LIPOPRINT®
A Breakthrough in Cholesterol Management
• Heart Disease is the leading cause of death in the United States

• Each year 1.5 million Americans suffer from a heart attack

• 500,000 deaths each year

• $140 billion will be spent annually on operations and medical bills
Scientific studies have clearly demonstrated that lipids and lipoproteins are strongly associated with CAD along with other genetic and environmental factors.
NCEP ATP III guidelines for desirable lipid levels:

- Cholesterol < 200 mg/dl
- Triglycerides < 150 mg/dl
- LDL Cholesterol < 130 mg/dl
- HDL Cholesterol > 40 mg/dl
THE GOOD AND THE BAD CHOLESTEROL

- LDL cholesterol  
  \((\text{Bad})\)
- HDL cholesterol  
  \((\text{Good})\)

www.aeb.org/food/eggs-health.html
Traditional Risk Factors Don’t Tell the Whole Story

• The fact is that approximately 50% of CAD occurs among individuals with normal lipid levels

• Only 30% of all heart attacks can be explained on the basis of total cholesterol measurements alone

• Even with the most current ATP III guidelines most cases of CAD go unexplained
• Many individuals with normal LDL and HDL cholesterol levels are still at risk from cardiovascular disease

• These suggest that LDL and HDL measurement are not the best indicators for cardiovascular disease risk
Lipoproteins have been classified into five major classes based on their density

- Chylomicrons
- VLDL (very low density lipoprotein)
- IDL (intermediate density lipoprotein)
- LDL “bad” (low density lipoprotein)
- HDL “good” (high density lipoprotein)
Classification of Lipoproteins

Chylomicron and Chylomicron Remnant
1000 nm

VLDL
Very Low Density Lipoprotein
70 nm

IDL
Intermediate Density Lipoprotein
40 nm

LDL
Low Density Lipoprotein
20 nm

HDL
High Density Lipoprotein
10 nm

"Bad"
(Non-HDL)

"Good"
Lipoprotein Metabolism
# LIPOPROTEIN PROPERTIES

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>CHYLOMICRON</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDL</th>
<th>HDL</th>
<th>LP(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Density (g/mL)</strong></td>
<td>&lt;0.95</td>
<td>0.95 - 1.006</td>
<td>1.006 - 1.019</td>
<td>1.019 - 1.063</td>
<td>1.063 - 1.210</td>
<td>1.040 - 1.130</td>
</tr>
<tr>
<td><strong>Diameter (nm)</strong></td>
<td>&gt;70</td>
<td>25 - 70</td>
<td>22 - 24</td>
<td>19 - 23</td>
<td>4 - 10</td>
<td>25 - 30</td>
</tr>
<tr>
<td><strong>Lipid: protein</strong></td>
<td>99:1</td>
<td>90:10</td>
<td>85:15</td>
<td>80:20</td>
<td>50:50</td>
<td>75:25</td>
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<tr>
<td><strong>Major lipids</strong></td>
<td>trig</td>
<td>trig</td>
<td>trig, chol. Esters</td>
<td>chol. Esters</td>
<td>phospholipids</td>
<td>chol. Esters, phospholipids</td>
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<tr>
<td><strong>Major Proteins</strong></td>
<td>A-I, B-48, C-I, II, III</td>
<td>B-100, C-I, II, III</td>
<td>B-100 E</td>
<td>B-100</td>
<td>A-I, II, C-I, II, III</td>
<td>(a) E, B-100</td>
</tr>
</tbody>
</table>

LDL particle is the primary carrier of cholesterol throughout the body

Bioch.szote.u-szegedhu/astrojan/Prot/Ld.jpg
HDL particle is responsible for the removal of excess cholesterol from the body.
LIPOPROTEIN CLASSES ARE HETEROGENEOUS

These lipoprotein classes are heterogeneous consisting of multiple subclasses within each class.

[Classification of Lipoproteins diagram]

Chylomicron and Chylomicron remnant

VLDL Very Low Density Lipoprotein

IDL Intermediate Density Lipoprotein

LDL Low Density Lipoprotein

HDL High Density Lipoprotein

"Bad" (Non-HDL)

"Good"

1000 nm

70 nm

40 nm

20 nm

10 nm

Medscape® www.medscape.com
LIPOPROTEIN CLASSES AND SUBCLASSES

- Chylomicrons: continuum of particles
- VLDL: continuum of particles
- IDL: three subclasses
- DL Seven subclasses
- HDL Up to ten subclasses
“GOOD” AND “BAD” LDL

- Large LDL subclasses 1 and 2 are the “good or normal” LDL that are responsible for the transport of cholesterol

