



HDL Subclass Analysis in Predicting Metabolic Syndrome in Koreans With High HDL Cholesterol Levels

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Background: High-density lipoprotein cholesterol (HDL-C) is a complex mixture of subclasses with heterogeneous atheroprotective activities. We analyzed HDL-C subclass in relation to cardiovascular risk and metabolic syndrome (MetS) in a population with high HDL-C levels.

Methods: A total of 300 Korean individuals with high HDL-C levels (≥ 2.331 mmol/L) were enrolled following a comprehensive general medical examination including body composition analysis. HDL3-C levels were measured using the HDL3-EX SEIKEN kit (Randox Ltd., Crumlin, UK) and non-HDL3-C levels were calculated by subtracting HDL3-C levels from total HDL-C levels.

Results: HDL3-C levels and HDL3-C proportion had a weak positive correlation with low-density lipoprotein cholesterol (LDL-C) and triglycerides ($r=0.21$, $r=0.25$; $r=0.26$, $r=0.34$, respectively, all $P<0.001$); in contrast, non-HDL3-C levels had a weak negative correlation with these parameters ($r=-0.17$ and $r=-0.25$, respectively, both $P<0.005$). HDL3-C levels and HDL3-C proportion were significantly higher in the MetS group ($N=8$) than in the non-MetS group (0.71 vs 0.63 mmol/L, $P=0.001$; 29.7 vs 25.8% , $P=0.001$, respectively); these were the only predictors of MetS among the lipid variables (areas under the curves [AUC]= 0.84 and 0.83 , respectively, both $P=0.001$).

Conclusions: In populations with high HDL-C levels, HDL-C subclass may provide a greater amount of information on cardiovascular risk and MetS than HDL-C levels alone.

Key Words: High-density lipoprotein cholesterol, Subclass, HDL3 cholesterol, Cardiovascular risk, Metabolic syndrome

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INTRODUCTION

High-density lipoprotein (HDL) cholesterol (HDL-C) is a well-known independent, inverse predictor of cardiovascular diseases (CVD); it has a modest but useful effect on refining risk estimation [1, 2]. However, the association may be more complicated under certain conditions (e.g., genetically altered HDL-

C, use of drugs that elevate HDL-C levels, and extremely high HDL-C levels) [3-5], and its effect may not be seen in some populations, particularly in those with relatively high HDL-C levels [6, 7]. Furthermore, recent studies have highlighted that very high HDL-C levels may not consistently indicate cardiovascular (CV) risks [5, 6].

HDL comprises several subclass particles, which differ in size,

density, and components: the two major subclasses, based on density gradient ultracentrifugation, are larger and buoyant particles of HDL2 (density 1.063–1.125 g/mL) and smaller and denser particles of HDL3 (density 1.125–1.210 g/mL) [8–14]. These HDL subclasses are thought to play different roles in atherosclerosis progression and regression, and total HDL-C levels

