**LP4 *NMR MetaboProfile*TM Analysis**

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**Background.** Since its introduction 20 years ago, *NMR LipoProfile* lipoprotein particle testing has employed an identical analytic procedure for obtaining 400 MHz proton NMR spectra of patient serum or plasma on the NMR Profiler1 and Vantera Clinical Analyzer2 platforms. However, there has been incremental optimization of the “back-end” computational process by which lipoprotein subclass concentrations are derived by deconvolution of the lipid methyl signal envelope contained within these spectra. In short, the measured lineshape of the plasma methyl signal envelope “whole” is modeled as the sum of its lipoprotein subspecies signal “parts” using non-negative linear least squares deconvolution. This calculation gives the signal area (amplitude) contributed by each lipoprotein subspecies, which after multiplication by their respective signal to particle conversion factors gives the subspecies particle concentrations. These have traditionally been grouped for simplicity into “large”, “medium, and “small” subclass categories of VLDL, LDL, and HDL.1 Optimization of the deconvolution algorithm over time, represented by versions LP1, LP2, and LP3, has primarily involved expanding the number of subspecies in the deconvolution model to better account for the wide compositional diversity of human lipoproteins.

***NMR MetaboProfile* Analysis.** New software has been developed for analysis of current or previously-measured and stored *NMR LipoProfile* spectra that uses a further-optimized deconvolution algorithm (LP4) to quantify lipoprotein subspecies, while simultaneously measuring a novel NMR inflammation biomarker (GlycA)3-7 plus several clinically-relevant metabolites including branched-chain amino acids (BCAA). The current software version reports 48 parameters, including the LP-IR insulin resistance index and a new diabetes risk multimarker (DRI) derived from a combination of LP-IR and BCAA. One impetus for developing the LP4 deconvolution algorithm was to enable more detailed epidemiologic investigation of disease relationships of different HDL particle subspecies, prompted by emerging evidence for the functional and proteomic diversity of different-size HDL particles. NMR has inherently high resolving power to discriminate HDL particles differing only slightly in size, but previous algorithms did not fully exploit this capability. The LP4 algorithm now measures 7 different HDL subspecies with improved precision, and corrects a prior systematic overestimation of the absolute concentrations of HDL particles (responsible for apoA-1/HDL-P ratios that were improbably low).8 New HDL subclass signal to particle conversion factors were determined by regression of the NMR subclass signal areas against plasma apoA-1 concentrations, producing “calibrated” HDL particle concentrations (cHDLP) that are ~30% lower than previous HDL-P values. Also corrected was an aspect of prior deconvolution models that led to systematic underestimation of LDL particle concentrations (owing to imperfect modeling of the plasma protein background signal). As a result, calibrated LP4 LDL particle concentrations (cLDLP) are now higher by about 350 nmol/L while remaining highly correlated (r~0.95) with previous LDL-P values and retaining equivalently strong associations with cardiovascular outcomes. Finally, linear regressions of subclass signal areas against independent chemical measures of cholesterol, triglycerides, and apolipoproteins from a large population sample have produced conversion factors enabling the reporting of NMR-derived lipid and apolipoprotein concentrations. A full description of the LP4 deconvolution process and its performance characteristics is not yet published. In the interim, the information below is intended to facilitate research use of LP4 *NMR MetaboProfile* data.

**Parameters and Nomenclature.** The 48 reported parameters are listed in Table 1 along with their reference ranges.

**TRLP (Triglyceride-rich lipoprotein particles)** - Large particle subclasses formerly named VLDL or chylomicrons are now called TRLP subclasses to explicitly acknowledge that NMR distinguishes particles only on the basis of size, not protein composition (apoB48/apoB-100) or metabolic origin (intestine, liver, remnant). The former IDL-P subclass, comprising particles intermediate in size between LDL and VLDL (24-29 nm), is now named “very small TRLP” since its lipid composition has more in common with triglyceride-rich particles than cholesterol-rich LDL particles.