- Small LDL subclasses 3 through 7 are “bad or abnormal” and are easily oxidized and promote cardiovascular disease
Continuous Gradient Ultracentrifugation (Gold Standard)

- Distribution of various LDL particles by their densities
- Used primarily in basic lipoprotein research

Gradient Gel Electrophoresis (GGE - Legacy Method)

- LDL subfraction distribution and major LDL peak particle size
- Qualitative LDL subfraction analysis
Nuclear Magnetic Resonance (NMR)

- Distribution LDL size determination by mathematical deconvolution of NMR signal
- Measures LDL particle number

Vertical Analytical Profile (VAP) centrifugation

- LDL LDL distribution determined by deconvolution of the elution rate curve
- Qualitative phenotype classification of LDL sizes
A test that measures all the lipoprotein classes and subclasses would be superior to the current lipid profile.
THE LIPOPRINT SYSTEM IS THE ONLY FDA-CLEARED DIAGNOSTIC TOOL FOR LDL SUBFRACTION TESTING
Intended Use:

The Quantimetrix Lipoprint System LDL Subfractions is a device intended to measure lipoprotein cholesterol (for lipoprotein fractions and subfractions from VLDL to HDL) in fasting serum or plasma with a Total Cholesterol concentration of ≥100 mg/dl.
Indications For Use:

Lipoprotein subfraction cholesterol measurements are used as an aid in evaluating lipid metabolism disorders when used in conjunction with other lipid tests, patient risk assessment and clinical evaluation.
**Test Principles:**

The Lipoprotein System utilizes non-denaturing, linear (non-gradient) polyacrylamide gel electrophoresis (PAGE) to separate the lipoprotein fractions and subfractions in serum or plasma on the basis of size.

The test uses a lipophilic dye that binds to the cholesterol in the lipoprotein particle prior to electrophoresis.

The electrophoresed gels are scanned to determine the relative area for each lipoprotein subfraction. The subfraction cholesterol is calculated by multiplying the relative area of each subfraction by the total cholesterol of the sample.
LIPOPRINT LDL SYSTEM

System components:
- Chamber
- Prep Rack
- Prep Light
- Power Supply
- Digital Scanner
- iMac + software
- Printer
Lipoprint Kit:
- Gel Tubes
- Loading Gel
- Buffers
• Only fasting (12 hours) samples should be used
• Serum or EDTA plasma may be used
• Do not use heparin as anticoagulant
• Samples can be kept for up to 7 days at 2 - 8 C
The four steps of the Lipoprint test:

- Electrophoresis
- Scan
- Analysis
- Report
**Loading Sample**

- Place gel tubes in rack
- Apply 25 µl serum or plasma to gel tube
- Add 200 µl Loading Gel solution
Mixing Sample

- Place cover over gel tubes
- Mix sample with Loading Gel by inverting the gel tubes
Photo-Polymerization

- Place gel tube in front of Preparation Light
- Photopolymerize gel tubes for 30 minutes

Stain binds to lipoprotein particles
Loading Chamber

- Remove gel tubes from Preparation Rack
- Load gel tubes on the upper chamber
**TEST PROCEDURE…**

**Electrophoresis**

- Add Electrolyte buffer to Upper and lower Chambers

- Electrophores for 1 hour at 3 ma per gel tube
LIPOPROTEIN SUBFRACTIONS ARE RESOLVED

VLDL

MID bands

LDL bands

HDL

large LDL → small LDL
Scan Gel Tubes

- Load electrophoresed gel tubes on Scanner
- Scan the gel tubes
A digitized image of the electrophoresed gel tubes is generated.
Automatic analysis of patient’s results

- Enter patient’s name and total cholesterol
- Run analysis of the scanned tubes
- Generate patient’s profile
- Print patient’s profile
Up to 12 lipoproteins bands are resolved according to size (1 VLDL, 3 Mid Bands, 7 LDLs and 1 HDL)

VLDL migrates at the top of the separating gel Rf = 0 and HDL migrates at the front Rf = 1

Mid Bands and LDL subfractions migrate at specific rates between VLDL and HDL
An easy-to-interpret color graph of the patient’s lipoprotein profile is generated and printed.
Run Analysis Of The Scanned Tubes

- The Lipoware software analyzes the samples
- Calculates the area percent for each subfraction
- Calculates the amount of cholesterol in each subfraction
- Creates a spreadsheet with all the results
UNDERSTANDING THE PROFILE
The Lipoprint System produces a “color-coded” easy-to-interpret patient profile, featuring lipoprotein subfraction distribution, cholesterol levels in each fraction, and a comparison to the NORMAL reference range.
NORMAL TYPE A PROFILE

VLDL: Primárne lipoprotein bohatý na triglyceridy.

