

ORIGINAL ARTICLE

High-Density Lipoprotein Subfraction Analysis by High-Density Lipoprotein Cholesterol Levels and Gender in Healthy Adults

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ABSTRACT

Background: Attempts to raise high-density lipoprotein cholesterol (HDL-C) levels pharmacologically have failed to reduce cardiovascular risk and some studies suggest that very high HDL-C levels may paradoxically be linked to adverse outcomes. These findings have shifted focus from HDL quantity to quality, emphasizing functional properties. Because direct functional assays are difficult to implement clinically, HDL subfraction analysis has emerged as a practical alternative for evaluating heterogeneity and potential function of HDL.

Methods: A total of 172 healthy adults (85 men and 87 women) were classified into five gender-specific HDL-C percentile groups. HDL subfractions were analyzed using a commercial polyacrylamide gel electrophoresis-based assay and compared across groups and genders. The profiles of 22 patients with dyslipidemia whose HDL-C levels corresponded to Group III were compared with healthy individuals in Group III (used as controls).

Results: Among men, increased HDL-C levels were associated with a higher proportion of small HDL subfraction, whereas large and intermediate HDL subfractions remained stable. In women, the large HDL subfraction increased and intermediate HDL subfraction decreased with increasing HDL-C levels. Despite similar HDL-C levels, patients with dyslipidemia had lower large HDL subfraction and higher intermediate HDL subfraction than healthy controls, with no difference in small HDL subfraction.

Conclusions: Gender-specific reference distributions for HDL subfractions across a wide HDL-C range were established using a polyacrylamide gel electrophoresis-based method. These findings suggest that HDL subfraction analysis may reflect qualitative differences beyond HDL-C levels and offer additional value for individualized cardiovascular risk assessment and future research on HDL function.

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KEYWORDS

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INTRODUCTION

High-density lipoprotein cholesterol (HDL-C) is traditionally referred to as “good cholesterol” and has been shown to be inversely associated with cardiovascular disease (CVD) risk in several epidemiologic studies [1-3]. However, largescale clinical trials attempting to increase HDL-C levels pharmacologically have failed to show clinical benefit [4-7], raising questions about

whether HDL-C plays a causal role in cardiovascular protection. Mendelian randomization studies have shown that individuals with genetically high HDL-C levels do not necessarily have a low risk of myocardial infarction [8,9]. More recently, the “U-shaped relationship” hypothesis has emerged, proposing that very high HDL-C levels may actually be associated with increased cardiovascular or overall mortality, and both low and very high HDL-C levels may increase CVD risk [10-12].

Based on these findings, recent studies have shifted focus toward the “quality” and “function” of HDL rather than the quantity of HDL-C [13-17]. Assays that measure HDL function, such as cholesterol efflux capacity, are considered key indicators for evaluating the protective effect of HDL against CVD but are difficult to perform in most clinical laboratories [13,18]. HDL particles are heterogeneous in size, density, composition, and biological activity. Although the exact mechanisms remain unclear, it is known that structural differences in HDL particles lead to functional diversity [16]. Therefore, many studies have attempted to analyze HDL subfractions as surrogate markers for functional assays that are otherwise impractical. Various techniques have been developed to analyze HDL subfractions, including ultracentrifugation, vertical auto-profile, nuclear magnetic resonance spectroscopy, gel electrophoresis, and ion mobility [16,19].

This study evaluated HDL subfraction profiles in healthy Korean men and women using the Lipoprint HDL subfractions testing system (Quantimetrix Corp., Redondo Beach, CA, USA), a commercial assay kit based on polyacrylamide gel electrophoresis (PAGE). This study aimed to characterize the distribution of HDL subfractions according to HDL-C levels, analyze gender differences, and explore the clinical implications by comparing patients with dyslipidemia who had similar HDL-C levels with healthy individuals.

MATERIALS AND METHODS

Subjects

Residual serum samples were collected from 172 healthy men and women (85 men and 87 women) who visited the Health Promotion Center of Kyung Hee University Hospital at Gangdong between April 2024 and May 2025. All participants had no history of underlying medical conditions or medication use, and their general laboratory results were within the reference ranges, except for the lipid test results. The participants were screened to ensure an even distribution of HDL-C levels across the entire range.