**cLDLP** - Three LDL subclasses (large, medium, small) are now reported instead of two (large, small). Total LDL particle concentration formerly included IDL-P, but is now calculated as the sum of the concentrations of the three LDL subclasses. Total concentrations of NMR-measured apoB-containing particles, given by the sum of the 5 TRLP and 3 cLDLP subclasses, now closely match those measured by apoB immunoassay (after converting mg/dL mass concentrations to nmol/L assuming a 512 kDa apoB molecular weight.)

**cHDLP** – The earlier LP3 algorithm accounted for the size diversity of HDL particles by including 26 HDL subpopulations in the deconvolution model, but these were summed for reporting purposes into only 3 subclasses (large, medium, small) to give acceptable precision.9  The LP4 deconvolution model includes 7 HDL subspecies and the concentrations of each are reported. The nomenclature assigns higher numbers (H1P, H2P, …H7P) to subspecies of greater particle size. Optimal clinical and research use of the 7 HDL subspecies is anticipated to result from combining (summing) particular subsets to improve measurement precision and strengthen disease associations. Since HDL particles perform diverse functions related to many disease states, the most advantageous grouping of these subspecies will likely differ according to the intended clinical or research application as would need to be informed by epidemiologic investigation.10 To facilitate comparison with the previous large, medium, and small LP3 subclass categories (which may not be clinically optimal), the reported corresponding grouping of LP4 subspecies is (H5P+H6P+H7P), (H3P+H4P), and (H1P+H2P), respectively.

**Mean Particle Sizes** – These mass-weighted mean particle diameters are analogous to previously reported sizes. Note that TRL size, as before, excludes particles larger than 100 nm from the calculation due to their extremely low concentration and outsize contribution to measurement imprecision.

**Derived Lipid and Apolipoprotein Concentrations** – Linear regression of the NMR subclass signal areas against serum lipid and apolipoprotein levels measured chemically in a large study population provided the coefficients (conversion factors) to generate NMR-derived concentrations of total cholesterol and triglycerides, TRL triglyceride, the cholesterol in TRL, LDL, and HDL subclass fractions, and apoB and apoA-1. NMR-derived concentrations of all of these parameters are highly correlated (r ~ 0.95) with those measured by standard methods.

**GlycA** – The GlycA NMR signal, reflecting the aggregated levels of several glycosylated acute phase proteins, is a useful new marker of systemic inflammation and appears to be a significant modulator of HDL subclass function(s) as related to mortality, CVD, and metabolic disease.3-7,10

**Branched-Chain Amino Acids (BCAA)** – The 3 branched-chain amino acids (valine, leucine, isoleucine) give rise to characteristic NMR signals that, via deconvolution, enable their accurate quantification as validated by comparison with LC/MS/MS values.11 Since levels of valine, leucine, and isoleucine are highly correlated, their sum (“BCAA”) is also reported for ease of epidemiologic investigation. Recent studies have documented associations of NMR-measured BCAA with diabetes and CVD outcomes.11,12

**Alanine + Glycine** – Alanine and glycine are also quantified accurately by NMR. Glycine is a particularly interesting metabolomic biomarker implicated in several aspects of diabetic pathophysiology.13

**Ketone Bodies** – The 3 ketone bodies (β-hydroxybutyrate, acetoacetate, acetone) give rise to resolved NMR signals that serve as the basis of their quantification. Since levels of the 3 ketone bodies are highly correlated, their reported sum (“KetBod”) may have advantages for epidemiologic analyses.

**Serum Protein** – The broad NMR signal from bulk serum proteins (reported in arbitrary units of signal area) offers an alternative to total serum protein or serum albumin as an inflammation or malnutrition marker.

**Small Molecule Metabolites** – NMR-derived concentrations of glucose and citrate are reported.

**NMR Multimarkers** – Multiple parameters obtained from a single *NMR MetaboProfile* spectrum have independent associations with various disease outcomes, and thus contribute additively to multivariable prediction models. The most efficient clinical use of such information is to combine the parameters related to a particular disease process or outcome into a single “multimarker” index or numerical score. The first such multimarker derived from *NMR LipoProfile* testing was LP-IR (Lipoprotein Insulin Resistance Index).14 A new multimarker of diabetes risk is now included called DRI (Diabetes Risk Index). Additional risk multimarkers for diverse clinical outcomes that include CVD, mortality, cancer, dementia, and liver and kidney disease are under development.

