Evaluation of Lipid, Lipoprotein, and Apolipoprotein Analysis by Nuclear Magnetic Resonance

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Abstract No. 184
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INTRODUCTION-1

Serum lipoproteins play a causative role in atherosclerotic cardiovascular disease (ASCVD).

Lipoproteins vary in size and density and in apolipoprotein, cholesterol (free and esterified), triglyceride (TG, and phospholipid content (FIGURES 1A and 1B). The analysis of the protein and lipid content of the different lipoprotein fractions and the particle number can point to the degree of ASCVD risk and guide risk management.

Standard clinical assays that employ enzymatic or immuno-turbidimetric methods to measure serum lipid and apolipoprotein concentrations are rapid and sensitive. They require, however, the use of multiple reagents and sometimes different analyzers and are not practical for analyzing the many components within lipoprotein particles.

High resolution nuclear magnetic resonance (NMR) is an ideal alternative analytical method. It can simultaneously assess the size, density, composition, and number of VLDL, IDL, LDL, and HDL particles and their subfractions.

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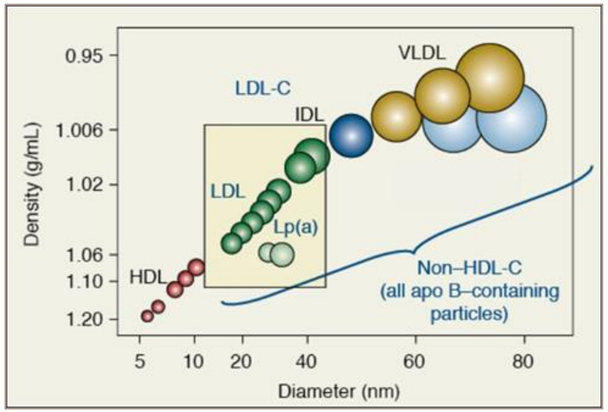

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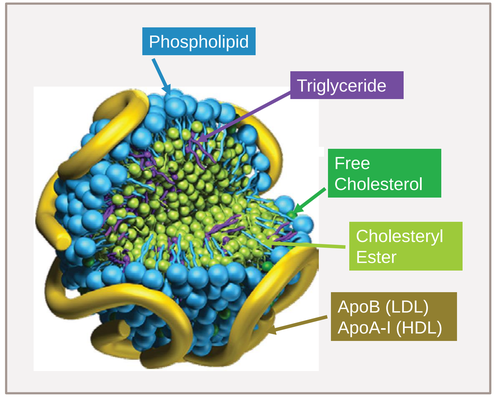

FIGURE 1. Lipoprotein particles. Panel A, Particles in order of size and density. Panel B, Representative particle showing major apolipoprotein and lipid components.

Click here for a larger view of FIGURE 1

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INTRODUCTION-2 NMR Analysis and ASCVD Risk Prediction

- **Patients with ASCVD often have elevated LDL particle number (LDL-P) and decreased HDL particle number (HDL-P).**
  - Women's Health Study (Blake GJ, Otvos JD, Rifai N, Ridker PM. *Circulation* 2002; 106:1930-1937)
  - Veteran Affairs High Density Lipoprotein Intervention Trial (Otvos JD, Collins D, Freedman DS et al. *Circulation* 2006; 113:1556-1563)
  - Framingham Offspring Study (Cromwell WC, Otvos JD, Keyes MG, et al. *J Clin Lipidol* 2007; 1:583-592)

- **LDL-P and HDL-P have been shown to be superior to LDL-C, HDL-C, and cholesterol efflux measurements in ASCVD risk assessment.**
  - Women's Health Study (Mora S., Otvos JD, Rifai N, et al. *Circulation* 2009; 119:931-939).
  - Multi-Ethnic Study of Atherosclerosis (Mackey RH, Grenland P, Goff DC Jr, et al. *J Am Coll Cardiol* 2012; 60:508-516).
  - JUPITER Trial (Khera AV, Demier OV, Adelman SJ, et al. *Circulation* 2017; 135:2494-2504).

