Supplementary methods – metabolomic/lipidomic measurements

Free fatty acids:

The derivatization procedure followed by LC-MS analysis was employed for the quantitative analysis of free fatty acids in serum samples. 10 µL of serum sample was mixed with 20 µL of isotopically labelled internal standard mixture and 20 µL of acetonitrile were added. The derivatization was performed by adding each 20 µL of 5 mM 2-hyrazinoquinoline, 20 µL of 5 mM triphenylphosphine and 20 µL od 5mM 2,2´-dipyridyl disulfide. Samples were incubated at 60 °C for 2 hours. The derivatization was stopped by putting the samples on ice and adding 100 µL water. After centrifugation the supernatant was used for the LC-MS analysis. A Vanquish UHPLC system (Thermo Scientific) coupled to an Orbitrap Fusion Lumos (Thermo Scientific) mass spectrometer was used for the LC-MS analysis. The chromatographic system was equipped with an Accucore C18 column, 2.6 µm, 150 x 2.1 mm inclusive a precolumn (Thermo Scientific) maintained at 50 °C. The separation was carried out using 0.15 % formic acid (v/v) with 10 mM ammonium formate in water as mobile phase A and 88 % (v/v) isopropanol 10 % (v/v) acetonitrile, 0.10 % (v/v) formic acid with 10 mM ammonium formate as mobile phase B at a flow rate of 0.4 mL/min. For elution of analytes the gradient of mobile phase B was applied with the total analysis time of 20 min.Sample injection volume was set to 2 µL. The mass spectrometer was operated in a positive electrospray ionization mode: spray voltage 3.5 kV; sheath gas flow rate 60 arb; auxillary gas flow rate 20 arb; capillary temperature 285 °C. For the analysis a full MS scan mode with a scan range m/z 150 to 520, resolution 120000, AGC target 2e5 and a maximum injection time 50 ms was applied. A ten point linear calibration curve with internal standardization and 1/x weighing was constructed for the quantification of the metabolites. The data processing was performed with the TraceFinder 4.1 software (Thermo Scientific).

Metabolite method:

10 µL of serum were mixed with 10 µL of an isotopically labeled internal standard mixture in a hydrophobic 96well filter plate. Aliquots of 300 µL of methanol were added and mixed for 20 min at 450 rpm. Afterwards, the sample extracts were collected in a 96-well plate by centrifuging the filter plate for 5 min at 500 g. A Vanquish UHPLC system (Thermo Scientific) coupled with an Orbitrap Q Exactive (Thermo Scientific) mass spectrometer was used for the LC-MS analysis. The chromatographic separation for samples was carried out on an ACQUITY UPLC BEH Amide, 1.7 µm, 2.1x100 mm analytical column (Waters) equipped with a VanGuard: BEH C18, 2.1x5mm pre-column (Waters). The column was maintained at a temperature of 40°C and 2 µL sample were injected per run. The mobile phase A was 0.15% formic acid (v/v) in water and mobile phase B was 0.15% formic acid (v/v) in 85% acetonitrile (v/v) with 10 mM ammonium formate. The gradient elution with a flow rate 0.4 mL/min was performed with a total analysis time of 17 min. The Orbitrap Q Exactive (Thermo Scinetific) mass spectrometer was operated in an electrospray ionization positive mode, spray voltage 3.5 kV, aux gas heater temperature 400°C, capillary temperature 350°C, aux gas flow rate 12. The metabolites of interest were analyzed using a full MS scan mode, scan range m/z 50 to 400, resolution 35000, AGC target 1e6, maximum IT 50ms. The Trace Finder 4.1 software (Thermo Scientific) was used for the data processing. Seven-point linear calibration curves with internal standardization and 1/x weighing was constructed for the quantification of metabolites.

Lipid method:

20 µL of the sample was transferred into a glass vial, 10 µL internal standard solution (SPLASH® Lipidomix®, Avanti Polar Lipids) and 120 µL methanol were added. After vortexing, 500 µL MTBE were added and the mixture was incubated in a shaker for 10 min at room temperature. A phase separation was induced by adding 145 µL MS-grade water. After 10 min of incubation at room temperature, the samples were centrifuged at 1000 xg for 10min. An aliquot of 450 µL of the upper phase (organic) was collected and dried in a

vacuum concentrator. The samples were reconstituted in 200 µL methanol and used for LC-MS analysis.

The LC-MS analysis was performed using a Vanquish UHPLC system (Thermo Fisher Scientific) combined with an Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer (Thermo Fisher Scientific). Lipid separation was performed by reversed phase chromatography employing an Accucore C18, 2.6 µm, 150 x 2 mm (Thermo Fisher Scientific) analytical column at a column temperature of 35 °C. As mobile phase A an acetonitrile/water (50/50, v/v) solution containing 10 mM ammonium formate and 0.1 % formic acid was used. Mobile phase B consisted of acetonitrile/isopropanol/water (10/88/2, v/v/v) containing 10 mM ammonium formate and 0.1% formic acid. The flow rate was set to 400 µL/min. A gradient of mobile phase B was applied to ensure optimal separation of the analysed lipid species. The mass spectrometer was operated in ESI-positive and -negative mode, capillary voltage 3500 V (positive) and 3000 V (negative), vaporize temperature 320 °C, ion transfer tube temperature 285 °C, sheath gas 60 arbitrary units, aux gas 20 arbitrary units and sweep gas 1 arbitrary unit. The Orbitrap MS scan mode at 120000 mass resolution was employed for lipid detection. The scan range was set to 250-1200 m/z for both positive and negative ionization mode, the AGC target was set to 2.0e5 and the intensity threshold to 5.0e3. The data analysis was performed using the TraceFinder software (ThermoFisher Scientific).

Supplementary figures – Metabolomics/Lipidomics

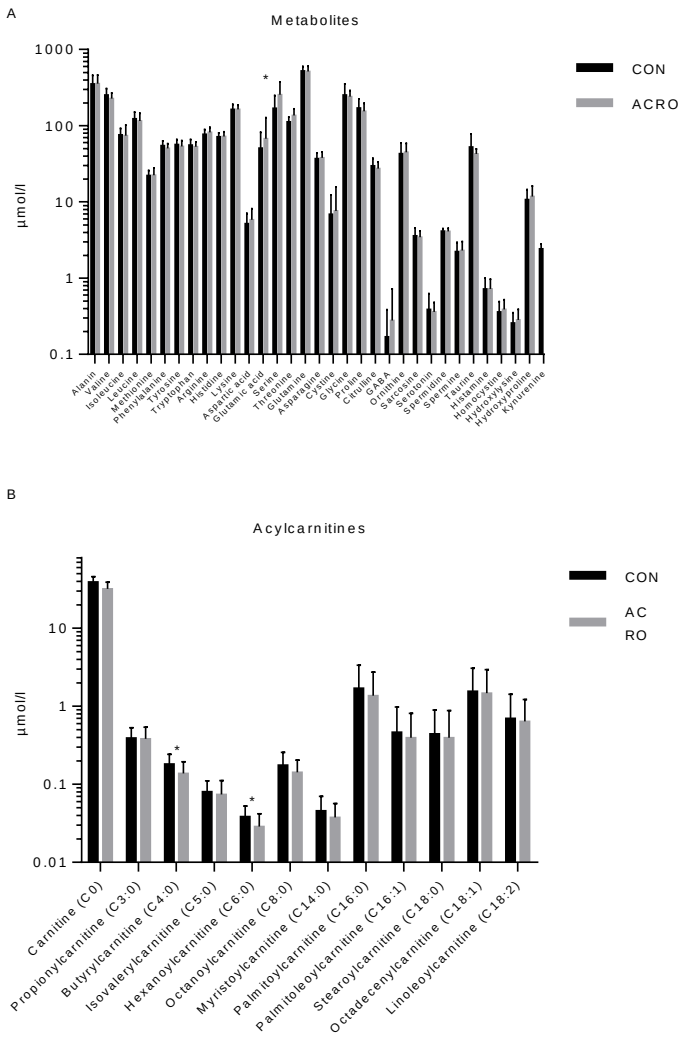

*Figure 1- -supplement: concentrations of metabolites and acylcarnitines; All data analysis performed with https://www.metaboanalyst.ca. mean + standard deviation; *p<0,05*

