

# **DIFFERENCES IN HDL SUBFRACTION DISTRIBUTION IN NORMOLIPIDEMIC VERSUS DYSLIPIDEMIC INDIVIDUALS**

**J. MORAIS, N. MUÑIZ**

**QUANTIMETRIX CORP., REDONDO BEACH, CA • USA**



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## ABSTRACT

High density lipoprotein (HDL) is known to be inversely associated with coronary heart disease (CHD) risk. HDL is a heterogeneous class of lipoproteins grouped into various subclasses based on particle size, density and composition. HDL heterogeneity has been demonstrated by various analytical methods such as density gradient ultracentrifugation, nuclear magnetic resonance (NMR), gradient gel electrophoresis (GGE), and high performance liquid chromatography (HPLC). Recent studies suggest that different HDL subclasses are significantly associated with CHD prevalence and that measurement of these subclasses could be a better indicator of CHD than measurement of total HDL alone. The objective of this study was to evaluate a new method for measuring differences in the distribution of HDL subfractions that could help to improve the assessment of CHD risk. The Lipoprint® HDL System is a convenient, non-denaturing, linear polyacrylamide gel electrophoresis method that separates HDL particles into multiple subclasses. This method was used to measure the various HDL subfractions in normolipidemic and dyslipidemic populations. Fasting serum samples from 234 participants (84 males and 150 females, ages 18-87) were used in the study. After electrophoresis using the Lipoprint HDL System, the HDL bands were separated and grouped into three major subfractions consisting of large-buoyant particles, intermediate particles and small-dense particles. Total cholesterol (TC), triglycerides (TRIG), HDL-cholesterol and LDL-cholesterol were measured using the Dade Dimension analyzer. Samples that met the National Cholesterol Education Program (NCEP, ATP III) guidelines for total cholesterol < 200 mg/dL, triglycerides < 150 mg/dL, HDL > 40 mg/dL and LDL < 130 mg/dL were classified as normolipidemic. Certain criteria (such as lipid lowering drugs, pregnancy, metabolic disorders, etc.) were used to further exclude samples. Out of 234 samples, 99 samples were identified as the normolipidemic group. Using SAS JMP statistical software to test the various subfractions for outliers, an additional 7 samples were excluded resulting in a total of 92 normal samples. The residual 127 samples were classified as dyslipidemic following exclusion of 8 outliers. The 95% confidence limits for cholesterol content of each subfraction was established as the normal range. In the normolipidemic group, the mean±SD (mg/dL) for large, intermediate and small HDL subfractions were 24±9 mg/dL, 31±5 mg/dL and 4±3 mg/dL, respectively. The mean±SD (mg/dL) for the dyslipidemic group were 15±9 mg/dL, 29±6 mg/dL and 5±2 mg/dL, respectively. Statistical significance was determined using a non-parametric approach using the Wilcoxon Rank Sums Test with both normal and chi square approximation. Interestingly, the means for both large and small HDL subfractions of the normolipidemic group showed a statistically significant difference ( $p < 0.0001$ ) when compared to that of the dyslipidemic group. However, the means for the intermediate HDL subfractions did not show a statistically significant difference ( $p < 0.0069$ ) between the normolipidemic and dyslipidemic groups. These data suggest that both large HDL and small HDL subfractions as measured by the Lipoprint HDL System may be a better indicator of CHD risk than intermediate HDL subfraction or total HDL.

## METHODS AND MATERIALS

Fasting human serum samples (N= 319) collected from 159 males and 160 females, aged 18 to 87 years were electrophoresed using the Lipoprint® HDL System to generate a profile of the HDL subfraction distribution.

The samples were classified as normolipidemic and dyslipidemic according to the NCEP ATP III guidelines where Total Cholesterol (TC) < 200 mg/dl, Triglyceride (Trig) < 150 mg/dl, HDL cholesterol (HDL-C) > 40 mg/dl, and LDL Cholesterol (LDL-C) < 130 mg/dl.

A Dimension RxL Analyzer (Dade Behring, Inc.) was used to quantify HDL-C (automated).