- **LDL-P and HDL_ can be favorably altered by lifestyle modification and cholesterol-lowering medication.**
  - Women's Health Study (Mora S, Otvos JD, Rifai N, et a. *Circulation* 2009; 119:931-939)

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OBJECTIVES

- **Validate the use of specialized NMR software for the measurement of serum total cholesterol, triglycerides, LDL-C, HDL-C, apoB, and apoA-I concentrations.**
- **Analyze the particle number and lipid and apolipoprotein content of all lipoprotein fractions except lipoprotein(a), comparing women with men.**

METHODS

- **Specimens**
  - Fasting serum from 1,458 men and women (median age 58.2 yr) who were recipients of Boston Heart testing as ordered by their healthcare providers in the United States. Subjects had to be fasting ≥8 hr.
  - Serum separated from whole blood collected in Greiner BioOne VACUETTE® Z-serum separator clot activator tubes (Greiner cat. no. 455071P) or equivalent.
- **NMR Analysis**
  - 400 µL serum diluted 1:1 with Bruker NMR buffer solution. Assay volume = 600 µL diluted serum.
  - 600 MHz NMR and Bruker B.I.LISA™ analytical software (Bruker, Rheinstetten, Germany).
  - Performed New York State testing criteria for laboratory developed assays: accuracy, precision, linearity, analytical sensitivity, analytical specificity, analyte stability, reportable range, reference range.
- **Comparator Analysis**
  - Total cholesterol, TG, small dense LDL-C (sdLDL-C), and HDL-C by standardized enzymatic methods (Roche, Indianapolis, IN).
  - ApoB and apoA-I by immunoturbidimetric assays (Roche, Indianapolis, IN).
  - LDL-P and HDL-P by 600 MHz NMR and Numares analytical software (Numares, Regensberg, Germany).

