

# **IMPACT OF TRIGLYCERIDE CONCENTRATION ON THE LEVELS OF LIPOPROTEIN FRACTIONS AND SUBFRACTIONS IN A RANDOM POPULATION**

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# ABSTRACT

The objective of this study was to determine the relationship between different triglyceride concentrations in a random population and the distribution of lipoprotein cholesterol in its fractions and subfractions. Serum was obtained from 271 fasting volunteers (107 male, 164 female) with a median age of 40 years (range: 18 - 84) and tested for total cholesterol (TC), triglycerides (TRIG), HDL-C and LDL-C on a Dade Dimension analyzer. All participants were required to fill out a questionnaire about their health status. Individuals that were either pregnant, used lipid lowering medication, had diabetes mellitus or a past myocardial infarction were excluded from the study. We used the Lipoprint™ System to generate lipoprotein profiles for all 271 individuals. Lipoprint is a linear gel electrophoresis method that separates lipoprotein particles into a maximum of 12 fractions and subfractions - VLDL, Midbands C, B and A (i.e. IDL), LDL-1 through 7 and HDL - and quantitates their cholesterol concentrations. The 271 samples were classified into one of four triglyceride intervals, namely TRIG <100 mg/dl (N=128, 47.2%), 100-150 mg/dl (N=76, 28%), 150-200 mg/dl (N=34, 12.6%) and >200 mg/dl (N=33, 12.2%). Subsequently we analyzed the samples in each group that exceeded the normal reference range (as stated in the Lipoprint LDL package insert) for any given lipoprotein fraction. The concentration of triglyceride-rich lipoprotein particles (VLDL, Midbands C and B) was the highest at high TRIG levels and it became proportionally less with lower TRIG concentrations. The same was true for the smaller and denser LDL particles (LDL-2 to 7). However, Midband A and large LDL (LDL-1) concentrations increased with decreasing TRIG levels. Surprisingly it was found that 31.9% (N=65) of all samples with normal triglycerides (<150 mg/dl) exhibited elevated concentrations of IDL cholesterol (Midband B) and/or small, dense LDL cholesterol (mainly LDL-3 and 4). These lipoprotein particles are believed to be highly atherogenic. This suggests that a lipoprotein profile analysis is warranted even for individuals with triglyceride levels that are normal according to the latest NCEP guidelines (ATP III).



# INTRODUCTION

Lipids and Lipoproteins have long been implicated as crucial components in the genesis of atherosclerosis. Lipoproteins are heterogeneous particles, consisting of multiple subclasses that vary with respect to particle size, density and chemical composition.

The National Cholesterol Education Program (NCEP) has been addressing the role of lipids and lipoproteins in its Adult Treatment Panel (ATP) guidelines. The ATP III guidelines also specify new desirable levels of triglycerides (< 150 mg/dl) whose role as a risk factor for Coronary Artery Disease (CAD) risk has only emerged recently.

The triglyceride-rich VLDL remnants and Intermediate Density Lipoproteins (IDL) are considered atherogenic. Furthermore, increased triglyceride levels in the core of LDL typically leads to the formation of small, dense LDL particles. These small, dense LDL particles are considered highly atherogenic unlike the large, buoyant (cholesterol-rich) LDL particles. This prompted the American Association of Clinical Endocrinologists (AACE) recently to point out that patients may carry these small, dense LDL particles despite normal LDL-C levels. In its comprehensive set of guidelines to reverse the current patterns of underevaluation and undertreatment of dyslipidemia, it is suggested to screen for small, dense LDL particles if triglyceride levels are moderately elevated (150 - 200 mg/dl). (1)

The Lipoprint® system allows for the separation of lipoprotein particle classes and subclasses -including VLDL, IDL, LDL and HDL - and the quantitation of their cholesterol content.

In view of these facts we investigated the impact of the triglyceride concentration on the levels of lipoprotein fractions and subfractions in a random population.



# METHODS AND MATERIALS

## Samples and Sample Analysis

Fasting blood samples were collected from volunteers. Serum was obtained after the blood had been allowed to clot for 30 minutes.

Samples that met the following exclusion criteria were rejected:

- o lipid lowering medication
- o recent heart attack
- o pregnancy
- o diabetes

The following analytes were measured for 271 samples:

- o Total Cholesterol (TC)
- o LDL Cholesterol
- o HDL Cholesterol
- o Triglyceride

Subsequently the lipoprotein particle distribution was analyzed and corresponding lipoprotein profiles were generated for each sample (N=271).

## Equipment

A Dimension XL analyzer (Dade, Inc.) was used to quantify TC, LDL, HDL and triglycerides.

A Lipoprint System (Quantimetrix Corp.) was used to generate Lipoprotein subfraction profiles.

Lipoprint is a linear polyacrylamide gel system for the separation of Lipoprotein particles according to size. (Figures 1 and 2)



