

Original Article

HDL₂/HDL₃ Ratio Changes, Metabolic Syndrome Markers, and Other Factors in a Japanese Population

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Aim and Methods: The high-density lipoprotein 2 cholesterol (HDL₂-C) to HDL₃-C ratio is associated with insulin resistance, high-molecular-weight adiponectin (HMW-Ad), and metabolic syndrome (MetS) components and is useful for evaluating MetS in Japanese individuals. We investigated potential associations between changes in HDL₂-C/HDL₃-C and changes in MetS components, insulin resistance, adipocytokine, lipids, and lifestyle habits in 892 Japanese subjects who underwent annual health examinations twice at a mean interval of 1.1 years. Study subjects were divided into three groups on the basis of HDL₂-C/HDL₃-C changes.

Results: Average changes in waist circumference (WC) and HDL-C were significantly lower and higher, respectively, in the 0 to <0.5 and ≥0.5 HDL₂-C/HDL₃-C change groups compared with those in the reference group (<0 HDL₂-C/HDL₃-C change). Among MetS components, average changes in HDL₂-C/HDL₃-C were associated with changes in WC and HDL-C. Average changes in HMW-Ad and the homeostasis model assessment of insulin resistance were significantly higher and lower, respectively, in the ≥0.5 HDL₂-C/HDL₃-C change group compared with those in the reference group. In addition, the average low-density lipoprotein cholesterol (LDL-C) gradually decreased as HDL₂-C/HDL₃-C increased. The average change in LDL-C was significantly lower in the 0 to <0.5 and ≥0.5 HDL₂-C/HDL₃-C change groups than in the reference group. Moreover, a ≥0.5 HDL₂-C/HDL₃-C change positively correlated with good lifestyle statuses in terms of smoking, physical activity, and alcohol consumption.

Conclusion: Changes in HDL₂-C/HDL₃-C inversely correlated with changes in WC, insulin resistance, and LDL-C and positively correlated with HMW-Ad and good lifestyle habits. Therefore, HDL₂-C/HDL₃-C changes comprise a useful marker for both MetS and atherogenic conditions in Japanese population.

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Key words: HDL subtype, Metabolic syndrome, Insulin resistance, Lifestyle habit, Annual health examination

Introduction

High-density lipoprotein (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, on the basis of their density after ultracentrifugation¹. The inverse association between the HDL cholesterol (HDL-C) concentration and coronary heart disease (CHD) is well known; however, the associations between HDL subclasses and CHD remain controversial².

Visceral obesity is part of a phenotype that includes dysfunctional subcutaneous adipose tissue expansion and ectopic triglyceride storage and is closely related to the clustering of cardiometabolic risk factors. The various metabolic alterations that closely associate with this condition include hypertriglyceridemia, increased free fatty acid availability, release of proinflammatory

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cytokines from adipose tissue, liver insulin resistance and inflammation, increased very low-density lipoprotein synthesis and secretion in the liver, reduced clearance of triglyceride-rich lipoproteins; the presence of small, dense low-density lipoprotein (LDL) particles; and reduced HDL-C levels³). Clinicians often face the burden of educating overweight and obese people regarding the health risks associated with obesity and the appropriate strategies for weight reduction⁴). The waist circumference (WC) is a simple marker of abdominal obesity and a strong predictor of morbidity and mortality that is independent of the body mass index (BMI)⁵⁻⁷). WC is advantageous because it allows a crude estimation of the absolute amount of visceral adipose tissue; in other words, a reduction in the waistline with weight loss is a clear sign of abdominal fat loss, which is almost always associated with increased insulin activity^{8, 9}). The improved insulin resistance resulting from chronic physical activity, which is independent of weight change, likely involves changes in body composition, particularly a reduction in visceral fat¹⁰).