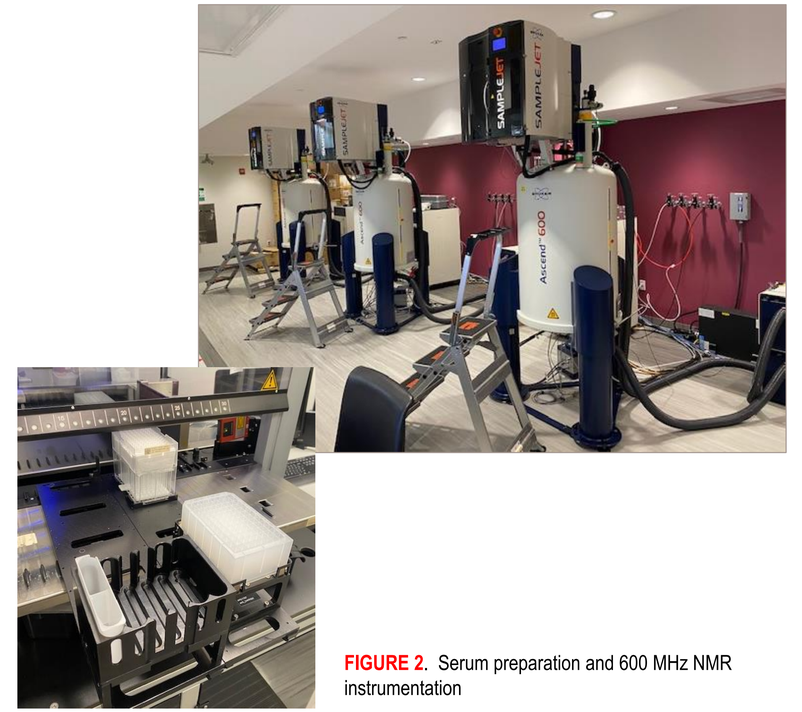

FIGURE 2. Serum preparation and 600 MHz NMR instrumentation

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RESULTS-1

- The NMR-derived concentrations of total cholesterol, TG, directed LDL-C, HDL-C, apoB, and apoA-I were highly correlated (R>0.95; P<0.00001) with standardized enzymatic and immunoturbidimetric values (FIGURE 2).
- LDL-P values derived by Bruker software analysis were highly correlated (R=0.920; P<0.0001) with LDL-P values derived by Numares software analysis (FIGURE 3).
- Female subjects had significantly higher serum total cholesterol, apoB, apoA-I and apoA-II concentrations (P<0.01) but lower serum TG concentrations (P=0.016) than male subjects (TABLE 1).
- Females had lower VLDL-C, VLDL-TG, and VLDL-apoB (P<0.00001) but higher LDL-C, LDL-FC, LDL-TG, LDL-apoB, and LDL-C (P<0.01), corresponding to VLDL and LDL particle number (TABLE 2).
- Compared to male subjects, female subjects had significantly lower VLDL-P and LDL3-P (P<0.00001) but markedly higher large buoyant LDL1-P and LDL2-P and total LDL-P (FIGURE 4).
- Small dense LDL6-P was modestly higher in females than in males (P=0.046), but in females there was a trend towards lower LDL6-C concentration (P=0.071).

RESULTS-2 Figure 2 NMR Analysis Correlated with Standardized Assays (*R*>0.95)

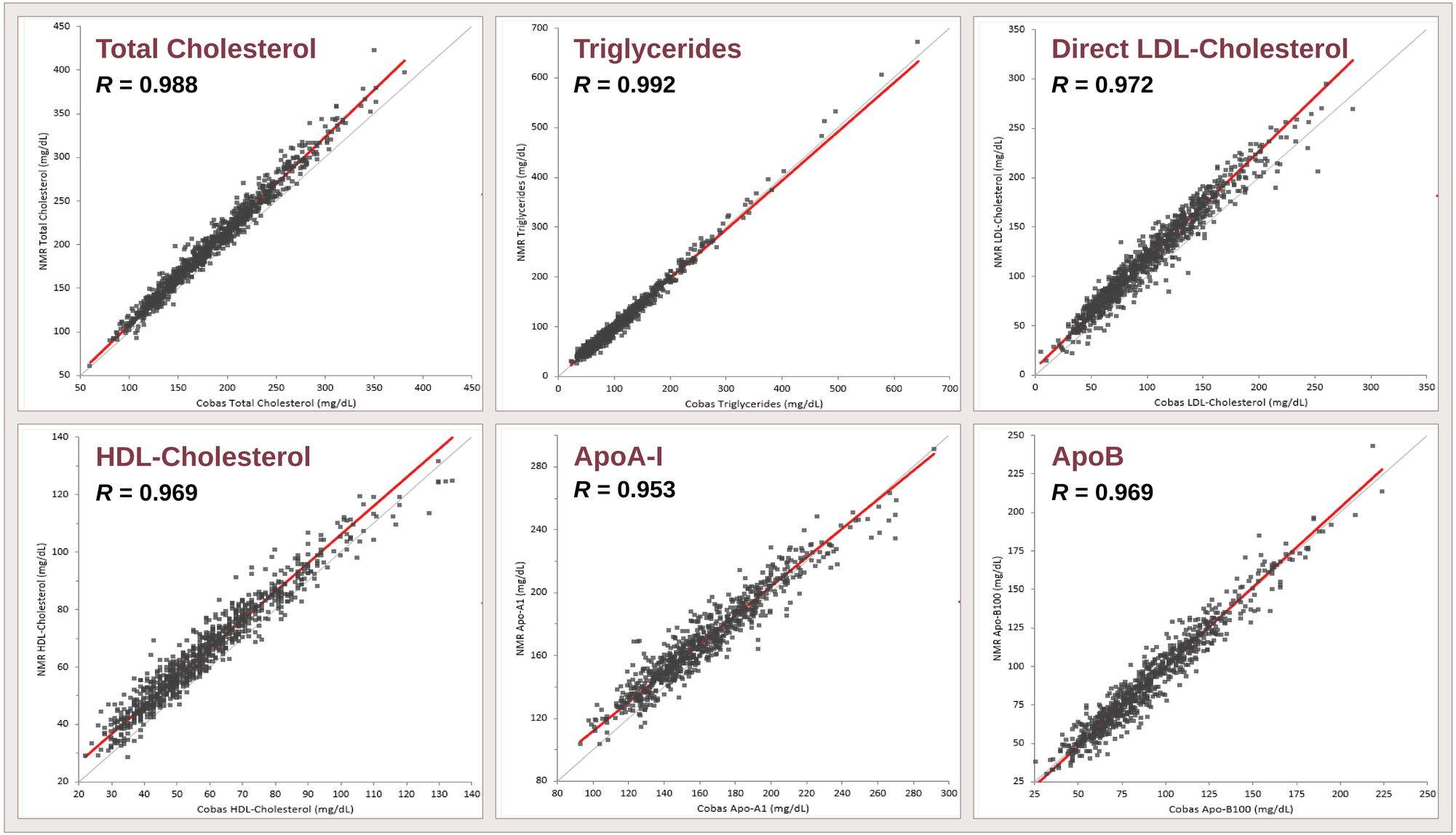


FIGURE 2. Validation of NMR-derived serum lipid and apolipoprotein concentrations.

