

LIPOPROTEIN SUBFRACTION TESTING WITH THE LIPOPRINT® SYSTEM - EASY, ACCURATE AND COMPREHENSIVE

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ABSTRACT

Lipoprint, the FDA cleared method for the determination of cholesterol in the subfractions of LDL, is an integrated system consisting of hardware, software and ready-to-use reagents.

The system components have been optimized to provide clinical laboratories with the only do-it-yourself LDL subfraction solution currently available in the market place. Precast linear gels separate the lipoprotein particles into up to 10 prestained visible bands, from VLDL to HDL, and allow for the precise and accurate determination of cholesterol in all lipoproteins. Comprehensive, yet intuitive reports contain all the lipoprotein cholesterol information in visual and numerical form, complete with normal reference ranges for all parameters.

The clinical utility of the system for screening and monitoring of patients is demonstrated in exemplary case studies. Unlike other methods, Lipoprint measures the cholesterol levels in each lipoprotein subfraction down to 1 mg/dL which allows for the unequivocal identification of the highly atherogenic small dense LDL and IDL subspecies. This makes Lipoprint a valuable adjunct in the determination of a wide spectrum of conditions, e.g. small dense LDL dyslipidemia associated with metabolic syndrome as well as type 3 dyslipidemia frequently present with large LDL particles and consequently classified as normal when using particle size or classification into pattern A and B only.



INTRODUCTION

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

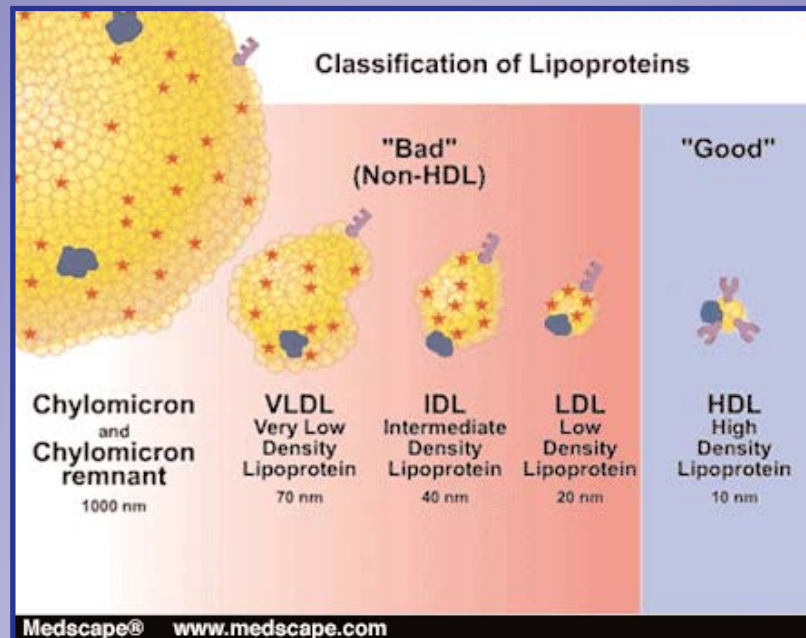


Figure 1. Lipoprotein particles

The National Cholesterol Education Program (NCEP) has been addressing the crucial role of lipoproteins in its Adult Treatment Panel (ATP) guidelines. In 2001 the latest iteration of these guidelines (ATP III) was released. Special emphasis was given to the level of Low Density Lipoprotein (LDL) cholesterol as a decision point for intervention, with or without drug treatment.

It is well known that under certain circumstances, e.g. in Metabolic Syndrome, triglycerides are elevated, HDL is decreased and the LDL particle population is shifted towards the smaller, denser particles.

This prompted the American Association of Clinical Endocrinologists (AACE) recently to draw attention to the fact that small, dense LDL particles seem to be especially atherogenic and commonly precede coronary artery disease (CAD). Patients may carry these particles despite normal LDL-C levels.