The Lipoprint® System (Quantimetrix Corp.) is a linear polyacrylamide gel system for the separation of lipoprotein particles according to charge and size (Figs. 1-2). It was used to generate the HDL subfraction profiles.

SAS JMP® software was used to analyze the clinical data.

Automated analysis of the banding pattern of the gel tube with Lipoware (lipoprotein analysis software) measures cholesterol concentration of the various HDL subfractions. (Figs. 3-4)



# LIPOPRINT SYSTEM



The Lipoprint HDL System consists of the reagent kit, preparation rack, preparation light, electrophoresis chamber, power supply, scanner and computer .

## TEST PRINCIPLE FIGURES

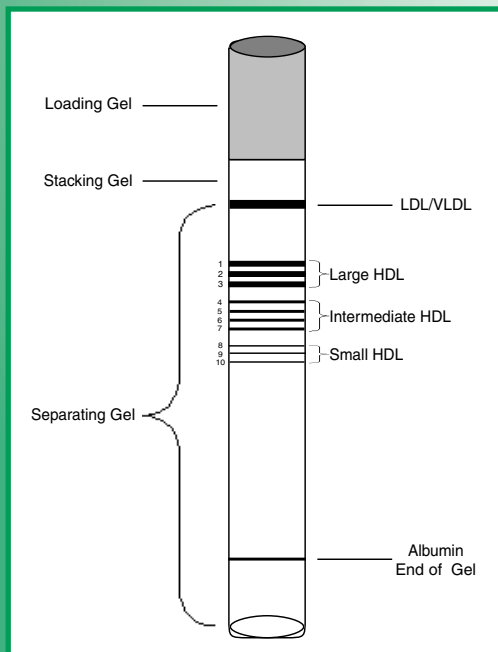


Figure 1. HDL Lipoprint® Gel Tube Schematic



Figure 2. HDL Lipoprint® Gel Tubes after Electrophoresis of a Normal, Intermediate & Abnormal Human Serum Sample.



# PROFILES

The Lipoprint HDL System can resolve up to ten subfractions of HDL. These subfractions have been grouped into three main subclasses: HDL 1-3 represent the Large HDL, HDL 4-7 represent the Intermediate HDL and HDL 8-10 represent the Small HDL.

