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【 ORIGINAL ARTICLE 】

The Association between the Triglyceride to High-density Lipoprotein Cholesterol Ratio and Low-density Lipoprotein Subclasses

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Abstract:

Objective The triglyceride (TG)/high-density lipoprotein cholesterol (HDL-C) ratio is related to insulin resistance (IR). However, information about whether or not the TG/HDL-C ratio is associated with low-density lipoprotein (LDL) subclasses in the Japanese population is limited.

Methods In total, 1,068 Japanese subjects who underwent an annual health examination and who were not taking medications were recruited. The association between the TG/HDL-C ratio and LDL subclasses was investigated using correlation, multiple regression, and receiver operating characteristic analyses.

Results A correlation analysis revealed that both malondialdehyde-modified low-density lipoprotein (MDA-LDL) and small dense low-density lipoprotein cholesterol (sdLDL-C) were positively associated with the TG/HDL-C ratio. Furthermore, a multiple linear regression analysis revealed that the TG/HDL-C ratio was positively associated with MDA-LDL and sdLDL-C in both men and women. The multiple logistic regression analysis also revealed that the TG/HDL-C ratio was positively associated with the upper tertile of MDA-LDL and sdLDL-C in men and women. The LDL-C levels increased with the increasing TG/HDL-C ratio. The MDA-LDL and sdLDL-C are known to be positively associated with LDL-C. However, within the same LDL-C range, both MDA-LDL and sdLDL-C levels increased with the TG/HDL-C ratio, except for MDA-LDL levels in the LDL-C <112 mg/dL group in women. These results further supported the notion that the TG/HDL-C ratio was positively associated with the MDA-LDL and sdLDL-C levels, especially in the higher LDL-C range, in both men and women. The optimal cut-off points of the TG/HDL-C ratio for the upper tertile of MDA-LDL and sdLDL-C were 1.85 and 2.03 in men and 0.88 and 1.30 in women, respectively.

Conclusion The TG/HDL-C ratio is positively associated with MDA-LDL and sdLDL-C in Japanese subjects. The relationship was particularly notable in subjects with high LDL-C levels.

Key words: triglyceride to high-density lipoprotein cholesterol ratio, malondialdehyde-modified low-density lipoprotein, small dense low-density lipoprotein cholesterol

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Introduction

A high level of low-density lipoprotein cholesterol (LDL-C) plays a key role in the development and progression of atherosclerosis and cardiovascular disease (CVD). Recently, the use of the triglyceride (TG)/high-density lipoprotein cholesterol (HDL-C) ratio as a compliment to LDL-C for predicting CVD has been proposed. The TG/HDL-C ratio is significantly correlated with insulin resistance (IR) (1-3) and may be a useful predictor for the development of diabetes (4), coronary heart disease (CHD), and cardiovascular mortality (5). Furthermore, it is a better screening index for IR than the homeostasis model assessment of insulin resistance (HOMA-IR) (6). However, few studies have investigated this ratio as an indicator of LDL subclasses, such as malondialdehyde-modified low-density lipoprotein (MDA-LDL) and small dense low-density lipoprotein cholesterol

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(sdLDL-C).

LDL comprises heterogeneous subclasses of particles with varying density, size, electrophoretic mobility, relative lipid-protein proportions, and binding affinity (7). According to size and density, LDL particles are fractionated into large, buoyant LDL (lbLDL; diameter ≥25.5 nm) and small, dense LDL (sdLDL; diameter <25.5 nm) (8). Recently, a direct method for the quantification of sdLDL-C using an auto-analyzer was established (9). sdLDL is directly correlated with serum TG and inversely with serum HDL-C (8).

High sdLDL-C levels are currently considered a risk factor for CVD. Compared with large LDL, sdLDL-C exhibits a lower affinity to LDL receptor and longer half-life in the plasma, binds to arterial proteoglycans more tightly, penetrates the arterial subendothelial space more easily, and is more susceptible to chemical modification, including oxidation (10). An increase in reactive oxygen species, which is often accompanied by various cardiovascular risk factors, such as dyslipidemia, hypertension, and diabetes mellitus, leads to the formation of oxidized LDL (11). MDA-LDL is a major form of oxidized LDL that plays a key role in the progression of atherosclerosis (10). Increased serum MDA-LDL levels were found to be associated with coronary artery disease (CAD) (12-14) and coronary artery calcification (15). Furthermore, serum MDA-LDL levels are positively correlated with the carotid intima-media thickness (13, 16). Therefore, it might be important to measure not only the LDL-C levels but also the MDA-LDL and sdLDL-C levels. However, measurements of LDL subclasses are not commonly performed and can be expensive in clinical settings. Given that both the TG/HDL-C ratio and LDL subclasses are associated with IR, atherosclerosis, and CVD, there is a possibility of a relationship between the serum TG/HDL-C ratio and LDL subclasses. However, such an association has not been assessed thus far.