Although HDL-C levels have been shown to be cardioprotective¹¹), recent data have questioned the cardioprotective nature of HDL. In patients at high cardiovascular risk, the inhibition of cholesteryl ester transfer protein, which increases HDL-C levels, has been shown to increase the risk of cardiovascular disease and death from cardiovascular causes¹²). Although patients receiving niacin treatment had significantly higher HDL-C levels at 2-year follow-up, the rate of adverse cardiovascular events in that group did not significantly differ from that in the placebo group¹³). Another study revealed that the carriers of a single nucleotide polymorphism in the endothelial lipase gene (LIPG Asn396Ser) had significantly higher levels of HDL-C; however, this polymorphism was not associated with a risk of myocardial infarction¹⁴).

Rohatgi *et al.* recently reported that the HDL-C efflux capacity is a relevant predictive biomarker of cardiovascular events¹⁵). Several reports indicated that elevated levels of prebeta-1 HDL, the sole acceptor for cholesterol effluxed from macrophages through the ABCA1 transporter, exhibited a strong positive correlation with CHD risk¹⁶⁻¹⁹). These results suggested that all HDL species might not equally contribute to cholesterol efflux. However, cholesterol efflux assays that require special equipment, techniques, and kits with limited availabilities are not widely accepted. Cholesterol efflux capacity will need to be compared with HDL particle concentration and other biomarker assays before becoming widely accepted as a risk-predicting method. For instance, HDL subclass measure-

ments indicated that HDL₃ levels were more predictive of carotid artery disease status than were HDL₂, HDL-C, or apolipoprotein A1 levels²⁰).

Recently, we demonstrated the importance of analyzing the levels of HDL-C subclasses, as well as the overall HDL-C levels, when evaluating an individual's lifestyle habits²¹⁻²³). In Japanese male subjects with ≤ 2 risk factors for metabolic syndrome (MetS), HDL-C increased as homeostasis model assessment of insulin resistance (HOMA-IR) decreased. However, in subjects with ≥ 3 risk factors for MetS, HOMA-IR increased when HDL-C was ≥ 90 mg/dL²¹). Female subjects who were not obese, did not smoke, and drank < 75 g alcohol/day had elevated HDL-C levels, which were associated with improved insulin sensitivity. Alcohol intake of > 75 g/day appeared to provide no advantages in terms of changes in HDL-C or HOMA-IR levels²²). HDL₂-C levels were associated with alcohol consumption, WC, smoking, exercise, and gender. However, HDL₃-C levels were only associated with alcohol consumption²³). Both HDL₂-C and HDL₃-C have been reported to exhibit inverse associations with the incidence of CHD. However, the relative values of these two HDL subclasses as risk predictors remain unknown²). Lagos *et al.* recently reported that lower HDL-C levels in patients with MetS resulted from reductions in both the large and small HDL subclasses²⁴). As the number of MetS components increases, the HDL phenotype includes a greater percentage of small HDL₃ and a lesser percentage of large HDL₂ molecules, resulting in a lower HDL₂/HDL₃ ratio. Furthermore, 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 individuals²⁵). Given our^{21-23, 25}) and other reports²⁴) and noting that HDL-C measurement alone is not satisfactory for risk assessment^{11-15, 20}), it becomes apparent that not only HDL-C but also HDL subclasses and ratios should be investigated with respect to CHD risk, obesity, MetS, and lifestyle habits. Because it is unknown whether the HDL subclass ratio varies with obesity and MetS, the present study examined whether changes in the HDL₂-C/HDL₃-C ratio after a mean 1.1-year follow-up period were associated with changes in MetS components, insulin resistance, adipocytokine, lipids, and lifestyle habits in Japanese adults who underwent annual health examinations.