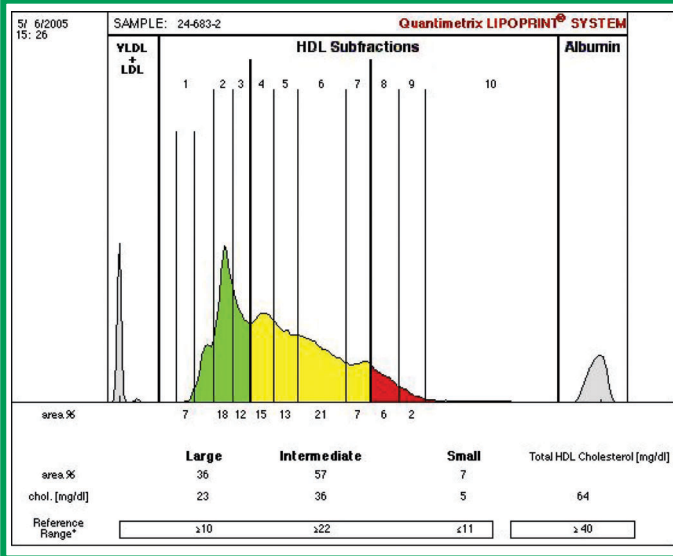


Figure 3. Lipoprint Report for a Normal Profile

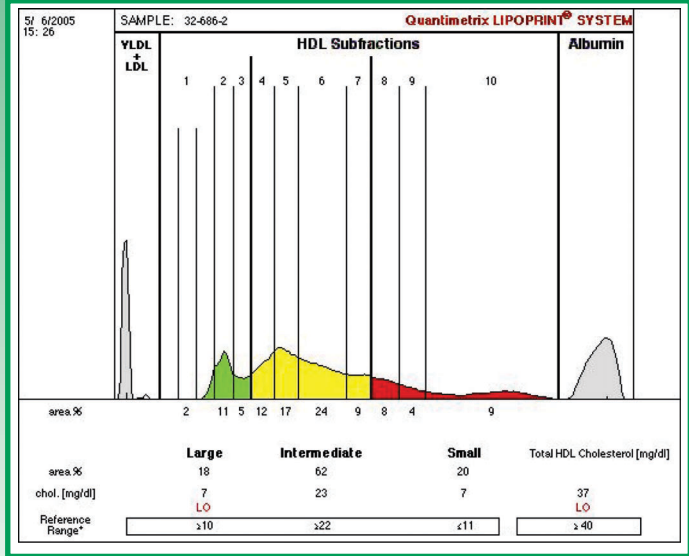


Figure 4. Lipoprint Report for an Abnormal Profile

## RESULTS

Reference ranges for the Large, Intermediate and Small HDL subclasses based on the normolipidemic group (NCEP ATPIII guidelines), N = 123 were developed using non-parametric approach. Samples with values that were outside the range, RR = upper quartile ± 1.5(interquartile range) were not considered for normal range determination.

From the residual 123 samples, the 95% range (2.5th percentile to 97.5th percentile) was defined as the normal reference range (Table 1).

The residual patient samples represented the dyslipidemic group (N = 191) where at least one lipid parameter is outside the desirable range as defined by the NCEP ATPIII guidelines. The ranges for the different HDL subclasses are tabulated in Table 2.

	HDL Large [mg/dl]	HDL Intermediate [mg/dl]	HDL Small [mg/dl]	HDL total [mg/dl]	CHOL total [mg/dl]
range	8 - 43	18 - 44	0 - 12	40 - 89	110 - 199
mean	21.7	30.4	4.3	56.5	166.2
SD	8.05	5.06	2.56	10.87	19.37
95% range	10.0- 41.9	22.0-41.9	1.0-11.0	41.0-79.9	118.5-197.8
N*	123	123	123	123	123