A comprehensive set of guidelines to address the current patterns of underevaluation and undertreatment of dyslipidemia, a major risk factor for CAD, was subsequently issued by the AACE. (1)

Recently, intermediate density lipoprotein (IDL) - which can be identified with the Lipoprint system - was also found to be significantly involved in the atherosclerotic process. (2)



THE PROBLEM

Coronary artery disease (CAD) is the leading cause of death in the US. Normal lipid levels have been established in the latest NCEP (ATP III) guidelines:

Cholesterol < 200 mg/dl
Triglycerides < 150 mg/dl
LDL-C < 130 mg/dl
HDL-C > 40 mg/dl

Yet:

- 50% of CAD occurs among individuals with normal lipid profiles (3)
- Individuals with SMALL, DENSE LDL are at threefold risk for CAD - independent of other risk factors (4)
- ATP III does not recommend measurement of small, dense LDL because "standard and inexpensive methodologies are not available for their measurement" (5)

THE SOLUTION

Lipoprint, the first and only FDA-cleared methodology for the determination of LDL subfractions, which allows for the rapid, accurate and inexpensive determination of LDL subfraction cholesterol in any laboratory.



Figure 2. Lipoprint system components



THE SOLUTION Cont.

- Lipoprint resolves up to 12 lipoprotein fractions and subfractions:
 - > VLDL
 - > Mid-bands C, B, A : IDL, Remnant Lipoproteins and Lipoprotein (a) (Lp(a))
 - > LDL subfractions (subclasses): Large (LDL-1, 2) and small-dense (LDL-3 to LDL-7)
 - > HDL

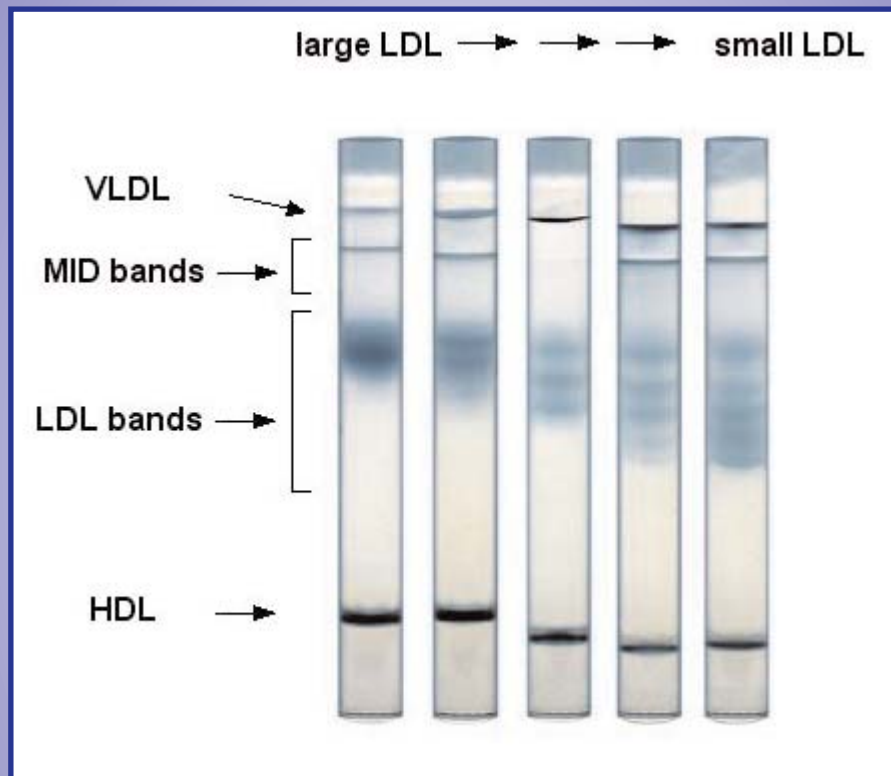


Figure 2a. Lipoprint gel tubes after electrophoresis

- Lipoprint is based on linear polyacrylamide gel electrophoresis
 - > separation of lipoprotein particles is achieved due to sieving effect of the gel matrix (without a gel gradient!)
 - > Lipoprotein distribution is comparable to that of the legacy method for lipoprotein separation: Continuous Gradient Ultracentrifugation



THE SOLUTION Cont.