The present study therefore investigated whether or not the TG/HDL-C ratio is associated with the MDA-LDL or sdLDL-C level in Japanese adults.

Materials and Methods

Subjects

A total of 1,329 subjects who underwent an annual health examination at the Health Evaluation and Promotion Center of Tokai University Hachioji Hospital between April 2011 and March 2015 were included in this cross-sectional study. After excluding 261 subjects who were taking medications for hypertension, diabetes mellitus, dyslipidemia, hyperuricemia, or chronic renal disease as well as those with a history of stroke, coronary artery disease, or chronic renal failure, 1,068 subjects were included in the final analysis. Medical histories were obtained using self-administered questionnaires and via interviews conducted by nurses.

Measurements

The waist circumference (WC) was measured at the level of the umbilicus during slight expiration while the participant was in standing position. Blood pressure (BP) was measured in the upper right arm using an automatic BP monitor device (TM-2655P; A&D, Tokyo, Japan) while the participant was seated. Blood samples were collected in heparin-coated tubes early in the morning after an overnight fast. The fasting plasma glucose (FPG) level was measured with the L-type Glu 2 kit using the hexokinase/glucose-6-phosphate dehydrogenase method (Wako Pure Chemicals, Osaka, Japan). The fasting immunoreactive insulin (FIRI) levels were measured using a fluorescence enzyme immunoassay (ST AIA-PACK IRI; Toso, Tokyo, Japan). The HOMA-IR was calculated as follows: FPG (mg/dL) × FIRI (μIU/mL)/405 (17). The serum high-sensitivity C-reactive protein (hsCRP) levels were measured using latex agglutination turbidimetry. The LDL-C levels were calculated using the Friedewald formula (18). The HDL-C and TG levels were measured using visible spectrophotometry (Determiner L HDL-C and Determiner L TG II, respectively; Kyowa Medex, Tokyo, Japan). The MDA-LDL levels were measured with an enzyme-linked immunosorbent assay using monoclonal antibodies specific for MDA-LDL (clone ML25) and to apolipoprotein B (clone AB16) (Sekisui Medical, Tokyo, Japan). The sdLDL-C levels were measured using a homogeneous method (sdLDL-Ex; Denka Seiken, Tokyo, Japan). The uric acid (UA) levels were measured with an L-Type UA M kit using the uricase-*N*-(3-sulfopropyl)-3-methoxy-5-methylaniline method (Wako Pure Chemicals).

Verbal consent for the use of anonymized health records was obtained from all study participants. The study protocol was approved by the Ethics Committee of the Tokai University School of Medicine.

Statistical analyses

Data were expressed as the mean ± standard deviation or median (interquartile range). Normality was examined using the Kolmogorov-Smirnov test. Bonferroni's multiple comparison test was used to compare mean values across three or more groups. Student's *t*-test was used to compare mean values between two groups. The associations between study variables were investigated using Pearson's correlation coefficient. The MDA-LDL and sdLDL-C levels were compared after stratifying subjects according to the sex, TG/HDL-C, and LDL-C levels. Since the reference ranges for MDA-LDL and sdLDL-C were uncertain, upper tertile values [157.2 (U/L) and 44.4 (mg/dL) for men, and 130.0 (U/L) and 35.5 (mg/dL) for women] were determined.

A multiple linear regression analysis was performed to identify the significant determinants of MDA-LDL or sdLDL-C. The age, body mass index (BMI), WC, systolic and diastolic BP, FPG, FIRI, TG/HDL-C ratio, and hsCRP were used as independent variables in the multiple linear regression analysis of MDA-LDL or sdLDL-C. We then per-

Table 1. Characteristics of Study Subjects.

	Men (n=651)	Women (n=417)
Age	55.5±12.1	57.6±11.7*
BMI (kg/m^2)	23.8±3.2	21.7±2.9**
Waist circumference (cm)	84.1±8.5	78.1±8.5**
Systolic BP (mmHg)	122.0±17.0	117.8±19.0**
Diastolic BP (mmHg)	78.5±12.8	71.6±12.3**
FPG (mg/dL)	102.9±18.9	98.2±18.6**
FIRI (μIU/mL)	6.24±4.64	5.08±2.99**
HOMA-IR	1.64±1.57	1.27±0.94**
TG (mg/dL)	103.0 [73.0,147.0]	73.0 [55.0,103.0]**
HDL-C (mg/dL)	59.4±14.5	76.4±16.8**
TG to HDL-C ratio	1.74 [1.13,2.89]	0.95[0.67,1.53]**
LDL-C (mg/dL)	121.6±30.7	127.2±33.3*
non-HDL-C (mg/dL)	146.3±34.1	143.9±35.9
UA (mg/dL)	6.3±1.2	4.6±0.94**
MDA-LDL (U/L)	142.4±43.0	121.0±41.0**
sdLDL-C (mg/dL)	40.0±16.8	32.9±13.2**
hsCRP (mg/dL)	0.04 [0.02,0.09]	0.03 [0.02,0.07]