Table 1. Characteristics of the study population

	Total (N=300)	Women (N=237)	Men (N=63)	P
Age (yr)	44.0 (37.0–51.0)	43.0 (36.0–48.0)	49.0 (41.0–54.5)	<0.001
SBP (mm Hg)	116.0 (104.0–124.0)	111.0 (102.0–120.0)	125.0 (119.0–133.0)	<0.001
DBP (mm Hg)	71.0 (63.0–79.0)	69.0 (61.0–77.0)	77.0 (69.0–80.5)	<0.001
Past medical history (%)				
Hypertension	11 (3.7)*	5 (2.1)	6 (9.5)	0.01
Diabetes mellitus	2 (0.7)†	1 (0.4)	1 (1.6)	0.4
Dyslipidemia	15 (5.0)‡	9 (3.8)	6 (9.5)	0.1
Body composition analysis				
Weight (kg)	54.0 (49.9–61.3)	52.7 (49.0–57.3)	66.0 (58.3–72.4)	<0.001
Height (cm)	162.4 (157.4–167.3)	160.4 (156.7–164.2)	171.3 (168.9–174.9)	<0.001
BMI (kg/m ²)	20.8 (19.3–22.7)	20.6 (19.2–22.1)	22.4 (20.6–23.5)	<0.001
Waist (cm)	72.0 (67.5–78.0)	71.0 (67.0–75.0)	79.0 (74.2–84.0)	<0.001
Waist/hip ratio	0.8 (0.8–0.8)	0.8 (0.8–0.8)	0.9 (0.8–0.9)	<0.001
Body fat mass (kg)	13.9 (11.9–16.2)	14.0 (12.2–16.6)	12.5 (9.2–15.2)	0.001
Body fat (%)	25.8 (21.2–29.0)	27.2 (23.9–30.0)	18.8 (15.3–21.3)	<0.001
VFA (cm ²)	51.5 (35.9–74.0)	47.0 (33.8–65.3)	91.0 (66.1–102.0)	<0.001
Laboratory analysis				
Hb (g/L)	135 (129–143)	133 (126–138)	151 (146–158)	<0.001
FPG (mmol/L)	4.9 (4.6–5.2)	4.8 (4.6–5.2)	5.2 (4.8–5.5)	<0.001
Hb A1c (%)	5.3 (5.1–5.5)	5.3 (5.1–5.5)	5.4 (5.2–5.5)	0.4
Total-C (mmol/L)	5.5 (5.1–6.1)	5.6 (5.1–6.1)	5.5 (4.8–6.0)	0.4
LDL-C (mmol/L)	2.7 (2.2–3.2)	2.7 (2.2–3.2)	2.7 (2.1–3.2)	0.6
HDL-C (mmol/L)	2.5 (2.4–2.5)	2.4 (2.4–2.5)	2.5 (2.4–2.6)	0.8
HDL3-C (mmol/L)	0.6 (0.6–0.7)	0.6 (0.6–0.7)	0.6 (0.6–0.7)	0.8
Non-HDL3-C (mmol/L)	1.8 (1.8–1.9)	1.8 (1.8–1.9)	1.8 (1.8–1.9)	0.5
HDL3-C proportion (%)	25.9 (24.3–27.4)	25.9 (24.4–27.5)	25.8 (23.9–27.4)	0.7
TG (mmol/L)	0.6 (0.5–0.8)	0.6 (0.5–0.8)	0.7 (0.5–0.8)	0.7
AST (ukat/L)	0.37 (0.32–0.45)	0.36 (0.31–0.43)	0.48 (0.39–0.63)	<0.001
ALT (ukat/L)	0.26 (0.21–0.36)	0.26 (0.19–0.31)	0.37 (0.28–0.55)	<0.001
Creatinine (μmol/L)	70.7 (61.9–79.6)	70.7 (61.9–70.7)	88.4 (79.6–97.2)	<0.001
GFR (mL/min/1.73 m ²)	85.0 (76.5–91.0)	85.0 (77.0–91.0)	82.0 (76.0–90.5)	0.3

Values are expressed as median (interquartile range), number (percentage), or proportion.

P is based on the comparison between men and women using the Mann-Whitney U test (quantitative variables) and Chi-square or Fisher's exact test (categorical variables); bold text indicates $P < 0.05$.

*Treated with single calcium channel blockers (N=4), single angiotensin II receptor blockers (N=2), calcium channel blockers with angiotensin II receptor blockers (N=2), or lifestyle modification only (N=3).

†Treated with sitagliptin (N=1) or lifestyle modification only (N=1).

‡Treated with atorvastatin (N=6), rosuvastatin (N=3), pitavastatin (N=2), or lifestyle modification only (N=4).

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; VFA, visceral fat area; Hb, hemoglobin; FPG, fasting plasma glucose; HbA1C, glycated hemoglobin; Total-C, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HDL3C, high-density lipoprotein 3 cholesterol; TG, triglycerides; AST, aspartate transaminase; ALT, alanine transaminase; GFR, glomerular filtration rate based on the Modification of Diet in Renal Disease Study equation.

do not always represent HDL particle numbers, subclass distribution, or HDL functions such as cholesterol efflux capacity [8]. Whether HDL subclasses play different roles in atherosclerotic CVD protection or whether measuring HDL subclasses improves risk prediction compared with measuring total HDL-C levels alone has not been determined [15–22].