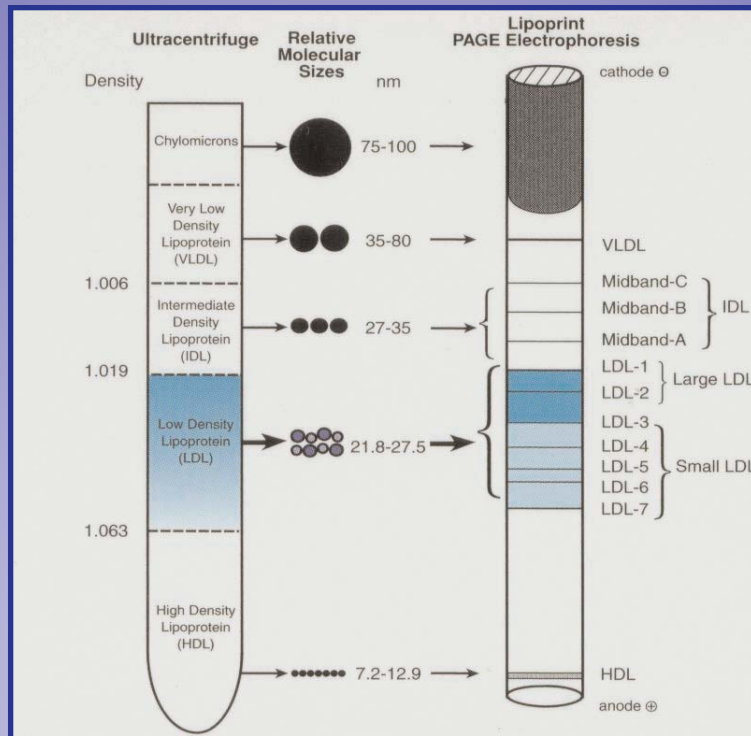


Figure 3. Ultracentrifuge vs. linear polyacrylamide gel

- Lipoprint has been validated extensively as indicated in the representative excerpts below:

1) S.V. Hirany, I. Jialal, et al., Am.J.Clin.Pathol. 119 (2003) 439-445

"We evaluated a LDL subfraction separation method using polyacrylamide tube gel electrophoresis (PTGE) (Lipoprint) and compared it with (...) polyacrylamide gradient gel electrophoresis (PGGE-REF). Excellent intra-assay and inter-assay coefficients of variation were obtained (<4%) for PTGE."

"(For 102 subjects) Excellent agreement between the methods was observed."

"The PTGE method is precise and compares favorably with PGGE-REF. It has the advantage of being simple, less expensive, and more suitable for use in the clinical lab."

2) J. P. McConnell., D. M. Hoefner., et al., Clin. Chem. 47 (2001) 266-274

"A quantitative method for the assessment of LDL particle size phenotype was developed using the Quantimetrix Lipoprint LDL System. The method can be performed in less than 3 h in batch mode and is suitable for routine use in clinical laboratories."

3) G.L. Vega, S.M. Grundy, et al., Am.J.Cardiol. 91 (2003) 956-960

"The present study was designed to examine the safety and efficacy of a combination of low-dose simvastatin and fenofibrate in the treatment of combined hyperlipidemia."

"LDL sizes were measured with the Lipoprint system (Quantimetrix Inc., Redondo Beach, California). (...)The coefficients of inter- and intraassay variation for this assay were 2%."

4) T. Kazumi et al., Atherosclerosis 142 (1999) 113-119

"We used continuous disc electrophoresis (Lipoprint System), which is rapid and easier to perform in assessing large numbers of samples (...). LDL-PPD measured using continuous electrophoresis was identical to peak particle diameter of LDL which was isolated by ultracentrifugation and was measured directly using an electron microscopy (sic!)."



THE SOLUTION Cont.

LIPOPRINT - THE ULTIMATE LIPOPROTEIN SUBFRACTION METHOD FOR ANY LAB

- Sample requirements:
 - > fasting serum or plasma - only 25µl needed
 - > may be refrigerated for up to 7 days
- Procedure:
 - 1) Application of serum/plasma (25 µl) to gel



Figure 4. Sample Application

- 2) Addition of loading gel (200 µl) and mixing by inversion



Figure 5. Mixing sample and loading gel



THE SOLUTION Cont.

3) Polymerization of loading gel (30 min.)



Figure 6. Photopolymerization

4) Electrophoresis at 3 mA/gel tube (1 hour)



Figure 7. Electrophoresis



THE SOLUTION Cont.