Variables are given as means±standard deviations or median [inter-quartile range]. BMI: body mass index, BP: blood pressure, FPG: fasting plasma glucose, FIRI: fasting immunoreactive insulin, HOMA-IR: homeostasis model assessment-insulin resistance, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, non-HDL-C: non high-density lipoprotein cholesterol, UA: uric acid, MDA-LDL: malondialdehyde-modified low-density lipoprotein, sdLDL-C: small-dense low-density lipoprotein cholesterol, hsCRP: high-sensitivity C-reactive protein

**p<0.01, *p<0.05 by paired t- test.

formed a multiple logistic regression analysis to calculate the odds ratios (ORs) for the upper tertile of MDA-LDL or sdLDL-C using the same variables utilized in the multiple linear regression analysis. A stepwise procedure was used to select variables for multiple regression analyses. A receiver operating characteristic (ROC) curve was prepared in order to evaluate the discriminatory ability for the variables, and the area under the curve (AUC) with its 95% confidence interval (CI) was calculated. In order to determine the optimal cut-off point of TG/HDL-C, the square root of [(1 - sensitivity)^2 + (1 - specificity)^2] was calculated, which was the point on the ROC curve with the shortest distance from the upper left corner.

All statistical analyses were performed using SAS studio software program, version 3.4 (SAS Institute, Cary, USA). All p values were two-tailed, and a p value of <0.05 was considered statistically significant.

Results

All variables evaluated in this study are shown in Table 1, with data stratified according to sex. Of the 1,068 subjects included, 417 (39.0%) were women. The mean age, median TG level, mean HDL-C level, and median the TG/HDL-C ratio of the men were 55.5 years, 103.0 mg/dL, 59.4 mg/dL, and 1.74, respectively. The mean age, median TG level, mean HDL-C level, and median the TG/HDL-C ratio of the women were 57.6 years, 73.0 mg/dL, 76.4 mg/dL, and 0.95, respectively. Most markers were accentuated in men, except for the age, HDL-C level, and LDL-C level.

The association between the logarithmic transformed the TG/HDL-C ratio [ln(TG/HDL-C)] and the MDA-LDL or sdLDL-C level was investigated using a correlation analysis. The Pearson's correlation coefficients of ln(TG/HDL-C) for the MDA-LDL level in men and women were 0.388 and 0.333, respectively (Fig. 1a, b). The Pearson's correlation coefficients of ln(TG/HDL-C) for the sdLDL-C level in men and women were 0.641 and 0.522, respectively (Fig. 1c, d). These results indicated that both MDA-LDL and sdLDL-C levels were positively associated with the TG/HDL-C ratio.

The determinants of MDA-LDL or sdLDL-C were identified by a multiple linear regression analysis (Table 2). Among the variables included in this study (age, BMI, WC, systolic and diastolic BP, FPG, FIRI, and TG/HDL-C ratio), the BMI and the TG/HDL-C ratio for men (Table 2a) and the age, WC, FPG and the TG/HDL-C ratio for women (Table 2b) were selected for MDA-LDL using a stepwise procedure. The analysis revealed that the BMI and the TG/HDL-C ratio were positively associated with the MDA-LDL level in men (Table 2a), while the age, WC, FPG level, and TG/HDL-C ratio were positively associated with the MDA-LDL level in women (Table 2b).

For sdLDL-C, the BMI and TG/HDL-C ratio in men (Table 2a) and the age, WC, diastolic BP, FPG level, and TG/HDL-C ratio in women (Table 2b) were selected using a stepwise procedure. The analysis revealed that the BMI and