# TEST PRINCIPLE FIGURES

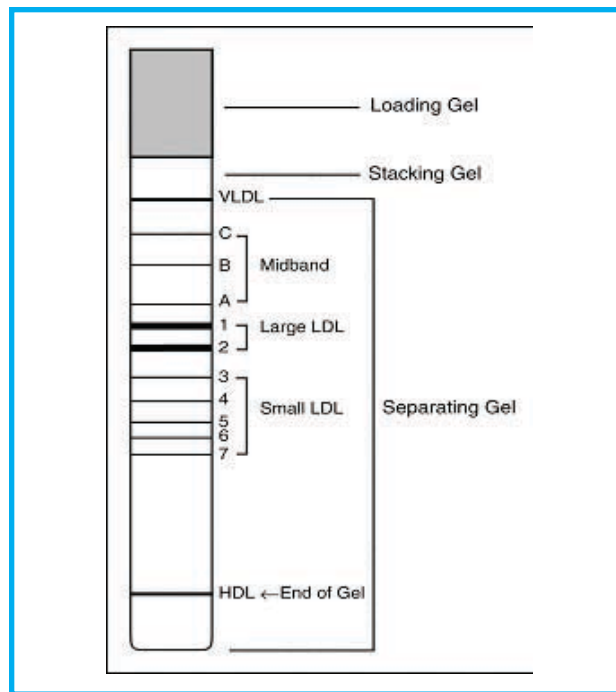


Figure 1. Lipoprint Gel Tube Schematic

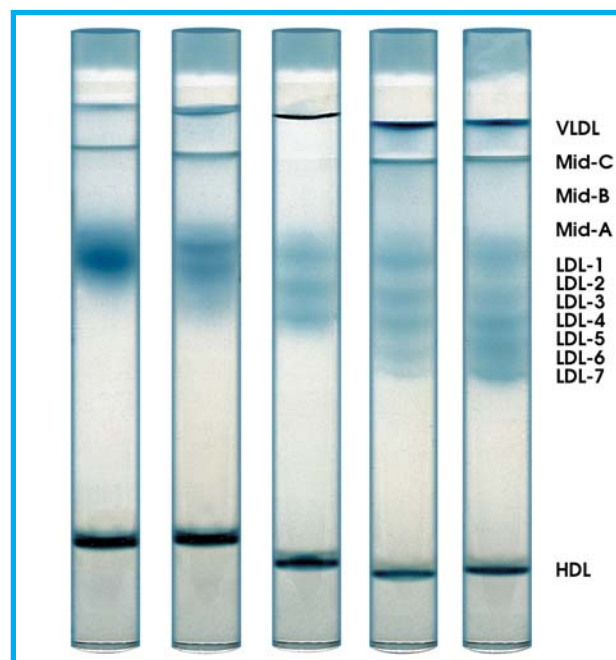


Figure 2. Lipoprint Gel Tubes after Electrophoresis - Progressively Disperse LDL Profile (from left)