5) Scanning of gel tubes



Figure 8. Scanner with gel tube tray

6) Analysis of the scan(s)

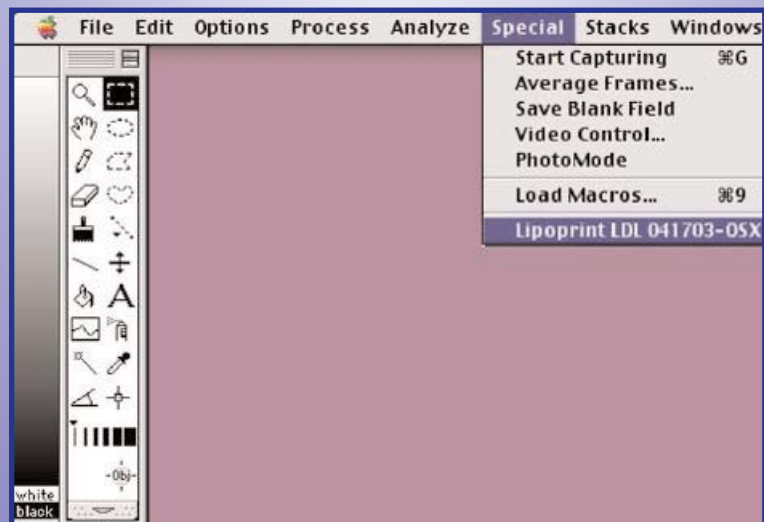


Figure 9. LipoWare analysis software



THE SOLUTION Cont.

total time: 2.5 hours for up to 48 samples

- Minimal user intervention - the software automatically identifies the relevant lipoprotein bands
- Comprehensive and easy to interpret reports - what you see is what you get