Values derived by 600 MHz NMR and Bruker B.I.LISA™ software correlated (Spearman *R*) highly (all *P* <0.00001) with concentrations obtained by standardized automated enzymatic and immunoturbidimetric assays. Corrections were applied to total cholesterol, LDL-C, HDL-C, and apoA-I to make absolute values consistent with standard chemically-derived values and national guidelines.

RESULTS-3 Figure 3 Bruker-derived LDL-P correlated with Numares-derived LDL-P

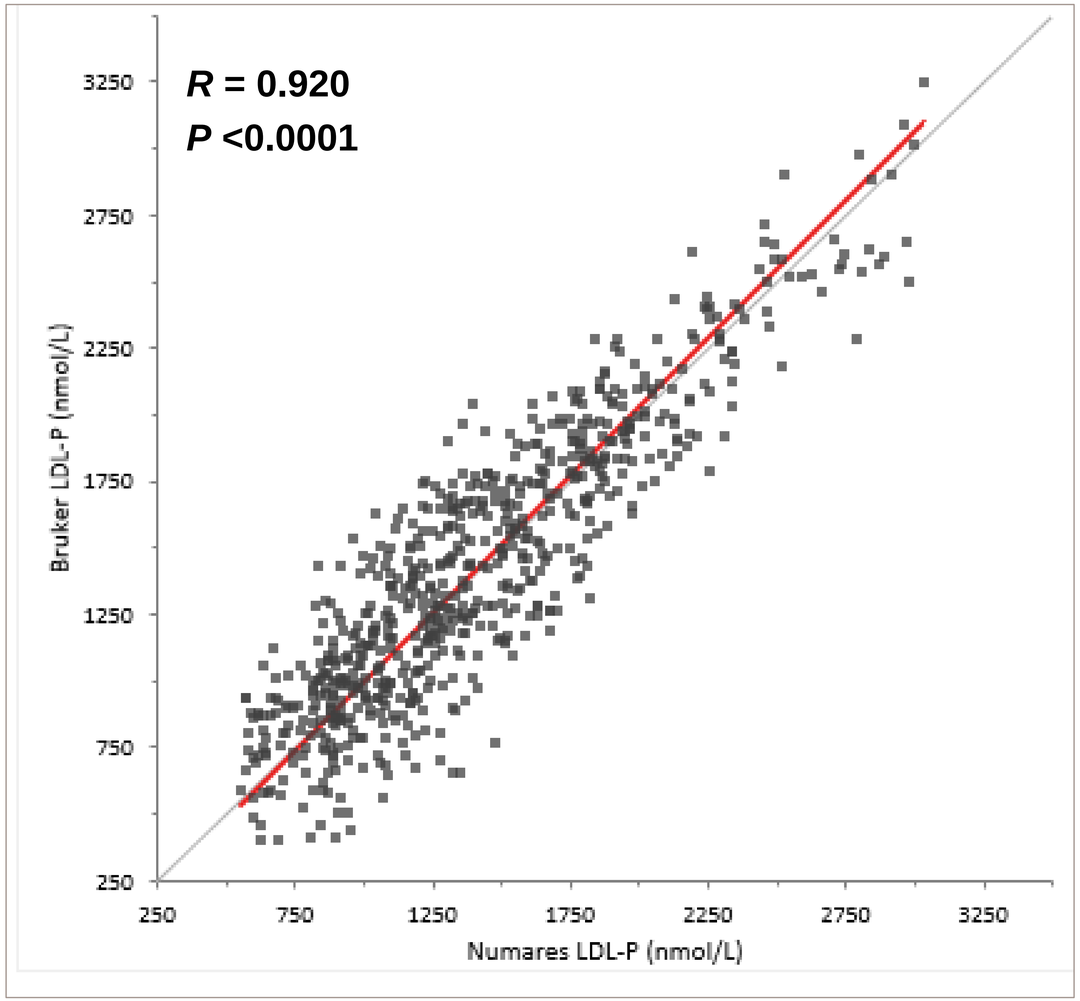


FIGURE 4. Validation of LDL particle number analysis.
LDL-P values derived by 600 MHz NMR and Bruker B.I.LISA™ software were highly correlated (Spearman *R*) with LDL-P values derived with Numares software.

