NMR biomarkers in the Mexico City Prospective Study

Introduction

Nightingale Health Plc. and the Nuffield Department of Population Health (NDPH) Wolfson laboratory (Oxford, UK) are performing NMR-based metabolomic biomarker profiling of participants from the Mexico City Prospective Study (MCPS). This first release covers lipid and metabolite biomarker data from just over 40,000 EDTA plasma samples from baseline recruitment (1998-2004). The second release, expected mid-2023, will cover all available EDTA plasma study samples, comprising ~150,000 from baseline recruitment and ~10,000 from resurvey (2015-2019). Metabolomic measures in the second phase will be quantified using an updated algorithm and will therefore form a completely separate data resource.

The remainder of this document details the methodology and metabolomics data present from the first phase.

Methods

Between September 2018 and October 2019, a subset of just over 40,000 baseline plasma samples were sub-aliquoted with a 110µL volume provided for NMR metabolomics using the Nightingale Health platform. This biomarker platform has been described recently in the context of profiling the UK Biobank: Julkunen et al, Nat Comms 2023 in press (https://www.medrxiv.org/content/10.1101/2022.06.13.22276332v2). For the present study, samples were shipped on dry ice in batches of ~5,000-10,000 samples, with the majority of samples analysed at Nightingale Health Plc (Helsinki and Kuopio, Finland), and the remainder analysed with the same protocol validated for use at the Nuffield Department of Population Health (NDPH) Wolfson laboratory (Oxford, UK). The Nightingale Health NMR metabolomics platform[1] generates spectra from which 225 biomarker measures are quantified as absolute concentrations or ratios (Figure 1, Appendix).

Details of the Nightingale Health NMR platform and experimentation have been described previously[1]. In brief, EDTA plasma samples were stored in a freezer at -80°C prior to analysis. Before preparation, frozen samples were slowly thawed at +4°C overnight, and then mixed gently and centrifuged (3 min, 3400´g, +4°C) to remove possible precipitate. Aliquots of each sample were transferred into 3-mm outer-diameter NMR tubes and mixed in 1:1 ratio with a phosphate buffer (75mM Na_2HPO4 in 80%/20% H_2O/D_2O, pH 7.4, including also 0.08% sodium 3-(trimethylsilyl) propionate-2,2,3,3-d4 and 0.04% sodium azide) automatically with an automated liquid handler (PerkinElmer Janus Automated Workstation in the Nightingale Health Laboratories; Beckman-Coulter Biomek NX^p Automated Liquid Handler the NDPH Wolfson Laboratory).

The prepared samples were held in a 96-position NMR rack and loaded onto a cooled sample changer, which maintains the temperature of samples waiting to be measured at +6°C. Two NMR spectra were recorded for each plasma sample using, in the Nightingale Health Laboratories, a 500 MHz NMR spectrometer (Bruker AVANCE IIIHD) and, in the NDPH Wolfson Laboratory, a 600MHz NMR spectrometer (Bruker AVANCE IIIHD). The first spectrum is a pre-saturated proton NMR spectrum, which features resonances arising mainly from proteins and lipids within various lipoprotein particles. The other spectrum is a T2-relaxation-filtered spectrum where most of the broad macromolecule and lipoprotein lipid signals are suppressed, leading to enhanced detection of low-molecular-weight metabolites. Automated quality

control of the spectral data was performed. The lipids and metabolites were quantified using Nightingale Health's proprietary software.