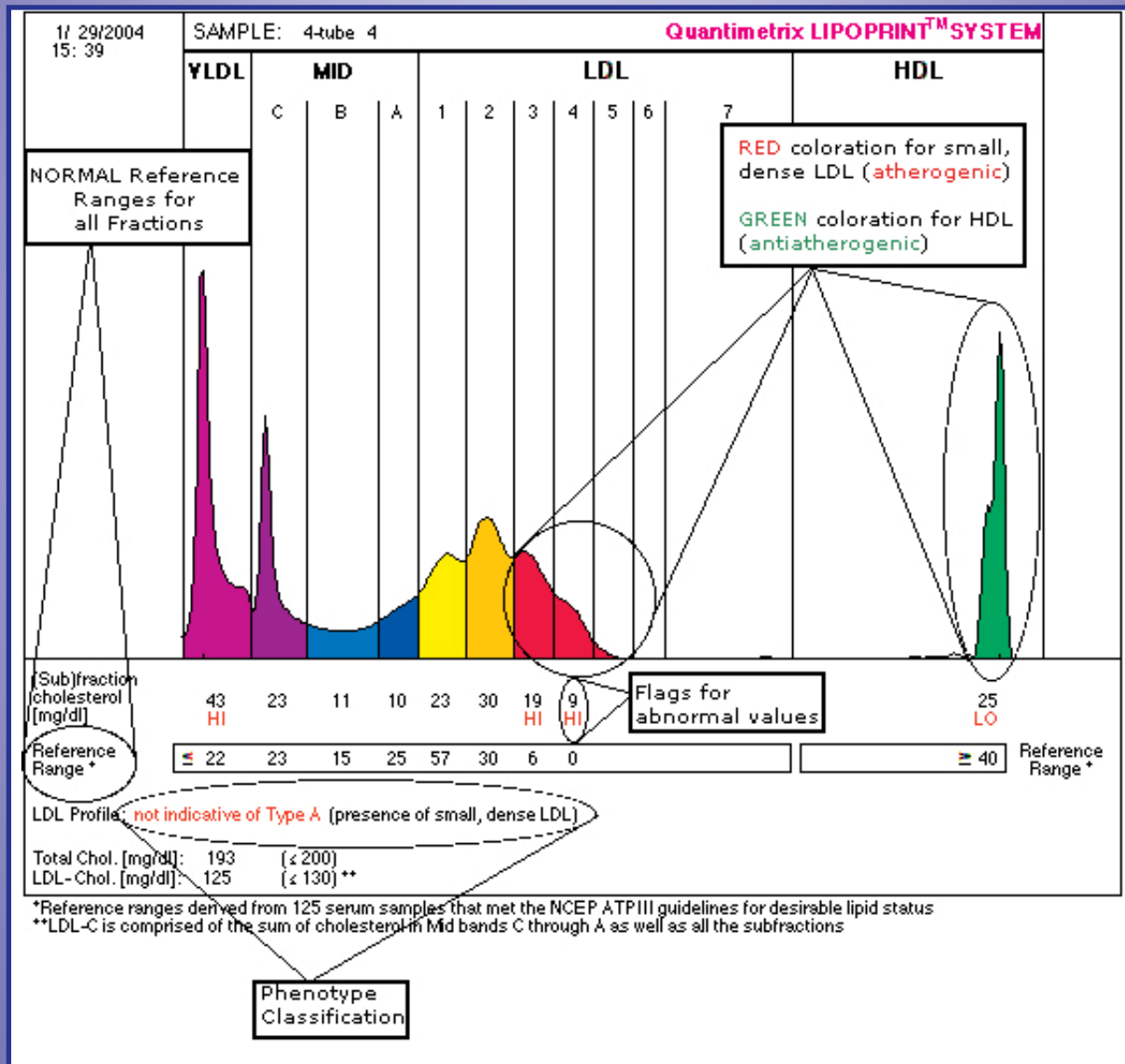


Figure 10. Lipoprint report



THE SOLUTION Cont.

LIPOPRINT RESULTS - QUANTITATIVE AND QUALITATIVE

- Lipoprint accurately quantifies lipoprotein cholesterol:
 - > Lipoprint determines the amount of cholesterol in each lipoprotein fraction rather than just particle size or particle number.
 - > HDL-C and LDL-C values obtained with Lipoprint correlate very well to those obtained with reference lab tests (N=80):

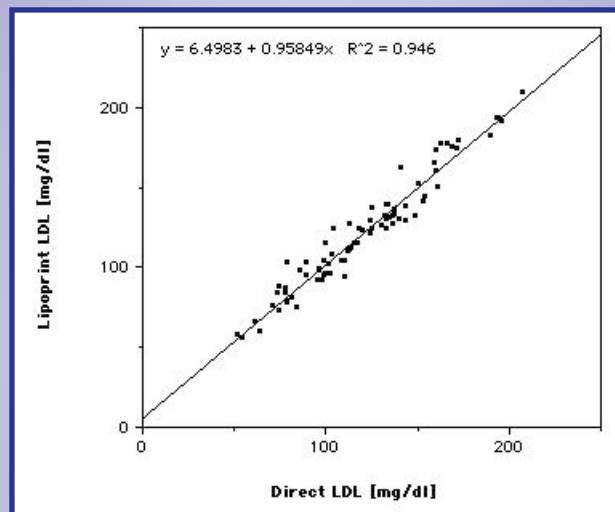


Figure 11. Lipoprint LDL vs. Direct LDL

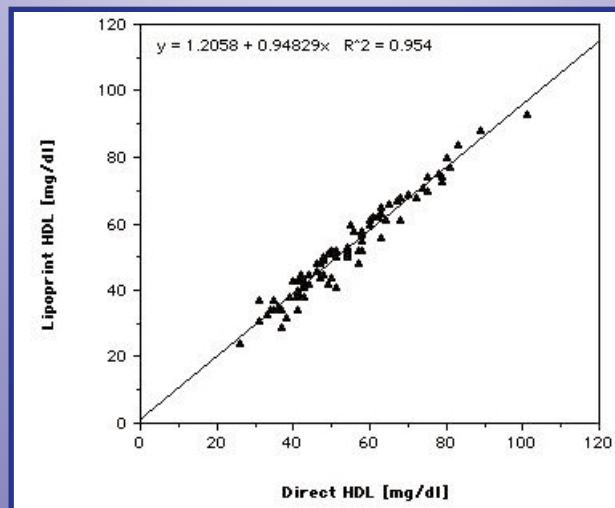


Figure 12. Lipoprint HDL vs. Direct HDL



THE SOLUTION Cont.