Table 1. Characteristics of study subjects at baseline and follow-up

	Men (<i>n</i> =524)			Women (<i>n</i> =368)		
	① Baseline	② Follow-up	Change (②-①)	① Baseline	② Follow-up	Change (②-①)
BMI (kg/m ²)	24.2 ± 3.0	24.1 ± 3.0	-0.1 ± 0.8*	22.5 ± 3.3	22.3 ± 3.1	-0.2 ± 1.0**
Waist circumference (cm)	85.8 ± 8.3	85.1 ± 8.2	-0.7 ± 3.2**	80.6 ± 9.4	80.3 ± 9.0	-0.4 ± 4.0
Systolic BP (mmHg)	124.0 ± 15.9	124.1 ± 16.1	0.1 ± 12.8	117.0 ± 18.3	118.1 ± 18.2	1 ± 12.7
Diastolic BP (mmHg)	78.2 ± 12.1	78.8 ± 11.6	0.6 ± 9.7	71.4 ± 12.1	71.7 ± 12.1	0.3 ± 9.9
FPG (mg/dL)	102.7 ± 11.8	103.0 ± 11.0	0.3 ± 8.0	98.7 ± 10.5	98.9 ± 14.7	0.2 ± 10.9
FIRI (μIU/mL)	6.56 ± 4.62	6.52 ± 4.60	-0.04 ± 3.01	5.68 ± 3.33	5.56 ± 3.08	-0.12 ± 2.63
HOMA-IR	1.69 ± 1.24	1.70 ± 1.32	0.01 ± 0.90	1.42 ± 0.93	1.40 ± 0.94	-0.02 ± 0.80
LDL-C (mg/dL)	122.0 ± 27.8	121.8 ± 27.0	-0.2 ± 21.6	129.4 ± 31.4	129.1 ± 30.8	-0.3 ± 21.8
TG (mg/dL)	129.4 ± 166.4	124.8 ± 100.0	-4.6 ± 110.3	96.3 ± 45.8	96.3 ± 65.7	0.0 ± 59.0
HDL-C (mg/dL)	59.0 ± 13.9	60.6 ± 14.4	1.58 ± 7.42**	72.9 ± 16.2	74.3 ± 16.9	1.4 ± 7.4**
HDL ₂ -C (mg/dL)	37.0 ± 11.8	38.4 ± 11.9	1.35 ± 7.41**	50.3 ± 15.5	50.2 ± 15.6	-0.1 ± 7.6
HDL ₃ -C (mg/dL)	21.8 ± 3.6	21.6 ± 3.5	-0.13 ± 2.80	21.5 ± 3.1	21.6 ± 3.4	0.1 ± 3.1
HDL ₂ -C/HDL ₃ -C	1.72 ± 0.60	1.79 ± 0.58	0.07 ± 0.41*	2.38 ± 0.79	2.37 ± 0.80	-0.01 ± 0.52
HMW-Ad (μg/mL)	2.87 ± 1.78	2.84 ± 1.83	-0.03 ± 0.69	5.53 ± 3.30	5.51 ± 3.15	-0.02 ± 1.31

Variables are given as means ± standard deviations.

BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; FIRI, fasting immunoreactive insulin; HOMA-IR, homeostasis model assessment-insulin resistance; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HDL₂-C, high-density lipoprotein 2 cholesterol; HDL₃-C, high-density lipoprotein 3 cholesterol; HMW-Ad, high-molecular-weight adiponectin

***p* < .001, **p* < .05 (① vs. ②) by paired *t*-test.

Methods

Subjects

A total of 937 subjects underwent baseline annual health examinations at the Health Evaluation and Promotion Center at Tokai University Hachioji Hospital between April 2011 and March 2014; follow-up health examinations were performed at the same institute between January 2012 and March 2015 at a mean follow-up interval of 1.1 years. These examinations included HDL subclass analyses. Medical history information was obtained through self-administered questionnaires and interviews conducted by nurses. After excluding 45 subjects who were undergoing treatment for diabetes mellitus, 892 subjects were included in this study. Among these 892 subjects, 134 were under medication for dyslipidemia, and 195 were using antihypertensive drugs at the time of follow-up.

At the first annual health examination, the results of the examinations were explained to all subjects, and well-trained medical doctors and nurses provided instructions to all patients regarding lifestyle improvements.

It should be noted that no fatal atherosclerotic vascular events were recorded during the 1.1-year study period.