# PROFILES

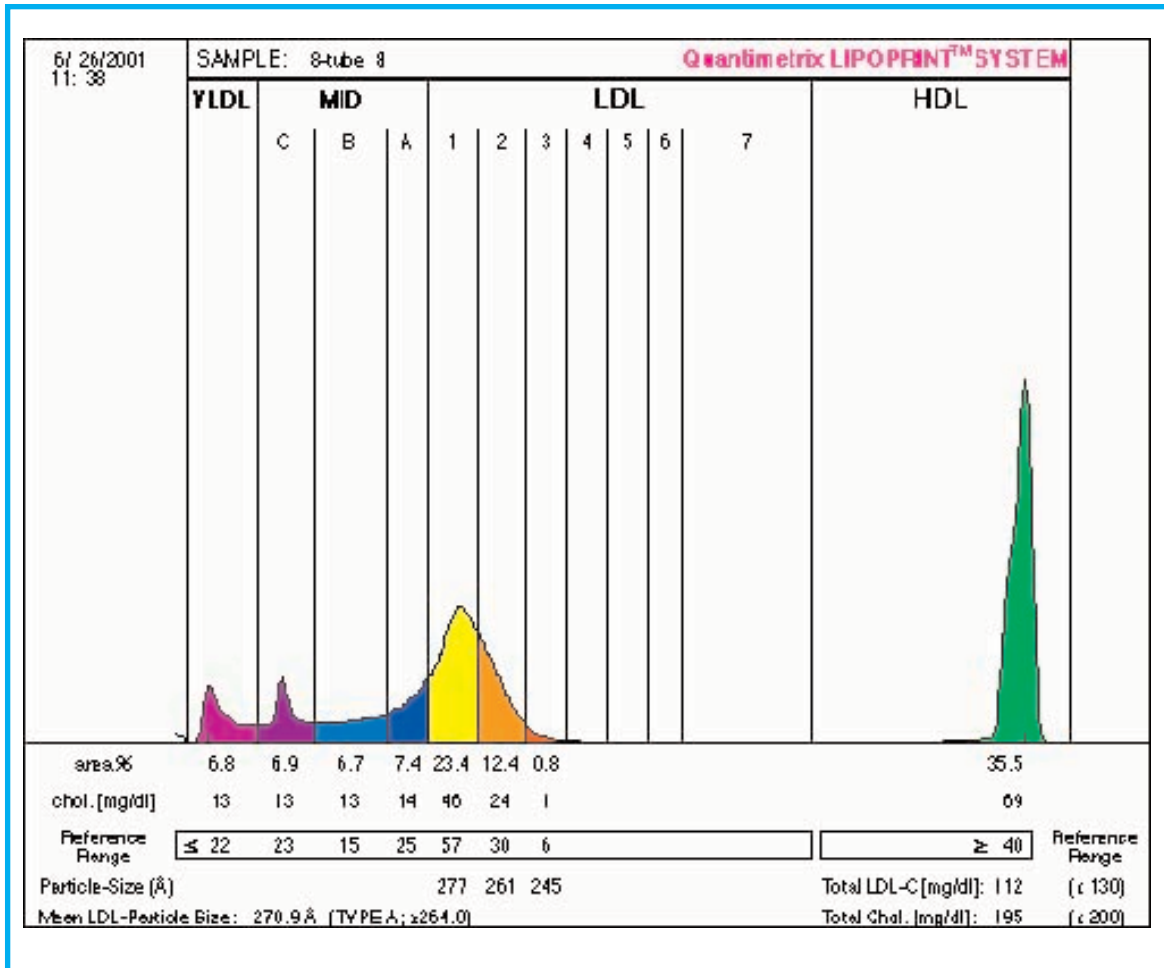


Figure 3. Lipoprint Report for a Normal Profile



# PROFILES Cont.

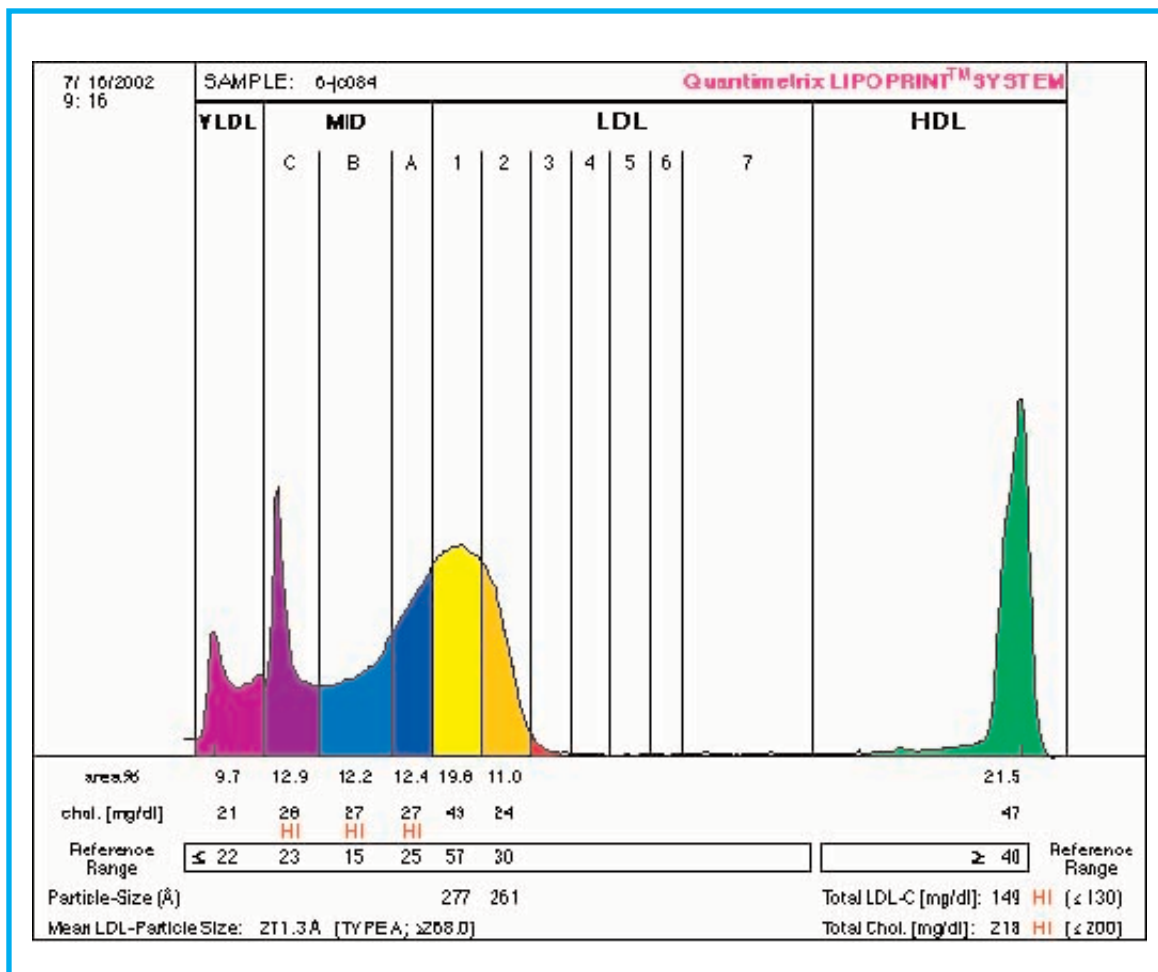


Figure 4. Lipoprint Report for a Profile with Elevated IDL (Mid-C, Mid-B and Mid-A)



# PROFILES Cont.

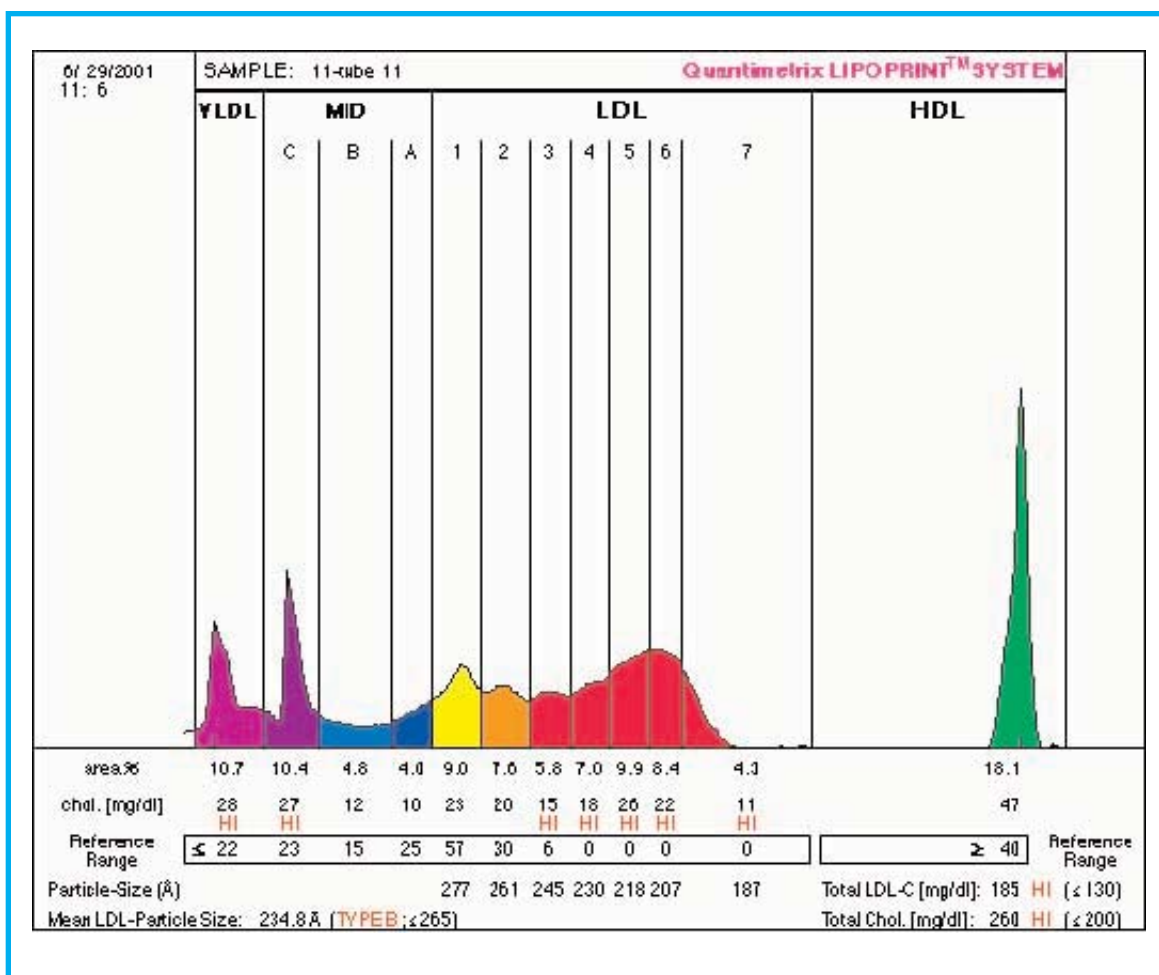


Figure 5. Lipoprint Report for a Profile with Elevated IDL (Mid-C, Mid-B and Mid-A)