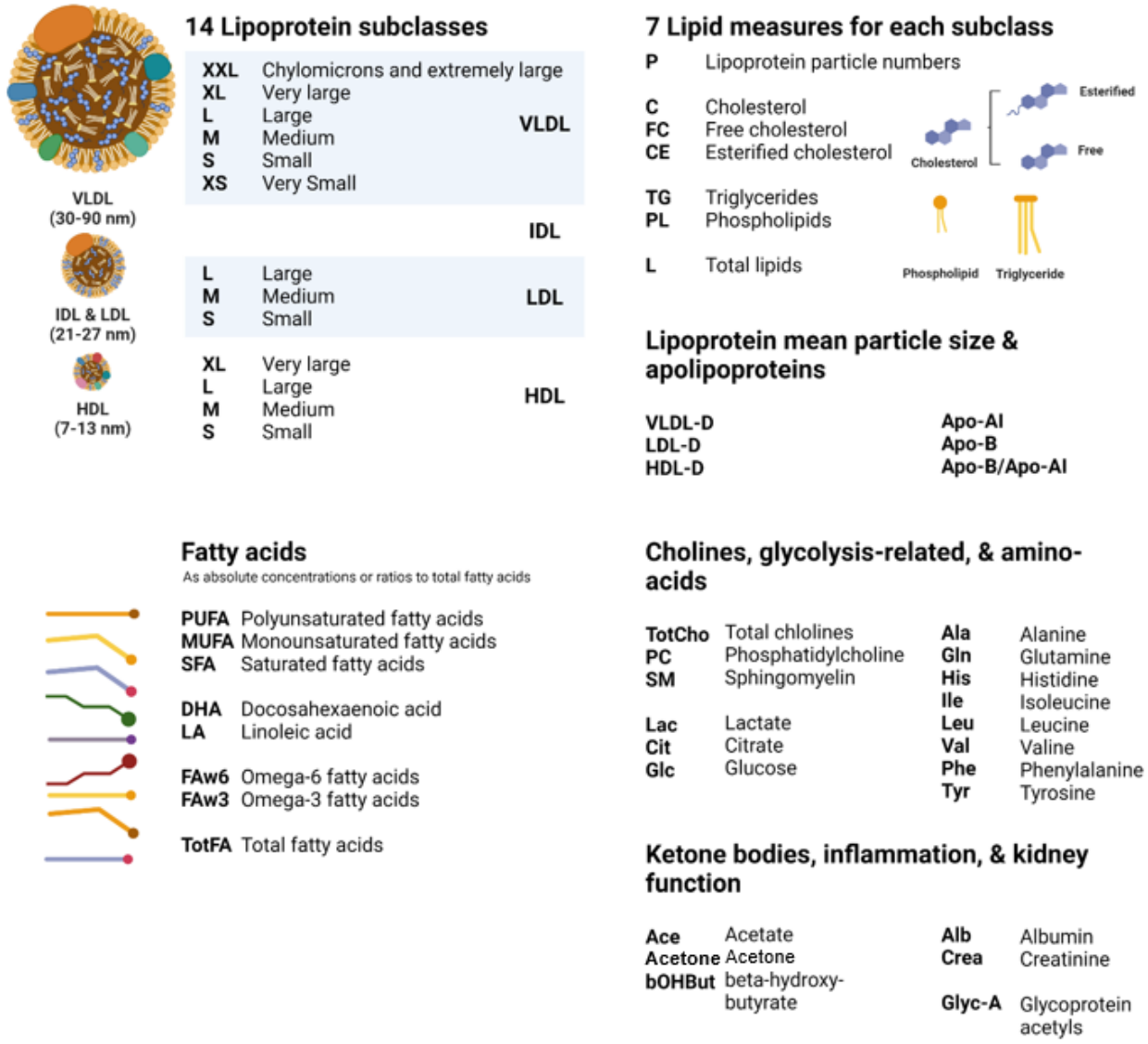

Figure 1: Plasma lipid and metabolomic measures quantified by nuclear magnetic resonance (NMR) spectroscopy

Abbreviations: Apo-A1, spolipoprotein A1; Apo B, spolipoprotein B; HDL, high density lipoprotein; HDL-D, HDL particle diameter; IDL, intermediate density lipoprotein; LDL, low density lipoprotein;LDL-D, LDL particle diameter; VLDL, very low density lipoprotein; VLDL-D, VLDL particle diameter. Source: Aguilar-Ramirez, D., Herrington, W.G., Alegre-Díaz, J. et al. Adiposity and NMR-measured lipid and metabolic biomarkers among 30,000 Mexican adults. Commun Med 2, 143 (2022). https://doi.org/10.1038/s43856-022-00208-2

Quality control assurance

Real-time performance monitoring of measurement consistency was performed. The first and last position of the 96-position NMR racks were designated as high- and low-level quality control (QC) samples respectively. The QC samples were aliquots from two pools of human EDTA plasma and were processed identically to the other samples. Coefficient of variation (CV) targets across eight metabolic measures (HDL Cholesterol, LDL Cholesterol, Total Cholesterol, Total Triglycerides, Glucose, Apo-A1, Apo-B, and Creatinine) were pre-specified and tracked using Levey-Jennings plots. For the majority of the metabolic biomarkers the CVs were below 5%. The automated NMR platform has multiple and standardized quality control checkpoints at both plate and batch level. An inter-laboratory comparison between Nightingale Health Ltd and CTSU's Wolfson Laboratory (using multiple sets of samples run in both laboratories) demonstrated good agreement.