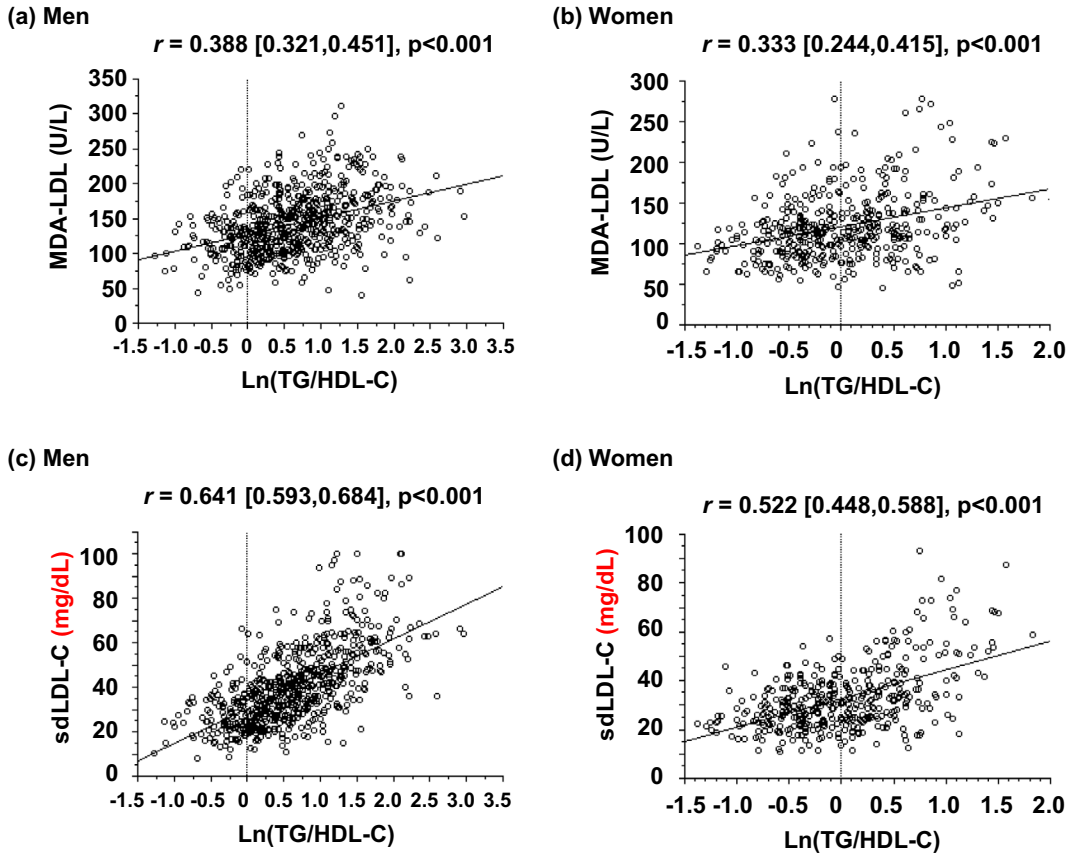

Figure 1. **Scatter plots and regression lines for the comparisons of MDA-LDL and sdLDL-C levels (a, c: men and b, d: women) and ln (TG/HDL-C). Pearson's correlation coefficient with 95% confidence intervals is indicated on the graph. MDA-LDL: malondialdehyde-modified low-density lipoprotein, sdLDL-C: small-dense low-density lipoprotein cholesterol, ln (TG/HDL-C): logarithmic transformed triglyceride to high-density lipoprotein cholesterol ratio**

Table 2. **Multiple Linear Regression Analysis for the MDA-LDL and sdLDL-C.**

(a) Men

	MDA-LDL				sdLDL-C			
	RC	SRC	t	p	RC	SRC	t	p
BMI	2.43897	0.17940	4.58	<0.0001	0.89203	0.16791	4.96	<0.0001
TG/HDL-C	4.99563	0.23936	6.11	<0.0001	4.13176	0.50659	14.97	<0.0001

Variable selection was made by a stepwise procedure. MDA-LDL: malondialdehyde-modified low-density lipoprotein, sdLDL-C: small-dense low-density lipoprotein cholesterol, RC: regression coefficient, SRC: standardized regression coefficient, BMI: body mass index, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol

(b) Women

	MDA-LDL				sdLDL-C			
	RC	SRC	t	p	RC	SRC	t	p
Age	0.72496	0.206.3	4.55	<0.0001	0.12239	0.10828	2.69	0.0074
WC	0.83574	0.17278	3.62	0.0003	0.18355	0.11814	2.72	0.0067
Diastolic BP	----	----	----	----	0.12097	0.11295	2.79	0.0056
FPG	0.32901	0.14922	3.26	0.0012	0.10325	0.14578	3.60	0.0004
TG/HDL-C	10.00933	0.20149	4.35	<0.0001	7.36252	0.46141	11.27	<0.0001

Variable selection was made by a stepwise procedure. MDA-LDL: malondialdehyde-modified low-density lipoprotein, sdLDL-C: small-dense low-density lipoprotein cholesterol, RC: regression coefficient, SRC: standardized regression coefficient, WC: waist circumference, BP: blood pressure, FPG: fasting plasma glucose, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol

Table 3. Multiple Logistic Regression Analysis for the Upper Tertile of MDA-LDL and sdLDL-C.

(a) Men

	Upper tertile of MDA-LDL					Upper tertile of sdLDL-C				
	RC	SE	OR	95% CI	p	RC	SE	OR	95% CI	p
Age	-0.0254	0.0078	0.975	0.960-0.990	0.0011	----	----	----	----	----
Systolic BP	0.0157	0.0054	1.016	1.005-1.027	0.0034	----	----	----	----	----
BMI	----	----	----	----	----	0.0867	0.0350	1.091	1.018-1.168	0.0133
TG/HDL-C	0.2687	0.0500	1.308	1.186-1.443	<0.0001	0.9297	0.0908	2.527	2.115-3.020	<0.0001

Variable selection was made by a stepwise procedure. MDA-LDL: malondialdehyde-modified low-density lipoprotein, sdLDL-C: small-dense low-density lipoprotein cholesterol, RC: regression coefficient, SE: standard error, OR: odds ratio, CI: confidence interval, WC: waist circumference, BP: blood pressure, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol

(b) Women

	Upper tertile of MDA-LDL					Upper tertile of sdLDL-C				
	RC	SE	OR	95% CI	p	RC	SE	OR	95% CI	p
Age	0.0442	0.0109	1.045	1.023-1.068	<0.0001	0.0248	0.0109	1.025	1.004-1.047	0.0225
WC	0.0580	0.0147	1.060	1.030-1.091	<0.0001	0.0455	0.0152	1.047	1.016-1.078	0.0028
Diastolic BP	----	----	----	----	----	0.0229	0.0100	1.023	1.003-1.043	0.0221
TG/HDL-C	0.6275	0.1569	1.873	1.377-2.547	<0.0001	0.9607	0.1701	2.613	1.873-3.647	<0.0001

Variable selection was made by a stepwise procedure. MDA-LDL: malondialdehyde-modified low-density lipoprotein, sdLDL-C: small-dense low-density lipoprotein cholesterol, RC: regression coefficient, SE: standard error, OR: odds ratio, CI: confidence interval, BP: blood pressure, BMI: body mass index, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol

TG/HDL-C ratio were positively associated with the sdLDL-C level in men (Table 2a), while the age, WC, diastolic BP, FPG and the TG/HDL-C ratio were positively associated with the sdLDL-C level in women (Table 2b). Collectively, the multiple linear regression analysis revealed that the TG/HDL-C ratio was positively correlated with the MDA-LDL and sdLDL-C levels in both men and women.

The determinants for the upper tertile of MDA-LDL level were analyzed using a multiple logistic regression analysis (Table 3). When we analyzed the same variables used in the multiple linear regression analysis (age, BMI, WC, systolic and diastolic BP, FPG, FIRI, and the TG/HDL-C ratio), age, systolic BP, and the TG/HDL-C ratio for men (Table 3a) and age, WC, and the TG/HDL-C ratio for women (Table 3b) were selected using a stepwise procedure. The results of the analysis revealed that systolic BP and the TG/HDL-C ratio for men (Table 3a) and age, WC, and the TG/HDL-C ratio for women (Table 3b) were positively associated, whereas age in men was negatively associated with the upper tertile of MDA-LDL.

The determinants for the upper tertile of sdLDL-C level were analyzed using a multiple logistic regression analysis (Table 3). When we analyzed the same variables used in the multiple linear regression analysis, the BMI and TG/HDL-C ratio for men (Table 3a) and the age, WC, diastolic BP, and TG/HDL-C ratio for women (Table 3b) were selected using a stepwise procedure. The results of the analysis revealed that the BMI and TG/HDL-C ratio for men (Table 3a) and the age, WC, diastolic BP, and TG/HDL-C ratio for women (Table 3b) were positively associated with the upper tertile of the sdLDL-C level. Collectively, this multiple logistic regression analysis revealed that the TG/HDL-C ratio was positively associated with the upper tertile of MDA-LDL and sdLDL-C levels in both men and women.

We previously reported that both the MDA-LDL and sdLDL-C levels were positively correlated with the LDL-C levels (19). Indeed, the MDA-LDL and sdLDL-C levels had a moderate to strong association with the LDL-C levels in men and women in the present study (Supplementary material 1). In addition, the LDL-C levels increased with the TG/HDL-C ratio in both men and women based on a comparison of the LDL-C levels after subjects were stratified according to sex and the TG/HDL-C ratio (Fig. 2). To rule out the possibility that the MDA-LDL and sdLDL-C levels increased due to elevated LDL-C levels, we compared the MDA-LDL and sdLDL-C levels after subjects were stratified according to the combination of LDL-C and the TG/HDL-C ratio (Fig. 3). Within the same LDL-C range, both the MDA-LDL and sdLDL-C levels increased with the TG/HDL-C ratio, except for the MDA-LDL levels in the LDL-C <112 mg/dL group of women. These results further supported the notion that the MDA-LDL and sdLDL-C levels were positively associated with the TG/HDL-C ratio, especially in the high-LDL-C range, in both men and women.

Fig. 4 shows the ROC curve for evaluating the discriminatory ability for 3rd tertile values of MDA-LDL and sdLDL-C. The AUCs (95% CIs) were 0.719 (0.678, 0.759) for MDA-LDL and 0.856 (0.826, 0.887) for sdLDL-C in men and 0.680 (0.627, 0.783) for MDA-LDL and 0.707 (0.654, 0.761) for sdLDL-C in women.

