Supplementary description of the NMR protocol

As described previously, [1,2] the frozen samples were slowly thawed at +4°C and mixed at a ratio of 1:1 with a sodium phosphate buffer (75 mM Na_2HPO_4 in 80%/20% H_2O/D_2O at a pH of 7.4 and also including 0.08% sodium 3-[trimethylsilyl]propionate-2,2,3,3-d_4 and 0.04% sodium azide). Each 96-well plate contained two quality control samples. The prepared samples were warmed to a stabilized temperature of 36.95°C just before the measurements were taken.

The NMR spectra were measured using a Bruker AVANCE III 500-Mhz spectrometer. The spectral data was acquired in two separate parts: a presaturated proton NMR spectrum, which features resonances arising mainly from proteins and lipids within various lipoprotein particles, and a T2-filtered spectrum where most of the broad macromolecule and lipoprotein lipid signals are suppressed, leading to enhanced detection of low-molecular-weight metabolites. [3] The first spectrum was recorded with 80k data points and 8 scans using Bruker noesypresat pulse sequence. The second spectrum was recorded with 64k data points and 24 or 16 scans using T2-relaxation filtered Bruker 1D CPMG pulse sequence. The characteristic molecular spectrums of the spectral windows are presented in [3].

The NMR spectra then underwent Fourier transformation, phase correction, overall signal check for missing/extra peaks, background control, baseline removal, and spectral area-specific signal alignments. [2] The quality control procedures included comparisons with the control samples and an extensive database of quantitative molecular data. Internal standards were not used, but the concentrations were calibrated to comply with external standards. [2] The quantification of metabolites is based on Bayesian modelling. [4,5] The lipid

quantification is calibrated against gel permeation high-performance liquid chromatography as briefly described in, [6] and the consistency with alternative approaches such as mass spectrometry and gas chromatography have been demonstrated. [7,8]

Supplementary references

1 Chrysafi P, Perakakis N, Farr OM, *et al.* Leptin alters energy intake and fat mass but not energy expenditure in lean subjects. *Nat Commun* 2020;**11**:1–15. doi:10.1038/s41467-020-18885-9

2 Soininen P, Kangas AJ, Würtz P, *et al.* Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Cardiovascular Epidemiology and Genetics. *Circ Cardiovasc Genet* 2015;**8**:192–206. doi:10.1161/CIRCGENETICS.114.000216

3 Soininen P, Kangas AJ, Wurtz P, *et al.* High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *Analyst* 2009;**134**:1781–5. doi:10.1039/b910205a

4 Vehtari A, Mäkinen V-P, Soininen P, *et al.* A novel Bayesian approach to quantify clinical variables and to determine their spectroscopic counterparts in 1H NMR metabonomic data. *BMC Bioinformatics* 2007;**8 Suppl 2**:S8. doi:10.1186/1471-2105-8-S2-S8

5 Würtz P, Kangas AJ, Soininen P, *et al.* Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Large-Scale Epidemiology: A Primer on -Omic Technologies. *Am J Epidemiol* 2017;**186**:1084–96. doi:10.1093/aje/kwx016

6 Würtz P, Soininen P. Reply to: "Methodological issues regarding: 'A third of nonfasting plasma cholesterol is in remnant lipoproteins: Lipoprotein subclass

profiling in 9293 individuals'". *Atherosclerosis* 2020;**302**:59–61. doi:10.1016/j.atherosclerosis.2020.03.028

7 Würtz P, Havulinna AS, Soininen P, *et al.* Metabolite profiling and cardiovascular event risk: A prospective study of 3 population-based cohorts. *Circulation* 2015;**131**:774–85. doi:10.1161/CIRCULATIONAHA.114.013116

8 Deelen J, Kettunen J, Fischer K, *et al.* A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals. *Nat Commun* 2019;**10**:1–8. doi:10.1038/s41467-019-11311-9