# RESULTS

All 271 samples were classified into one of four possible groups according to their triglyceride concentrations:

Group	N	Triglycerides (mg/dl)
1	128	< 100
2	76	100 - 150
3	34	150 - 200
4	33	> 200

The above groups were further analyzed in terms of Lipoprotein particle distribution, namely for VLDL, IDL (Midbands C, B and A using the Lipoprint convention), and LDL subclasses 1-3.

The cholesterol concentrations of the samples in each of the four groups was illustrated for the respective lipoprotein fraction/subfraction (the red boxes span from the lower quartile to the upper quartile, the red lines represent the 10% and the 90% quantiles). (Figs. 6-1 to 6-4)



# RESULTS Cont.

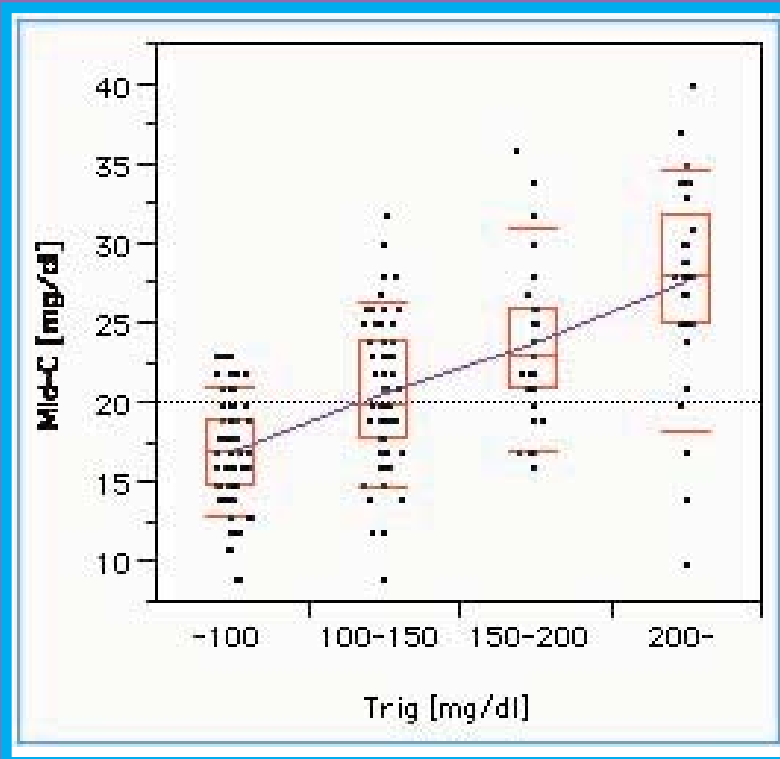
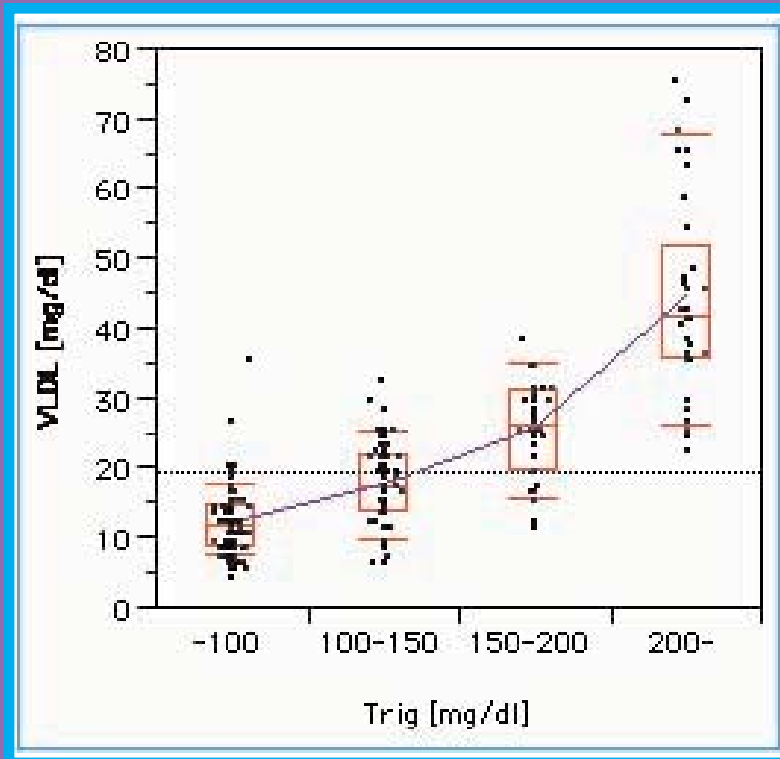


