

MEASUREMENT AND DISTRIBUTION OF HDL SUBCLASSES WITH THE NEW LIOPRINT[®] HDL METHOD

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ABSTRACT

HDL cholesterol is believed to exert a protective role in preventing coronary artery disease (CAD) due to its role in the reverse cholesterol transport. However, HDL is not a homogeneous entity but contains multiple HDL subclasses consisting of particles of different size, density and composition. The importance and function of these subclasses have not been well understood, not to a small degree due to the lack of suitable and convenient methods of identifying and quantifying these subclasses. Lipoprint HDL is a linear gel electrophoresis method that separates HDL particles into a maximum of 10 subclasses of decreasing particle size - HDL-1 through 10 - and quantitates their cholesterol concentrations. The relative distribution of HDL cholesterol is also displayed in the resulting HDL profile.

Using the Lipoprint platform, the distribution of HDL cholesterol over the various subclasses was determined for fasting serum samples of a random population. Other traditional lipid parameters were determined for these samples using a laboratory analyzer (Total cholesterol (TC), triglycerides (Trig), HDL-C, and LDL-C) and the Lipoprint LDL test (LDL particle size). The results were analyzed in terms of non-parametric association of the various HDL subclasses with the above lipid parameters. It was found that HDL-2 through HDL-4 showed a significant positive correlation with HDL-C (Spearman Rho: 0.63, $p < 0.0005$). No significant correlation was found with either TC or LDL-C. While HDL-2 through HDL-4 showed some positive correlation with LDL particle size (0.54, $p = 0.002$) an inverse relationship was found for HDL-7 through 10 (-0.51, $p = 0.0004$). Interestingly, a significant inverse correlation was also found between triglyceride concentration and HDL-1 through 4 (-0.64, $p = 0.0005$). The situation for the small HDL particles (HDL-7 to 10) was exactly opposite (0.64, $p < 0.0005$) compared to that of the larger HDL particles.



METHODS AND MATERIALS

Frozen human serum samples (N=270) were thawed and electrophoresed using the new Lipoprint® HDL method to generate a profile of the HDL subfraction distribution.

HDL subfraction cholesterol for the 270 samples were correlated (non-parametric measures of association) with the traditional NCEP lipid parameters, Total Cholesterol (TC), Triglyceride (Trig), HDL cholesterol (HDL-C) , LDL Cholesterol (LDL-C) as well as LDL particle size.

Equipment

A Dimension RXL Analyzer (Dade Behring, Inc.) was used to quantify TC, Trigs, HDL-C (direct) and LDL-C (direct).

The Lipoprint System (Quantimetrix Corp.) was used to generate the HDL subfraction profiles as well as the LDL particle size.

Lipoprint is a linear polyacrylamide gel system for the separation of lipoprotein particles according to size. (Figs. 1-3)

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. 4-6)



LIOPRINT SYSTEM

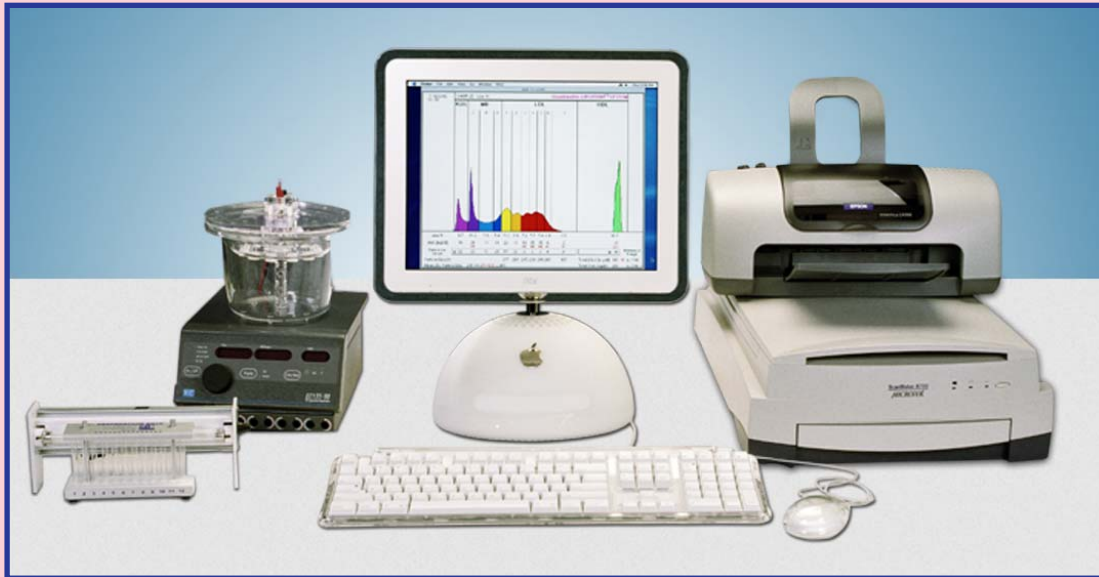


Figure 1. The Lipoprint System consists of reagent kit, electrophoresis chamber, preparation rack, polymerization light, power supply, digital scanner, computer, software and color printer

TEST PRINCIPLES

- Lipoprint uses the discontinuous linear polyacrylamide gel electrophoresis technique (not gradient gel).
- The Separating Gel and the Stacking Gel are pre-cast in a 75 mm glass tube ready to use.
- The patient sample reacts with a lipophilic dye in the Loading gel that binds to the cholesterol in the lipoprotein particles.
- Electrophoresis is conducted at a current of 3 mA. per tube for approximately one hour. The pre-stained lipoproteins bands are separated on the basis particle size.
- The electrophoresed gels are digitally scanned and analyzed using Lipoware (proprietary software).
- A color profile of the HDL cholesterol distribution is generated.

HDL subfraction profiles generated with the Lipoprint platform contained up to 10 HDL subfractions. As can be seen in Figs. 4 - 6 samples with similar total HDL concentrations may exhibit widely different HDL profiles. To simplify the analysis we tentatively lumped subfractions 1-3 together as well as subfractions 8-10 and termed them "large HDL" and "small HDL", respectively.