Quality Data Flags

Nightingale Health's proprietary software integrates quality procedures verifying sample quality by reporting quality data flags, which can indicate signs of degradation and contamination issues. The quality data flags include:

- Low glucose / high pyruvate / high lactate
    - The circulating glucose concentration in a population is wel-defined with a very low probability for deviating samples, particularly on the low concentration side of the distribution. However, if, during the sample collection process, blood is kept at the room temperature (with the cells), glucose will be metabolised to pyruvate/lactate.
- Isopropyl alcohol
    - Isopropyl alcohol signals detected. Prevents quantification of creatinine.
- Abnormal macromolecule A
    - Signals from abnormal macromolecule(s)/protein(s). Prevents quantification of phenylalanine, acetate, acetone, glutamine and pyruvate.
- Low glutamine / high glutamate
    - In these samples most of glutamine appears to have degraded into glutamate. This can take place, during the sample collection process, if the sample is kept at room temperature for a prolonged period of time, there are multiple freeze-thaw cycles, or there are oxidative conditions / oxidation occurs in the sample. In these samples glutamine is not quantified.
- Low protein content
    - Based on the distribution of albumin concentration in large populations a sample is tagged if potential dilution of the sample is suspected.
- High ethanol
    - Small quantities of ethanol can sometimes be introduced in the sample either from disinfectants used in the blood donation/collection process or during the sample storage or preparation procedures; in these cases glycerol and sometimes β-hydroxybutyrate cannot be quantified. It is also good to note that high alcohol in the sample might (metabolically artificially) increase the acetate concentration.
- Diluted sample
    - Based on the distribution of albumin concentration in large populations a sample is marked if potential dilution of the sample is suspected. This can also relate to degradation of the sample integrity.

Information for data processing and analysis

NMR metabolites

Of the 225 measures provided per participant (Figure 1, Appendix), 128 are direct measures and 97 derived (i.e. via post-estimation algorithms). Many of the direct measures have dependencies. For example, the total lipids (L) in small (S) low-density lipoproteins (LDL) is the sum of the free and esterified cholesterol (FC and CE, respectively), the triglycerides (TG), and the phospholipids (PL) within this lipoprotein subclass (i.e. S-LDL-L = S-LDL-FC + S-LDL-CE + S-LDL-TG + S-LDL-PL). On the other hand, most of the derived measures are ratios between two direct measures, which represent the proportion of a given lipoprotein lipid, such as the triglycerides in small LDL (i.e. S-LDL-TG), of the total amount of lipids in the relevant lipoprotein subclass (i.e. S-LDL-L). As a result of these characteristics, some metabolomic measures are considered several times within the NMR-biomarkers platform. For instance, the esterified cholesterol in small LDL (S-LDL-CE) – a direct measure – is also considered within the cholesterol in small LDL (S-LDL-C), within the cholesterol in LDL (LDL-C), within the total cholesterol (TC), and within the esterified cholesterol to total lipids ratio in small LDL.

Among the NMR metabolite variables, missing values are denoted as follows:

- Zero (0) values indicate very low concentration (i.e. below the quantification threshold of the platform, and therefore not necessarily true null values). It is suggested by Nightingale Health to replace these with the lowest non-zero observed value of the relevant NMR measure.
- Not Defined (coded -1): Derived value or ratio cannot be given due to low concentration in original measures
- Tag (coded -2): Value cannot be quantified due to detected irregularity in sample, see quality data flags section above
- Not available (coded -3): Value was rejected by automatic sample and measurement quality control.

It should be noted that, in data produced prior to 2020, the Nightingale Health platform incorrectly labelled acetone as acetoacetate. MCPS were made aware of this in December 2024 and it has been corrected in all currently available datasets. However, any first release NMR data provided by MCPS in 2024 or earlier will have this variable mislabelled.

Participants with/without NMR metabolomics measures

Samples were processed in the order in which they were received in Oxford, approximately following the temporal pattern in which participants were recruited to the study. Study recruitment began in the Coyoacán district and subsequently moved to the Iztapalapa district (the less affluent of the two districts). Consequently, participants with NMR data available in the first release are not a random subset of the MCPS study population, and are more likely to be participants from Coyoacán than Iztapalapa. Mean age, weight, height and BMI are not different between those MCPS participants with vs without NMR data, but there are some differences in other characteristics (likely associated with the differing socio-demographic and lifestyle profiles of the two districts).