To establish gender-specific HDL-C percentiles, the lipid test results of 123 healthy men and 138 healthy women who met the aforementioned criteria were analyzed. Based on this, the study participants were categorized into five groups: Group I (< 2.5th percentile), Group II (2.5 - 25th percentile), Group III (25 - 75th

percentile), Group IV (75 - 97.5th percentile), and Group V (> 97.5th percentile) (Table 1).

To compare the HDL subfraction profiles between patients with dyslipidemia and Group III, used as healthy controls, residual serum samples were also collected from 22 patients with dyslipidemia whose HDL-C levels fell within the 25th - 75th percentiles. The dyslipidemia group consisted of eight men and 14 women, with a mean age of 57.1 ± 5.0 years. All patients received statin therapy, and nine of them were taking antihypertensive medications.

This study was approved by the Institutional Review Board of Gangdong Kyung Hee University Hospital (Seoul, Korea; Institutional Review Board approval No.: KHNMC 2024-06-018), which waived the need for informed consent for the use of leftover human specimens.

Lipid measurements and HDL subfraction analysis

Using the residual blood collected after 12 hour of fasting, total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C), and triglyceride levels were measured using a Roche-Hitachi Cobas 8000 c702 analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan).

HDL subfraction analysis was performed using the Lipoprint HDL Subfractions Testing System (Quantimetrix, USA) according to the manufacturer’s instructions. The principle of this testing system is PAGE, which separates HDL into 10 bands based on particle size, categorized into three subfractions: large (bands 1 - 3), intermediate (bands 4 - 7), and small (bands 8 - 10) subfractions.

Statistical analysis

All statistical analyses were performed using SPSS version 29.0 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). Polynomial contrast analysis was used to evaluate trends in HDL subfractions across the HDL-C groups for each gender. Independent *t*-tests were used to compare the HDL subfractions between genders within each HDL-C group. Two-way analysis of variance (ANOVA) was used to evaluate the main and interaction effects of the HDL-C group and gender on the HDL subfraction composition. A *p*-value < 0.05 was considered statistically significant. All graphs were generated using Python 3.8 with the matplotlib library (version 3.8.4).

RESULTS

Demographic and lipid characteristics

A total of 172 healthy men and women were categorized into five HDL-C groups based on gender-specific percentiles. The demographic and lipid profiles of each group are summarized in Table 2. There were no significant differences in age between the HDL-C groups. TC levels progressively increased with increasing HDL-C levels (*p* = 0.01), and triglyceride levels showed a de-

Table 1. Classification of study participants by gender-specific percentiles of high-density lipoprotein cholesterol (HDL-C) levels.

	Percentile	HDL cholesterol (mg/dL)	
		Men (n = 85)	Women (n = 87)
Group I	~ 2.5th	~ 36.1 (n = 10)	~ 42.4 (n = 8)
Group II	2.5th ~ 25th	36.1 ~ 45.7 (n = 18)	42.4 ~ 57.0 (n = 20)
Group III	25th ~ 75th	45.7 ~ 65.1 (n = 30)	57.0 ~ 80.0 (n = 23)
Group IV	75th ~ 97.5th	65.1 ~ 81.9 (n = 12)	80.0 ~ 110.6 (n = 27)
Group V	97.5th ~	81.9 ~ (n = 15)	110.6 ~ (n = 9)

Table 2. Demographic and lipid characteristics of gender-specific high-density lipoprotein cholesterol (HDL-C) percentile groups in healthy adults.

		Total	Group I	Group II	Group III	Group IV	Group V	p
n	total	172	18	38	53	39	24	
	men	85	10	18	30	12	15	
	women	87	8	20	23	27	9	
Age	men	44.6 ± 11.1	40.8 ± 11.6	43.6 ± 9.3	44.4 ± 12.2	47.6 ± 8.0	46.4 ± 13.0	0.64
	women	45.7 ± 9.9	49.0 ± 8.1	43.0 ± 10.1	45.0 ± 9.4	46.1 ± 10.5	49.2 ± 9.9	0.46
TC (mg/dL)	men	218.9 ± 42.7	194.9 ± 48.2	217.7 ± 32.5	213.0 ± 43.3	215.4 ± 39.4	250.7 ± 38.3	0.01
	women	227.9 ± 53.1	199.9 ± 67.9	208.5 ± 35.3	221.8 ± 60.7	241.6 ± 43.0	270.6 ± 51.3	0.01
HDL-C (mg/dL)	men	60.4 ± 23.2	31.1 ± 3.8	40.9 ± 2.9	57.3 ± 4.8	73.8 ± 4.2	98.8 ± 17.4	< 0.01
	women	75.4 ± 26.1	36.1 ± 6.6	50.0 ± 3.5	69.2 ± 5.4	97.3 ± 8.2	117.6 ± 6.6	< 0.01
LDL-C (mg/dL)	men	128.8 ± 40.4	106.2 ± 50.2	132.2 ± 30.9	130.0 ± 42.7	126.6 ± 42.1	138.7 ± 36.8	0.40
	women	129.1 ± 42.1	126.5 ± 61.3	127.5 ± 32.2	129.1 ± 49.8	127.5 ± 35.0	139.6 ± 48.2	0.96
TG (mg/dL)	men	126.9 ± 94.2	275.9 ± 161.1	166.2 ± 72.2	97.7 ± 41.7	71.1 ± 21.1	83.5 ± 44.1	< 0.01
	women	85.5 ± 43.4	111.6 ± 47.6	109.4 ± 48.6	86.3 ± 45.8	64.2 ± 26.7	71.0 ± 25.6	< 0.01

TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein, TG: triglyceride.

creasing trend ($p < 0.01$), whereas LDL-C levels did not differ between the HDL-C groups. These trends were similar in both men and women.

HDL subfraction profile by HDL-C level and gender
The distribution of HDL subfractions varied significantly according to HDL-C group and gender (Table 3). In men, the proportions of large and intermediate HDL did not differ between groups, even with increasing HDL-C levels ($p = 0.25$ and $p = 0.21$, respectively), but the proportion of small HDL showed a significant upward trend, increasing from 6.7% in Group I to 14.7% in Group V ($p = 0.03$). In women, the proportion of large HDL increased gradually from 41.4% (Group I) to 52.1% (Group V) with increasing HDL-C levels ($p < 0.001$), whereas the proportion of intermediate HDL decreased from 49.1% (Group I) to 38.0% (Group V) ($p < 0.001$). The proportion of small HDL did not differ between groups ($p = 0.71$).

In Groups I and II, HDL subfraction distributions did not differ between genders, but Groups III to V showed distinct gender differences. In Groups III to V, women had a significantly higher proportion of large HDL ($p < 0.01$) and a significantly lower proportion of intermediate HDL ($p < 0.01$) than those of men. The proportion of small HDL was significantly lower in women than in men in Groups III and V ($p < 0.01$). In Group V, which included individuals with very high HDL-C levels, men had the highest proportion of small HDL, but their proportions of large and intermediate HDL were similar to those in the other groups. By contrast, women had the highest proportion of large HDL and lowest proportion of intermediate HDL in Group V; however, the proportion of small HDL was similar to that in the other groups (Figure 1).

To assess the independent and interactive effects of HDL-C group and gender on HDL subfraction composi-

Table 3. Distribution of high-density lipoprotein subfractions by high-density lipoprotein-cholesterol percentile group and gender.

		Total	Group I	Group II	Group III	Group IV	Group V	p †
n	total	172	18	38	53	39	24	
	men	85	10	18	30	12	15	
	women	87	8	20	23	27	9	
Large%	men	39.1 ± 9.9	43.2 ± 12.6	36.2 ± 10.9	38.0 ± 8.9	41.1 ± 9.0	40.6 ± 9.2	0.25
	women	46.5 ± 9.8	41.4 ± 15.2	39.2 ± 10.4	47.8 ± 7.6	50.4 ± 6.5	52.1 ± 5.6	< 0.01
	p ‡		0.79	0.39	< 0.01	< 0.01	< 0.01	
Inter%	men	47.5 ± 6.7	49.3 ± 5.2	49.7 ± 7.6	47.4 ± 6.2	46.6 ± 6.6	44.7 ± 7.0	0.21
	women	42.7 ± 7.2	49.1 ± 8.8	48.8 ± 6.5	42.0 ± 5.2	38.6 ± 4.5	38.0 ± 5.3	< 0.01
	p ‡		0.95	0.70	< 0.01	< 0.01	0.02	
Small%	men	13.2 ± 7.2	6.7 ± 8.7	13.8 ± 10.1	14.5 ± 5.8	12.3 ± 3.1	14.7 ± 4.6	0.03
	women	10.7 ± 4.9	9.2 ± 6.6	11.9 ± 5.7	10.1 ± 4.5	11.0 ± 4.5	9.9 ± 2.9	0.71
	p ‡		0.52	0.47	< 0.01	0.38	0.01	

†: Between high-density lipoprotein-cholesterol groups (linear trend analysis using one-way analysis of variance with polynomial contrasts).