Measurements

The 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 blood pressure monitor (TM-2655P; A&D, Tokyo, Japan) while the subject was seated. Blood samples were collected early in the morning after an overnight fast. Fasting immunoreactive insulin (FIRI) was measured using a fluorescence enzyme immunoassay (ST AIA-PACK IRI; Toso, Tokyo, Japan). The HOMA-IR was calculated as follows: fasting plasma glucose (FPG) (mg/dL) × FIRI (mU/mL)/405²⁶. LDL-C, HDL-C, and triglyceride (TG) levels were measured using visible spectrophotometry (Determiner L LDL-C, Determiner L HDL-C, and Determiner L TG II, respectively; Kyowa Medex, Tokyo, Japan). The HDL₂-C and HDL₃-C levels were determined via ultracentrifugation. Briefly, after plasma was centrifuged using L-60 (Beckman Coulter, Brea, USA) at 223,00 × *g* for 4 hours at plasma density (*d* = 1.063 kg/L) and at a solvent density of 1.125 kg/L, adjusted by adding solid KBr, 40% volume from the top was aspirated, yielding of HDL (a) and HDL₃ (b) fractions. The cholesterol concentration of each fraction was measured, and HDL₂-C was calculated as follows; [(a) - (b) × 1.54] × 0.6²⁷. HMW-Ad was measured using a chemiluminescent enzyme immunoassay based on a monoclonal anti-

Table 2. Changes in parameters stratified by changes in HDL₂-C/HDL₃-C

	Changes in HDL ₂ -C/HDL ₃ -C			Total (n=892)
	<0 (n=404)	0-<0.5 (n=370)	≥0.5 (n=118)	
BMI (kg/m ²)	0.0 ± 0.8	-0.2 ± 0.8*	-0.5 ± 1.2**	-0.1 ± 0.9
Waist circumference (cm)	0.0 ± 3.3	-0.7 ± 3.6**	-1.9 ± 4.0**	-0.5 ± 3.5
Systolic BP (mmHg)	0.8 ± 12.3	0.4 ± 13.4	-0.8 ± 12.4	0.5 ± 12.9
Diastolic BP (mmHg)	-0.3 ± 9.8	1.5 ± 9.8**	-0.1 ± 9.8	0.5 ± 9.8
FPG (mg/dL)	0.9 ± 8.9	-0.1 ± 10.9	-0.6 ± 7.9	0.3 ± 9.3
TG (mg/dL)	2.1 ± 48.9	-6.5 ± 132.4	-7.2 ± 40.8	-2.7 ± 92.6
HDL-C (mg/dL)	-0.7 ± 6.8	2.4 ± 6.9*	6.5 ± 7.9*	1.5 ± 7.4

Variables are given as means ± standard deviations.

HDL₂-C, high-density lipoprotein 2 cholesterol; HDL₃-C, high-density lipoprotein 3 cholesterol; BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol

***p* < .01, **p* < .05 by Dunnett's multiple comparison test, change in HDL₂-C/HDL₃-C < 0 used as a reference.

Table 3. Lifestyle status stratified by changes in HDL₂-C/HDL₃-C

		Changes in HDL ₂ -C/HDL ₃ -C			Total (n=892)
		<0 (n=404)	0-<0.5 (n=370)	≥0.5 (n=118)	
Smoking	Current smoker	51 (13%)	51 (14%)	8 (7%)	110 (12%)
	Stop smoking	7 (2%)	4 (1%)	2 (2%)	13 (1%)
Exercise	No exercise	243 (60%)	200 (54%)	62 (53%)	505 (57%)
	Initiated exercise	33 (8%)	26 (7%)	16 (14%)	75 (8%)
Physical activity	Inactive	204 (50%)	159 (43%)	44 (37%)	407 (46%)
	Became active	38 (9%)	46 (12%)	18 (15%)	102 (11%)
Alcohol consumption	Drank alcohol everyday both at the baseline and follow-up	102 (25%)	96 (26%)	17 (14%)	215 (24%)

Variables are given as number of subjects (percentage of total).