Metabolic syndrome (MetS) is a cluster of multi-dimensional CV risk factors related to abdominal obesity, type 2 diabetes, dyslipidemia, and hypertension [23, 24]. Given that low HDL-C levels are one of the five components of MetS diagnosis, it is debatable whether it is necessary to subclassify HDL for a more accurate diagnosis of MetS. A few studies have explored the association between HDL and MetS components, mostly using ultracentrifugation methods, in patient populations [25–28].

Recently, a fully-automated homogeneous assay for HDL3-C measurement has become commercially available; compared with the classic ultracentrifugation method, it allows for simpler, more rapid, and more effective measurement of HDL3-C levels, making it suitable for routine clinical practice [10–12]. We analyzed HDL-C subclass in relation to the CV risk and MetS in a Korean population with high HDL-C levels, using a fully automated direct HDL3-C assay. We hypothesized that HDL-C subclass analysis would provide additive value for assessing the CV risk and MetS even in a rather homogenous population with high HDL-C levels.

MATERIALS AND METHODS

Study populations and design

This cross-sectional study was conducted according to the World Medical Association Declaration of Helsinki, and the protocol was approved by the institutional review board of Konkuk University Medical Center (KUMC), Seoul, Korea. Between March and December 2018, we enrolled a total of 300 adult individuals (237 women and 63 men) with high HDL-C levels (≥ 2.331 mmol/L) based on their annual general medical examination (GME) program, which included body composition analysis and routine blood analysis. We retrospectively reviewed their medical records including routine check-up results and self-administered questionnaires; no gender difference was observed in their lipid profiles and they were considered free of coronary heart disease (CHD). Individual characteristics are presented in Table 1.

Body composition was analyzed using InBody770 (InBody Co., Ltd., Seoul, Korea) based on the direct segmental multi-frequency bioelectrical impedance method [29]. The following parameters were determined: body weight (kg), skeletal muscle mass (kg), body mass index (BMI, kg/m²), waist circumference (cm), waist/hip ratio, body fat mass (kg), body fat (%), and visceral fat area (cm²).

MetS was defined according to the National Cholesterol Education Program Adult Treatment Panel III [23] and modified Asian criteria [24] as the presence of any three of the following five

Table 2. Correlations between HDL subclass and parameters reflecting metabolic syndrome components or lipid profiles

	HDL-C		HDL3-C		Non-HDL3-C		HDL3-C proportion	
	r	P	r	P	r	P	r	P
BMI	-0.09	0.1	0.07	0.2	-0.14	0.02	0.13	0.02
Waist	-0.08	0.2	0.09	0.1	-0.13	0.02	0.14	0.01
Waist/hip ratio	-0.02	0.7	0.07	0.2	-0.06	0.3	0.08	0.2
Fat mass	-0.08	0.2	0.08	0.2	-0.12	0.04	0.14	0.02
Body fat	-0.06	0.3	0.08	0.2	-0.11	0.05	0.13	0.02
VFA	-0.03	0.6	0.08	0.2	-0.07	0.2	0.1	0.1
SBP	0.02	0.7	0.12	0.03	-0.04	0.5	0.12	0.03
DBP	0.05	0.4	0.17	0.004	-0.03	0.6	0.15	0.009
FPG	-0.01	0.9	0.07	0.3	-0.04	0.5	0.08	0.2
HbA1c	-0.02	0.8	0.02	0.7	-0.03	0.6	0.04	0.5
Total-C	0.18	0.002	0.43	<0.001	-0.03	0.6	0.37	<0.001
LDL-C	0.03	0.7	0.21	<0.001	-0.17	0.004	0.26	<0.001
TG	-0.11	0.07	0.25	<0.001	-0.25	<0.001	0.34	<0.001

Bold text indicates $P < 0.05$.

Abbreviations and units: see Table 1.