***LP-IR (Lipoprotein Insulin Resistance Index)* –** This is the LP4 version of the original LP-IR (0-100 score) that combines 6 TRL, LDL, and HDL subclass and size parameters associated with insulin resistance.14-17 The new LP-IR score is virtually identical to the former LP3 version (correlation 0.96) and is equally strongly associated with insulin resistance measured by HOMA-IR, frequently-sampled intravenous glucose tolerance testing, and euglycemic clamp.

***DRI (Diabetes Risk Index)* –** This multimarker (1-100 score) combines LP-IR and BCAA levels to function as a simple and effective indicator of risk of future diabetes, independent of glycemic status.

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**Table 1. LP4 *NMR MetaboProfile* Parameters and Reference Range Values\***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Particle**  **Subclass** | **Description** | **Diameter**  **Estimate**  **(nm)** | **Reference Range Values**  **Range = 5th – 95th percentile**  **Mean (SD) Median Range** | | |
| **TG-Rich Lipoprotein Particle (TRLP) Concentrations (nmol/L)** | | | |  |  |  |
| TRLP |  | Total TRLP | 24- 240 | 125.2 (61.6) | 116 | 42 - 239 |
|  | VL-TRLP | Very Large TRLP | 90-240 | 0.4 (1.0) | 0.1 | 0 – 1.6 |
|  | L-TRLP | Large TRLP | 50-89 | 2.9 (6.5) | 0.5 | 0 – 12.8 |
|  | M-TRLP | Medium TRLP | 37-49 | 17.9 (16.2) | 14.2 | 0.3 – 48.4 |
|  | S-TRLP | Small TRLP | 30-36 | 56.5 (37.5) | 50.3 | 7.3 – 124.4 |
|  | VS-TRLP | Very Small TRLP | 24-29 | 47.5 (46.9) | 34.8 | 0 – 142.3 |
| **LDL Particle (LDLP) Concentrations (nmol/L)** | | | |  |  |  |
| cLDLP |  | Total cLDLP | 19-23 | 1454 (393) | 1402 | 891 - 2150 |
|  | L-cLDLP | Large LDLP | 21.5-23 | 309 (223) | 266 | 17 - 748 |
|  | M-cLDLP | Medium LDLP | 20.5-21.4 | 676 (405) | 656 | 0 - 1377 |
|  | S-cLDLP | Small LDLP | 19-20.4 | 469 (431) | 361 | 13 - 1318 |
| **Calibrated HDL Particle (cHDLP) Concentrations (μmol/L)** | | | |  |  |  |
| cHDLP |  | Total cHDLP | 7.5-13 | 24.0 (3.0) | 23.7 | 19.2 – 29.3 |
|  | L-cHDLP | Large HDLP (H5+H6+H7) |  | 2.5 (1.9) | 2.0 | 0.2 – 6.3 |
|  | M-cHDLP | Medium HDLP (H3+H4) |  | 7.7 (2.7) | 7.5 | 3.7 – 12.6 |
|  | S-cHDLP | Small HDLP (H1+H2) |  | 13.8 (3.4) | 13.8 | 8.1 – 19.6 |
|  | H7P | HDLP subspecies (12 nm) | 12.0 | 0.29 (0.41) | 0.15 | 0 – 1.09 |
|  | H6P | HDLP subspecies (10.8 nm) | 10.8 | 0.99 (1.22) | 0.53 | 0 – 3.64 |
|  | H5P | HDLP subspecies (10.3 nm) | 10.3 | 1.16 (1.02) | 0.91 | 0 – 3.02 |
|  | H4P | HDLP subspecies (9.5 nm) | 9.5 | 2.42 (1.31) | 2.24 | 0.70 – 4.59 |
|  | H3P | HDLP subspecies (8.7 nm) | 8.7 | 5.29 (2.27) | 5.16 | 1.58 – 9.23 |