Figure 6-1. Lipoprotein Particle Distribution vs. Triglyceride Ranges



# RESULTS Cont.

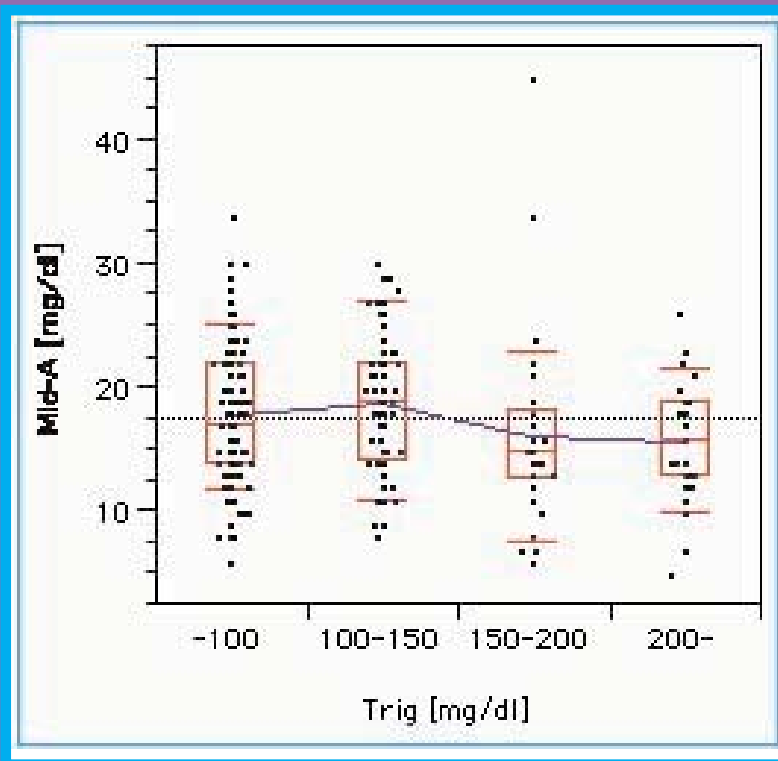
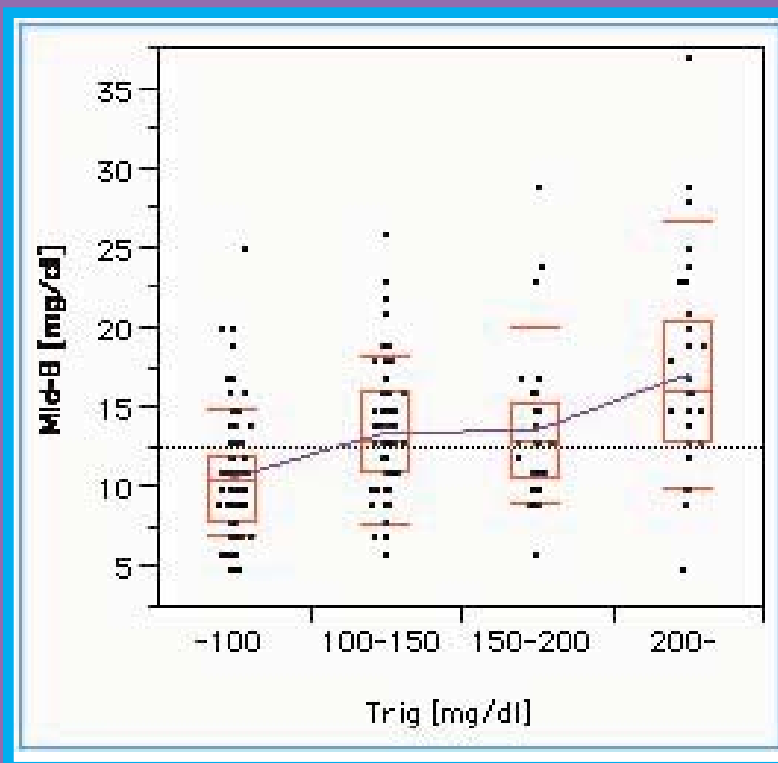


Figure 6-2. Lipoprotein Particle Distribution vs. Triglyceride Ranges



## RESULTS Cont.

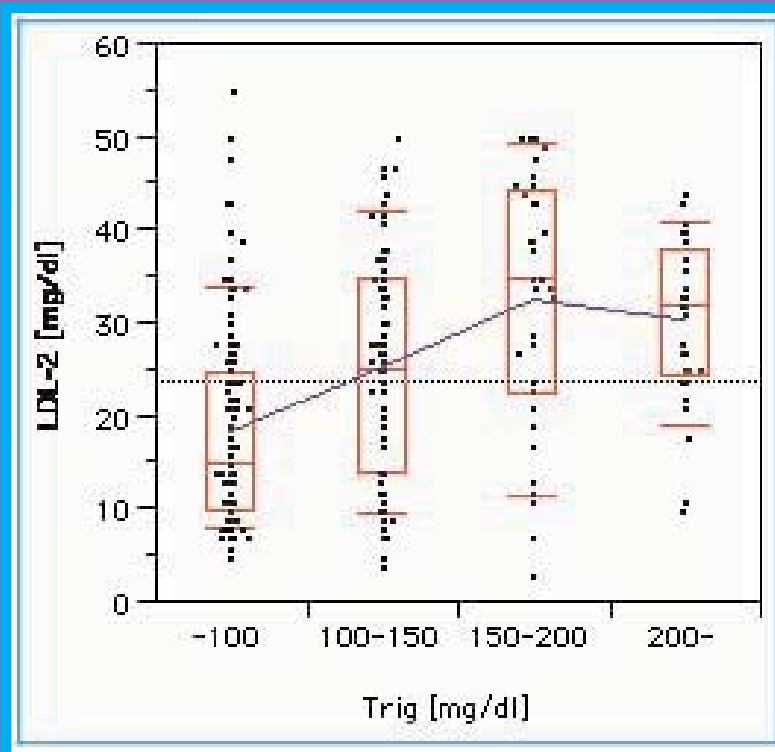
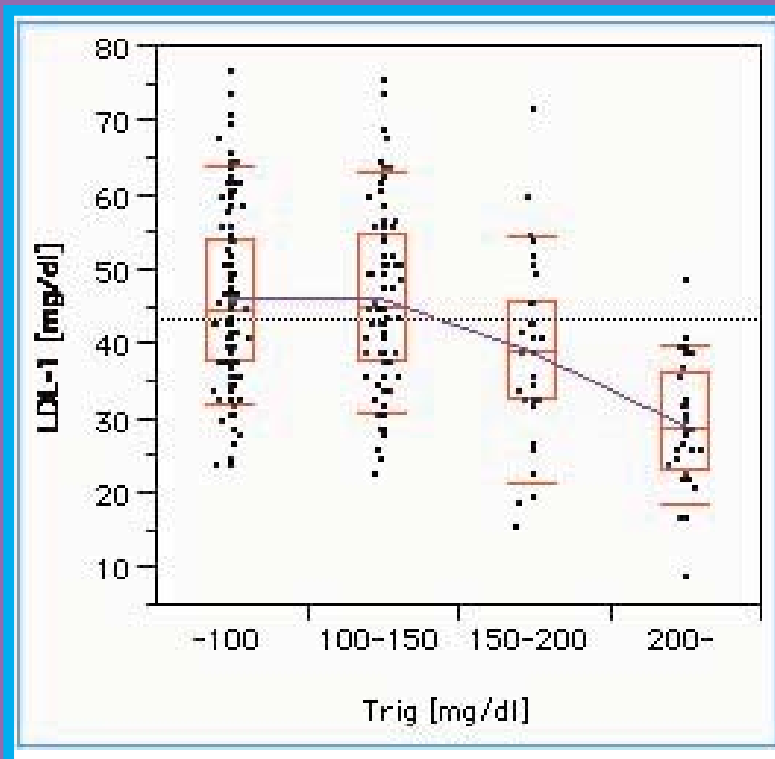