‡: Between men and women (*t*-test).

Large%: large high-density lipoprotein %, Intermediate%: intermediate high-density lipoprotein %, Small%: small high-density lipoprotein.

Table 4. Comparison of lipid parameters and HDL subfractions between dyslipidemia group and healthy controls (Group III).

	Dyslipidemia group (n = 22)	Group III (n = 53)	P
TC (mg/dL)	185.2 ± 43.8	216.8 ± 51.2	0.01
LDL-C (mg/dL)	108.4 ± 42.2	129.6 ± 45.5	0.049
TG (mg/dL)	114.2 ± 56.0	92.7 ± 43.5	0.12
Large%	33.6 ± 6.9	42.3 ± 9.7	< 0.01
Inter%	53.5 ± 3.2	45.1 ± 6.3	< 0.01
Small%	12.8 ± 4.9	12.6 ± 5.7	0.89

tion, a two-way ANOVA was performed. Significant main effects were observed for both HDL-C group and gender on large and intermediate HDL ($p < 0.01$ for both). A significant interaction effect was also found for large HDL ($p = 0.04$), indicating that the relation between HDL-C levels and the proportion of large HDL differed by gender.

Comparison with patients with dyslipidemia

When comparing Group III (healthy controls) and patients with dyslipidemia whose HDL-C levels corresponded to Group III, the dyslipidemia group had lower TC ($p = 0.01$) and LDL-C ($p = 0.049$) levels than those of Group III. Regarding HDL subfraction distribution, the dyslipidemia group had a low proportion of large HDL ($p < 0.01$) and high proportion of intermediate

HDL ($p < 0.01$), whereas the proportion of small HDL did not differ between the two groups ($p = 0.89$).

DISCUSSION

This study analyzed the distribution of HDL subfractions according to HDL-C levels in healthy Korean adults and compared the results with those of patients with dyslipidemia. HDL subfraction distributions varied significantly by HDL-C level and gender, suggesting that subfraction analysis may reveal qualitative differences not captured by HDL-C levels alone.

HDL particles are highly heterogeneous in size and composition, and structural differences influence their functional properties and clinical relevance. A recent re-

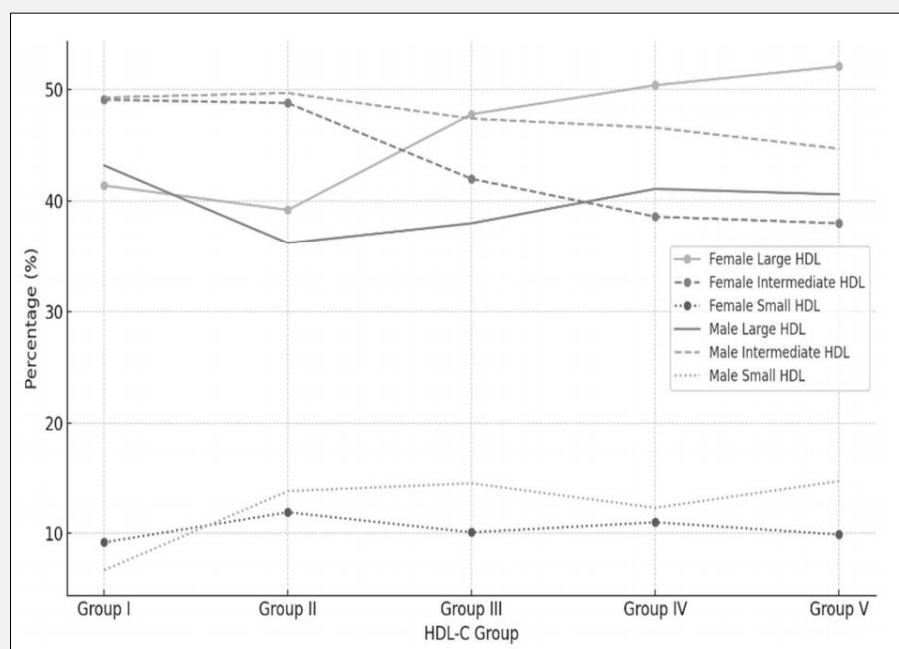


Figure 1. Gender-specific distribution of HDL subfractions across HDL-C percentile groups. Proportions of large, intermediate, and small HDL subfractions are plotted according to gender across HDL-C Groups I - V. HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol.

view highlighted the ongoing debate about whether large HDL or small HDL is more cardioprotective. Some studies report that large HDL plays a major protective role, whereas others argue that small HDL is the primary protective factor. These conflicting results may stem from differences in study populations, analytical methods, and classification criteria, and no consensus has been reached to date [20].