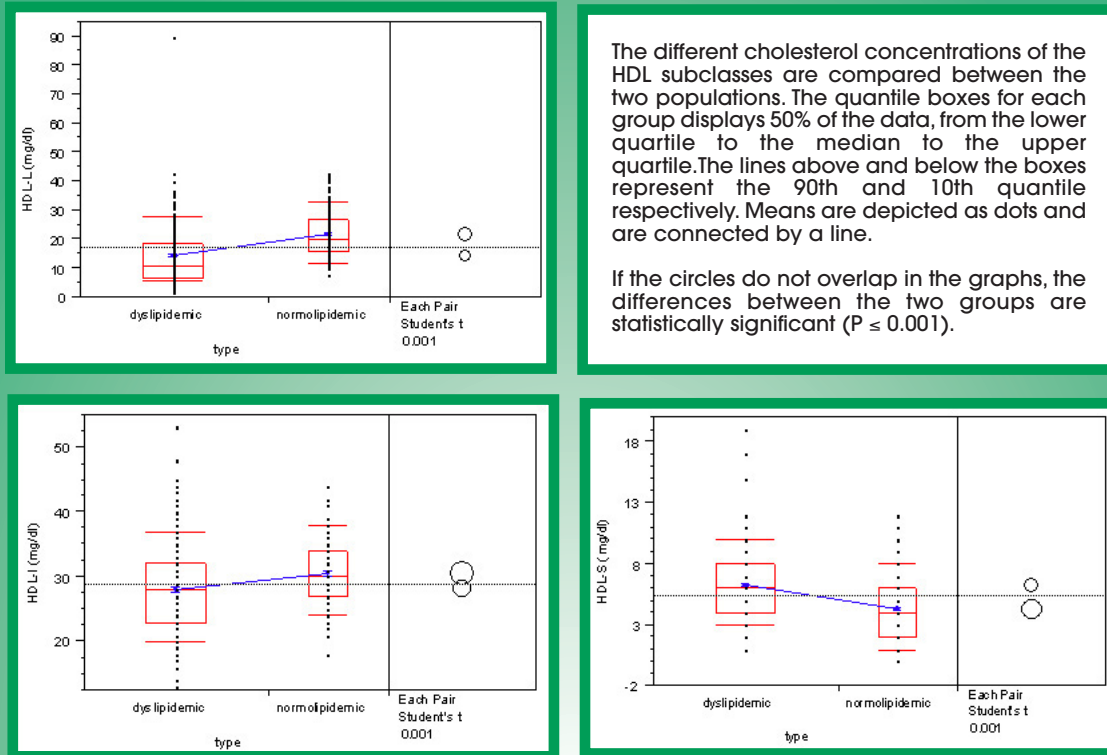
Table 1. Normolipidemic population

	HDL Large [mg/dl]	HDL Intermediate [mg/dl]	HDL Small [mg/dl]	HDL total [mg/dl]	CHOL total [mg/dl]
range	2 - 90	13 - 53	1 - 19	21-122	94 - 322
mean	14.5	28.1	6.2	49.0	213.9
SD	10.3	7.01	3.05	15.54	39.17
95% range	3.8 - 37.0	16.8 - 43.2	1.0 - 12.6	27.0-85.8	124.6-299.4
N*	191	191	191	191	191

Table 2. Dyslipidemic population



# COMPARISON OF THE NORMOLIPIDEMIC, N = 123 VS. DYSLIPIDEMIC POPULATION, N = 191



The different cholesterol concentrations of the HDL subclasses are compared between the two populations. The quantile boxes for each group displays 50% of the data, from the lower quartile to the median to the upper quartile. The lines above and below the boxes represent the 90th and 10th quantile respectively. Means are depicted as dots and are connected by a line.

If the circles do not overlap in the graphs, the differences between the two groups are statistically significant ( $P \leq 0.001$ ).

Figure 5. Plot of HDL-L, HDL-I, and HDL-S vs. sample type

## SMALL/LARGE HDL RATIO (S/L HDL)

- It was found that the ratio of Small/Large HDL (S/L HDL) provides a convenient surrogate for the HDL subfraction profile. A large ratio indicates a presumably undesirable profile that is shifted towards the small HDL subfractions (e.g. Fig. 4) and vice versa.
- Furthermore, we reasoned that such a ratio might be a valuable clinical tool to identify samples that have a desirable HDL profile even though their total HDL level is abnormal (<40 mg/dl). Likewise, the ratio may also identify samples that have an undesirable HDL profile even though their total HDL level is in the normal (>40 mg/dl) or even optimal range ( $\geq 60$  mg/dl) according to NCEP (ATP III).

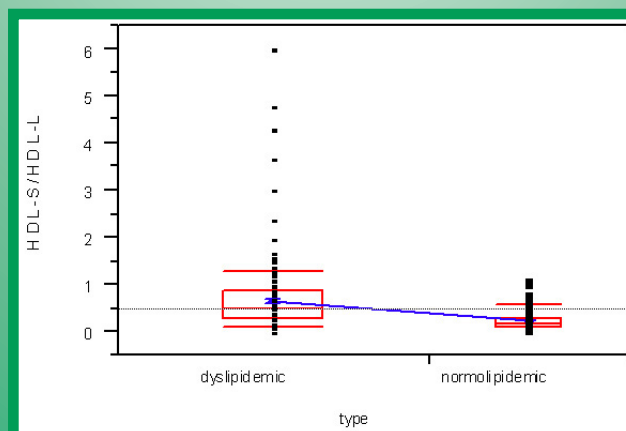


Figure 6. Ratio of HDL-S to HDL-L vs. sample type



# PROPOSED REFERENCE RANGE FOR S/L HDL

- To establish such a reference range we selected the group with optimal HDL levels (>60 mg/dl, negative risk factor according to NCEP) and stratified it to contain only samples that were also normal in regard to the latest (ATP III) guidelines, i.e. TC < 200 mg/dl, Trigs < 150 mg/dl, LDL-C < 130 mg/dl.
- From the distribution of all the qualifying ratios (N= 48, Fig. 7) a 95% confidence interval was determined as 0 - 0.3 (spanning quantiles 2.5% to 97.5%), after removing five outliers. This S/L HDL range seems to reflect the HDL subfraction profiles.
- Subsequently, the ratio was used to analyze the number of samples for each group within and outside the reference range (0-0.3).