RESULTS-4 Table 1 Serum Lipids and Apolipoproteins in Males and Females

TABLE 1. NMR-derived concentrations of lipids and apolipoproteins in fasting serum

	All Subjects (n=1076)	**Males** (n=504; 46.8%)	**Females** (n=572; 53.2%)	**% Difference***	***P* Value†**
Total cholesterol	182.18 (74.09)	167.04 (76.3)	192.01 (72.22)	+14.95	3.14×10^{-13}
Triglycerides	85.28 (56.63)	89.39 (67.51)	83.14 (51.24)	–6.99	0.016
ApoB	87.92 (43.67)	86.04 (45.90)	90.68 (43.46)	+5.39	0.0055
ApoA-I	158.78 (37.16)	146.47 (30.03)	169.71 (34.48)	+15.87	1.13×10^{-45}
ApoA-II	34.07 (7.73)	32.29 (7.40)	35.50 7.13)	+9.94	2.81×10^{-22}

Data are presented as median (IQR).

*Median percent difference, female subjects vs male subjects.

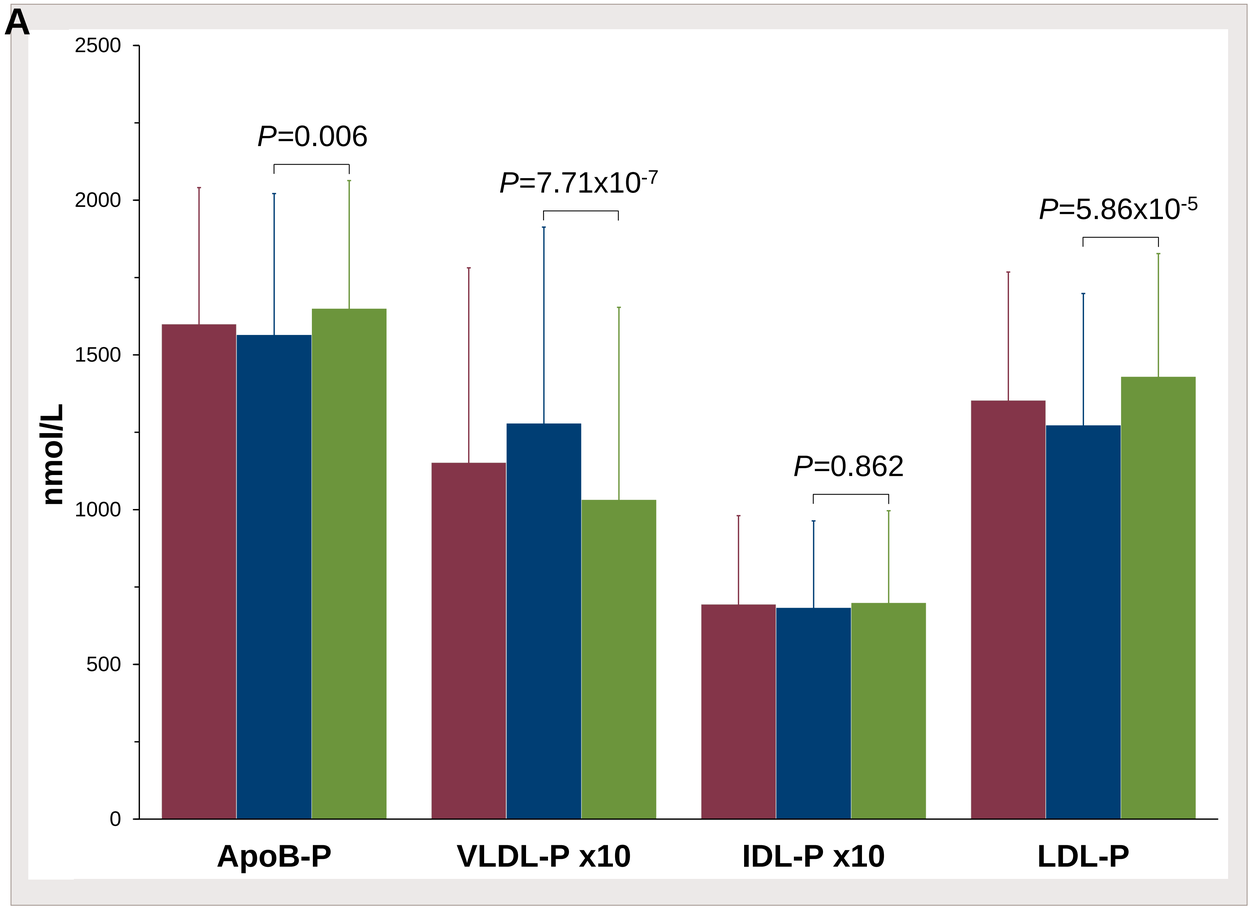
†*P* value for females subjects vs male subjects, as determined by the Kruskal-Wallis test.