TEST PRINCIPLE FIGURES

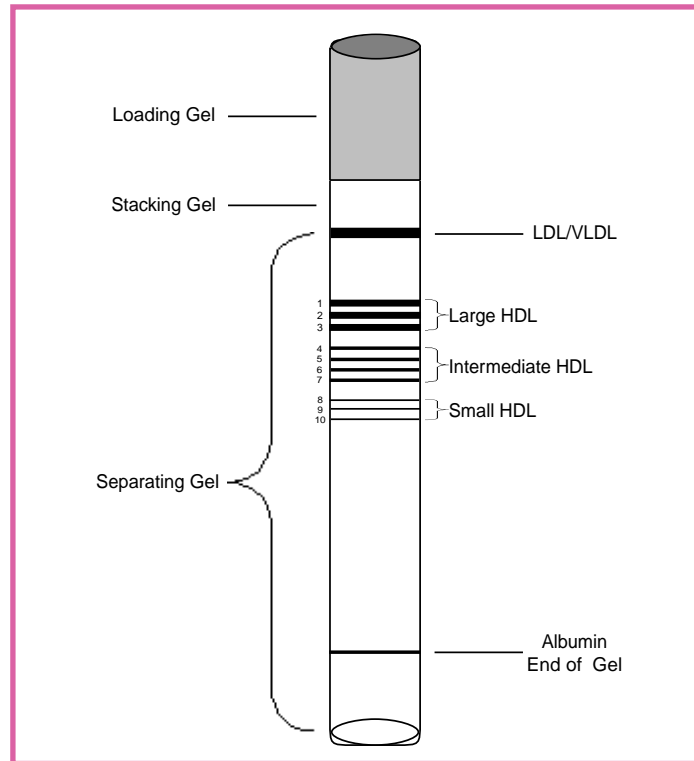


Figure 2. HDL Lipoprint® Gel Tube Schematic

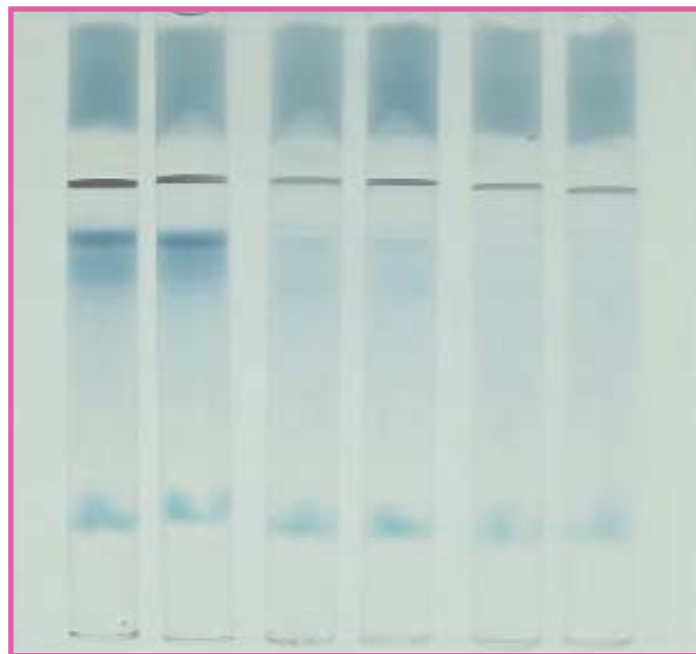


Figure 3. HDL Lipoprint® Gel Tubes after Electrophoresis of a Normal, Intermediate & Abnormal Human Serum Sample