Table 3. Comparison between the two groups with and without metabolic syndrome (MetS)

	MetS (N=8)	Non-MetS (N=292)	P
Age (yr)	52.5 (48.5–59.0)	44.0 (37.0–50.0)	0.003
Gender, women	6 (75.0%)	231 (79.1%)	1.0
SBP (mm Hg)	138.0 (134.0–146.0)	115.0 (103.0–123.5)	<0.001
DBP (mm Hg)	85.5 (79.0–89.5)	70.5 (62.5–79.0)	<0.001
MetS components* (%)			
Obesity	8 (100.0)	26 (8.9)	<0.001
Hypertension	8 (100.0)	48 (16.4)	<0.001
Glucose	8 (100.0)	28 (9.6)	<0.001
HDL-C	0 (0.0)	0 (0.0)	1.0
Triglycerides	0 (0.0)	2 (0.7)	1.0
Alcohol consumption† (%)			
None	3 (37.5)	91 (31.2)	0.8
Adequate	4 (50.0)	144 (49.3)	> 0.9
Intermediate	0 (0.0)	43 (14.7)	0.2
Heavy alcohol use	1 (12.5)	8 (2.7)	0.1
Body composition analysis			
Weight (kg)	64.1 (59.6–73.2)	53.8 (49.8–60.9)	0.002
BMI (kg/m ²)	24.2 (23.4–26.0)	20.7 (19.2–22.5)	<0.001
Waist (cm)	82.8 (81.8–88.2)	72.0 (67.5–77.5)	<0.001
Waist/hip ratio	0.86 (0.84–0.90)	0.82 (0.77–0.85)	0.007
Body fat mass (kg)	19.4 (18.0–22.3)	13.7 (11.9–15.9)	<0.001
Body fat (%)	32.5 (28.0–35.5)	25.6 (21.1–28.7)	0.006
VFA (cm ²)	91.5 (66.5–109.0)	51.0 (35.5–72.8)	0.006
Laboratory analysis			
Hb (g/L)	141 (138–146)	135 (129–143)	0.2
FPG (mmol/L)	5.7 (5.6–6.2)	4.9 (4.6–5.2)	<0.001
Hb A1c (%)	5.8 (5.2–6.2)	5.3 (5.1–5.5)	0.05
Total-C (mmol/L)	5.8 (5.4–6.6)	5.5 (5.1–6.0)	0.2
LDL-C (mmol/L)	2.8 (2.2–3.8)	2.7 (2.2–3.2)	0.6
HDL-C (mmol/L)	2.4 (2.4–2.6)	2.5 (2.4–2.5)	> 0.9
HDL3-C (mmol/L)	0.7 (0.7–0.8)	0.6 (0.6–0.7)	0.001
Non-HDL3-C (mmol/L)	1.7 (1.7–1.8)	1.8 (1.8–1.9)	0.09
HDL3-C proportion (%)	29.7 (27.3–30.0)	25.8 (20.3–27.3)	0.001
TG (mmol/L)	0.8 (0.6–1.0)	0.6 (0.5–0.8)	0.2
AST (ukat/L)	0.65 (0.32–1.30)	0.37 (0.32–0.44)	0.08
ALT (ukat/L)	0.75 (0.28–1.56)	0.26 (0.20–0.36)	0.01
Creatinine (umol/L)	70.7 (61.9–79.6)	70.7 (61.9–79.6)	0.7
GFR (mL/min/1.73 m ²)	80.0 (73.5–85.5)	85.0 (77.0–91.0)	0.2

Values are expressed as median (inter-quartile range) or number (percentage).

Bold text indicates $P < 0.05$.

*National Cholesterol Education Program Adult Treatment Panel III with Asian modification: (1) abdominal obesity: waist circumference ≥ 90 cm (men) or ≥ 80 cm (women) in Asian individuals; (2) blood pressure $\geq 130/85$ mm Hg or medication; (3) fasting glucose ≥ 5.55 mmol/L or drug treatment; (4) HDL-C < 1.036 mmol/L (men) or < 1.295 mmol/L (women) or drug treatment; (5) TG ≥ 1.695 mmol/L or drug treatment [23, 24].