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

body to human HMW-Ad (Fujirebio, Tokyo, Japan). Smoking, habitual exercise, physical activity, and alcohol drinking were recorded as the self-reported current smoking status (continued smoking and initiated smoking after the first annual examination), current exercise activity (continued exercise < 2 times/week and initiated exercise > 2 times/week after the first annual examination), current physical activity (continued activity < 1 hour/day and initiated activity > 1 hour/day after the first annual examination), and current alcohol consumption (drank alcohol every day at both the baseline and follow-up), respectively. All subjects provided verbal consent for the use of anonymized health records in the study analysis. The study protocol was approved by the institutional ethics com-

mittee of the Tokai University School of Medicine.

Statistical Analysis

The significance of pairwise comparisons was determined using a paired *t*-test. Associations between changes in the HDL₂-C/HDL₃-C ratio and mean changes in various parameters and comparisons of the mean values among more than two groups were performed using the Dunnett's multiple comparisons test; subjects with HDL₂-C/HDL₃-C ratio changes < 0 were used as the reference group. Statistical analyses were performed using the SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA). All *P* values were two-tailed, and a *P*-value of < 0.05 was considered statistically significant.

Results

Table 1 lists the baseline and follow-up characteristics of the subjects. The mean ages at baseline were 59.1 years for men and 58.3 years for women. Among men, the HDL-C, HDL₂-C, and HDL₂-C/HDL₃-C ratio were significantly higher, and BMI and WC were significantly lower at follow-up than those at baseline. Among women, the HDL-C was significantly higher and BMI was significantly lower at follow-up than that at baseline.

Table 2 shows the changes in parameters stratified according to changes in the HDL₂-C/HDL₃-C ratio. Study subjects were divided into three groups according to the changes in this ratio. The numbers of subjects with HDL₂-C/HDL₃-C ratio changes of <0, 0 to <0.5, and ≥0.5 were 404 (female: 186, 46.0%), 370 (female: 127, 34.3%), and 118 (female: 53, 44.9%), respectively. The mean average HDL₂-C/HDL₃-C ratio changes among subjects in the <0, 0 to <0.5, and ≥0.5 groups were -0.34 ± 0.34 , 0.22 ± 0.14 , and 0.75 ± 0.23 (mean \pm SD), respectively.

The changes in BMI and WC were significantly smaller in the 0 to <0.5 and ≥0.5 HDL₂-C/HDL₃-C ratio change groups than those in the reference group (<0 HDL₂-C/HDL₃-C ratio change group). Changes in HDL-C were significantly higher in the 0 to <0.5 and ≥0.5 HDL₂-C/HDL₃-C ratio change groups than those in the reference group.

Table 3 presents lifestyle status data stratified by changes in the HDL₂-C/HDL₃-C. Differences were observed in the ≥0.5 HDL₂-C/HDL₃-C ratio change group when compared with <0.5 and 0 to <0.5 HDL₂-C/HDL₃-C ratio change groups, particularly a lower percentage of current smokers, lower percentage of subjects who exercised <2 times/week, higher percentage of subjects who initiated exercise, lower percentage of subjects with <1 hour/day of physical activity, higher percentage of subjects who initiated >1 hour/day of physical activity, and lower percentages of subjects who drank alcohol everyday both at the baseline and follow-up. Overall, higher changes in the HDL₂-C/HDL₃-C ratio positively correlated with good lifestyle habits.

Fig. 1 shows the stratification of average changes in HDL₂-C and HDL₃-C according to the changes in the HDL₂-C/HDL₃-C ratio. A gradual average increase in HDL₂-C was observed as the HDL₂-C/HDL₃-C ratio increased. This average change in HDL₂-C was significantly higher in the 0 to <0.5 and ≥0.5 HDL₂-C/HDL₃-C ratio change groups than in the reference group. In contrast, a gradual average decrease in HDL₃-C was observed as the HDL₂-C/HDL₃-C ratio