Mid Band C, B, A: Intermediate density lipoprotein (IDL)

LDL subfrakcie 1 a 2: Nájdite’né takmer vo všetkých ľudských sérich, veľké čiastočky, vzostupný LDL (Žltá)

HDL ten Dobrý Cholesterol (Zelená)

Celkový cholesterol: Meraný nezávisle

Fenotyp: Type A (žiadúci)
LIPOPRINT®

ABNORMAL TYPE B PROFILE

Cholesterol mg/dl: Quantifies the concentration of lipoprotein fractions and their subfractions

Subfractions 3 – 7: Small dense LDL, highly atherogenic (Red)

Phenotype: Not indicative of Type A (Predominantly small, dense LDL)

HDL: Direct measurement of total HDL (Green)

Reference Ranges: Flags all parameters that are above normal range – values based on ATP III

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Reference Ranges: Flags all parameters that are above normal range – values based on ATP III

Phenotype: Not indicative of Type A (Predominantly small, dense LDL)
Triglyceride Enriched Lipoproteins

- Chylomicron Remnants
- VLDL Remnants
- Intermediate Density Lipoproteins
TYPE III DYSLIPIDEMIAS (ELEVATED IDL)

LIPOPRINT®

Quantimetrix LIPOPRINT® SYSTEM

21 16 2022
V. 15

SAMPLE 1-pot

YLDL  MID  LDL  HDL

C  B  A  1  2  3  4  5  6  7

162 24 20 18 16 14 12 10 8 6

Reference Range

Particle Size (Å) 277.5 261.1
Mean LDL Particle Size 271.3 (TYPE A, >75%)
Total Cholesterol (mg/dL) 140 (±10)

Quantimetrix LIPOPRINT® SYSTEM

SAMPLE 2-pot

YLDL  MID  LDL  HDL

C  B  A  1  2  3  4  5  6  7

152 24 20 18 16 14 12 10 8 6

Reference Range

Particle Size (Å) 277.5 261.1
Mean LDL Particle Size 271.3 (TYPE A, >75%)
Total Cholesterol (mg/dL) 140 (±10)
Only FDA-cleared diagnostic test for separation and measurement of lipoprotein fractions and subfractionation

Measures cholesterol (mg/dL) in all 12 lipoprotein fraction and subfraction

- VLDL - associated with hypertriglyceridemia
- Mid A, B, and C (IDL and VLDL remnants) Increased CVD risk
- LDL 1 and 2 - associated with hypercholesterolemia
- LDL 3 through 7 - associated with 3X increase of CVD
- HDL - good cholesterol
- LDL - bad Cholesterol
• Normal reference ranges based on the National Cholesterol Education Program Adult Treatment Panel (NCEP ATP III)
• Cholesterol values outside the normal reference range are flagged in red for ease of interpretation
• Color coded profile for ease of interpretation by doctors and patients.
• Lipoprotein Profile Classification – predominance of large LDL is classified as Type A and predominance of small dense LDL is classified as Not Indicative of Type A
• Test is accurate, easy, quick turn around time and can be performed at any laboratory
• Test is reimbursable by most insurances (CPT Code 83701)
WHY MEASURE LDL SUBFRACTIONS?
• LDL subfraction testing conveys a level of CVD risk not possible with conventional lipid profiles
• This risk could be present even with normal lipid levels
Different profiles require different treatment