PROFILES

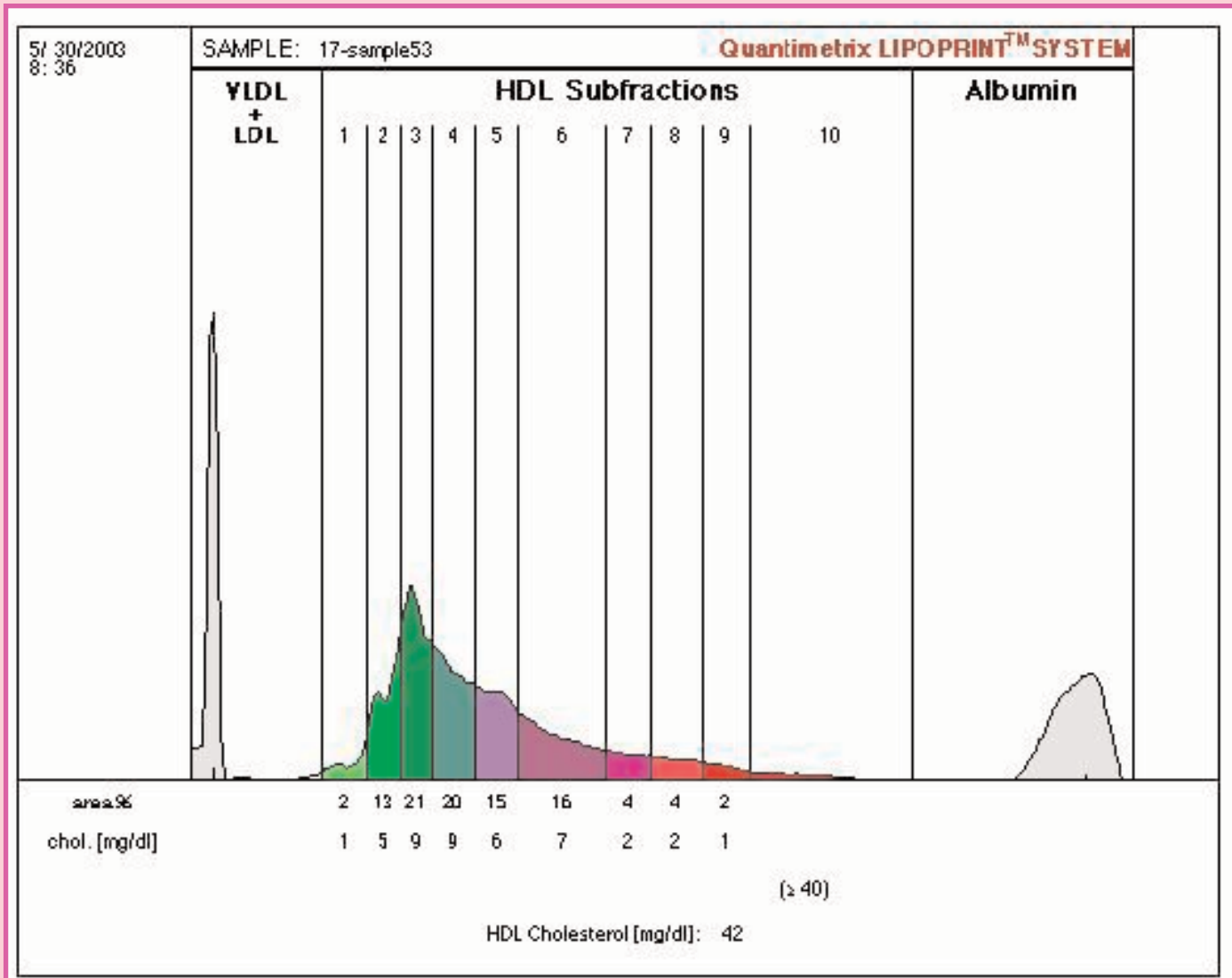


Figure 4. Lipoprint Report for a Normal Profile



PROFILES Cont.

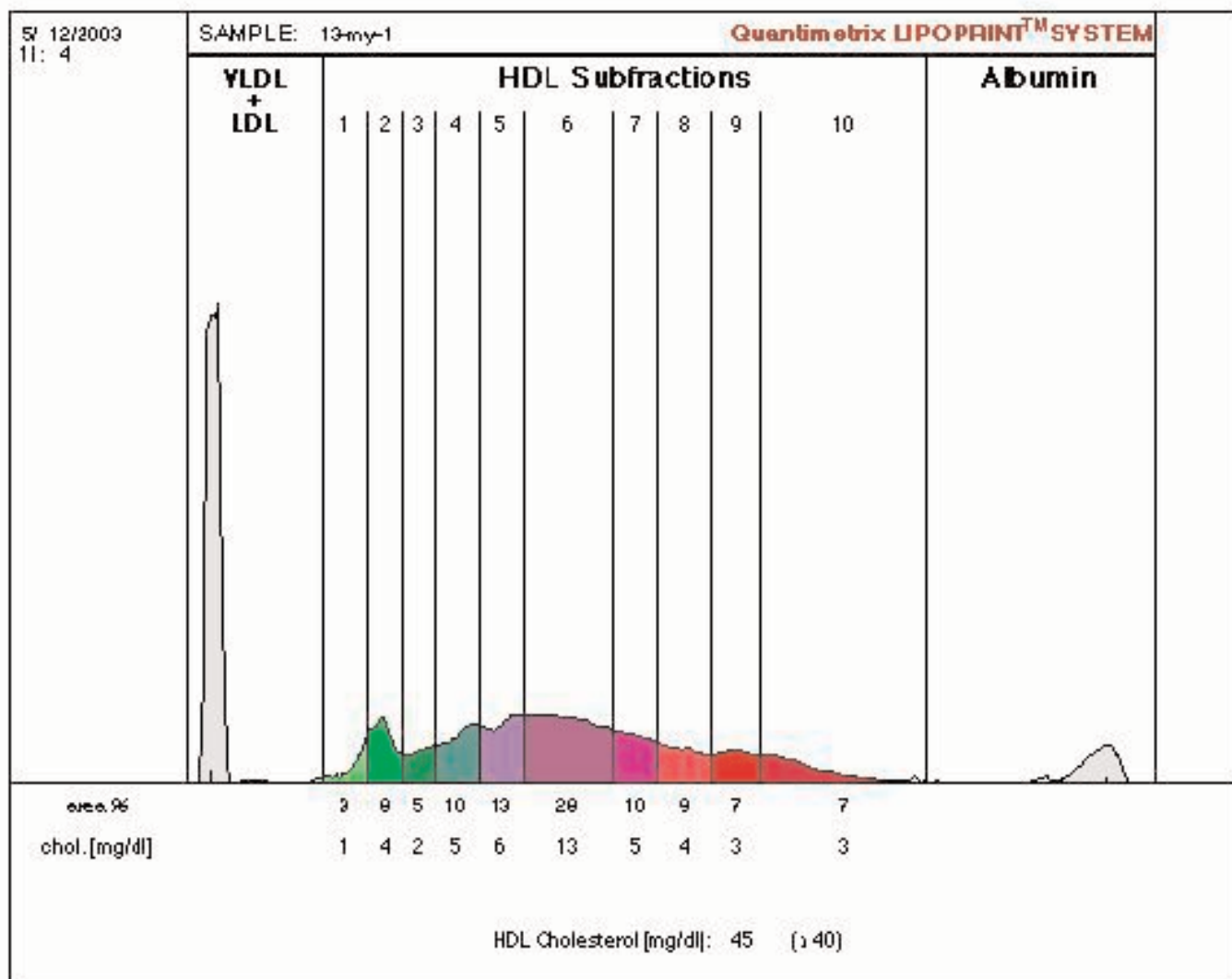


Figure 5. Lipoprint Report for an Intermediate Profile



PROFILES Cont.