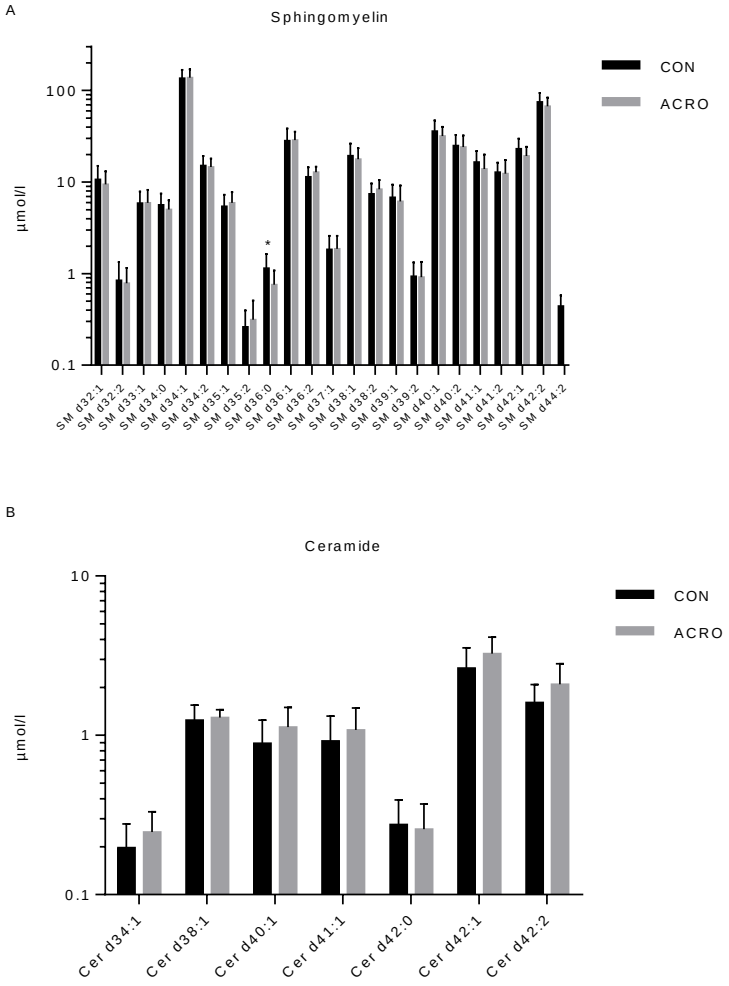

*Figure 2-supplement: concentrations of sphingomyelins and ceramides; All data analysis performed with https://www.metaboanalyst.ca. mean + standard deviation; *p<0,05*

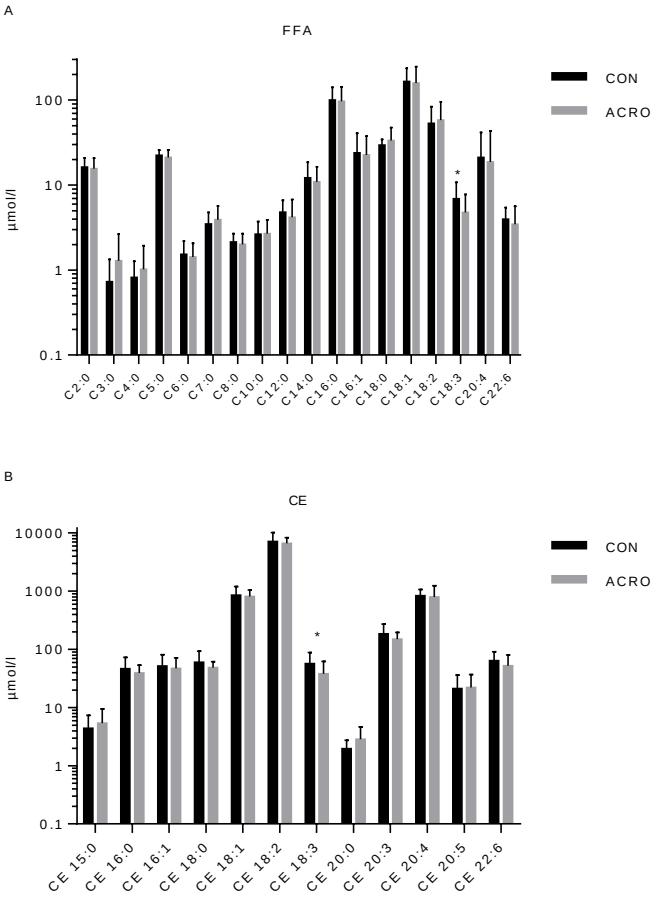

*Figure 3-supplement: Concentrations of free fatty acids (FFA) and Cholesteylesters (CE); All data analysis performed with https://www.metaboanalyst.ca. mean + standard deviation; *p<0,05*

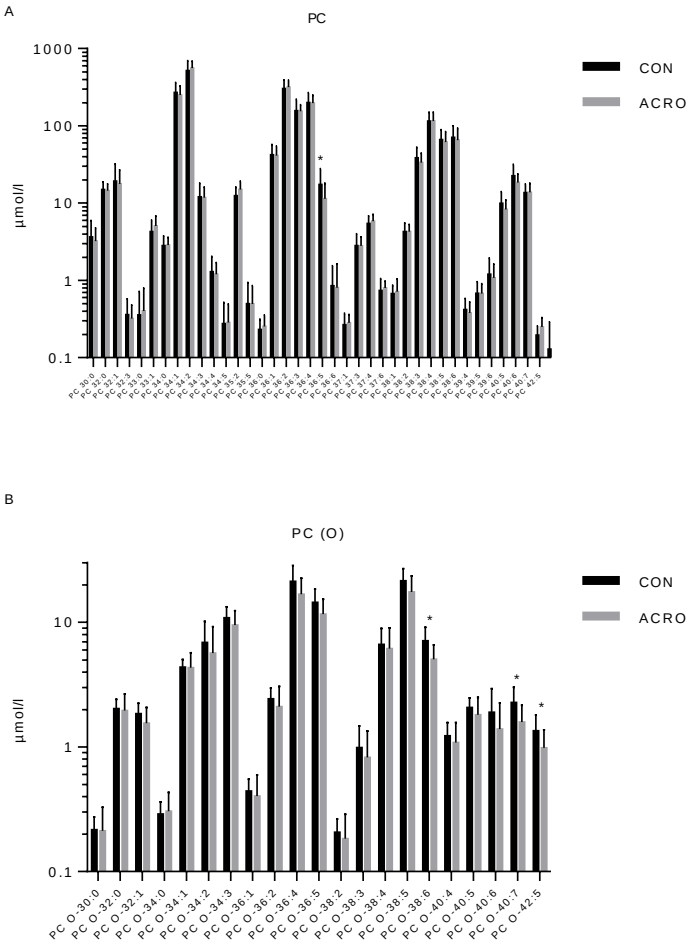

*Figure 4-supplement: Concentrations of phosphatidylcholine (PC) and glycerophospholipidcholine (PC(O)); All data analysis performed with https://www.metaboanalyst.ca. mean + standard deviation; *p<0,05*

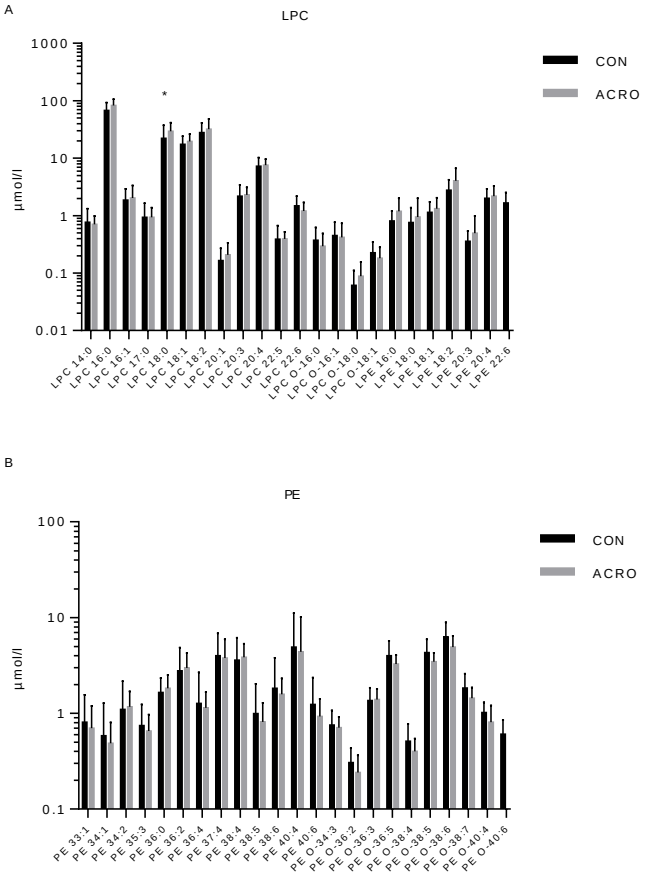

*Figure 5-supplement: concentrations of lysophosphatidylcholine (LPC) and phosphatidylethanolamine (PE); All data analysis performed with https://www.metaboanalyst.ca. mean + standard deviation; *p<0,05*

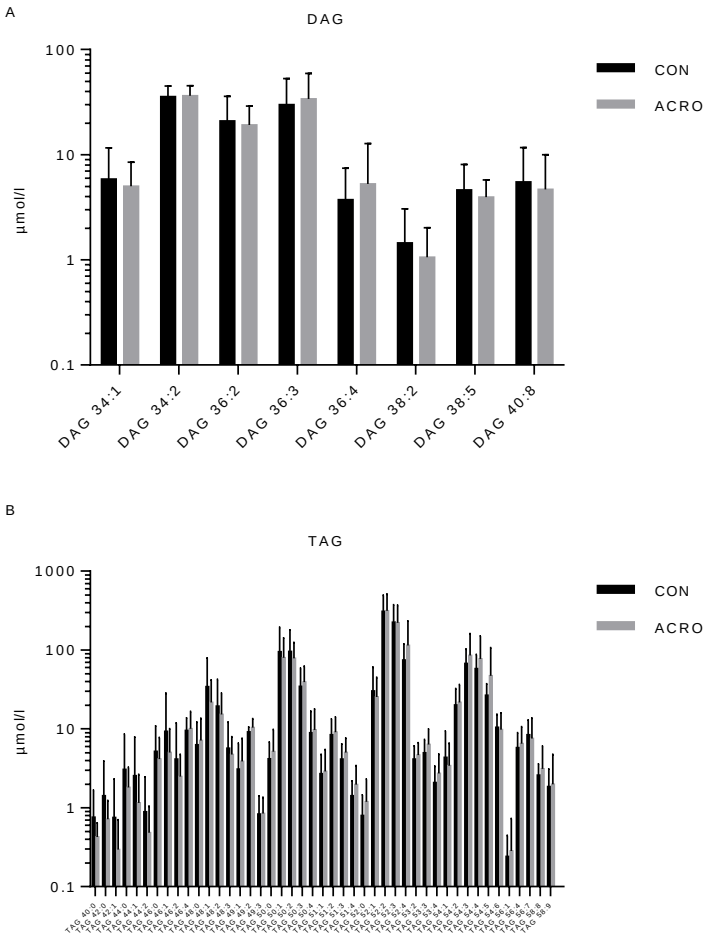

*Figure 6-supplement: concentrations of diacylglycerol (DAG) and triacylglycerol (TAG); All data analysis performed with https://www.metaboanalyst.ca. mean + standard deviation; *p<0,05*