Comparison with chemical assays

Overall, traits quantified in MCPS participants by NMR are consistent with measurements from standard chemical assays. However, these correlations are lower than those reported from cohorts with North European,[2] UK,[3,4] and East Asian[5] populations.

NMR measured total and LDL cholesterol mean values are, on average, lower than those from clinical assays. Nightingale Health Ltd. have indicated software bias related to sample volume in the biomarker quantification version 2016 (MCPS samples were 100 uL instead of 350 uL conventionally used for NMR measurements in most previously-assayed cohorts) is the main reason for this discrepancy. However, as these traits chiefly represent a continuum rather than a cut-off risk, scaling variables (e.g. to standard deviation units) allows for appropriate epidemiological assessments. The second NMR metabolite data release, on all study samples and due mid-2023, will be based on an updated quantification algorithm, version 2020, that does not have this issue.

From an epidemiological perspective, the consistency of the associations between biomarkers and disease is as equally relevant as is the analytical consistency in absolute concentrations.[3,6]

Fasting duration

Initial analyses of this data show variation in levels of multiple metabolites according to fasting duration. It is increasingly accepted that non-fasting sampling is acceptable, if not preferred, for epidemiological studies as it is more representative of the usual levels of such biomarkers than fasting measures.[7]

Batch

Batch number runs from 1 to 8, where batches 1 through 7 were analysed at Nightingale Health Ltd (Finland), and batch 8 at NDPH (Oxford). Each batch comprised ~5000 samples. The metabolomics profiling platform is known for high repeatability over time and absence of batch effects. Nonetheless, researchers may wish to consider performing sensitivity analyses to explore this.

References

1. Soininen P, Kangas AJ, Wurtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ Cardiovasc Genet*. 2015;8(1):192-206.
2. Vehtari A, Mäkinen V-P, Soininen P, Ingman P, Mäkelä SM, Savolainen MJ, et al. A novel Bayesian approach to quantify clinical variables and to determine their spectroscopic counterparts in 1H NMR metabonomic data. *BMC Bioinformatics*. 2007 May;8(S2):S8.
3. Würtz P, Kangas AJ, Soininen P, Lawlor DA, Davey Smith G, Ala-Korpela M. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Large-Scale Epidemiology: A Primer on -Omic Technologies. *American Journal of Epidemiology*. 2017 Nov 1;186(9):1084–96.
4. Fraser A, Macdonald-Wallis C, Tilling K, Boyd A, Golding J, Davey Smith G, et al. Cohort Profile: The Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. *International Journal of Epidemiology*. 2013 Feb 1;42(1):97–110.
5. Holmes MV, Millwood IY, Kartsonaki C, Hill MR, Bennett DA, Boxall R, et al. Lipids, Lipoproteins, and Metabolites and Risk of Myocardial Infarction and Stroke. *Journal of the American College of Cardiology*. 2018 Feb;71(6):620–32.
6. Balling M, Langsted A, Afzal S, Varbo A, Davey Smith G, Nordestgaard BG. A third of nonfasting plasma cholesterol is in remnant lipoproteins: Lipoprotein subclass profiling in 9293 individuals. *Atherosclerosis*. 2019 Jul;286:97–104.
7. Nordestgaard BG. A Test in Context: Lipid Profile, Fasting Versus Nonfasting. *Journal of the American College of Cardiology.* 2017 Sep;70(13):1637–46.