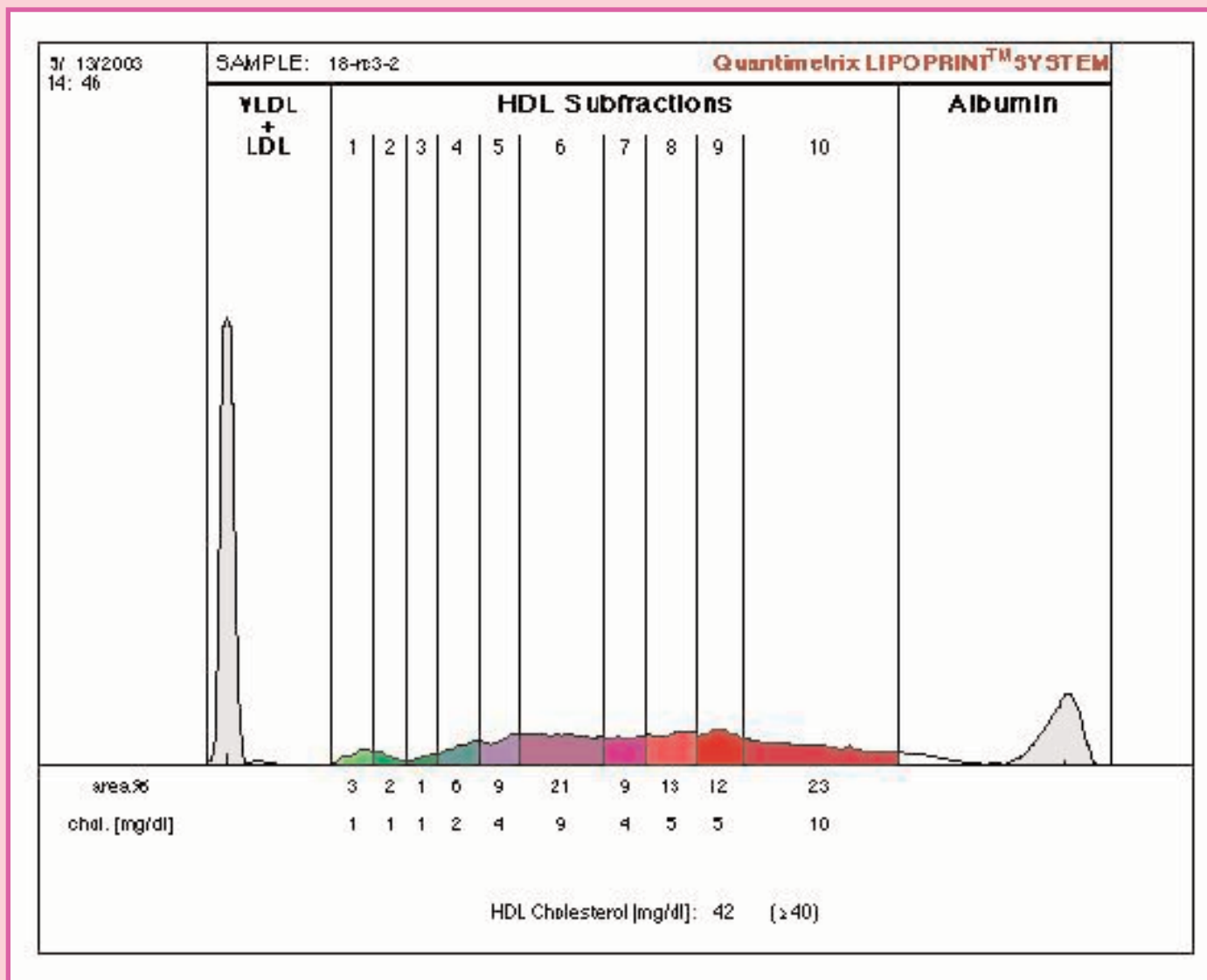


Figure 6. Lipoprint Report for an Abnormal Profile



RESULTS

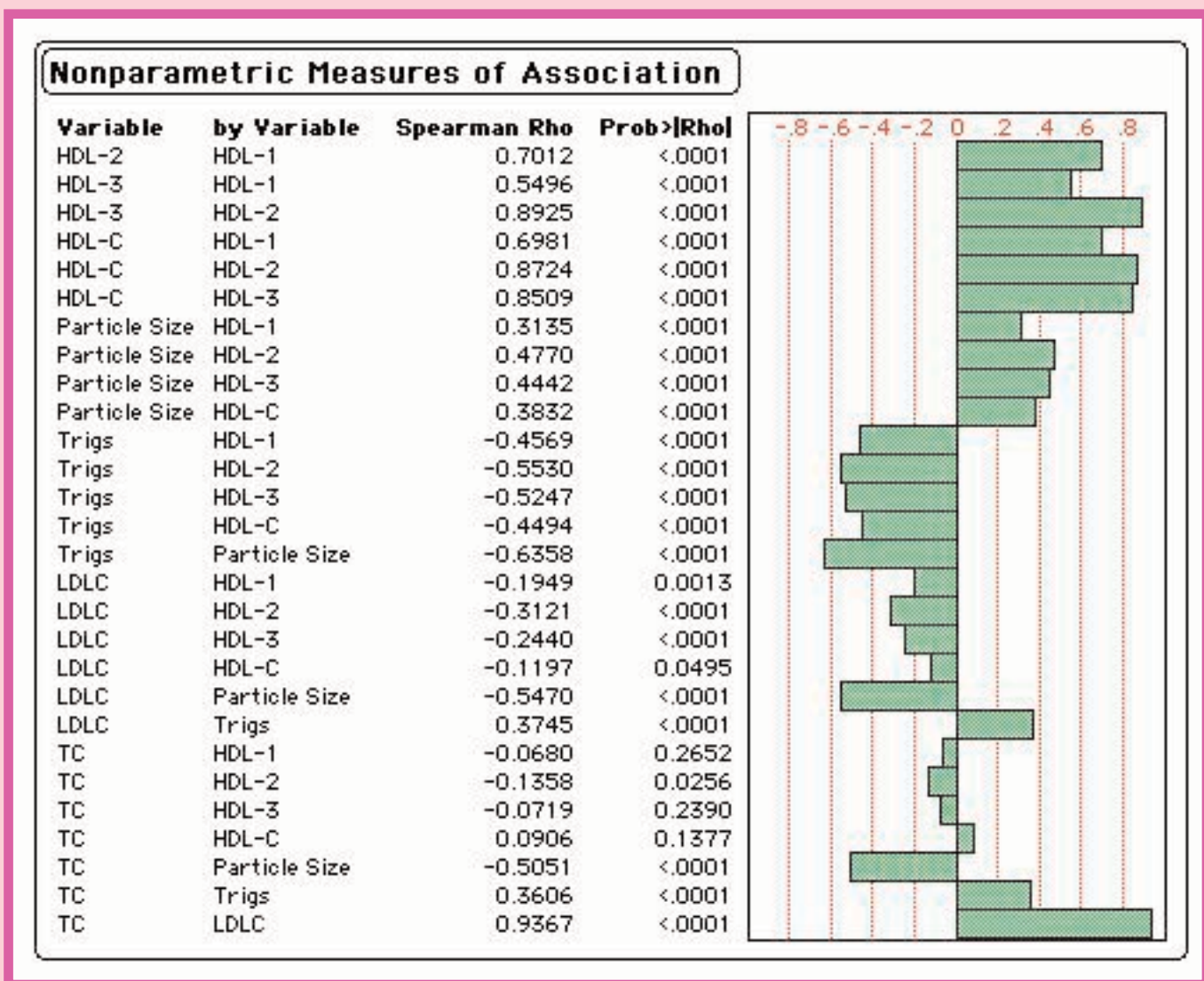


Figure 7. Non-parametric Measures of Association for HDL-1 to HDL-3 Subfractions



RESULTS Cont.

Nonparametric Measures of Association

Variable	by Variable	Spearman Rho	Prob> Rho
HDL-5	HDL-4	0.7792	<.0001
HDL-6	HDL-4	0.6263	<.0001
HDL-6	HDL-5	0.7843	<.0001
HDL-7	HDL-4	0.3047	<.0001
HDL-7	HDL-5	0.3533	<.0001
HDL-7	HDL-6	0.6720	<.0001
HDL-C	HDL-4	0.8664	<.0001
HDL-C	HDL-5	0.7529	<.0001
HDL-C	HDL-6	0.7708	<.0001
HDL-C	HDL-7	0.6175	<.0001
Particle Size	HDL-4	0.4592	<.0001
Particle Size	HDL-5	0.4793	<.0001
Particle Size	HDL-6	0.3094	<.0001
Particle Size	HDL-7	0.1135	0.0626
Particle Size	HDL-C	0.3832	<.0001
Trigs	HDL-4	-0.5373	<.0001
Trigs	HDL-5	-0.4418	<.0001
Trigs	HDL-6	-0.2328	0.0001
Trigs	HDL-7	-0.0717	0.2406
Trigs	HDL-C	-0.4494	<.0001
Trigs	Particle Size	-0.6358	<.0001
TC	HDL-4	0.0129	0.8323
TC	HDL-5	-0.0071	0.9070
TC	HDL-6	0.1354	0.0261
TC	HDL-7	0.1900	0.0017
TC	HDL-C	0.0906	0.1377
TC	Particle Size	-0.5051	<.0001
TC	Trigs	0.3606	<.0001
LDLC	HDL-4	-0.1620	0.0076
LDLC	HDL-5	-0.1523	0.0122