Results-5 Table 2 Sex Differences in Lipoprotein Particle Lipids and Apolipoprotein Values

Parameter	All Subjects (n=1076)	Males (n=504; 46.8%)	Females (n=572; 53.2%)	% Difference	*P* Value*
VLDL-C	8.79 (13.07)	11.15 (15.44)	7.38 (12.77)	–33.81	8.55×10^{-8}
VLDL-TG	55.86 (49.18)	62.12 (59.47)	49.95 (42.83)	–19.59	4.73×10^{-6}
VLDL-apoB	6.33 (5.82)	7.03 (6.11)	5.67 (5.61)	–19.34	7.75×10^{-7}
IDL-C	7.25 (8.02)	7.44 (7.73)	7.05 (8.00)	–5.24	0.260
IDL-TG	5.28 (7.95)	5.36 (9.07)	5.25 (6.87)	–2.05	0.424
IDL-apoB	3.81 (2.09)	3.75 (2.64)	3.84 (2.99)	+2.44	0.866
LDL-C	98.80 (32.05)	92.81 (58.56)	101.06 (60.54)	+8.89	2.12×10^{-3}
LDL-FC	–	33.99 (17.11)	37.20 (18.48)	+9.44	8.40×10^{-6}
LDL6-C†	20.83 (12.33)	23.72 (15.09)	25.13 (13.75)	+5.94	0.071
LDL-TG	22.80 (8.35)	22.14 (8.02)	23.79 (8.21)	+7.45	2.74×10^{-5}
LDL-apoB	74.38 (42.91)	69.98 (42.30)	78.59 (43.01)	+12.30	5.84×10^{-5}
HDL-C	55.80 (21.71)	49.06 (17.94)	62.03 (20.69)	+26.44	1.93×10^{-42}

Data are presented as median (IQR). Median percent difference, female subjects vs. male subjects.
**P* value for comparison of female subjects vs. male subjects, as determined by Kruskal-Wallis test.
†Equivalent to sdLDL-C (*d* 1.044-1.063 g/mL).