Appendix – list of NMR metabolite variables returned by platform

Variable	Biomarker name	Units	Figure 1*
XXL-VLDL-P	Concentration of chylomicrons and extremely large VLDL particles	mol/l	Y
XXL-VLDL-L	Total lipids in chylomicrons and extremely large VLDL	mmol/l	Y
XXL-VLDL-PL	Phospholipids in chylomicrons and extremely large VLDL	mmol/l	Y
XXL-VLDL-C	Total cholesterol in chylomicrons and extremely large VLDL	mmol/l	Y
XXL-VLDL-CE	Cholesterol esters in chylomicrons and extremely large VLDL	mmol/l	Y
XXL-VLDL-FC	Free cholesterol in chylomicrons and extremely large VLDL	mmol/l	Y
XXL-VLDL-TG	Triglycerides in chylomicrons and extremely large VLDL	mmol/l	Y
XL-VLDL-P	Concentration of very large VLDL particles	mol/l	Y
XL-VLDL-L	Total lipids in very large VLDL	mmol/l	Y
XL-VLDL-PL	Phospholipids in very large VLDL	mmol/l	Y
XL-VLDL-C	Total cholesterol in very large VLDL	mmol/l	Y
XL-VLDL-CE	Cholesterol esters in very large VLDL	mmol/l	Y
XL-VLDL-FC	Free cholesterol in very large VLDL	mmol/l	Y
XL-VLDL-TG	Triglycerides in very large VLDL	mmol/l	Y
L-VLDL-P	Concentration of large VLDL particles	mol/l	Y
L-VLDL-L	Total lipids in large VLDL	mmol/l	Y
L-VLDL-PL	Phospholipids in large VLDL	mmol/l	Y
L-VLDL-C	Total cholesterol in large VLDL	mmol/l	Y
L-VLDL-CE	Cholesterol esters in large VLDL	mmol/l	Y
L-VLDL-FC	Free cholesterol in large VLDL	mmol/l	Y
L-VLDL-TG	Triglycerides in large VLDL	mmol/l	Y
M-VLDL-P	Concentration of medium VLDL particles	mol/l	Y
M-VLDL-L	Total lipids in medium VLDL	mmol/l	Y
M-VLDL-PL	Phospholipids in medium VLDL	mmol/l	Y
M-VLDL-C	Total cholesterol in medium VLDL	mmol/l	Y
M-VLDL-CE	Cholesterol esters in medium VLDL	mmol/l	Y
M-VLDL-FC	Free cholesterol in medium VLDL	mmol/l	Y
M-VLDL-TG	Triglycerides in medium VLDL	mmol/l	Y
S-VLDL-P	Concentration of small VLDL particles	mol/l	Y
S-VLDL-L	Total lipids in small VLDL	mmol/l	Y
S-VLDL-PL	Phospholipids in small VLDL	mmol/l	Y
S-VLDL-C	Total cholesterol in small VLDL	mmol/l	Y
S-VLDL-CE	Cholesterol esters in small VLDL	mmol/l	Y
S-VLDL-FC	Free cholesterol in small VLDL	mmol/l	Y
S-VLDL-TG	Triglycerides in small VLDL	mmol/l	Y
XS-VLDL-P	Concentration of very small VLDL particles	mol/l	Y
XS-VLDL-L	Total lipids in very small VLDL	mmol/l	Y
XS-VLDL-PL	Phospholipids in very small VLDL	mmol/l	Y
XS-VLDL-C	Total cholesterol in very small VLDL	mmol/l	Y
XS-VLDL-CE	Cholesterol esters in very small VLDL	mmol/l	Y
XS-VLDL-FC	Free cholesterol in very small VLDL	mmol/l	Y
XS-VLDL-TG	Triglycerides in very small VLDL	mmol/l	Y
IDL-P	Concentration of IDL particles	mol/l	Y
IDL-L	Total lipids in IDL	mmol/l	Y
IDL-PL	Phospholipids in IDL	mmol/l	Y
IDL-C	Total cholesterol in IDL	mmol/l	Y
IDL-CE	Cholesterol esters in IDL	mmol/l	Y
IDL-FC	Free cholesterol in IDL	mmol/l	Y
IDL-TG	Triglycerides in IDL	mmol/l	Y