LDLC	HDL-6	-0.0259	0.6717
LDLC	HDL-7	0.0360	0.5562
LDLC	HDL-C	-0.1197	0.0495
LDLC	Particle Size	-0.5470	<.0001
LDLC	Trigs	0.3745	<.0001
LDLC	TC	0.9367	<.0001

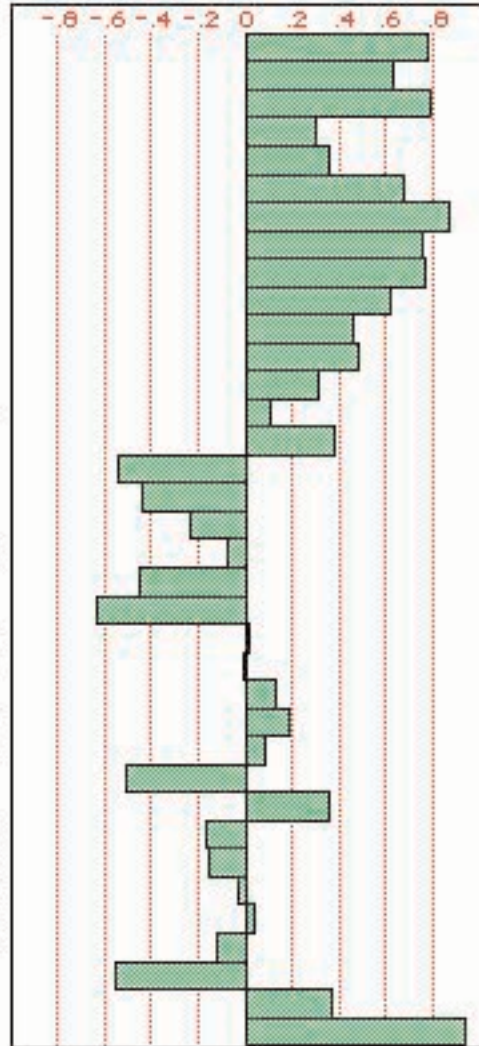


Figure 8. Non-parametric Measures of Association for HDL-4 to HDL-7 Subfractions



RESULTS Cont.

Nonparametric Measures of Association

Variable	by Variable	Spearman Rho	Prob> Rho
HDL-9	HDL-8	0.8539	<.0001
HDL-10	HDL-8	0.5571	<.0001
HDL-10	HDL-9	0.7493	<.0001
TC	HDL-8	0.3250	<.0001
TC	HDL-9	0.3853	<.0001
TC	HDL-10	0.3907	<.0001
HDL-C	HDL-8	0.5146	<.0001
HDL-C	HDL-9	0.3236	<.0001
HDL-C	HDL-10	-0.0531	0.3846
HDL-C	TC	0.0906	0.1377
LDLC	HDL-8	0.1800	0.0030
LDLC	HDL-9	0.2813	<.0001
LDLC	HDL-10	0.3885	<.0001
LDLC	TC	0.9367	<.0001
LDLC	HDL-C	-0.1197	0.0495
Trigs	HDL-8	0.0171	0.7796
Trigs	HDL-9	0.1094	0.0727
Trigs	HDL-10	0.2891	<.0001
Trigs	TC	0.3606	<.0001
Trigs	HDL-C	-0.4494	<.0001
Trigs	LDLC	0.3745	<.0001
Particle Size	HDL-8	-0.0586	0.3373
Particle Size	HDL-9	-0.1788	0.0032
Particle Size	HDL-10	-0.3664	<.0001
Particle Size	TC	-0.5051	<.0001
Particle Size	HDL-C	0.3832	<.0001
Particle Size	LDLC	-0.5470	<.0001
Particle Size	Trigs	-0.6358	<.0001