|  | H2P | HDLP subspecies (7.8 nm) | 7.8 | 11.34 (2.79) | 11.30 | 6.8 – 16.0 |
|  | H1P | HDLP subspecies (7.4 nm) | 7.4 | 2.45 (1.89) | 2.23 | 0 – 6.0 |
| **Mean Particle Sizes (nm)** | | | |  |  |  |
| TRLZ | --- | TRL Size | 30-100 | 44.0 (8.4) | 42.3 | 33.8 – 60.9 |
| LDLZ | --- | LDL Size | 19-22.5 | 21.0 (0.5) | 21.1 | 20.1 – 21.7 |
| HDLZ | --- | HDL Size | 7.4-13 | 8.98 (0.44) | 8.9 | 8.3 – 9.8 |
| **Derived Lipid and Apolipoprotein Concentrations (mg/dL)** | | | |  |  |  |
| TG |  | Total Triglycerides |  | 119.3 (89.8) | 96 | 43 - 276 |
| TC |  | Total Cholesterol |  | 193.8 (36.5) | 191 | 140 - 256 |
| TRLTG |  | TRL Triglycerides |  | 99.1 (88.7) | 75 | 25 - 238 |
| TRLC |  | TRL Cholesterol |  | 24.7 (12.3) | 23 | 8 - 48 |
| LDLC |  | LDL Cholesterol |  | 110.5 (30.7) | 108 | 63 - 163 |
| HDLC |  | HDL Cholesterol |  | 61.1 (14.4) | 59 | 41 - 88 |
| ApoB |  | Apolipoprotein B |  | 87.1 (23.6) | 84 | 53 - 127 |
| ApoA-1 |  | Apolipoprotein A-1 |  | 156.8 (27.8) | 154 | 116 - 209 |
| **Inflammation Biomarker** | | |  |  |  |  |
| GlycA |  | GlycA (μmol/L) |  | 402.4 (65.8) | 393 | 307 - 524 |
| **Branched-Chain Amino Acids (μmol/L)** | | | |  |  |  |
| BCAA |  | Total BCAA |  | 456.8 (112.5) | 438 | 309 - 658 |
| Val |  | Valine |  | 238.9 (53.3) | 234 | 166 - 332 |
| Leu |  | Leucine |  | 167.2 (43.4) | 162 | 110 - 239 |
| Ileu |  | Isoleucine |  | 50.5 (24.4) | 46 | 19 - 96 |
| **Alanine + Glycine (μmol/L)** | | | |  |  |  |
| Ala |  | Alanine |  | 454.2 (106.3) | 443 | 305 - 639 |
| Glycine |  | Glycine |  | 274.9 (82.8) | 261 | 164 - 424 |
| **Ketone Bodies (μmol/L)** | | | |  |  |  |
| KetBod |  | Total Ketone Bodies |  | 199.3 (125.3) | 165 | 87 - 422 |
| Β-HB |  | β-hydroxybutyrate |  | 136.7 (89.8) | 114 | 54 - 296 |
| AcAc |  | Acetoacetate |  | 31.5 (23.7) | 26 | 8 - 69 |
| Acetone |  | Acetone |  | 31.1 (20.7) | 27 | 9 - 69 |
| **Serum Protein (arbitrary units)** | | |  |  |  |  |
| Prot |  | Serum Protein |  | 531.9 (33.8) | 532 | 476 - 587 |
| **Small Molecule Metabolites** | | | |  |  |  |
| Glu |  | Glucose (mg/dL) |  | 78.9 (13.6) | 77 | 62 - 102 |
| Ctr |  | Citrate (μmol/L) |  | 103.7 (25.4) | 103 | 65 - 148 |
| **NMR Multimarkers (score 1-100)** | | | |  |  |  |
| LP-IR |  | Lipoprotein Insulin Resistance Index | | 36.0 (24.5) | 33 | 3 - 83 |
| DRI |  | Diabetes Risk Index | | 45.4 (18.3) | 43 | 20-79 |

\*Reference range values are from a representative sampling (n=698) of the general population, comprised of apparently healthy men (n=284) and women (n=414) aged 18 to 84 years (mean 39 years).