†Data was available for 294 subjects. Adequate is defined as one standard drink (14 g of ethanol, as found in five ounces of wine or 12 ounces of beer) per day or seven standard drinks per week with no more than three drinks per occasion. Heavy alcohol use is defined as binge drinking (four drinks for women and five drinks for men) on five or more days in the past month [31]. There was a weak positive correlation with alcohol grade vs HDL3-C ($r = 0.13$, $P = 0.03$), but not with HDL-C ($r = 0.04$, $P = 0.5$; data not shown).

Abbreviations: see Table 1.

traits: (1) Abdominal obesity in Asians, defined as a waist circumference ≥ 90 cm in men and ≥ 80 cm in women; (2) fasting serum triglyceride (TG) levels ≥ 1.695 mmol/L or drug treatment for elevated TG; (3) serum HDL-C levels < 1.036 mmol/L in men and < 1.295 mmol/L in women or drug treatment for low HDL-C levels; (4) blood pressure (BP) $\geq 130/85$ mm Hg or drug treatment for elevated BP; (5) fasting plasma glucose (FPG) level ≥ 5.55 mmol/L or drug treatment for elevated FPG level. As the HDL-C levels in all individuals were > 2.331 mmol/L, MetS was diagnosed based on the other four criteria.

Lipid analysis

Blood samples were drawn after at least 12 hours of overnight fasting following the routine GME protocol and were centrifuged within 1 hour. The serum samples were stored at -80°C until

use. Total cholesterol (Total-C), low-density lipoprotein cholesterol (LDL-C), HDL-C, and TG levels were measured using commercially available enzymatic reagents (Kyowa Kirin Co., Ltd., Tokyo, Japan) on a chemistry analyzer (TBA-200FR NEO, Toshiba Medical Systems Co., Tokyo, Japan). HDL3-C levels were measured on the same analyzer using the HDL3-EX “SEIKEN” kit (Randox Laboratories Ltd., Crumlin, UK), according to the manufacturer’s protocol. Briefly, this fully automated direct HDL subclass assay consists of two reaction steps. First, the cholesterol released from the lipoprotein particles is degraded to water and oxygen through hydrolysis by cholesterol esterase, oxidation by cholesterol oxidase, and the subsequent catalase reaction. Second, a specific surfactant selectively reacts with HDL3-C. Compared with the ultracentrifugation method, this assay showed a much stronger correlation ($r=0.92$ based on the manufacturer’s