Figure 6-3. Lipoprotein Particle Distribution vs. Triglyceride Ranges



## RESULTS Cont.

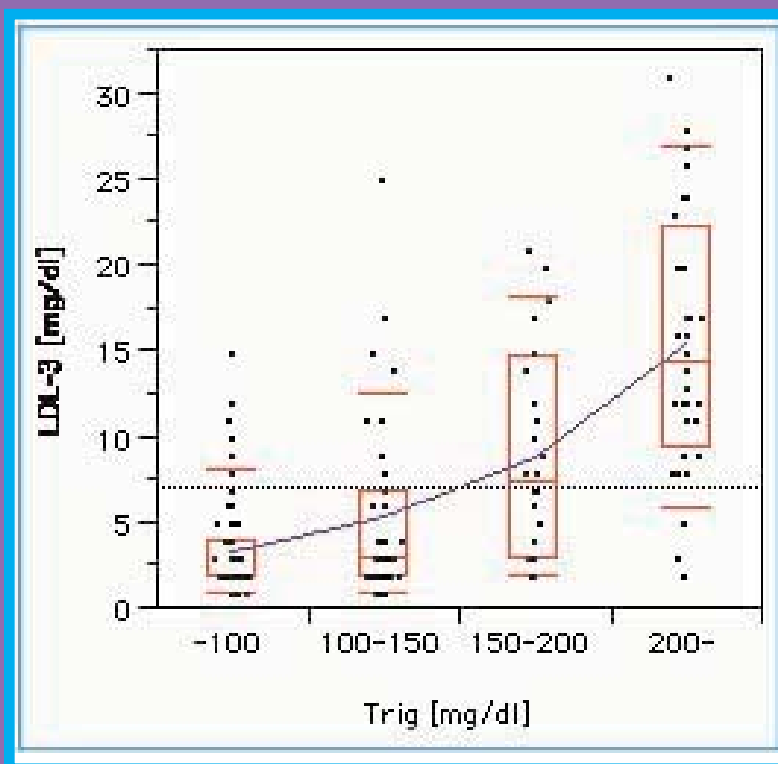


Figure 6-4. Lipoprotein Particle Distribution vs. Triglyceride Ranges

As expected the levels of triglyceride-rich particles (VLDL, Midband C and B) as well as the levels of the cholesterol-depleted particles (in increasing measure from LDL-2 to 3 and 4) showed a positive correlation with triglyceride levels.

This relationship became even clearer when the percentage of samples with elevated values for the respective lipoprotein classes/subclasses was determined across the different triglyceride groups.

(Note: this percentage was determined using the normal cholesterol range for each lipoprotein class/subclass as previously established using the Lipoprint system (2))



# RESULTS Cont.

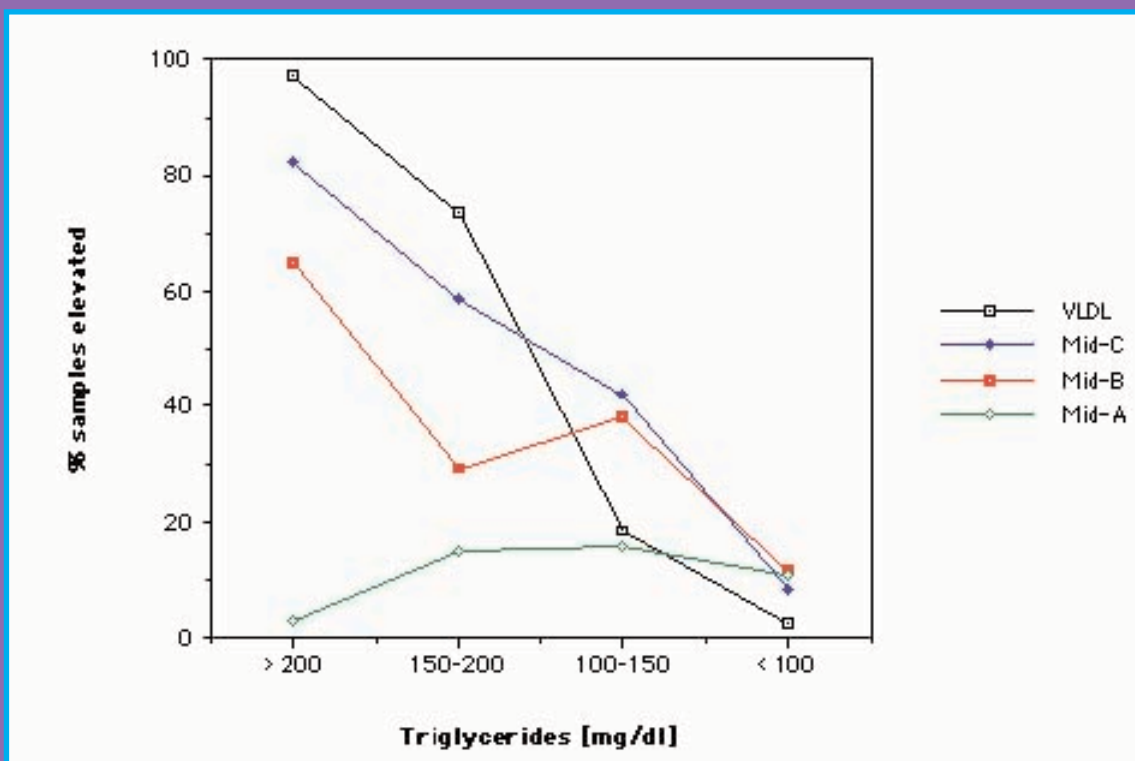


Figure 7. Percentage of Samples with Elevated Lipoprotein Concentration (VLDL, Mid-C, Mid-B and Mid-A) vs. Triglyceride Ranges