- Lipoprint also provides profile classification for comparability with the legacy methods
 - > Traditionally lipoprotein profiles have been classified as "Type A"(normal), "Intermediate" and "Type B" (abnormal)
 - > Qualitative measure

Issues:

- > "Type B" is not recognized by the FDA , therefore Lipoprint uses "A" and "non-A"

LIPOPRINT		Gradient Gel (GGE)			NMR		
Phenotype	N	Classification		Agreement	Classification		Agreement
		A	Non-A		A	Non-A	
A	16	14	2	88%	15	1	94%
Non-A	35	4	31	89%	11	24	69%

Table 1. Phenotype classification agreement between methods (Adapted from: J.P. McConnell et al., Clin.Chem. 47 (2001) 266-274)

- > Phenotype doesn't take into account IDL - Lipoprint quantifies IDL cholesterol (Mid-Bands)
- > Type III dyslipidemia would be classified as normal (Type A) if relying on the phenotype; the problem here is elevated IDL which can easily be determined and visualized using Lipoprint

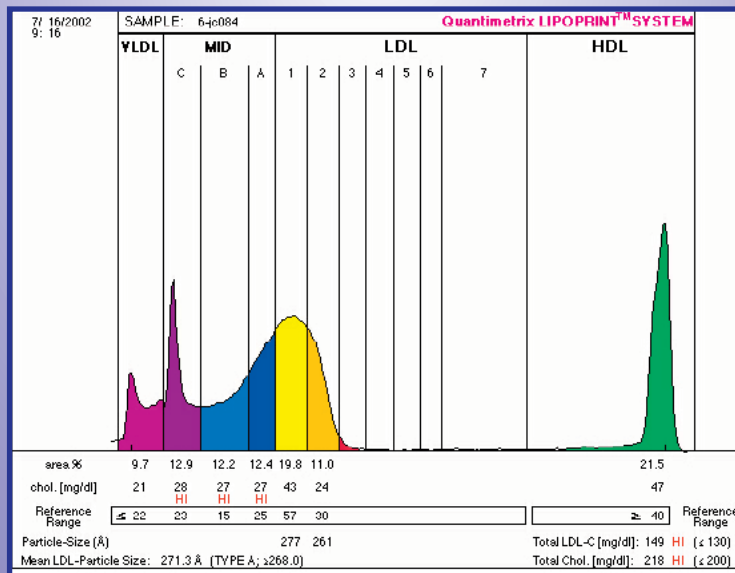


Figure 13. Sample with elevated IDL AND normal phenotype

- > Lipoprint provides normal reference ranges for the cholesterol contained in each fraction and subfraction. These ranges were established from a normal population conforming to the latest NCEP lipid guidelines (ATP III)



THE SOLUTION Cont.

LIPOPRINT - CLINICAL UTILITY

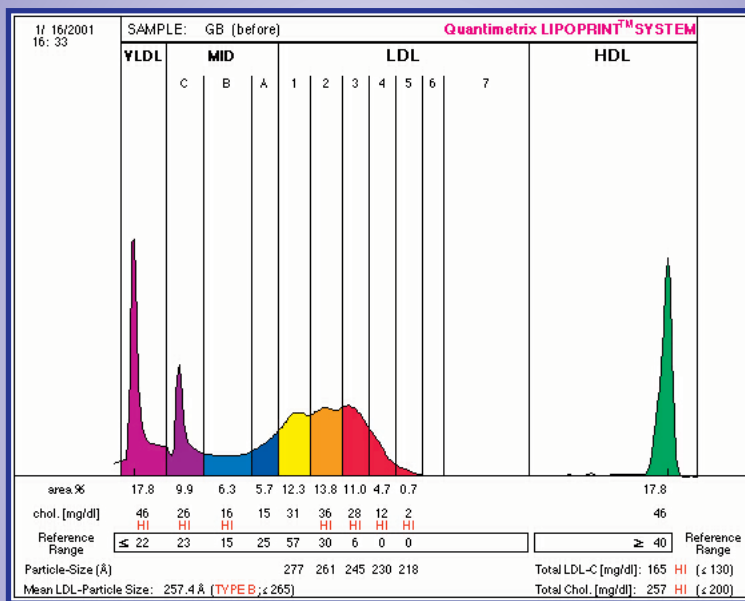
- Lipoprint profiles were determined at baseline and after 2-3 months of therapeutic intervention:

Example 1

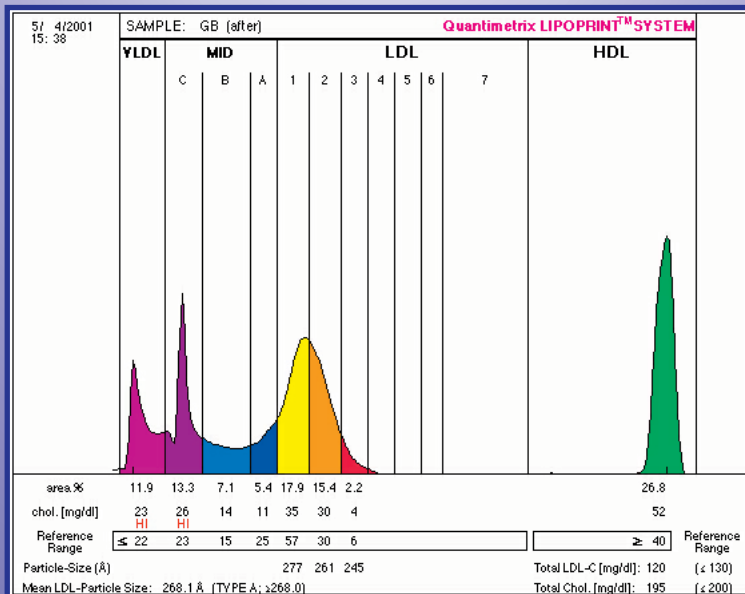
GB, a 65 year old caucasian male had his Lipoprint profile done. The profile contained small, dense LDL particles (red portion of the profile) indicating a lipid disorder.

After 3 months of niacin therapy combined with dietary changes and increased exercise the patient presented with a vastly improved profile. The lipoprotein distribution was normal and only two cholesterol values were slightly outside the normal reference range.

GB Profile Before Intervention:



GB Profile After Intervention:



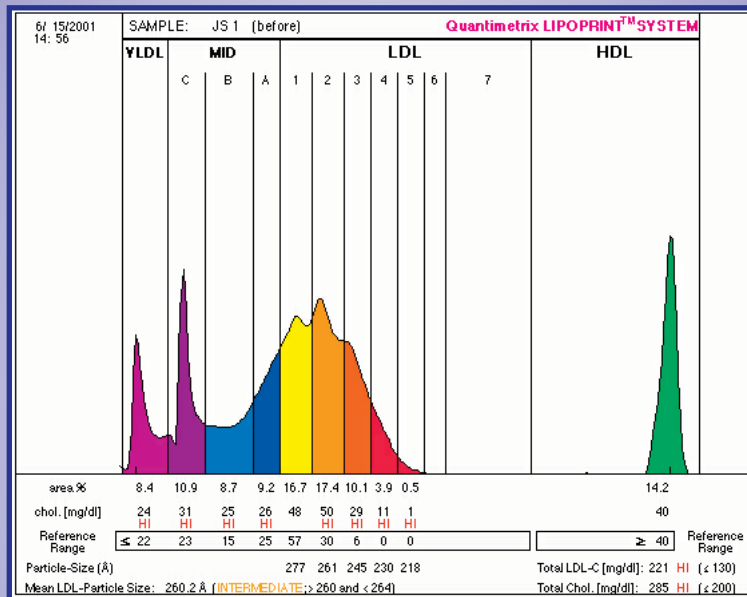
THE SOLUTION Cont.

Example 2

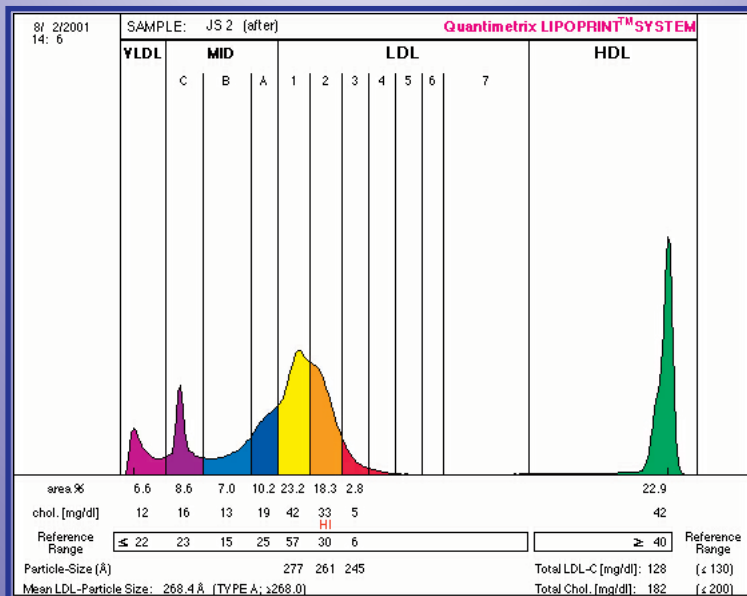
JS, a 42 year old Indian male had his Lipoprint profile done. The profile contained high levels of cholesterol in many fractions and the distribution of lipoprotein particles was shifted toward the smaller and denser particles (red portion of the profile) indicating a lipid disorder.