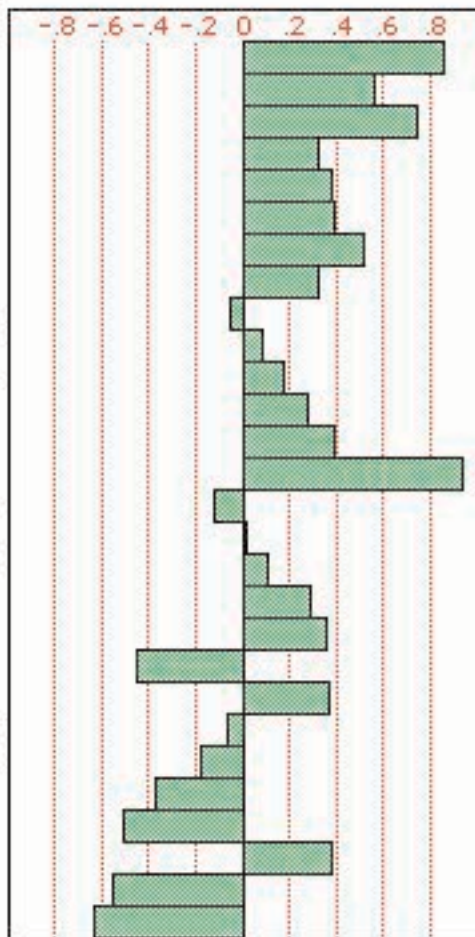


Figure 9. Non-parametric Measures of Association for HDL-8 to HDL-10 Subfractions



RESULTS Cont.

SMALL/LARGE HDL RATIO (S/L HDL)

- It was found that the ratio of small/large 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. 6) and vice versa.
- Furthermore we reasoned that such a ratio might be a valuable clinical tool to 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 (>60 mg/dl) according to NCEP (ATP III).

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 NCEP (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= 46, Fig. 10) a 95% confidence interval was determined, after removing one outlier. A S/L HDL range that seems to reflect the optimal HDL subfraction profiles was determined as 0.1 - 0.64.



RESULTS Cont.

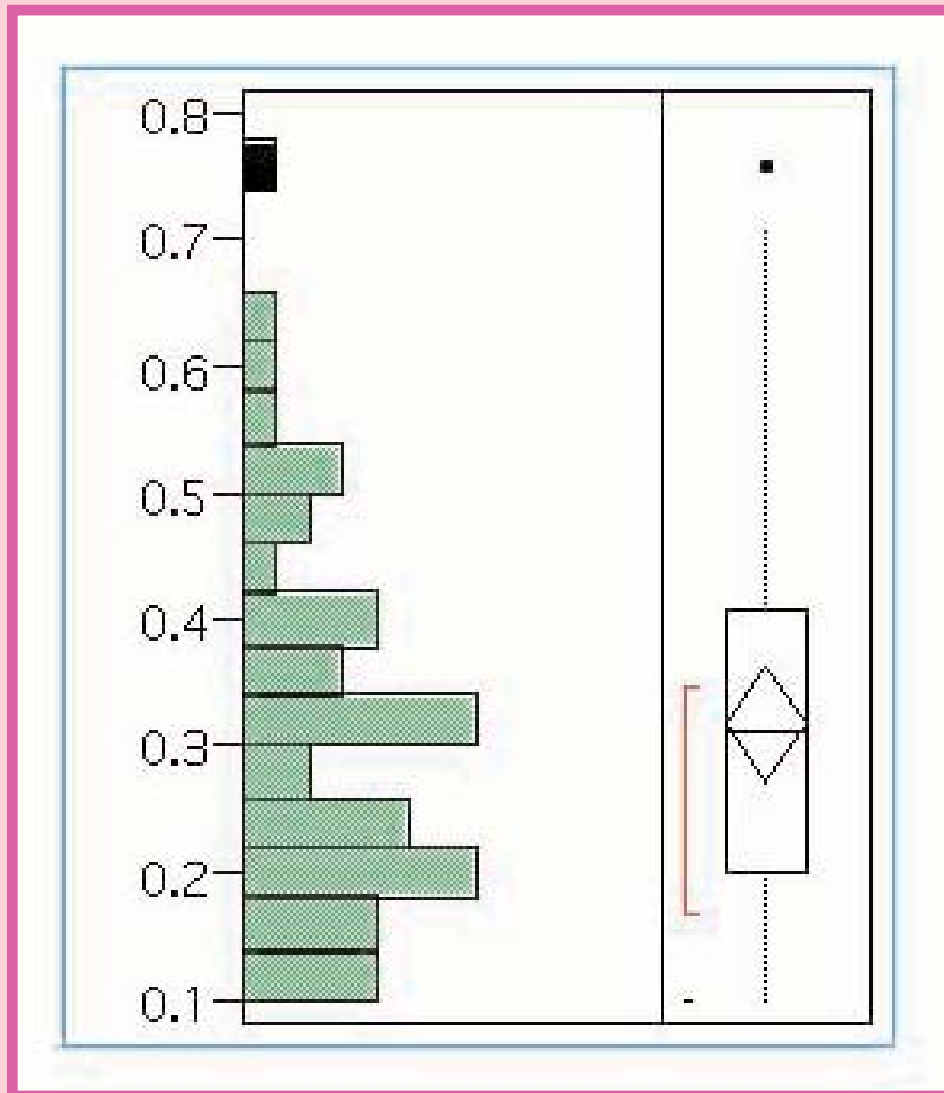


Figure 10. Distribution of Small HDL to large HDL ratio of Samples with HDL > 60.

After identifying and removing any outliers, the 95% confidence interval for the ratio was established (spanning quantiles 2.5% to 97.5%) as 0.1 - 0.64.

Subsequently, the ratio was used to analyze the number of samples for each group within and outside the reference range (0.1-0.64).



RESULTS Cont.