The optimal cut-off points of the TG/HDL-C ratio yielding the minimum value of the square root of $[(1 - \text{sensitivity})^2 + (1 - \text{specificity})^2]$ for the upper tertile of MDA-LDL and sdLDL-C were 1.85 and 2.03 in men and 0.88 and 1.30

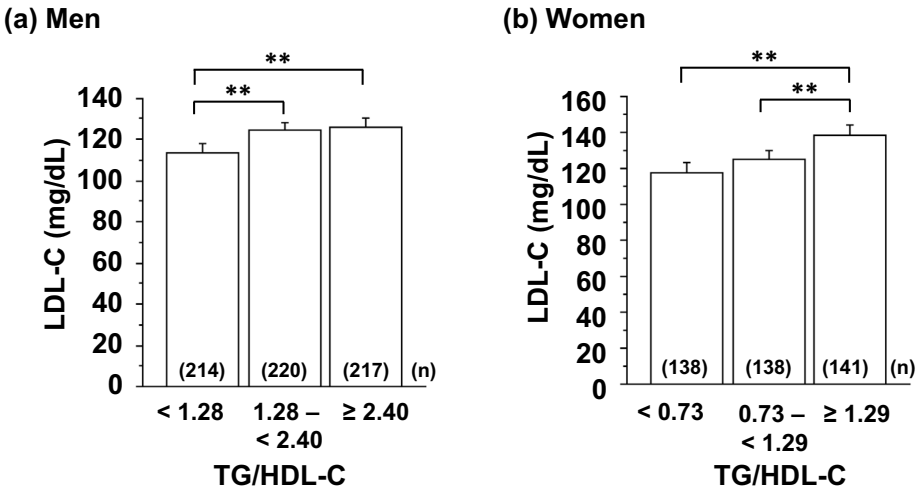

Figure 2. **A bar graph of the mean LDL-C values with 95% confidence intervals after stratifying the subjects according to sex and TG/HDL-C values. LDL-C: low-density lipoprotein cholesterol, TG/HDL-C: triglyceride to high-density lipoprotein cholesterol ratio. **p<0.01 according to Bonferroni's multiple comparison test**

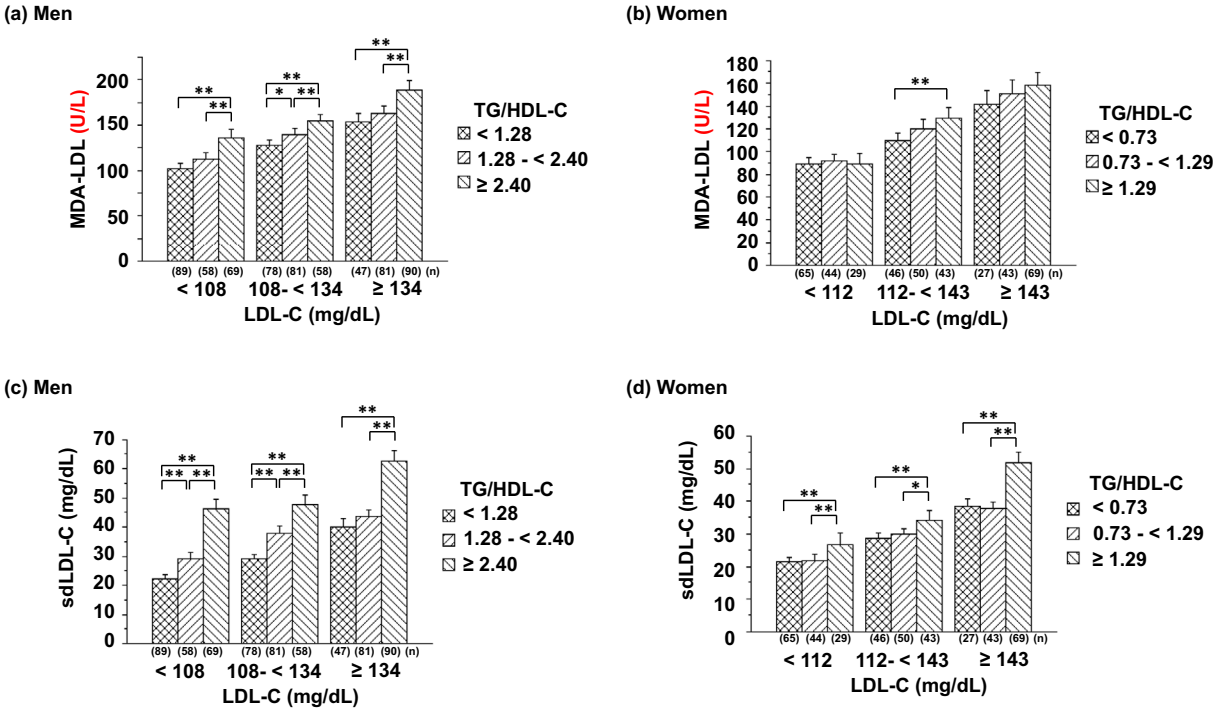

Figure 3. **A bar graph of the mean MDA-LDL and sdLDL-C levels (a, c: men and b, d: women) with 95% confidence intervals after stratifying the subjects according to sex, the TG/HDL-C ratio, and LDL-C values. MDA-LDL: malondialdehyde-modified low-density lipoprotein, sdLDL-C: small-dense low-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TG/HDL-C: triglyceride to high-density lipoprotein cholesterol ratio. *p<0.05, **p<0.01 according to Bonferroni's multiple comparison test.**

in women, respectively. The optimal cut-off points were also the points that maximized the product of the sensitivity and specificity, with a sensitivity and specificity for MDA-LDL of 0.644 and 0.703 in men and 0.543 and 0.748 in women, respectively. The optimal cut-off points with sensitivity and specificity for sdLDL-C of 0.778 and 0.803 in men, and 0.786 and 0.553 in women, respectively.