Variable	Biomarker name	Units	Figure 1*
L-LDL-P	Concentration of large LDL particles	mol/l	Y
L-LDL-L	Total lipids in large LDL	mmol/l	Y
L-LDL-PL	Phospholipids in large LDL	mmol/l	Y
L-LDL-C	Total cholesterol in large LDL	mmol/l	Y
L-LDL-CE	Cholesterol esters in large LDL	mmol/l	Y
L-LDL-FC	Free cholesterol in large LDL	mmol/l	Y
L-LDL-TG	Triglycerides in large LDL	mmol/l	Y
M-LDL-P	Concentration of medium LDL particles	mol/l	Y
M-LDL-L	Total lipids in medium LDL	mmol/l	Y
M-LDL-PL	Phospholipids in medium LDL	mmol/l	Y
M-LDL-C	Total cholesterol in medium LDL	mmol/l	Y
M-LDL-CE	Cholesterol esters in medium LDL	mmol/l	Y
M-LDL-FC	Free cholesterol in medium LDL	mmol/l	Y
M-LDL-TG	Triglycerides in medium LDL	mmol/l	Y
S-LDL-P	Concentration of small LDL particles	mol/l	Y
S-LDL-L	Total lipids in small LDL	mmol/l	Y
S-LDL-PL	Phospholipids in small LDL	mmol/l	Y
S-LDL-C	Total cholesterol in small LDL	mmol/l	Y
S-LDL-CE	Cholesterol esters in small LDL	mmol/l	Y
S-LDL-FC	Free cholesterol in small LDL	mmol/l	Y
S-LDL-TG	Triglycerides in small LDL	mmol/l	Y
XL-HDL-P	Concentration of very large HDL particles	mol/l	Y
XL-HDL-L	Total lipids in very large HDL	mmol/l	Y
XL-HDL-PL	Phospholipids in very large HDL	mmol/l	Y
XL-HDL-C	Total cholesterol in very large HDL	mmol/l	Y
XL-HDL-CE	Cholesterol esters in very large HDL	mmol/l	Y
XL-HDL-FC	Free cholesterol in very large HDL	mmol/l	Y
XL-HDL-TG	Triglycerides in very large HDL	mmol/l	Y
L-HDL-P	Concentration of large HDL particles	mol/l	Y
L-HDL-L	Total lipids in large HDL	mmol/l	Y
L-HDL-PL	Phospholipids in large HDL	mmol/l	Y
L-HDL-C	Total cholesterol in large HDL	mmol/l	Y
L-HDL-CE	Cholesterol esters in large HDL	mmol/l	Y
L-HDL-FC	Free cholesterol in large HDL	mmol/l	Y
L-HDL-TG	Triglycerides in large HDL	mmol/l	Y
M-HDL-P	Concentration of medium HDL particles	mol/l	Y
M-HDL-L	Total lipids in medium HDL	mmol/l	Y
M-HDL-PL	Phospholipids in medium HDL	mmol/l	Y
M-HDL-C	Total cholesterol in medium HDL	mmol/l	Y
M-HDL-CE	Cholesterol esters in medium HDL	mmol/l	Y
M-HDL-FC	Free cholesterol in medium HDL	mmol/l	Y
M-HDL-TG	Triglycerides in medium HDL	mmol/l	Y
S-HDL-P	Concentration of small HDL particles	mol/l	Y
S-HDL-L	Total lipids in small HDL	mmol/l	Y
S-HDL-PL	Phospholipids in small HDL	mmol/l	Y
S-HDL-C	Total cholesterol in small HDL	mmol/l	Y
S-HDL-CE	Cholesterol esters in small HDL	mmol/l	Y
S-HDL-FC	Free cholesterol in small HDL	mmol/l	Y
S-HDL-TG	Triglycerides in small HDL	mmol/l	Y
XXL-VLDL-PL_%	Phospholipids to total lipds ratio in chylomicrons and extremely large VLDL	%	N
XXL-VLDL-C_%	Total cholesterol to total lipids ratio in chylomicrons and extremely large VLDL	%	N