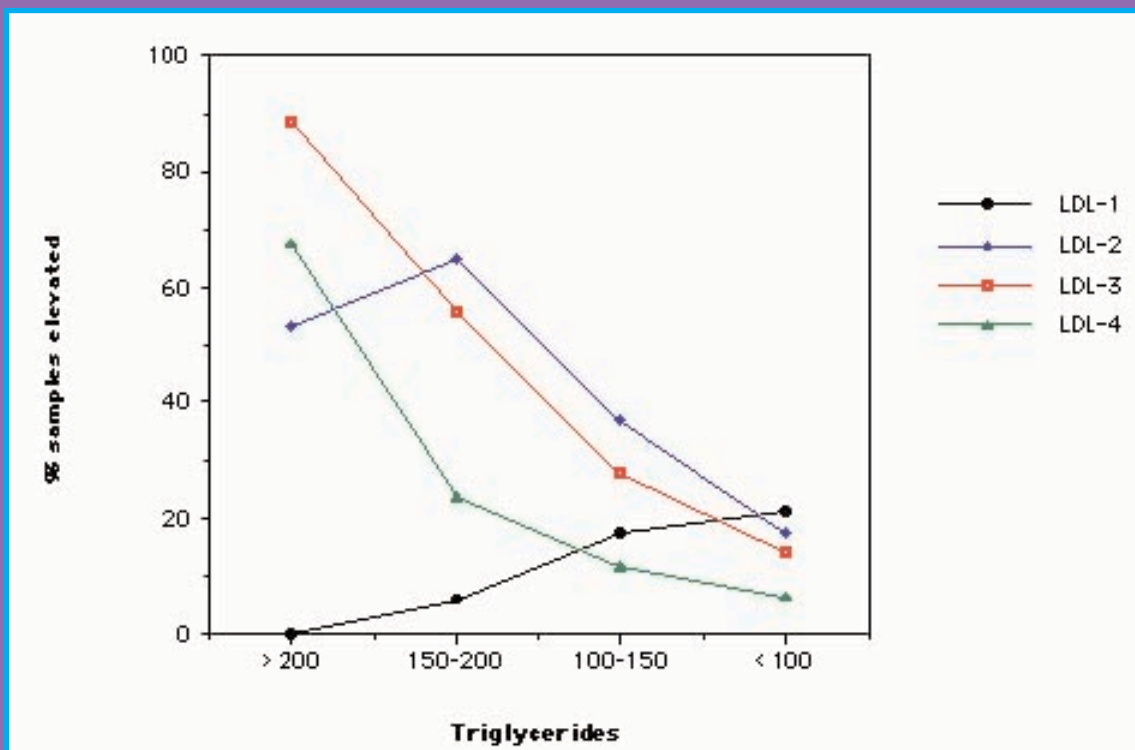


Figure 8. Percentage of Samples with Elevated Lipoprotein Concentration (LDL-1 Through LDL-4) vs. Triglyceride Ranges