Since the LDL size is negatively regulated by the serum TG levels and is significantly reduced in hypertriglyceridemic subjects, we compared the discriminatory ability for the variables of the TG/HDL-C ratio and TG (Supplementary material 2). Based on the results of ROC analyses,

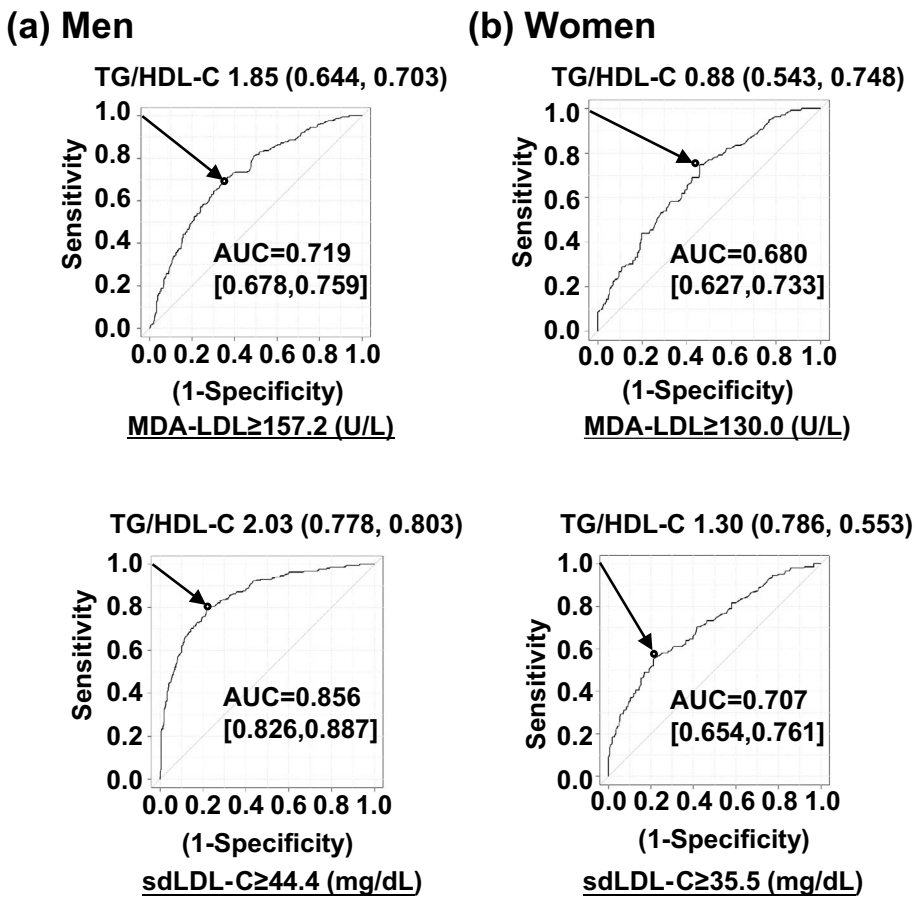

Figure 4. **The ROC curves of the TG/HDL-C ratio for predicting the upper tertile of the MDA-LDL and sdLDL-C levels. The AUC with its 95% CI and optimal cut-off point (sensitivity, specificity) for the TG/HDL-C ratio are shown in the graph. ROC: receiver operator characteristic, TG/HDL-C: triglyceride to high-density lipoprotein cholesterol ratio, MDA-LDL: malondialdehyde-modified low-density lipoprotein, sdLDL-C: small-dense low-density lipoprotein cholesterol, AUC: area under the curve, CI: confidence interval**

the discriminatory abilities of the TG/HDL-C ratio and TG for MDA-LDL and sdLDL-C were higher in men than in women. In addition, both the TG/HDL-C ratio and TG showed a higher discriminatory ability for sdLDL-C than MDA-LDL, especially in men. The cut-off points of the TG/HDL-C ratio and TG for high MDA-LDL and sdLDL-C were both higher in men than in women, and those cut-off points for sdLDL-C were higher than for MDA-LDL in both men and women. Taken together, these findings suggest that the TG/HDL-C ratio was a useful predictor for not only high sdLDL-C but also MDA-LDL compared to TG in both men and women.

Discussion

We showed that the TG/HDL-C ratio is associated with both MDA-LDL and sdLDL-C levels in Japanese subjects. These results suggest that the TG/HDL-C ratio may be useful for assessing the risk of CVD. To evaluate atherogenic LDL, assessments may focus on not only LDL-C levels but also the TG/HDL-C ratio.