RESULTS-6 Figure 4 Sex Differences in Lipoprotein Particle Number



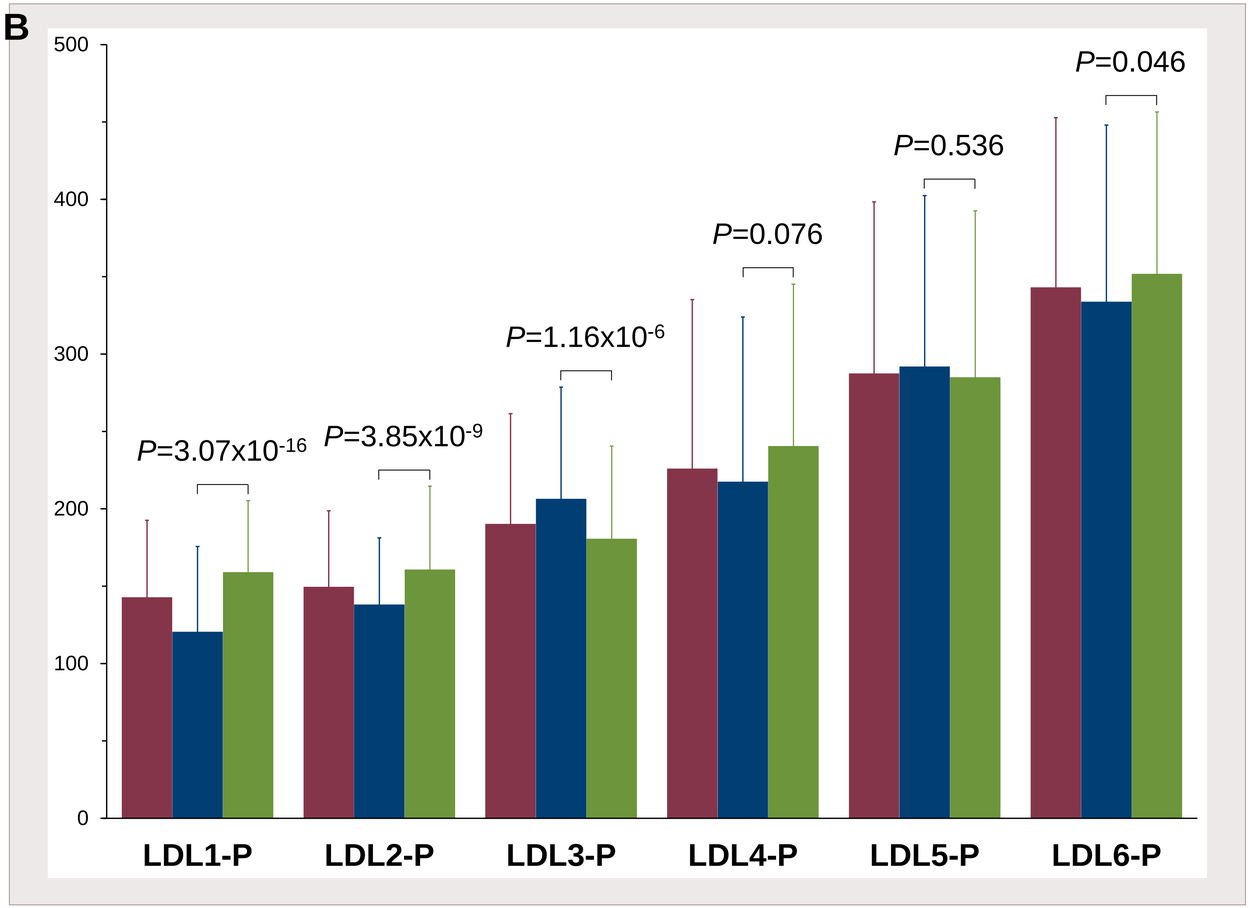


FIGURE 4. Lipoprotein particle number derived by Bruker B.I.LISA™ software. Panel A, ApoB-containing lipoprotein particles in serum. Panel B, LDL subfractions in serum. Data are expressed as median (25^{th}-75^{th} percentile). *P* for comparison of female subjects vs. male subjects, as determined by Kruskal-Wallis test. Red bars represent all subjects; blue bars, male subjects, green bars, female subjects.

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CONCLUSIONS

- Our data demonstrate that NMR lipoprotein analysis can provide, in a single serum test, a quantitative analysis of the number and the lipid apolipoprotein content of all lipoprotein fractions except lipoprotein(a).
- With 600 MHz NMR technology and Bruker analytical software, we can assess, for clinical and diagnostic purposes, 27 measured and 6 calculated lipoprotein parameters that meet the criteria for laboratory developed tests.
- For research purposes, with the Bruker software, we can measure lipoprotein particle number and the concentration of cholesterol, free cholesterol, triglycerides, phospholipids, apoB, apoA-I, and apoA-II in serum and in 5 VLDL, 6 LDL, and in HDL fractions.
- NMR methodology holds great promise for ASCVD risk assessment, the detection of lipoprotein disorders, and clinical decision-making.

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