In this study, as HDL-C levels increased, women showed an increase in the proportion of large HDL and a decrease in the proportion of intermediate HDL. By contrast, men showed no change in the proportions of large and intermediate HDL but exhibited a significant increase in the proportion of small HDL. This indicates that rising HDL-C levels may lead to different qualitative changes in HDL subfraction composition between genders. Gender differences were further confirmed statistically. A two-way ANOVA showed that both HDL-C group and gender had significant effects on HDL subfraction distribution, with a significant interaction effect for large HDL ($p = 0.04$). This suggests that the relation between HDL-C levels and the proportion of large HDL is gender-specific, emphasizing the need for gender-specific interpretation of high HDL-C levels. However, because the functional roles of HDL subfractions are not yet clearly defined, these findings should be inter-

preted with caution. Both large and small HDL may contribute to cardiovascular protection. Therefore, the present results should not be considered conclusive evidence favoring the superiority of one subfraction over another.

HDL-C has traditionally been considered a cardioprotective factor; however, recent studies have reported that very high HDL-C levels may be associated with increased cardiovascular risk [10-12]. In the men in this study, Group V, which had very high HDL-C levels, showed the highest proportion of small HDL and relatively constant proportions of large and intermediate HDL across groups. By contrast, the women in Group V had the highest proportion of large HDL-C and lowest proportion of intermediate HDL-C; however, the proportion of small HDL-C was similar to that in the other groups. These findings suggest that even within the same HDL-C percentile, the distribution of HDL subfractions differs between men and women, indicating that very high HDL-C levels may have different clinical implications in each gender.

When comparing patients with dyslipidemia whose HDL-C levels corresponded to the Group III percentiles with the healthy control group (Group III), a clear difference in HDL subfraction distribution was observed. The dyslipidemia group had a lower proportion of large

HDL and higher proportion of intermediate HDL than those of the healthy Group III, whereas the proportion of small HDL did not differ between the two groups. These results suggest that even at similar HDL-C levels, the composition of HDL particles - that is, their qualitative characteristics - may vary depending on metabolic status and medication use. In particular, a decrease in the proportion of large HDL subfraction without a corresponding increase in the proportion of small HDL subfraction may reflect compositional differences that are difficult to detect from HDL-C levels alone. These compositional differences may capture potential qualitative variations not revealed by HDL-C levels, and HDL subfraction analysis may serve as a useful adjunct to personalized cardiovascular risk assessment.

This study had several limitations. First, its cross-sectional design made it difficult to establish causality. Second, although healthy participants with various HDL-C levels were carefully selected, unmeasured variables, such as diet, physical activity, or genetic factors, may have influenced HDL composition. Third, the sample size of the dyslipidemia group was relatively small and could not be stratified by gender, which limited the generalizability of the comparisons. Fourth, this study used gender-specific percentiles to group men and women separately, which may reduce comparability with studies using absolute HDL-C concentrations. However, given the known differences in HDL-C distribution between genders, this approach may be appropriate. Finally, the Lipoprint system used in this study may yield results that differ from those of other HDL subfractionation methods; therefore, caution should be exercised when comparing results across studies.

Despite these limitations, this is one of the first studies to systematically characterize the distribution of HDL subfractions across the entire HDL-C range in healthy men and women using a commercially available PAGE-based assay. By providing a gender-specific reference distribution of HDL subfractions according to HDL-C level, this study may serve as a valuable resource for future investigations aimed at elucidating the clinical significance and function of HDL subfractions.

The distribution of HDL subfractions showed distinct differences by HDL-C level and gender. In women, increasing HDL-C was associated with a high proportion of large HDL and low proportion of intermediate HDL; in men, the proportion of small HDL increased, whereas large and intermediate HDL remained unchanged. In addition, even at similar HDL-C levels, the dyslipidemia group exhibited a different HDL subfraction distribution compared with that of the healthy control group. These results suggest that HDL subfraction analysis can help identify qualitative differences not detected by HDL-C levels alone and may serve as a supplementary tool for cardiovascular risk assessment and personalized lipid management strategies.

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Declaration of Interest:

The authors declare no conflict of interest.

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