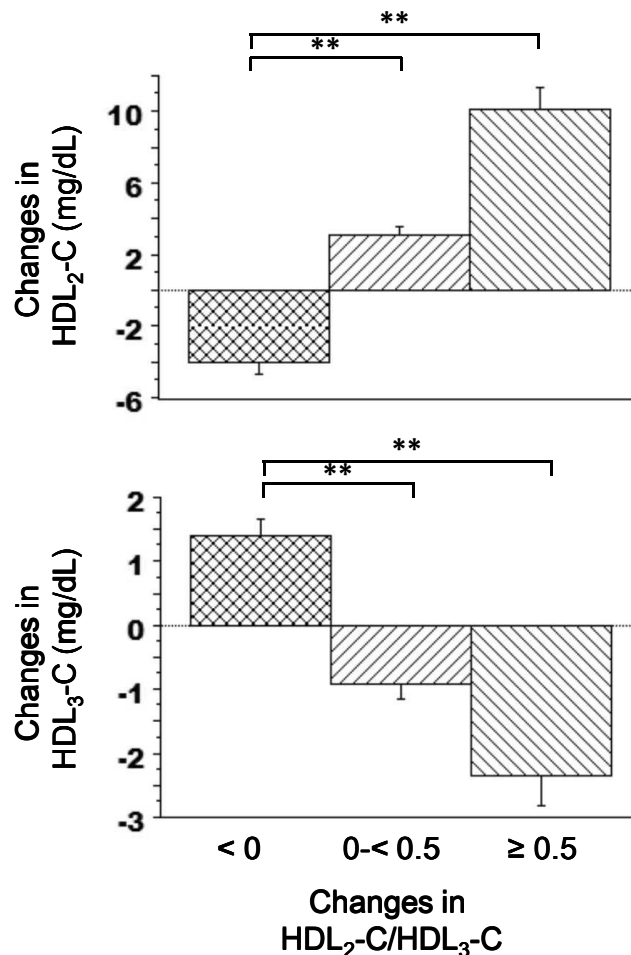


Fig. 1. Changes in the mean HDL₂-C and HDL₃-C values stratified by changes in the HDL₂-C/HDL₃-C ratio. Error bars represent 95% confidence intervals. ** $P < 0.01$ (Dunnett's multiple comparisons test). HDL₂-C, high-density lipoprotein 2 cholesterol; HDL₃-C, high-density lipoprotein 3 cholesterol.

increased. The average change in HDL₃-C was significantly lower in the 0 to <0.5 and ≥0.5 HDL₂-C/HDL₃-C ratio change groups than in the reference group.

Fig. 2 presents a stratification of the average changes in HWM-Ad according to the changes in the HDL₂-C/HDL₃-C ratio. HWM-Ad gradually increased as the HDL₂-C/HDL₃-C ratio increased. The average change in HWM-Ad was significantly higher in the ≥0.5 HDL₂-C/HDL₃-C ratio change group than in the reference group.

Fig. 3 presents a stratification of the average changes in HOMA-IR according to the changes in the HDL₂-C/HDL₃-C ratio. The average change in HOMA-IR was significantly lower in the ≥0.5 HDL₂-C/HDL₃-C ratio change group than in the reference group.

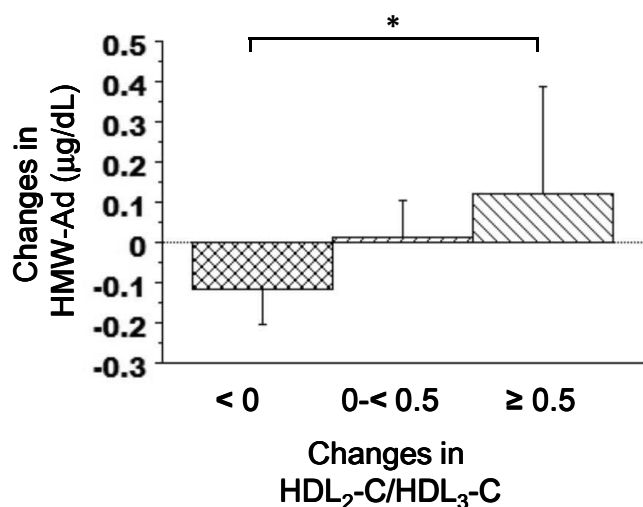


Fig. 2. Changes in the mean HMW-Ad values stratified by changes in the HDL₂-C/HDL₃-C ratio. Error bars represent 95% confidence intervals. * $P < 0.05$ (Dunnett's multiple comparisons test). HMW-Ad, high-molecular-weight adiponectin; HDL₂-C, high-density lipoprotein 2 cholesterol; HDL₃-C, high-density lipoprotein 3 cholesterol.