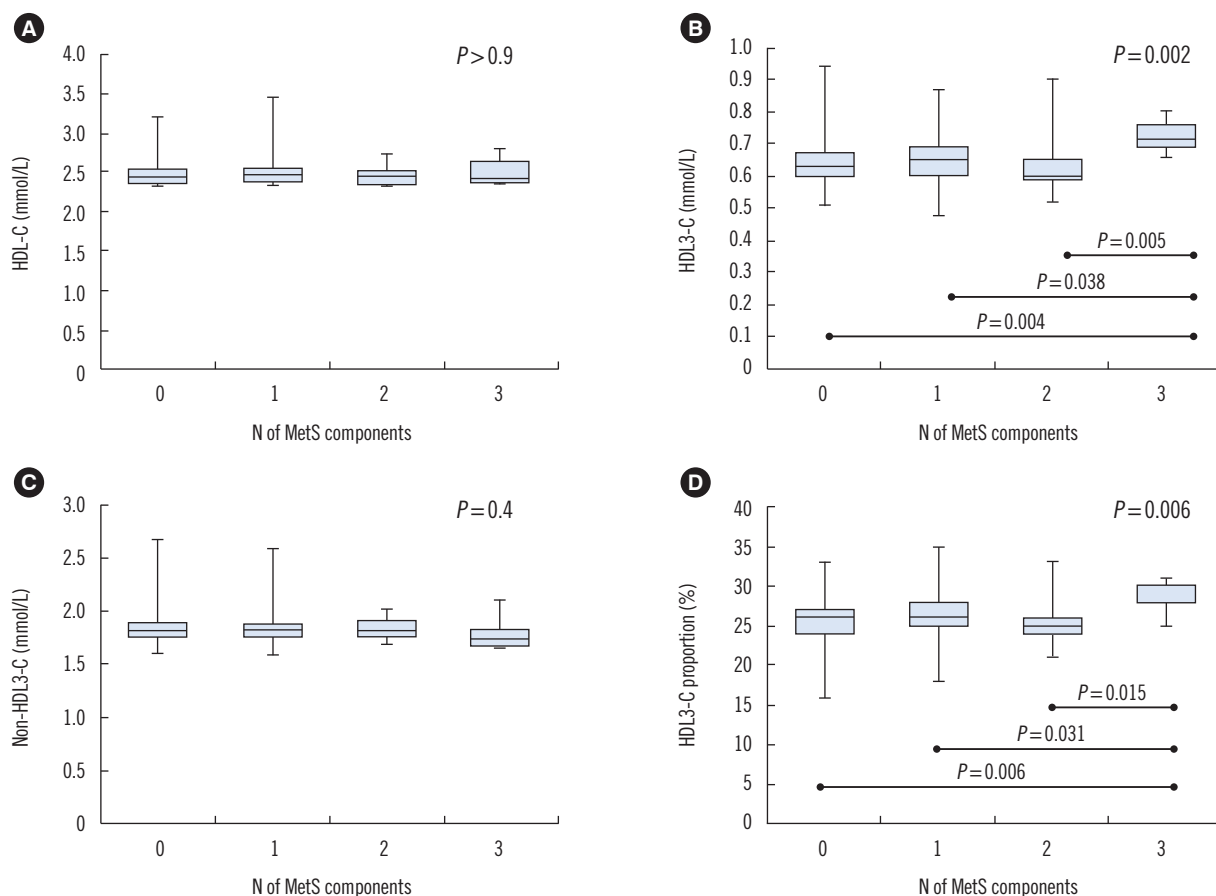


Fig. 1. Box-and-whisker plots for the distribution of HDL-C (A), HDL3-C (B), non-HDL3-C (C), and HDL3-C proportion (D) stratified by the number of MetS components (0, N=201; 1, N=78; 2, N=13; and 3, N=8). HDL3-C levels and HDL3-C proportion were significantly higher in the three MetS component group than in the other groups ($P < 0.05$, Kruskal-Wallis test with post-hoc Dunn test; P with statistical significance is indicated). The central box represents the values from the lower to upper quartile (25–75 percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value.

Abbreviations: HDL-C, high-density lipoprotein cholesterol; HDL3-C, high-density lipoprotein 3 cholesterol; MetS, metabolic syndrome.

claim; $r=0.90$ with a mean difference of 0.13 mmol/L according to Gómez, *et al.* [12]). The manufacturer claimed that the assay range was from 4 to 60 mg/dL (0.1036–1.554 mmol/L) and that the CVs (%) for total precision were 1.6% and 1.4% (Control I and II, respectively). Non-HDL3-C levels were calculated by subtracting HDL3-C levels from total HDL-C levels.

Statistical analysis

We performed the statistical analysis using dBSTAT (DBSTAT version 5.0 Chuncheon, Korea: DBSTAT Co; 2010. <http://dbstat.com>) and SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as medians (interquartile ranges) for continuous variables or numbers (percentages) for categorical or binary variables. We assessed the correlation between continuous variables using Pearson's correlation coefficient (r), and the correlation was interpreted as negligible (<0.1), weak (0.1–0.39), moderate (0.40–0.69), strong (0.70–0.89), or very strong (≥ 0.9) based on the absolute magnitude [30]. We compared clinical, body composition, and laboratory data of individuals with or without MetS using the Mann-Whitney U-test (continuous variables) and chi-square test or Fisher's exact test (categorical variables). Individuals were assigned to one of four groups by the number of MetS components (0, $N=201$; 1, $N=78$; 2, $N=13$; and 3, $N=8$) and compared using the Kruskal-Wallis test with a post hoc Dunn test. To identify predictors of MetS, we analyzed receiver-operating characteristic (ROC) curves and compared the areas under the curves (AUC). P (2-tailed) <0.05 was considered statistically significant.