Variable	Biomarker name	Units	Figure 1*
XXL-VLDL-CE_%	Cholesterol esters to total lipids ratio in chylomicrons and extremely large VLDL	%	N
XXL-VLDL-FC_%	Free cholesterol to total lipids ratio in chylomicrons and extremely large VLDL	%	N
XXL-VLDL-TG_%	Triglycerides to total lipids ratio in chylomicrons and extremely large VLDL	%	N
XL-VLDL-PL_%	Phospholipids to total lipds ratio in very large VLDL	%	N
XL-VLDL-C_%	Total cholesterol to total lipids ratio in very large VLDL	%	N
XL-VLDL-CE_%	Cholesterol esters to total lipids ratio in very large VLDL	%	N
XL-VLDL-FC_%	Free cholesterol to total lipids ratio in very large VLDL	%	N
XL-VLDL-TG_%	Triglycerides to total lipids ratio in very large VLDL	%	N
L-VLDL-PL_%	Phospholipids to total lipds ratio in large VLDL	%	N
L-VLDL-C_%	Total cholesterol to total lipids ratio in large VLDL	%	N
L-VLDL-CE_%	Cholesterol esters to total lipids ratio in large VLDL	%	N
L-VLDL-FC_%	Free cholesterol to total lipids ratio in large VLDL	%	N
L-VLDL-TG_%	Triglycerides to total lipids ratio in large VLDL	%	N
M-VLDL-PL_%	Phospholipids to total lipds ratio in medium VLDL	%	N
M-VLDL-C_%	Total cholesterol to total lipids ratio in medium VLDL	%	N
M-VLDL-CE_%	Cholesterol esters to total lipids ratio in medium VLDL	%	N
M-VLDL-FC_%	Free cholesterol to total lipids ratio in medium VLDL	%	N
M-VLDL-TG_%	Triglycerides to total lipids ratio in medium VLDL	%	N
S-VLDL-PL_%	Phospholipids to total lipds ratio in small VLDL	%	N
S-VLDL-C_%	Total cholesterol to total lipids ratio in small VLDL	%	N
S-VLDL-CE_%	Cholesterol esters to total lipids ratio in small VLDL	%	N
S-VLDL-FC_%	Free cholesterol to total lipids ratio in small VLDL	%	N
S-VLDL-TG_%	Triglycerides to total lipids ratio in small VLDL	%	N
XS-VLDL-PL_%	Phospholipids to total lipds ratio in very small VLDL	%	N
XS-VLDL-C_%	Total cholesterol to total lipids ratio in very small VLDL	%	N
XS-VLDL-CE_%	Cholesterol esters to total lipids ratio in very small VLDL	%	N
XS-VLDL-FC_%	Free cholesterol to total lipids ratio in very small VLDL	%	N
XS-VLDL-TG_%	Triglycerides to total lipids ratio in very small VLDL	%	N
IDL-PL_%	Phospholipids to total lipds ratio in IDL	%	N
IDL-C_%	Total cholesterol to total lipids ratio in IDL	%	N
IDL-CE_%	Cholesterol esters to total lipids ratio in IDL	%	N
IDL-FC_%	Free cholesterol to total lipids ratio in IDL	%	N
IDL-TG_%	Triglycerides to total lipids ratio in IDL	%	N
L-LDL-PL_%	Phospholipids to total lipds ratio in large LDL	%	N
L-LDL-C_%	Total cholesterol to total lipids ratio in large LDL	%	N
L-LDL-CE_%	Cholesterol esters to total lipids ratio in large LDL	%	N
L-LDL-FC_%	Free cholesterol to total lipids ratio in large LDL	%	N
L-LDL-TG_%	Triglycerides to total lipids ratio in large LDL	%	N
M-LDL-PL_%	Phospholipids to total lipds ratio in medium LDL	%	N
M-LDL-C_%	Total cholesterol to total lipids ratio in medium LDL	%	N
M-LDL-CE_%	Cholesterol esters to total lipids ratio in medium LDL	%	N
M-LDL-FC_%	Free cholesterol to total lipids ratio in medium LDL	%	N
M-LDL-TG_%	Triglycerides to total lipids ratio in medium LDL	%	N
S-LDL-PL_%	Phospholipids to total lipds ratio in small LDL	%	N
S-LDL-C_%	Total cholesterol to total lipids ratio in small LDL	%	N
S-LDL-CE_%	Cholesterol esters to total lipids ratio in small LDL	%	N
S-LDL-FC_%	Free cholesterol to total lipids ratio in small LDL	%	N
S-LDL-TG_%	Triglycerides to total lipids ratio in small LDL	%	N
XL-HDL-PL_%	Phospholipids to total lipds ratio in very large HDL	%	N
XL-HDL-C_%	Total cholesterol to total lipids ratio in very large HDL	%	N
XL-HDL-CE_%	Cholesterol esters to total lipids ratio in very large HDL	%	N