# RESULTS Cont.

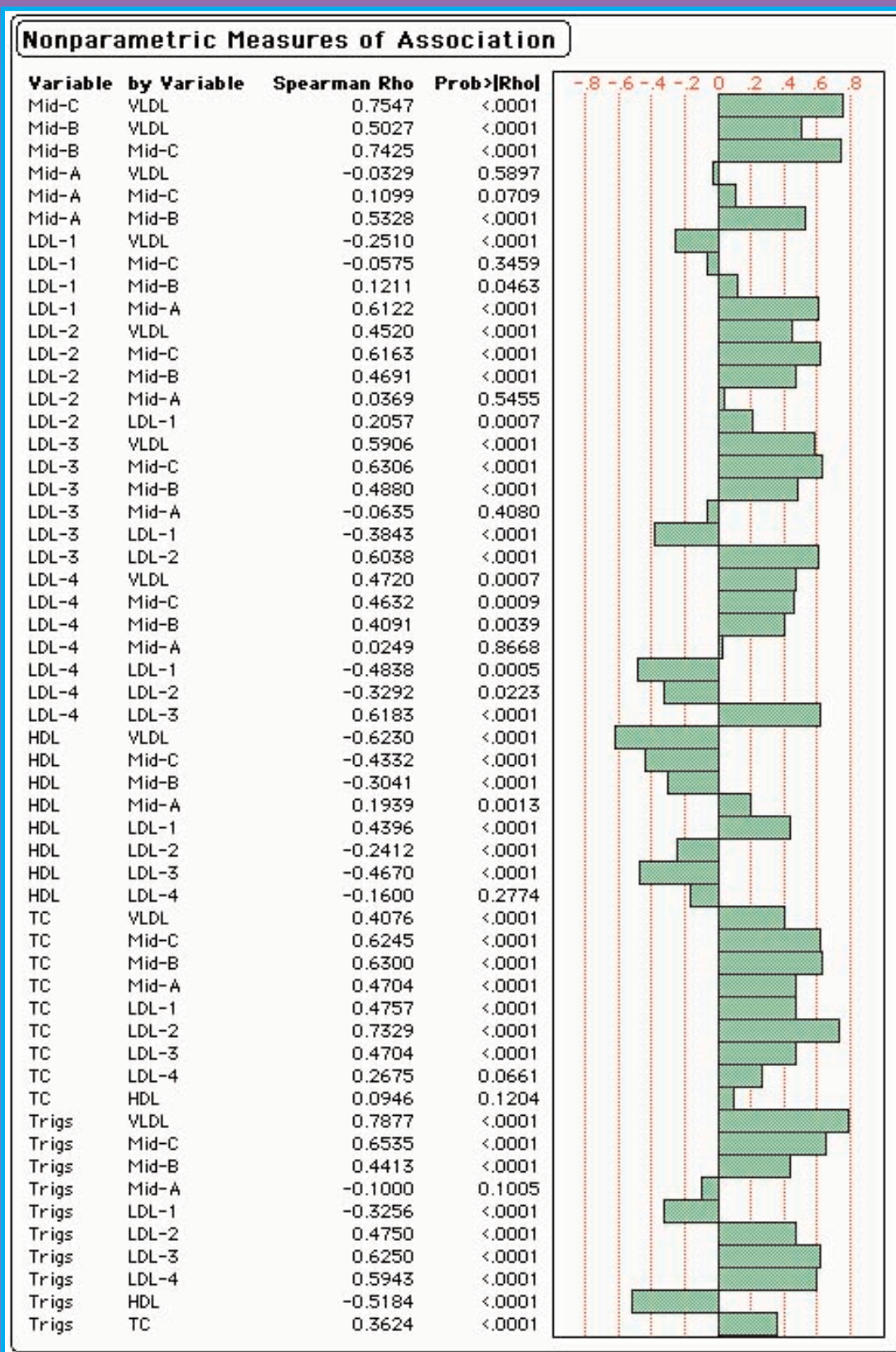


Figure 9. Multiple Correlation of Lipoprint Parameters



## RESULTS Cont.

The analysis of nonparametric association (Fig. 9) confirms and expands on the previous findings.

### Normotriglyceridemic Samples:

The total sample population contained 204 samples (75.3%) with desirable triglyceride concentrations (<150 mg/dl, according to ATP III). Of those 71 samples (34.8%) were found to have either elevated levels of IDL cholesterol (Midbands B and C) or LDL-3 cholesterol. Only 18 samples (8.8%) had low HDL levels (< 40 mg/dl). Figure 10 gives a breakdown of the percentage for each selected lipoprotein parameter.

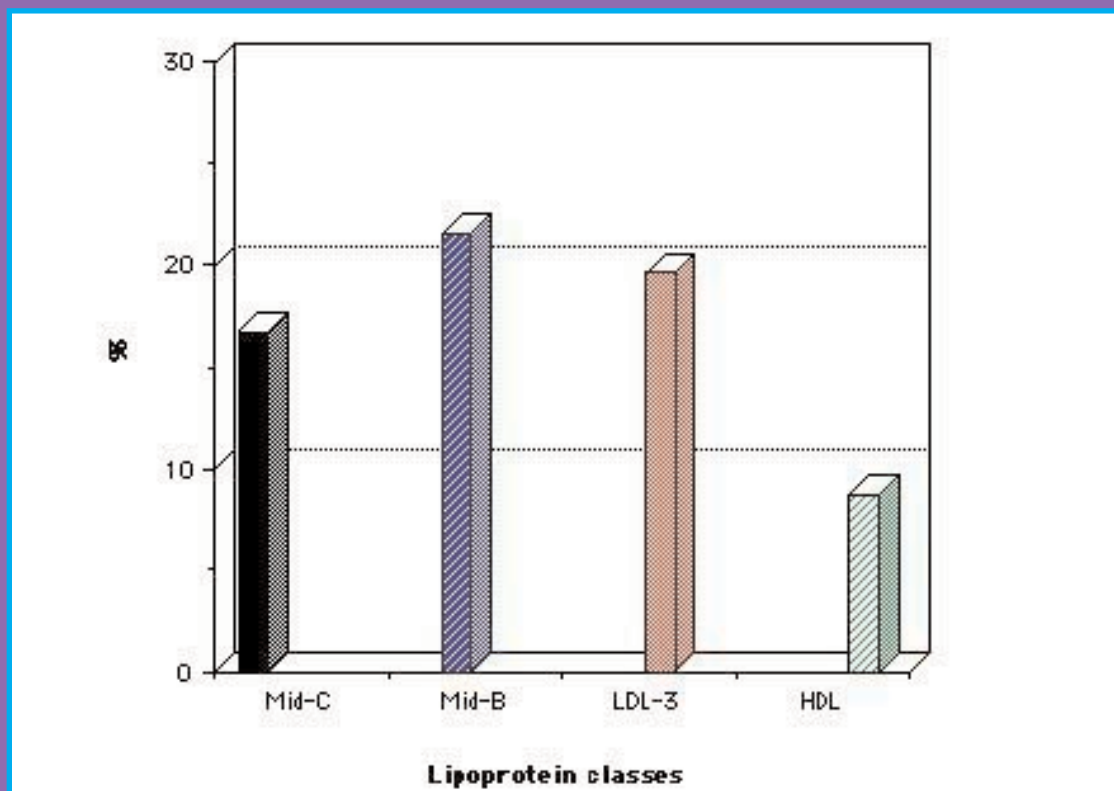


Figure 10. Samples Outside the Normal Reference Range for Select Parameters for a Normotriglyceridemic Population (Triglycerides < 150 mg/dl)



# CONCLUSIONS

- Concentrations of triglyceride-rich particles (VLDL; IDL: Mid-C and Mid-B; and small, dense LDL: LDL-3) showed a high degree of correlation with triglycerides, just as expected. However, Midband-A concentrations as well as the concentrations of large LDL (LDL-1) showed an inverse correlation with triglyceride levels.
- Given the fact that the highly atherogenic particles are triglyceride-rich (VLDL remnants, IDL and small, dense LDL) it was quite surprising to find a considerable number of samples containing elevated levels of those particles in a normotriglyceridemic population (trig < 150 mg/dl).
- The present findings suggest that lipoprotein subfraction testing using the Lipoprint system may be advisable even for samples with low triglyceride levels and not just those with elevated triglyceride levels as suggested in the AACE guidelines.

# REFERENCES

1. AACE LIPID GUIDELINES, *Endocr. Pract.*, 6(2), 162-205 (2000).
2. MUÑIZ N, DUNCAN D, NEYER G, Normal reference ranges for serum lipoproteins and their subfractions for the Lipoprint LDL System. Presented