- Statins reduce the levels of total Cholesterol and the number of lipoprotein particle
- Niacin and fibrates change the particle distribution from small atherogenic to large less atherogenic
- Combined therapy
- The same is true for dietary therapy
<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Generic Name</th>
<th>Brand Name</th>
<th>Dose Range (mg/d)</th>
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<tbody>
<tr>
<td>Nicotinic acid (niacin)</td>
<td>Nicotinic acid</td>
<td>Niaspan</td>
<td>500-2,000</td>
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<tr>
<td>HMG-CoA reductase inhibitors (statins)</td>
<td>Atorvastatin</td>
<td>Lipitor</td>
<td>10-80</td>
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<td></td>
<td>Simvastatin</td>
<td>Zocor</td>
<td>5-80</td>
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<tr>
<td></td>
<td>Lovastatin</td>
<td>Mevacor</td>
<td>10-80</td>
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<tr>
<td></td>
<td>Pravastatin</td>
<td>Pravachol</td>
<td>10-80</td>
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<td></td>
<td>Fluvastatin</td>
<td>Lescol</td>
<td>20-80</td>
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<tr>
<td></td>
<td>Rosuvastatin</td>
<td>Crestor</td>
<td>5-40</td>
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<tr>
<td>Bile acid sequestrants</td>
<td>Cholestyramine</td>
<td>Questran</td>
<td>4-24 grams</td>
</tr>
<tr>
<td></td>
<td>Colesevelam</td>
<td>Questran Light</td>
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<tr>
<td></td>
<td>Colestipol</td>
<td>WelChol</td>
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<td></td>
<td></td>
<td>Colestid</td>
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<tr>
<td>Fibric acid derivatives</td>
<td>Gemfibrozil</td>
<td>Lopid</td>
<td>2-16 grams in divided doses</td>
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<tr>
<td></td>
<td>Fenofibrate</td>
<td>Tricor</td>
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<tr>
<td>Combination</td>
<td>Niacin (extended release)-lovastatin</td>
<td>Adviscor</td>
<td>500/20</td>
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<tr>
<td></td>
<td>Ezitimibe-simvastatin</td>
<td>Vytorin</td>
<td>10/10-10/80</td>
</tr>
<tr>
<td>Cholesterol absorption inhibitors</td>
<td>Ezitimibe</td>
<td>Zetia</td>
<td>10</td>
</tr>
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</table>

Help doctors and patients monitor the efficacy of treatment over time

Before treatment

After treatment
CASE STUDIES
GB, a 65-year-old Caucasian male had his Lipoprint profile done in January 2001 (Figure 1). 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 the vastly improved profile (Figure 2). The lipoprotein distribution was normal and only two cholesterol values were slightly outside the normal reference range.

![Figure 2. GB's after 3 Months of Intervention](image)
JS, a 42 year old Indian male had his Lipoprint profile done in June 2001 (Figure 3). The profile contained high levels of cholesterol in many fractions and a distribution of lipoprotein particles shifted toward the smaller and denser particles (red portion of the profile) indicating a lipid disorder.

Figure 3. JS's Baseline Lipoprint Profile
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 still somewhat elevated (Figure 4).
The following Lipoprint profile was obtained 1.5 months before the individual, a fifty-year-old, apparently healthy (non-smoker) Hispanic male, suffered a fatal myocardial infarction.

Figure 5. RP’s Baseline Profile, 6 Weeks before His Fatal Infarct
COMPETING TESTS
LIPOSCIENCE – NMR PROFILE

LIPOPRINT

NMR LipoProfile®

Lipoprint, Inc.
3001 New Bern Avenue
Raleigh, NC 27659
www.lipoprofile.com

Page 1 of 2

<table>
<thead>
<tr>
<th>Patient Name</th>
<th>Sex</th>
<th>Age</th>
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<tbody>
<tr>
<td></td>
<td>M</td>
<td>63</td>
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<tr>
<th>Data Collected</th>
<th>Data Received</th>
<th>Date Reported</th>
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<tr>
<td>8-24-99</td>
<td>8-25-99</td>
<td>8-26-99</td>
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<table>
<thead>
<tr>
<th>LIPOPROTEIN PANEL</th>
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<tbody>
<tr>
<td>LDL Particle Concentration</td>
</tr>
<tr>
<td>LDL Particle Size</td>
</tr>
<tr>
<td>Large HDL Concentration</td>
</tr>
<tr>
<td>Large VLDL Concentration</td>
</tr>
</tbody>
</table>

Coronary Heart Disease (CHD) Risk Categories:
- Optimal: less than 190 mg/dL
- Desirable: 190 - 199 mg/dL
- Borderline: 200 - 239 mg/dL
- High Risk: greater than 240 mg/dL

The risk categories for LDL particle concentration correspond to NCEP categories for LDL cholesterol (on a percentile equivalence basis).

<table>
<thead>
<tr>
<th>Subclass Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL Subclasses</td>
</tr>
<tr>
<td>LDL Subclasses</td>
</tr>
</tbody>
</table>

SUBCLASS LEVELS

Lipoprotein subclass levels (mg/dL) are given in parentheses above each bar. The height of the bar gives the percent of the population with a peak or lower levels.

NMR LIPO PANEL

Total Cholesterol (mg/dL)
- Optimal: less than 200
- Desirable: 200 - 239
- Borderline-High: 240 - 259
- High: greater than 240

LDL Cholesterol (mg/dL)
- Optimal: less than 100
- Desirable: 100 - 129
- Borderline: 130 - 159
- High Risk: greater than 160

HDL Cholesterol (mg/dL)
- Optimal: greater than 50
- Desirable: 40 - 50
- Borderline-High: 30 - 40
- High: less than 30

Triglycerides (mg/dL)
- Optimal: less than 150
- Desirable: 150 - 199
- Borderline-High: 200 - 249
- High: greater than 250

*Goal for secondary prevention (patients with established CHD or diabetes)

www.lipoprofile.com
More Information

Easier Interpretation
PHENOTYPED FROM TYPE A...

