Online Supplement

**Septic Shock Non-Survivors Have Persistently Elevated Acylcarnitines Following Carnitine Supplementation**

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**Measurement of acylcarnitines by liquid chromatography-mass spectroscopy**

The column used was a Waters HSS T3 1.8 µ, 2.1 x 100 mm (Milford, MA), mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. The LC flow rate was 0.25 mL/min, and the gradient consisted of a 12 min linear ramp from 0 to 100% B, a 3 minute wash at 100%B, and an 8-minute re-equilibration period at 0%B (total run time 20 min). The injection volume was 5 µL and the column temperature was 50 °C. Detection was performed using multiple reaction monitoring (MRM) in positive ion mode, using precursor/product ion transitions specified in the literature (1). Mass spectrometer parameters were as follows: capillary voltage 4000 V, gas temperature 325 °C, gas flow 10 L/min, nebulizer pressure 40 psi.
Figure E1: Temporal trends of relative concentrations acylcarnitines followed similar trends as quantified acylcarnitines. Overall, carnitine treated non-survivors had higher levels compared with survivors and placebo treatment did not influence acylcarnitine levels. Data are median (IQR) of the sample volume corrected peak area of each acylcarnitine in 8 carnitine-treated survivors, 7-8 carnitine-treated non-survivors, 3 placebo-treated survivors and 8-12 placebo treated non-survivors.

Figure E2: Representative liquid chromatograms of (LC)-mass spectroscopy (MS)/MS of L-carnitine, acetylcarnitine, and internal standard compounds from a representative extracted serum sample. MRM transitions used for quantitation are given in each chromatogram. LC-MS conditions are described in the manuscript text.

Figure E3: A representative gas chromatogram (GC) separation of standard fatty acid methyl esters containing saturated and unsaturated components. The GC parameters and the running procedures are described in the manuscript text.

References: