Supplementary material S1

**Material and methods**

*Blood sample processing*

Five ml of blood collected by vein puncture into 6ml K3EDTA vacutainers (Vacuette) was transferred into 15ml tubes and centrifuged at 500 x g, 4°C, with acceleration and deceleration at moderate speed. Top 2.5 ml of platelet-rich plasma (PRP) was transferred into new 15ml tube. The rest of PRP and buffy coat ring was discarded and 1 ml of erythrocyte sediment was carefully pipetted from the bottom into new 15ml tube and washed with 10 ml of cold PBS. Both PRP and erythrocytes containing tubes were centrifuged at 3000 x g, 4°C, with acceleration and deceleration at maximum speed. Supernatant from erythrocytes was removed and both erythrocytes plasma were aliquoted and stored at -80°C.

*Determination of the total malondialdehyde in erythrocyte lysates and plasma using stable isotope dilution assay based on liquid chromatography-tandem mass spectrometry.*

Internal standard of 1,3-dideuteromalondialdehyde (MDA-D2) stock solution was prepared by acid hydrolysis of 1,3-D2-1,1,3,3-tetraethoxypropane (Cambridge Isotope Laboratories, Tewksbury MA, USA).

*MDA in erythrocyte lysates.*

100 μl of washed erythrocytes was mixed with 10 μl of diluted internal standard MDA-D2 (10 μM) and lysed with four volumes of cold distilled water in a refrigerator at 4°C for 15 min; the cell debris were removed by centrifugation. The concentration of hemoglobin was measured at 540 nm using extinction coefficient = 8.5. The supernatant was hydrolyzed with NaOH (1M final concentration) at the presence of butylated hydroxytoluene (6 mM final concentration) for 30 min at 60°C. To the hydrolysate 3M HClO­4 was added for protein precipitation and samples were centrifuged. The supernatant was derivatized by 5 mM 2,4-dinitrophenylhydrazine (DNPH) using 30 min shaking on Vibrax in the dark at laboratory temperature. The reaction mixture was centrifuged and 20 μl was injected onto HPLC column Nucleosil C18 ec (125 x 3 mm, 5 μm) (Macherey-Nagel, Düren, Germany) at 40 °C using isocratic mobile phase composed of 0.1% of formic acid in 50% acetonitrile (v/v). The flow rate was 400 μl/min.

The HPLC system was connected to the mass spectrometer QTRAP 4000 (Sciex, Prague, Czech Republic). MDA and MDA-D2 DNPH derivatives (MDA-DNPH and MDA-D2-DNPH) were detected in positive multiple reaction monitoring (MRM) mode. MDA-DNPH was monitored at m/z 235->189 and MDA-D2-DNPH at m/z 237->191. MDA and MDA-D2 DNPH derivatives eluted at 3.00 min. Analyst v.1.6 from SCIEX was used for the acquisition and analysis of data.

*MDA in plasma.*

MDA in plasma was analyzed by the same procedure as MDA in erythrocyte lysates. Briefly, 10 μl of diluted internal standard MDA-D2 (10 μM) was added to 100 μl of EDTA plasma and was hydrolyzed with NaOH (1M final concentration) at the presence of butylated hydroxytoluene (6 mM final concentration) for 30 min at 60°C. After protein precipitationwith HClO4, centrifugation, and supernatant derivatization with DNPH the sample was centrifuged and 20 μl of supernatant was analyzed using LC-MS/MS.

*Lipidomics analysis*

Aliquots of plasma (50 uL) were precipitated by 150 uL of 2-propanol (Merck, Darmstadt) in order to remove proteins and the clear supernatant after centrifugation (10 min at 24 000 g) was transferred into a vial with insert for analysis. Erythrocyte were diluted by deionized water to concentration 1E9 prior to 2-propanol precipitation and then processed in the same fashion as plasma samples. A small portion of each supernatant was collected to a separate vial to form a pooled quality control (QC) sample.

For the lipidomic analysis, U-HPLC (Infinity 1290, Agilent) coupled to a high-resolution mass spectrometer with a hyphenated quadrupole time-of-flight mass analyzer (6560 Ion Mobility Q-TOF LC/MS; Agilent) with the Agilent Jet Stream (AJS) electrospray (ESI) source were employed.

The mass analyzer was operated at following conditions at both ionization modes: Gas temperature 350 °C, Drying Gas 12 L/min, Nebulizer pressure 40 psig, Sheath gas temperature 350 °C, Sheath gas flow 11 l/min, Nozzle voltage 250 V, fragmentor voltage 380 V, octopole radiofrequency voltage 750 V. The capillary voltage was 3000 V in ESI+, 4000 V in ESI-. For all samples, the data were acquired over the m/z range of 100 – 1700 at the rate of 1 spectrum/s. The m/z range was autocorrected on reference masses 121.0509 and 922.0098 for positive mode and 119.0363 and 980.0164 in negative mode. To obtain the fragmentation spectra of lipids, the QC sample was run several times in auto MS/MS mode with collision energies 10, 20 and 40 eV, with acquisition rate of 5 spectra/s for full spectra and 7 spectra/sec for fragmentation spectra. Top 5 ions were chosen for fragmentation with dynamic exclusion for 0.1 min after fragmentation event.

For lipidomic fingerprinting an Acquity BEH C18 (1.7 μm, 2.1 mm x 150 mm (Waters, USA)) was used for chromatographic separation. The chromatographic system used with ESI+ detection was: A – 10 mM ammonium formate and 0.1 % formic acid in acetonitrile:water (60:40, v/v); B – 10 mM ammonium formate and 0.1 % formic acid in 2-propanol:acetonitrile (90:10, v/v). For chromatographic separation of plasma detected in ESI- mode, following mobile phases were used: A - 10 mM ammonium acetate and 0.1% acetic acid in acetonitrile:water (60:40); B - 10 mM ammonium acetate and 0.1% acetic acid in 2- propanol:acetonitrile (90:10, v/v). The flow rate was constant at 0.300 mL ∙ min−1. The mobile phase gradient is described in Supplementary Table S2. The column temperature was maintained at 60°C, the injection volume was increased to 1 μL in ESI+ mode and 5 μL in ESI-mode. The autosampler was kept at 10 °C.

QCsample was run every 10 samples for system stability assessment. The samples were measured in MS mode, the fragmentation experiments were run on the QC sample at the end of the batch at 10, 20 and 40 eV.

*Lipid identification and statistical analyses*

The mass spectrometry data was processed LipidMatch suite (DOI 10.1186/s12859-017- 1744-3) which uses MZmine 2 for feature extraction and an R script for lipid identification based on in-silico fragmentation databases. At least a class-specific fragment was required for lipid identification. The data matrix of plasma lipids was first truncated by removing all the features which were present in the lipid infusions (peak area > 5000), allowing for the assessment of intermediary metabolites only. For erythrocyte lipids this procedure was not necessary as the membrane lipids and cell interior lipids must be metabolized prior incorporation into erythrocyte lipidome. Lipidomics data processing was performed in both web-based and R based MetaboAnalyst (metaboanalyst.ca) packages followed by SIMCA (Umetrics). Patients´ and controls´ lipidomics data were subject to a two-dimensional hierarchical cluster analysis (HCA). In MetaboAnalyst, sum normalization, log transformation and pareto scaling were used for signal processing. These data matrices were first sum normalized in R and then loaded to SIMCA, where statistical models were built. When building OPLS-DA (Orthogonal PLS-DA) models in SIMCA logarithmic transformation and pareto scaling was used. Fragmentation spectra of the VIP score based significant compounds were double checked both against the in-silico fragmentation library and against METLIN and LIPIDMAPS databases for their identities to be confirmed.