### Quantimetrix LIPOPRINT® SYSTEM

<table>
<thead>
<tr>
<th>Sample: 8-tube 8</th>
<th>YLDL</th>
<th>MID</th>
<th>LDL</th>
<th>HDL</th>
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<tbody>
<tr>
<td></td>
<td>C</td>
<td>B</td>
<td>A</td>
<td>1</td>
</tr>
</tbody>
</table>

| Area (%) | 6.8 | 6.9 | 6.7 | 7.4 | 23.4 | 12.4 | 0.8 | 35.5 |
| cholest. [mg/dl] | 13 | 13 | 13 | 14 | 46 | 24 | 1 | 69 |

**Reference Range**
- LDL-C [mg/dl]: 112 (≤ 130)
- Total Cholesterol [mg/dl]: 195 (≤ 200)

**Particle-Size (Å)**
- Mean LDL-Particle Size: 270.9 Å (TYPE A; ≥ 264.0)
- Total LDL-C [mg/dl]: 112 (≤ 130)
- Total Cholesterol [mg/dl]: 195 (≤ 200)
### TO TYPE B

**LIPOPRINT®**

**Sample:** 11-tube 11

<table>
<thead>
<tr>
<th>YLDL</th>
<th>MID</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>B</td>
<td>A</td>
<td>1</td>
</tr>
</tbody>
</table>

**Reference Range**

- **Chol. [mg/dL]:**
  - **Reference Range:** ≤ 22, 23, 15, 25, 57, 30, 6, 0, 0, 0, 0
  - **HI: 47**

- **Particle-Size (Å):**
  - **Mean LDL-Particle Size:** 234.8 Å (TYPE B; ≤ 265)
  - **Total LDL-C [mg/dL]:** 185 HI (≤ 130)
  - **Total Chol. [mg/dL]:** 260 HI (≤ 200)
COMING SOON!!!

LIPOPRINT HDL
Up to ten subfractions may be identified

- Large HDL (subfractions 1 to 3)
- Intermediate HDL (subfractions 4 to 7)
- Small HDL (subfractions 8 to 10)
Lipoprint HDL Subfractions

VLDL/LDL  --------> 
Large HDL  --------> 
Intermediate HDL --> 
Small HDL  --------> 
Albumin  --------->
Primarily Large HDL particles

HDL Subfractions

- Large
- Intermediate
- Small

Reference Range:

- HDL Chol. (mg/dl) ≤ 40
- Ratio (Small/Large) ≥ 0.4

*Reference ranges derived from 123 serum samples that met the NCEP ATP III guidelines for desirable lipid status.
Primarily Intermediate HDL particles
Primarily Small HDL particle

**Graph:**
- HDL Subfractions:
  - Large: 17%
  - Intermediate: 57%
  - Small: 26%
- Cholesterol (mg/dL):
  - Reference Range: LO 210, HI 222
  - Ratio (Small/Large): HI > 0.3

*Reference ranges derived from 123 serum samples that met the NCEP ATP III guidelines for desirable lipid status.*
Other Applications

Animal Research
(Normal □ diet vs. high fat diet)
Shouldn’t you use Lipoprint?

<table>
<thead>
<tr>
<th>Sample: 11-tube 11</th>
<th>Quantimetrix LIPOPRINT™ SYSTEM</th>
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<tbody>
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<tr>
<td>1</td>
<td>2</td>
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<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
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<td>7</td>
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<tr>
<th>area.%</th>
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<th>10.4</th>
<th>4.0</th>
<th>4.0</th>
<th>9.0</th>
<th>9.0</th>
<th>5.0</th>
<th>7.6</th>
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<th>9.0</th>
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<td>chol.[mg/dl]</td>
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<td>27</td>
<td>12</td>
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<td>23</td>
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<td>15</td>
<td>18</td>
<td>26</td>
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<td>Reference Range</td>
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<td>15</td>
<td>25</td>
<td>27</td>
<td>30</td>
<td>30</td>
<td>6</td>
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<td>Particle-Size (Å)</td>
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<td>Mean LDL-Particle Size</td>
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<tr>
<td>Total LDL-C [mg/dl]</td>
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<td>HI</td>
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<tr>
<td>Total Chol. [mg/dl]</td>
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The End