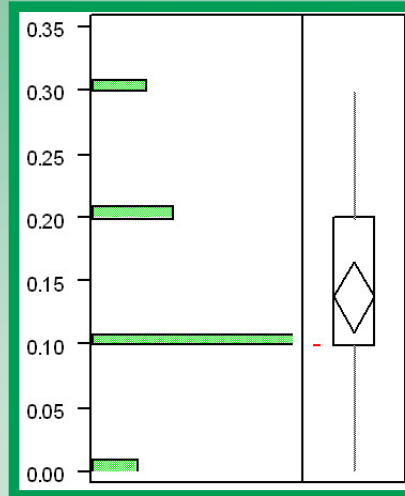


Figure 7. Distribution of Small HDL to Large HDL ratio of samples with HDL > 60 (and normal lipid parameters).

## APPLYING THE S/L HDL RATIO

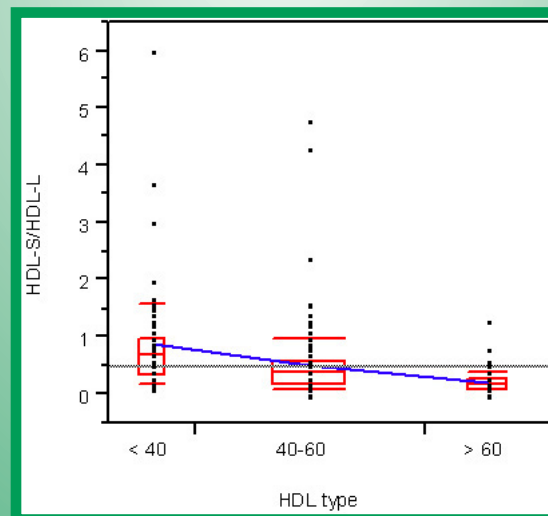


Figure 8. Ratio of Small HDL to Large HDL subfractions vs. HDL ranges

Figure 8 illustrates the relationship of the S/L HDL ratio for the three major HDL-C groups : HDL < 40, N = 62; 40 < HDL < 60, N = 166 and HDL ≥ 60, N = 91.

As expected, the means of the ratio of Small HDL (HDL-S) to Large HDL (HDL-L) decreases with increasing total HDL cholesterol.



# APPLYING THE S/L HDL RATIO Cont.

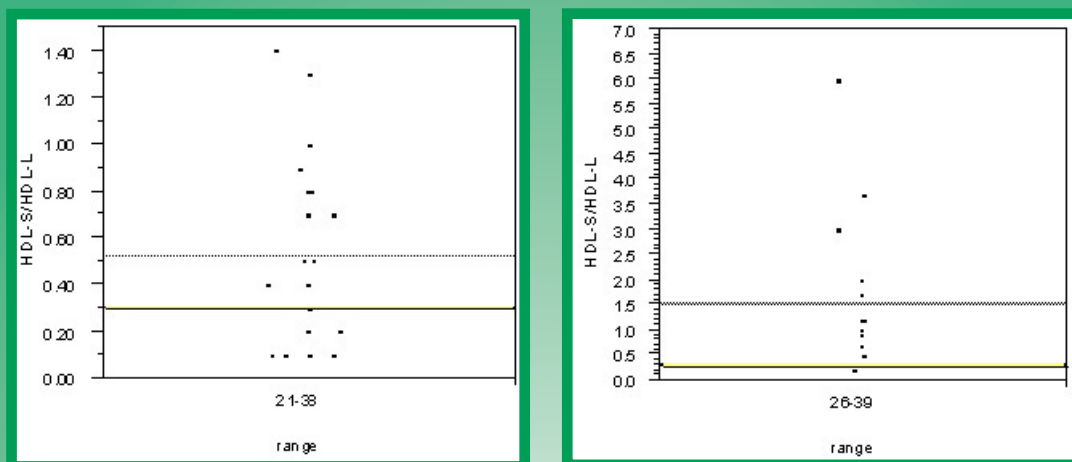


Figure 9. Quantification of the percentage of samples above the 0.3 cut-off range.

