Supplemental Appendix

Protocol for the quantification of plasma esterified 3-hydroxy fatty acids

Plasma esterified 3-hydroxy fatty acids (3-OH FAs) were quantified as a proxy of total plasma LPS burden using liquid chromatography tandem mass spectrometry (LC-tandem MS), as previously described by Pais de Barros [13] et al. with modifications.
Two aliquots of human plasma (100 µL) spiked with 4 pmol of internal standard (3-hydroxytridecanoic acid, 1 pmol/µL in ethanol) were used for unesterified and total 3OHFAs quantification.
Unesterified (free) 3OHFAs were extracted with ethanol (200 µL), hydrogen chloride 50 mM (300 µL), endotoxin free water (400 µL), and ethyl-acetate/hexane 3:2 v:v (5 mL).
For total 3OHFAs quantification, plasma was hydrolyzed with hydrochloride acid 8M (300 µL) for 3 hours at 90°C. Fatty acids were further extracted with 600 µL of endotoxin free water and ethyl-acetate/hexane 3:2 v:v (5 mL).
Samples were mixed for 5 minutes and then centrifuged for 5 min at 1700 g at room temperature. Organic phases (4,5 mL) were recovered using a Fluent 780 pipetting instrument equipped with a flexible 8 pipetting channel arm and DiTi LIHA 1000µL tips (TECAN, France).
Samples were then evaporated under vacuum using a Savant SC250 EXP Speedvac concentrator (ThermoScientific). Dried extracts were solubilized with ethanol (200 µL) and transferred to injection vials with 300 µL glass inserts, evaporated under vacuum and finally solubilized with ethanol (50µL).
Samples (3 µl) were injected on a SBC18 2,1 x 50 mm, 1,8 µm column maintained at 45°C at a flow rate of 0,4 ml/min. Mobile phases used were A: water containing formic acid (0,1%) and ammonium formate (5 mM) – B : Acetonitrile/water 95/5 containing formic acid (0,1%) and ammonium formate (5 mM).
Fatty acids were separated using an isocratic step at 45 % B for 0,5 min followed by a linear gradient up to 100% B in 2,5 min and a final isocratic step at 100 % B for 5 min.
Tandem MS detection was achieved with a 6490 triple quadrupole (Agilent Thechnologies) equipped with an ESI-JetStream source (Gas Temp 290°C, Gas Flow 19 L/min, Nebulizer 20 psi, SheathGasHeater 175°C, SheathGasFlow 12 L/min, Capillary 2000V, VCharging 2000V, CA voltage 2V). Acquisitions were performed in selected single reaction (SRM) negative mode. Deprotonated 3OHFAs [M-H] were monitored based on the product ion of m/z=58,9 Da which correspond to the CH3COO fragment (collision energies ranging from 8 to 20 V depending on to length of the fatty acid chain).
Human plasma calibration standards (100 µL) containing 0, 0,5, 1, 2, 4, 8, 16, 32 and 64 pmol of each of 3OHFA from C10 to C18 were prepared and treated as samples. Area under curve for each 3OHFA was determined and area to IS ratios were calculated. Linear calibration was used for calculations.
LPS-derived/esterified 3-OH FAs were calculated as the difference between total 3-OH FAs and unesterified 3-OH FAs. The resulting esterified 3-OH FA concentrations, expressed in picomolars per milliliter, are a proxy of total plasma LPS burden.