In this study, we analyzed the association between the TG/HDL-C ratio and MDA-LDL or sdLDL-C level using correlation and multiple regression analyses. Since the TG/HDL-C ratio varies between men and women based on a previous report (20), we analyzed the data after subjects had been stratified according to sex. The analysis of Pearson's correlation coefficient for MDA-LDL and sdLDL-C levels revealed that the correlation coefficients were higher in men than in women. The multiple linear regression analysis indicated that the standardized regression coefficients for MDA-LDL and sdLDL-C were higher in men than in women. Based on the ORs in the multiple logistic regression analysis, the TG/HDL-C ratio was a stronger determinant for the upper tertile of both MDA-LDL and sdLDL-C levels in women than in men. However, gender differences in the relationship between the TG/HDL-C ratio and the MDA-LDL or sdLDL-C/upper tertile of MDA-LDL or sdLDL-C level in these analyses were not notable. The exact reason for the gender differences in this study was unclear; however, it is speculated that higher HDL-C and LDL-C levels in women, which are particularly increased after menopause, than in men might have been involved.

Why the TG/HDL-C ratio more strongly influenced the

sdLDL-C than the MDA-LDL levels was unclear based on the Pearson's correlation coefficient, standardized regression coefficient, and OR. The LDL size was negatively regulated by the serum TG levels and was significantly reduced in hypertriglyceridemic subjects. The formation of sdLDL is closely associated with IR and hypertriglyceridemia (21). It is well recognized that the TG and HDL-C levels are inversely related, so high TG and low HDL-C levels lead to a high TG/HDL-C ratio and high sdLDL-C levels. Although sdLDL is prone to oxidization, oxidized LDL results from the exposure of LDL to several oxidizing agents, enzymes, and products of myeloperoxidase (22), suggesting that not all MDA-LDL came from sdLDL. This may have contributed to the TG/HDL-C ratio differently affecting the determination of the MDA-LDL and sdLDL-C levels.

The LDL-C and MDA-LDL or sdLDL-C levels had a moderate to strong association in both men and women (Supplementary material 1). The mean LDL-C level was significantly higher in women than in men, while the mean MDA-LDL and sdLDL-C levels were significantly higher in men than in women (Table 1). Furthermore, some subjects with LDL-C levels in the lowest and middle tertiles had relatively elevated MDA-LDL and sdLDL-C levels among both men and women (Fig. 3). Thus, the estimation of the atherogenic MDA-LDL or sdLDL-C levels based on the LDL-C level alone is insufficient. Accordingly, the use of MDA-LDL or sdLDL-C levels combined with the LDL-C level and the TG/HDL-C ratio might provide a better estimation than using the LDL-C level alone.

Since the quantitation of MDA-LDL and sdLDL-C levels is not a routine test, its use in the estimation of CVD risk is not common in healthy subjects. In contrast, the TG, HDL-C, and LDL-C levels are routinely measured, suggesting that the use of both the TG/HDL-C ratio and LDL-C level to identify individuals who are at an increased risk for CVD may be more practical than using the TG/HDL-C ratio or LDL-C per se. Furthermore, we recently reported that TG/HDL-C ratio is associated with IR, components of metabolic syndrome (MetS), and lifestyle habits (23). The TG/HDL-C ratio might therefore be a useful indicator for evaluating CVD and also suggest ways to prevent and improve atherosclerotic disorders.

The discrimination abilities of the TG/HDL-C ratio for MDA-LDL and sdLDL-C were as good as those of TG in both men and women (Supplementary material 2). However, the cut-off points of TG for MDA-LDL and sdLDL-C were both <150 mg/dL. It is well recognized that the diagnostic criteria of MetS and hypertriglyceridemia include a TG level ≥150 mg/dL. For this reason, TG cut-off points of <150 mg/dL can be ignored and may not be practical in clinical settings.

The major limitation of this study is its cross-sectional design, as it inhibited the assessment of the causal relationship between the TG/HDL-C ratio and LDL subclasses. All participants in this study were middle-aged Japanese individuals; therefore, the effect of ethnicity on the relationship between the TG/HDL-C levels and LDL subclasses was not assessed. Finally, our dataset was small; therefore, our findings might not be generalizable to all Japanese individuals.

In conclusion, the TG/HDL-C ratio is associated with LDL subclasses in healthy Japanese subjects. The quantification of the MDA-LDL and sdLDL-C levels is not a routine test; given our data, the routine use of triage tests for CVD based on an increased TG/HDL-C ratio might help identify subjects with metabolic abnormalities. Since both TG and HDL-C levels are routinely evaluated, the TG/HDL-C ratio might be a useful indicator for LDL subclasses, particularly when the TG/HDL-C ratio and LDL-C levels are both considered for the evaluation.

The author states that he has no Conflict of Interest (COI).

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