from oxidative damage by free radicals and therefore exerts antioxidative and anti-inflammatory properties²⁵. Circulating HDL particles, particularly small, dense, protein-rich HDL3, may provide potent

protection of LDL *in vivo* from oxidative damage by free radicals in the arterial intima, resulting in inhibited generation of proinflammatory oxidized lipids²⁵. It has yet to be established whether different levels of uric acid may directly modify the lipid profile, including HDL.

It is unclear how HDL3 modify the association between UA and MetS components. It is well recognized that oxidative stress increased in MetS, low physical activity, and smoking¹⁷. Under chronic inflammation status, such as in active condition of rheumatoid arthritis, HDL-C levels decrease and the composition of HDL changes²⁶. UA contributes to the overall inflammatory state of subjects. Unexpectedly, the results revealed association between hsCRP and UA by multiple linear regression, not in multiple logistic regression analysis. This is probably due to the characteristics of the study subjects, as none received medication for hyperuricemia, hypertension, diabetes mellitus, dyslipidemia, or chronic renal disease, all of which are associated with inflammation. Indeed, mean hsCRP level (0.118 mg/dL) in the current study was much lower than previously reported²¹. Therefore, it is probable that under milder oxidative stress where inflammation marker did not reflect the stress, HDL3 might protect against such stress, resulted in compensatory increased HDL3 level.

The association between serum UA and CVD has long been recognized. However, it has not been definitively established whether serum UA is merely a marker for risk or a causative agent in CVD. Many studies have found that serum UA independently associated with CVD risk and events^{27, 28}. On the other hand, compelling arguments have also been reported that the apparent association is primarily resulted from the strong collinear interaction of serum UA and established CVD risk factors^{29, 30}. Some of the variation in the study results likely relates to differences in population characteristics and statistical methods.

The limitations of our study include that it was cross-sectional in nature, which prevented the establishment of a causal relationship. The subjects in this study were middle-aged Japanese men; therefore, it is possible that the relationship between UA levels and clinical markers were affected by gender, and ethnicity. Finally, the results were calculated from the data of only a fraction of the subjects who underwent annual health examinations; therefore, the findings might not be generalizable to all Japanese men.

In conclusion, both HDL2-C and HDL3-C were associated with serum UA levels in subjects who are not receiving treatment for hyperuricemia, hypertension, diabetes mellitus, dyslipidemia, or chronic renal disease. In particular, high HDL3-C was associated with a high UA in relationship to MetS in Japanese men.

The authors state that they have no Conflict of Interest (COI).

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