RESULTS

HDL subclass distribution and correlations with other parameters

The correlations between HDL subclass and GME parameters reflecting MetS components or lipid profiles are presented in Table 2. Non-HDL3-C levels showed a weak negative correlation with body component parameters (BMI, waist circumference, and fat mass) and lipid profiles (LDL-C and TG levels). In contrast, HDL3-C levels and HDL3-C proportion showed weak positive correlations with these parameters, especially with LDL-C and TG levels ($r=0.21$ and $r=0.25$; $r=0.26$ and $r=0.34$, respectively, all $P<0.001$).

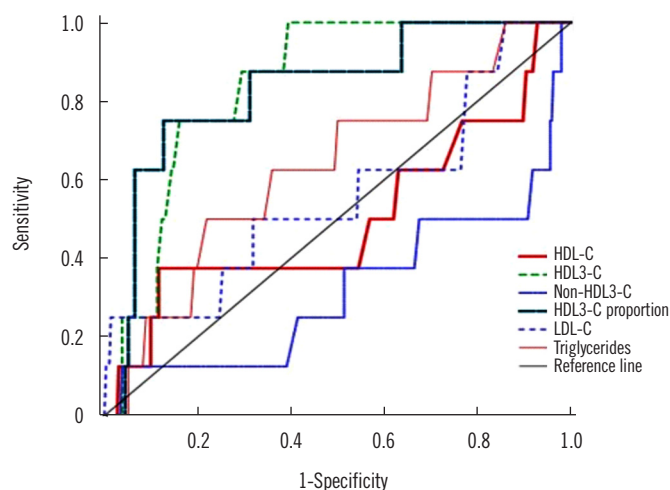
MetS

Of the 300 individuals, eight (2.7%) were diagnosed as having MetS (Table 3) [31]. No differences in total HDL-C levels were

observed between the two groups; however, HDL3-C levels and HDL3-C proportion were significantly higher in the MetS group than in the non-MetS group (0.71 vs 0.63 mmol/L, $P=0.001$; 29.7% vs 25.8%, $P=0.001$, respectively).

HDL3-C levels and HDL3-C proportion differed significantly across the four groups according to the number of MetS components ($P=0.002$ and $P=0.006$, respectively); in each group, they were significantly higher in the three MetS component group than in the other groups (Fig. 1).

ROC curve analysis demonstrated that only HDL3-C levels and HDL3-C proportion could predict MetS in a population with high HDL-C levels (both $P=0.001$; Fig. 2). Using an optimal cutoff level of 0.653 mmol/L, HDL3-C levels predicted MetS with 100% sensitivity and 60.6% specificity. Using an optimal cutoff level of 40%, HDL3-C proportion predicted MetS with 75.0% sensitivity and 87.3% specificity.



Variable	AUC (95% CI)	P
HDL-C (mmol/L)	0.50 (0.26–0.74)	>0.9
HDL3-C (mmol/L)	0.84 (0.76–0.93)	0.001
Non-HDL3-C (mmol/L)	0.32 (0.09–0.55)	0.09
HDL3-C proportion	0.83 (0.68–0.98)	0.001
LDL-C (mmol/L)	0.56 (0.34–0.79)	0.6
Triglycerides (mmol/L)	0.63 (0.44–0.83)	0.2

Fig. 2. Receiver operator characteristic curves of lipid profiles for predicting MetS in individuals with high HDL-C levels (≥ 2.331 mmol/L). HDL3-C levels and HDL3-C proportion were significant ($P=0.001$) and comparable ($P=0.9$) for predicting MetS.

Abbreviations: MetS, metabolic syndrome; HDL-C, high-density lipoprotein cholesterol; HDL3-C, high-density lipoprotein 3 cholesterol; LDL-C, low-density lipoprotein cholesterol; AUC, area under the curve; CI, confidence interval.