APPLYING THE S/L HDL RATIO

- Fig. 11 illustrates the relationship of the S/L HDL ratio for the three major HDL-C groups
All samples were classified into groups of HDL < 40 (N = 45); 40 < HDL < 60 (N=136) and HDL > 60 (N = 89).
As expected, the ratio of small HDL (HDL-S) to large HDL (HDL-L) decreases with increasing HDL cholesterol.

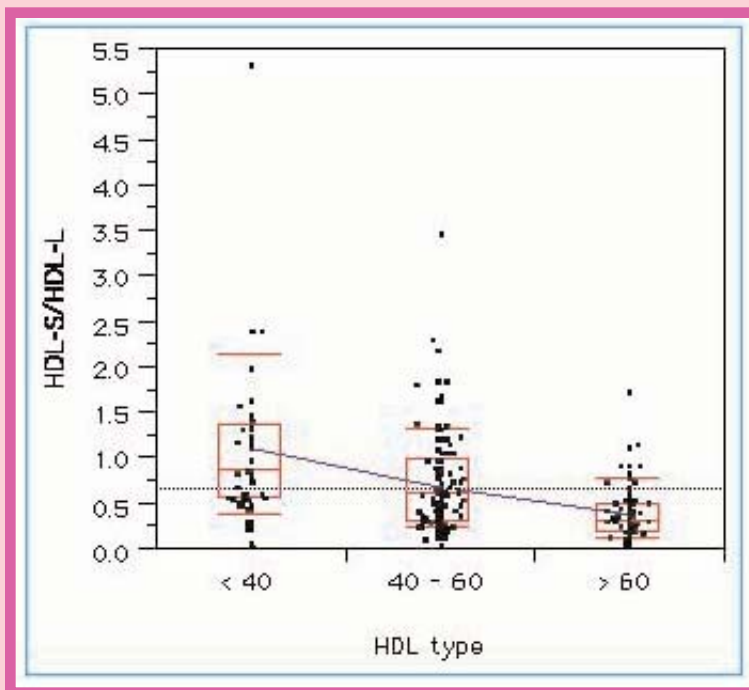


Figure 11. Ratio of small HDL to large HDL subfractions vs. HDL ranges.

- Fig. 12 quantifies the percentage of samples above the 0.64 cut-off. Significant percentages of samples exceed the cut-off in all instances.
- In Fig. 13 only samples in each of the HDL groups were considered that conformed otherwise to the normal NCEP (ATP III) values for the relevant lipid parameters (TC, LDL-C, Trigs). This is where the potential value of the ratio becomes apparent: 16% of samples that were completely normal according to the NCEP guidelines were identified as having an undesirable HDL subfraction profile. Surprisingly, 67% of samples with HDL-C <40 mg/dl and otherwise normal lipid parameters exhibited a desirable HDL subfraction profile.



RESULTS Cont.

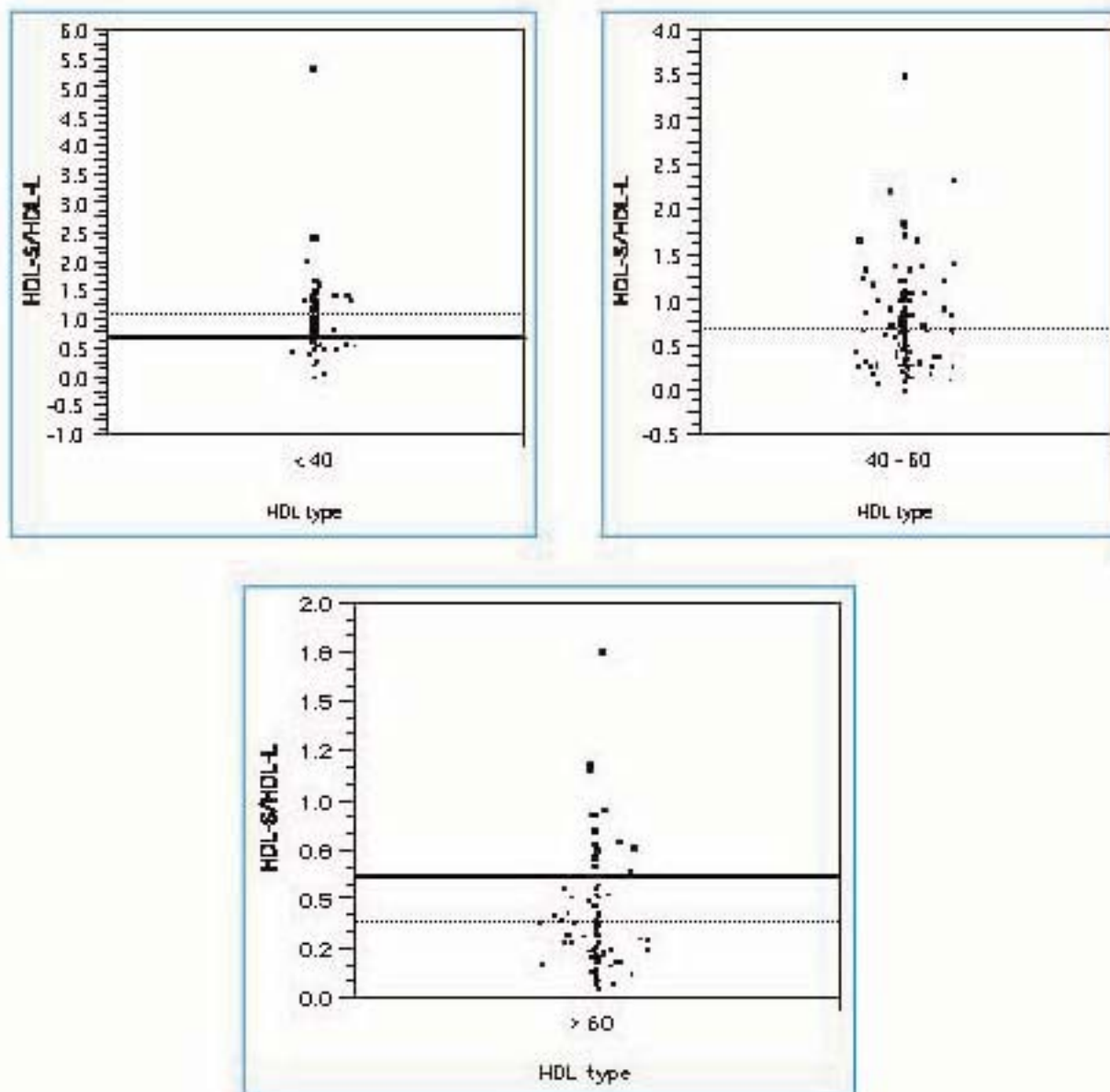


Figure 12. For samples with HDL < 40 (N=45), 67% exceed the reference range (0.64), for samples with HDL between 40-60 (N=136), 44% exceed the reference range and for samples with HDL > 60 (N=89), 16% exceed the reference range.