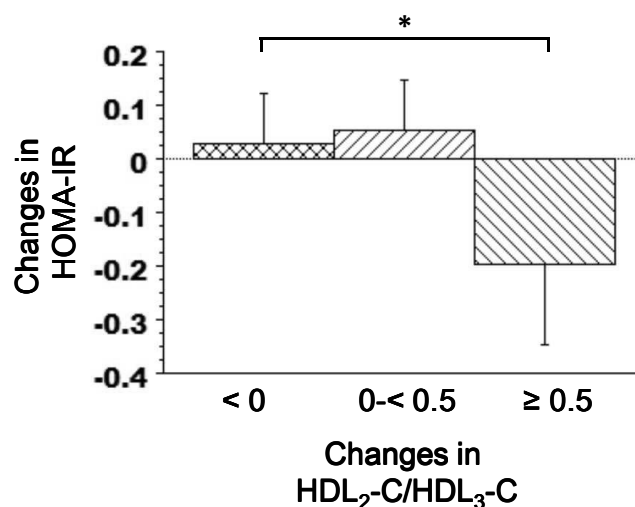


Fig. 3. Changes in the mean HOMA-IR values stratified by changes in the HDL₂-C/HDL₃-C ratio. Error bars represent 95% confidence intervals. * $P < 0.05$ (Dunnett's multiple comparisons test). HOMA-IR, homeostasis model assessment of insulin resistance; HDL₂-C, high-density lipoprotein 2 cholesterol; HDL₃-C, high-density lipoprotein 3 cholesterol.

C/HDL₃-C ratio change group than in the reference group.

Fig. 4 presents a stratification of the average changes in LDL-C according to the changes in the HDL₂-C/HDL₃-C ratio. LDL-C gradually decreased as the HDL₂-C/HDL₃-C ratio increased. The average change in LDL-C was significantly lower in the 0 to < 0.5 and ≥ 0.5 HDL₂-C/HDL₃-C ratio change groups than in the reference group.

Discussion

In this study, we found that changes in HDL₂-C/HDL₃-C inversely correlated with changes in WC, insulin resistance, and LDL-C and positively correlated with HMW-Ad and good lifestyle habits. These results suggest that an evaluation of HDL-C alone is not sufficient and that changes in the HDL₂-C/HDL₃-C ratio serve as a useful marker of not only MetS but also atherogenic conditions in Japanese individuals. To the best of our knowledge, this is the first study to describe associations between changes in the HDL₂-C/HDL₃-C ratio and changes in MetS components, HMW-Ad, insulin resistance, LDL-C, and lifestyle habits.

Insulin resistance is considered an underlying abnormality in most cases of type 2 diabetes; because diabetes treatment leads to improved insulin resis-

tance²⁸), we excluded subjects who were undergoing treatment for diabetes mellitus.

Subjects in the ≥ 0.5 HDL₂-C/HDL₃-C ratio change group exhibited improvements in obesity and increased HDL-C and HMW-Ad levels. In contrast, changes in BP and FPG were not associated with changes in the HDL₂-C/HDL₃-C ratio. A gradual decrease in the average TG was observed as the HDL₂-C/HDL₃-C ratio increased, but this difference was not significant between the reference and other groups. However, further obesity amelioration might improve the BP, FPG, and TG values. Further studies will be needed to clarify this speculation.