DISCUSSION

To the best of our knowledge, this is the first HDL subclass analysis using a fully automated homogenous assay for HDL3-C in a population with high HDL-C levels (≥ 2.331 mmol/L). In general, high HDL-C levels alone have been considered a negative risk factor for CHD and a GME setting naturally excludes acute cardiac diseases. We hypothesized that HDL-C subclass analysis would provide additive value to for assessing the CV risk and MetS in this population and demonstrated a potential additive value in subclassifying HDL-C compared with total HDL-C levels; the following were noted: (1) different correlations between HDL subclasses and BMI, waist circumference, fat mass, LDL-C levels, and TG levels; (2) improved prediction of MetS with HDL3-C levels (AUC=0.84) and HDL3-C proportion (AUC=0.83), but not with total HDL-C levels (AUC=0.5).

Interestingly, with regard to body composition and lipid parameters, an inverse correlation was observed between HDL subclasses (HDL3-C vs non-HDL3-C) and well-established CVD risk factors; in contrast to non-HDL3-C, HDL3-C levels and HDL3-C proportion showed a weak positive correlation with BP, LDL-C levels, and TG levels (Table 2). Given that the role of HDL subclass in atheroprotective effects has not been consistently determined, it was interesting to observe this contrasting relationship in this homogenous population, especially as all individuals in this study group had high HDL-C levels. Our findings indicate the potential value of HDL subclass analysis in a wide range of individuals. The use of proportions or ratios might be preferable to using simple subclass levels in order to generalize across the full spectrum of HDL-C levels [26]. Although a causal relationship could not be elucidated [32, 33], we demonstrated the dynamic changes in HDL3-C proportion, suggesting its potential role as a biomarker for atherosclerotic CVD [25].

Our study is unique in terms of study population; we enrolled individuals with high HDL-C levels, without prior CHD. Of note, HDL3-C levels or HDL3-C proportion demonstrated an additive value for MetS prediction compared with total HDL-C alone, even in this population; HDL subclass distribution successfully refined the risk of MetS, as a higher HDL3-C proportion indicated MetS (Figs. 1 and 2). Despite a strong inverse correlation between HDL-C levels and MetS, higher levels of HDL3-C predicted MetS in a contradictory manner; this “HDL3-C paradox” might be an important concept for understanding lipid dynamics related to MetS [25-28]. For example, exercise increases total HDL-C, mostly HDL2 or the “good HDL” subfraction via reduced hepatic lipase activity and elevated lecithin-cholesterol acyltransferase activity

(conversion of HDL3 into HDL2); this may decrease an individual’s susceptibility to MetS [27].

Our study has several limitations. This was a small-sized, single-center study on an Asian population. Accordingly, the results may not be extrapolated, particularly to other geographical regions or ethnicities. We wanted to explore the heterogeneity of HDL-C subclass even in a relatively homogenous and specific population with high HDL-C levels. Owing to this narrow study population, only 3% of the individuals were diagnosed as having MetS, and there is a possibility that we failed to identify some changes with potential significance [34]. Additionally, confounding factors (e.g., diet, exercise, smoking, alcohol, or genetic factors) were not controlled and non-HDL3-C levels were not measured but calculated by subtracting HDL3-C levels from total HDL-C levels. A longitudinal study involving a larger number of individuals with a wider range of HDL3-C levels would be necessary to elucidate the clinical value of HDL-C subclass analysis.

In conclusion, we demonstrated that in a population with high HDL-C levels, HDL-C subclasses can refine the CV risk and MetS risk profiles better than HDL-C levels alone. The clinical utility of HDL-C subclass analysis would be further facilitated using a fully automated assay. Further studies spanning a full-range HDL-C population are warranted to determine the subclass distribution and major adverse CV events more broadly and to support and generalize the present findings.

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AUTHOR CONTRIBUTIONS

HSY, SJK, and MH designed the research; HK and MH performed experiments; HSY, HK, SJK, and SS analyzed the data; HSY and MH wrote the manuscript; SDS served as a scientific advisor; all authors reviewed and approved the manuscript.

CONFLICT OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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