RESULTS Cont.

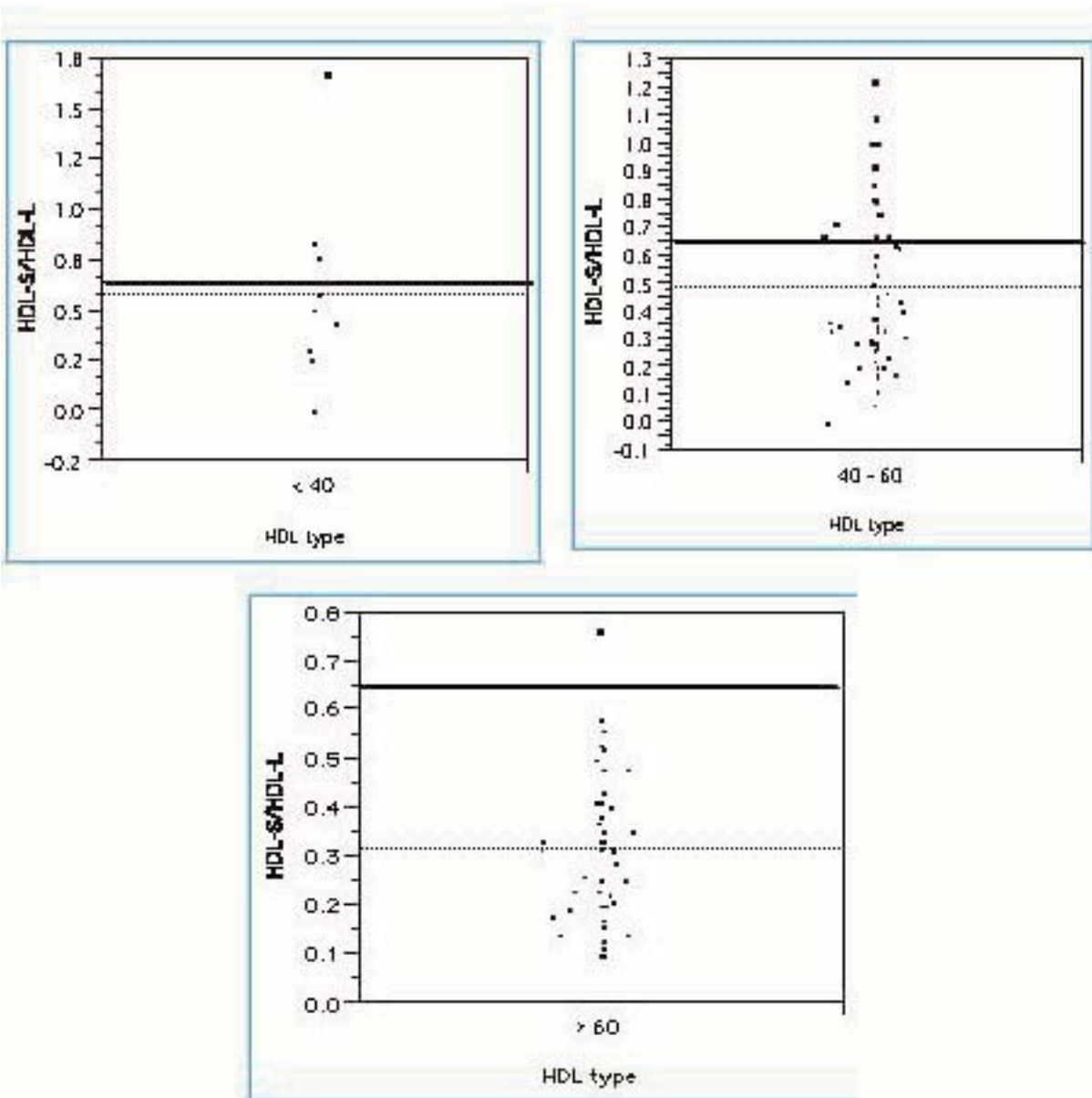


Figure 13. These samples were stratified using the normal ATPIII values (NCEP guidelines). For samples with HDL < 40 (N=9), 33% exceed the reference range (0.64), for samples with HDL between 40-6 (N=71), 25% exceed the reference range and for samples with HDL > 60 (N=46), 2% exceed the reference range.



CONCLUSIONS

- The large HDL subfractions, HDL-1 through HDL-3 showed a significant positive correlation with HDL-C but no strong correlation with LDL particle size. A slight inverse relationship was found with triglyceride and no correlation with total cholesterol and LDL-C.
- The intermediate HDL subfractions, HDL-4 through HDL-7 also exhibited a significant positive correlation with HDL-C. HDL-4 through HDL-5 only showed slightly positive correlation with LDL particle size and also a slight negative correlation with triglyceride. No correlation was found with total cholesterol and LDL-C.
- The small HDL subfractions, HDL-8 through HDL-10 didn't exhibit strong correlations with any of the traditional lipid parameters.
- The small HDL to large HDL ratio (S/L HDL) emerged as an interesting tool to evaluate the HDL subfraction distribution and the associated profile (normal, intermediate or abnormal) across a wide patient population. 16% of all samples that were normal according to NCEP (ATP III) exhibited S/L HDL above the cut-off while 67% of all samples with HDL < 40 mg/dl (and otherwise normal lipid values) were below the cut-off.
- 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-C, and vice versa.