Variable	Biomarker name	Units	Figure 1*
XL-HDL-FC_%	Free cholesterol to total lipids ratio in very large HDL	%	N
XL-HDL-TG_%	Triglycerides to total lipids ratio in very large HDL	%	N
L-HDL-PL_%	Phospholipids to total lipds ratio in large HDL	%	N
L-HDL-C_%	Total cholesterol to total lipids ratio in large HDL	%	N
L-HDL-CE_%	Cholesterol esters to total lipids ratio in large HDL	%	N
L-HDL-FC_%	Free cholesterol to total lipids ratio in large HDL	%	N
L-HDL-TG_%	Triglycerides to total lipids ratio in large HDL	%	N
M-HDL-PL_%	Phospholipids to total lipds ratio in medium HDL	%	N
M-HDL-C_%	Total cholesterol to total lipids ratio in medium HDL	%	N
M-HDL-CE_%	Cholesterol esters to total lipids ratio in medium HDL	%	N
M-HDL-FC_%	Free cholesterol to total lipids ratio in medium HDL	%	N
M-HDL-TG_%	Triglycerides to total lipids ratio in medium HDL	%	N
S-HDL-PL_%	Phospholipids to total lipds ratio in small HDL	%	N
S-HDL-C_%	Total cholesterol to total lipids ratio in small HDL	%	N
S-HDL-CE_%	Cholesterol esters to total lipids ratio in small HDL	%	N
S-HDL-FC_%	Free cholesterol to total lipids ratio in small HDL	%	N
S-HDL-TG_%	Triglycerides to total lipids ratio in small HDL	%	N
VLDL-D	Mean diameter for VLDL particles	nm	Y
LDL-D	Mean diameter for LDL particles	nm	Y
HDL-D	Mean diameter for HDL particles	nm	Y
Serum-C	Serum total cholesterol	mmol/l	N
VLDL-C	Total cholesterol in VLDL	mmol/l	N
Remnant-C	Remnant cholesterol	mmol/l	N
LDL-C	Total cholesterol in LDL	mmol/l	N
HDL-C	Total cholesterol in HDL	mmol/l	N
HDL2-C	Total cholesterol in HDL2	mmol/l	N
HDL3-C	Total cholesterol in HDL3	mmol/l	N
EstC	Esterified cholesterol	mmol/l	N
FreeC	Free cholesterol	mmol/l	N
Serum-TG	Serum total triglycerides	mmol/l	N
VLDL-TG	Triglycerides in VLDL	mmol/l	N
LDL-TG	Triglycerides in LDL	mmol/l	N
HDL-TG	Triglycerides in HDL	mmol/l	N
TotPG	Total phosphoglycerides	mmol/l	N
TG/PG	Ratio of triglycerides to phosphoglycerides		N
PC	Phosphatidylcholine and other cholines	mmol/l	Y
SM	Sphingomyelins	mmol/l	Y
TotCho	Total cholines	mmol/l	Y
ApoA1	Apolipoprotein A-I	g/l	Y
ApoB	Apolipoprotein B	g/l	Y
ApoB/ApoA1	Ratio of apolipoprotein B to apolipoprotein A-I		Y
TotFA	Total fatty acids	mmol/l	Y
UnSat	Estimated degree of unsaturation		Y
DHA	22:6, docosahexaenoic acid	mmol/l	Y
LA	18:2, linoleic acid	mmol/l	Y
FAw3	Omega-3 fatty acids	mmol/l	Y
FAw6	Omega-6 fatty acids	mmol/l	Y
PUFA	Polyunsaturated fatty acids	mmol/l	Y
MUFA	Monounsaturated fatty acids; 16:1, 18:1	mmol/l	Y
SFA	Saturated fatty acids	mmol/l	Y
DHA/FA	Ratio of 22:6 docosahexaenoic acid to total fatty acids	%	Y

Variable	Biomarker name	Units	Figure 1*
LA/FA	Ratio of 18:2 linoleic acid to total fatty acids	%	Y
FAw3/FA	Ratio of omega-3 fatty acids to total fatty acids	%	Y
FAw6/FA	Ratio of omega-6 fatty acids to total fatty acids	%	Y
PUFA/FA	Ratio of polyunsaturated fatty acids to total fatty acids	%	Y
MUFA/FA	Ratio of monounsaturated fatty acids to total fatty acids	%	Y
SFA/FA	Ratio of saturated fatty acids to total fatty acids	%	Y
Glc	Glucose	mmol/l	Y
Lac	Lactate	mmol/l	Y
Cit	Citrate	mmol/l	Y
Ala	Alanine	mmol/l	Y
Gln	Glutamine	mmol/l	Y
His	Histidine	mmol/l	Y
Ile	Isoleucine	mmol/l	Y
Leu	Leucine	mmol/l	Y
Val	Valine	mmol/l	Y
Phe	Phenylalanine	mmol/l	Y
Tyr	Tyrosine	mmol/l	Y
Ace	Acetate	mmol/l	Y
Acetone	Acetone	mmol/l	Y
bOHBut	3-hydroxybutyrate	mmol/l	Y
Crea	Creatinine	mmol/l	Y
Alb	Albumin	signal area	Y
Gp	Glycoprotein acetyls, mainly a1-acid glycoprotein	mmol/l	Y

*indicates whether variable is present in Figure 1