Although insulin resistance drives the overproduction of very low-density lipoproteins, and subsequently LDL, an increased number of LDL particles is not currently a part of the definition of MetS²⁹). Therefore, it is important to note that subjects with MetS do not always have elevated LDL-C levels. In this study, we observed a gradual average decrease in LDL-C as the HDL₂-C/HDL₃-C ratio increased, suggesting that an improvement in insulin resistance correlated with a decrease in the LDL-C level. Subjects with MetS have been described to exhibit a qualitative LDL abnormality³⁰). Moreover, a 4%–9% reduction in body weight has been shown to decrease the small-dense LDL level by 4.7%³¹). Our results indicated significantly greater decreases in BMI and WC in the 0

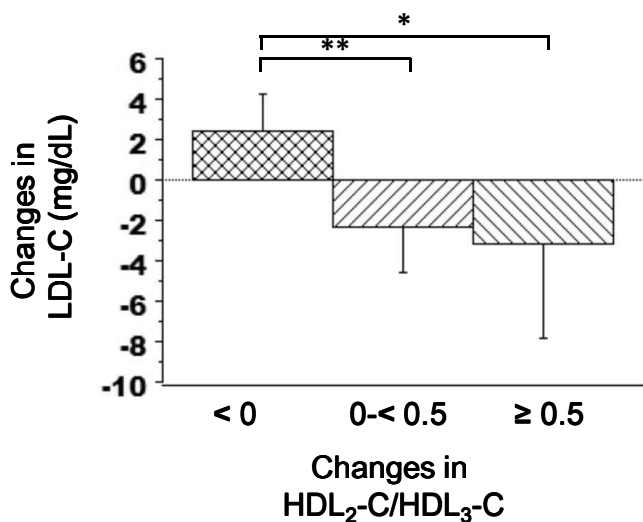


Fig. 4. Changes in the mean LDL-C values stratified by changes in the HDL₂-C/HDL₃-C ratio. Error bars represent 95% confidence intervals. ** $P < 0.01$, * $P < 0.05$ (Dunnnett's multiple comparisons test).

LDL-C, low-density lipoprotein cholesterol; HDL₂-C, high-density lipoprotein 2 cholesterol; HDL₃-C, high-density lipoprotein 3 cholesterol.

to <0.5 and ≥ 0.5 HDL₂-C/HDL₃-C ratio change groups than in the reference group. Therefore, the gradual average decrease in LDL-C, which occurred as the HDL₂-C/HDL₃-C ratio increased, was likely due to decreased levels of LDL subclasses (e.g., small-dense and/or oxidized LDL). It would be interesting to determine whether this is true in future studies.

When subjects were stratified according to changes in the HDL₂-C/HDL₃-C ratio, the ≥ 0.5 HDL₂-C/HDL₃-C ratio change group had a lower smoking rate and a lower percentage of subjects who drank alcohol every day, compared with the <0.5 and 0 to <0.5 HDL₂-C/HDL₃-C ratio change groups. The same group contained fewer inactive subjects and fewer subjects who did not exercise, as well as a higher percentage of subjects who initiated exercise after the first annual examination. Collectively, these findings indicated that subjects with good lifestyle habits had increased HDL₂-C/HDL₃-C ratios. Further studies are needed to investigate the relationship between improvements in lifestyle habits and changes in the HDL₂-C/HDL₃-C ratio.

We observed similar results after the exclusion of subjects who were under treatment for dyslipidemia ($n = 134$) (data not shown).

Limitations of our study were the lack of detailed information regarding treatments for hypertension and dyslipidemia. The possibility of confounding by

mutations in lipid-related genes also exists, although these data were not available in the present study. The subjects of this study were middle-aged Japanese individuals, and it is possible that the associations between HDL₂-C/HDL₃-C ratio changes and MetS components, HMW-Ad, insulin resistance, and lipids will be affected by age and ethnicity.

Conclusion

We found that changes in HDL₂-C/HDL₃-C inversely correlated with changes in WC, insulin resistance, and LDL-C and positively correlated with HMW-Ad and good lifestyle habits. Our data suggest that monitoring changes in the HDL subclass ratio might help to identify changes in MetS, HMW-Ad, insulin resistance, LDL-C, and lifestyle habits, at least in Japanese populations.

Conflicts of Interest

There are no conflicts of interest to declare.

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