Supplemental material: Methods for metabolomics analysis of amino acids, tricarboxylic acid cycle (TCA) intermediates, phospholipids and acylcarnitines.

Amino acids. Aliquots of the supernatant were prepared using derivatization as previously reported [1]. For internal standardization, a labeled amino acid standards set (set A, Cambridge Isotope Laboratories) was mixed with L-Asparagine (15N2, 98%, Cambridge Isotope Laboratories) and L-Tryptophan (Indole-D5, 98%, Cambridge Isotope Laboratories) and added to the precipitation reagent. AA butylester were determined by ion-pair liquid chromatography coupled to mass spectrometry detection (LC-MS/MS). 10 µL of the prepared sample were injected into the HPLC system (HPLC 1100, Agilent, Waldbronn, Germany) and chromatographic separation was performed with a XBridge C18 column (Waters GmbH, Eschborn, Germany). MS detection was performed with an API 2000 triple quadrupole instrument (Sciex, Darmstadt, Germany) with an APCI source operating in positive ion ionization mode. Data acquisition on the mass spectrometer was controlled by Analyst 1.6.2 software (AB Sciex, Darmstadt, Germany). Data handling and quantification were also performed with Analyst 1.6.2 software (AB Sciex, Darmstadt, Germany).

TCA intermediates. Metabolites of the TCA cycle and keto-acids were measured by a modified LC-MS/MS method based on previously published methods [2, 3]. D3-methylmalonic acid was used as internal standard. 100 µL of the supernatant were evaporated to dryness and re-suspended in 50 µL water. 5 µL of the extracted samples were injected to an Agilent 1200 HPLC and molecular species were separated on a Kinetex F5 core-shell HPLC column, 150 x 2.1 mm, 2.6 µm particle size (Phenomenex, Aschaffenburg, Germany). The mobile phase A was water with 1% formic acid and mobile phase B was composed of methanol/ isopropanol (50:50) with 1% formic acid. The gradient elution at a flow rate of 250

µL/min was held constant for 1 minute with 1% B, raised to 65% B within 6 minutes, and turned back to initial conditions of 1%B within 0.5 minutes. Re-equilibration was held for 5 minutes at 1% B. The triple quadrupole mass spectrometer (AB Sciex API4000; Applied Biosystems, Darmstadt, Germany) was operated in negative scheduled multiple reaction monitoring mode using electrospray ionization (ESI).

Phospholipids. Phospholipids were analyzed via flow-injection mass spectrometry (FIA-MS/MS). 30 µL of the centrifuged supernatant were mixed for 20 min at 600 rpm with 500 µl methanol containing 1.2 mM ammonium acetate and then used for FIA-MS/MS analysis. Samples were analyzed with a triple quadrupole mass spectrometer (QTRAP4000, Sciex, Darmstadt, Germany) with an electrospray ionization (ESI) source which was used in positive ionization mode. The MS was coupled to a LC system (Agilent, Waldbronn, Germany). MS/MS analysis was run in MRM with 184 Da (choline head group) as product ion for the PL. Analyst 1.6.2 software, followed by in-house processing with the statistical program R (R Project for Statistical Computing, http://www.r-project.org/), was used to post-process the entire analytical process.

Acylcarnitines. The supernatant was injected by flow-injection analysis into a LC-MS/MS system with isocratic elution with 76% isopropanol, 19% methanol and 5% water. The mass spectrometer was equipped with electrospray ionization and operated in positive ionization mode.

References

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