After 2 months of a mainly vegetarian diet with emphasis on soy protein and increased exercise the patient presented with an essentially normal profile - only LDL-2 was slightly elevated.

JS Profile Before Intervention:



JS Profile After Intervention:

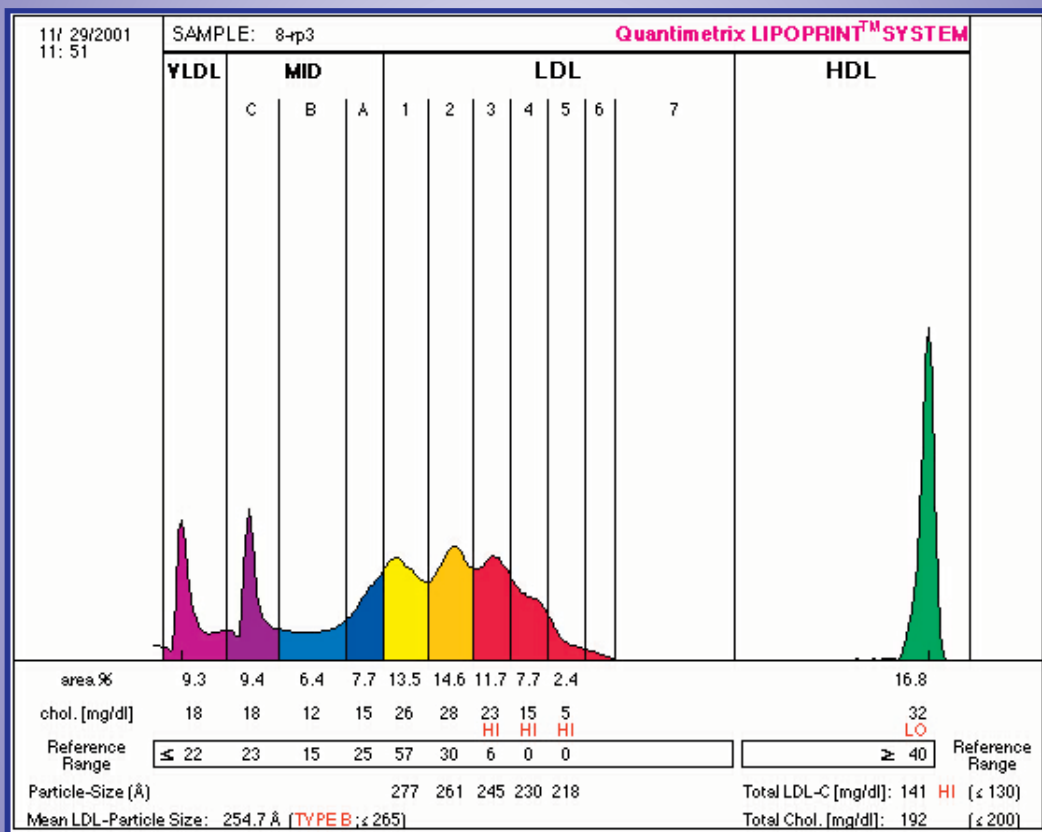


THE SOLUTION Cont.

Example 3

The following profile was obtained 1.5 months before a 50 year old apparently healthy (non smoker) Hispanic male, suffered a fatal myocardial infarction.

Fatal MI:



CONCLUSION

- Lipoprint has been extensively validated as an accurate, inexpensive and easy-to-use laboratory device for the visualization of lipoprotein fractions and subfractions, including the atherogenic IDL.
- Lipoprint determines the amount of cholesterol contained in each of these fractions and flags those results that exceed the normal reference range. These features, and the fact that the normal reference ranges for all fractions were derived from a population conforming to the latest NCEP guidelines (ATP III), set Lipoprint apart from all other lipoprotein subfraction methodologies.
- Lipoprint has all the makings of a "standardized and inexpensive methodology" for lipoprotein subfraction testing as called for in the ATP III report



REFERENCES

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