These samples were stratified using the ATPIII values (NCEP guidelines). For samples with HDL < 40 (but otherwise normal lipid values) (N=21), 62% exceed the reference range (0.3). For samples with HDL < 40 (with abnormal lipid values) (N=15), 73% exceed the reference range.

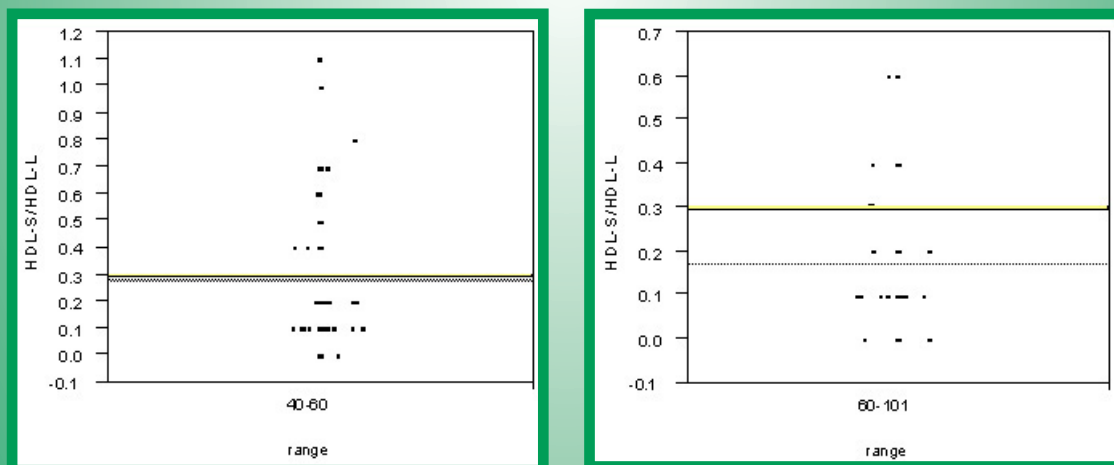


Figure 10. Quantification of the percentage of samples above the 0.3 cut-off range.

These samples were stratified using the ATPIII values (NCEP guidelines). For samples with HDL between 40-60 (N=80), 28% exceed the reference range. For samples with HDL > 60 (N=48), 10% exceed the reference range.



# CONCLUSIONS

- Comparison of the HDL subclasses, Large HDL (HDL-L), Intermediate HDL (HDL-I) and Small HDL (HDL-S) between the normolipidemic and dyslipidemic samples showed that there is significant difference between the means of the two samples ( $P < 0.001$ ) for the HDL-L and HDL-S but not for the HDL-I ( $P < 0.0015$ ).
- The Large HDL subclass (HDL-L) showed an inverse relationship with CHD risk factors while the Small HDL subclass (HDL-S) exhibited a direct relationship with CHD risk factors.
- The Small HDL to Large HDL ratio (S/L HDL) emerged as an interesting tool to evaluate the HDL subclass distribution and the associate profile (normal or abnormal) across a wide patient population. With the stratified samples using NCEP (ATP III) guidelines, 27% of all samples with HDL  $< 40$  mg/dl (and abnormal lipid parameters) and 38% of all samples with HDL  $< 40$  mg/dl (and otherwise normal lipid values) were within the reference range.
- Interestingly, 28% of all samples that are normal and have  $40 < \text{HDL} < 60$  exhibited S/L HDL outside the reference range while the same normal group of samples with optimal levels of HDL ( $> 60$  mg/dl) displayed 10% that are outside the reference range.
- While further clinical data is needed to evaluate if these findings have clinical significance we feel that this ratio has the potential to become a valuable adjunct to determining a patient's risk for heart disease, even at optimal levels of HDL, and vice versa.