Electronic Supplementary Material for

"*In vitro* Analysis of Metabolites from the Untreated Tissue of *Torpedo californica* Electric Organ by Mid-Infrared Laser Ablation Electrospray Ionization Mass Spectrometry"

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Quantitation of metabolites

The concentration values of typical metabolites measured by LAESI method using external calibration method were significantly below to the reported values in the literature. Subsequent experiments on spiking of d_9-choline into the solution obtained from freeze/thaw process of torpedo electric organ tissue revealed noteworthy ion-suppression effects in the LAESI process, similar to the effect known in the ESI process. In order to obtaining reasonable quantitative figures, we have moved to spiking of the tissue with deuterium labeled metabolites. Stock solutions (100 mM) of d_9-choline and acetyl-d_3-carnitine were prepared in water, and appropriate volumes of the stock solutions were used to spike deuterium labeled metabolites in the tissue at different concentrations. After spiking, the tissue was frozen in liquid nitrogen and crushed using a mortar and pestle, and repeated grinding in liquid nitrogen ultimately resulted in a homogenous tissue paste. A small portion of the tissue paste was loaded on a microscope slide for recording LAESI spectra. The spectrum obtained for the ground tissue without spiked metabolite (spectrum not shown) is similar to that obtained for the untreated tissue (as shown in Figure 1), which indicates that little change has happened in its metabolite composition during pulverization with liquid nitrogen. Figure S1 and S2 show the typical LAESI spectra of the grounded tissue spiked with d_9-choline (m/z 113) and acetyl-d_3-carnitine (m/z 207), respectively.

To quantitate choline, ion counts for the protonated d_9-choline (m/z 113) and another metabolite ion (m/z 90) closer to the mass value of spiked chemical that do not change from sample to sample were measured; the ratio of m/z 113/90 is plotted against the spiked concentration of d_9-choline. Figure S3 shows the calibration curve on a log-log scale and the corresponding linear regression with an R = 0.99 correlation coefficient. The concentration of choline in the electric organ tissue, estimated from the ratio of m/z 104/90, yielded $4.8\pm0.5\times10^{-4}$ M. This corresponds to a weight concentration of 50±5 μg/g of tissue. Similarly, concentration of acetylcarnitine was measured by spiking with acetyl-d_3-carnitine. The ratio m/z 207/189 was plotted against the spiked concentration of acetyl-d_3-carnitine. Figure S4 shows the calibration curve on a log-log to scale and the corresponding linear regression with an R = 0.99 correlation coefficient. The concentration of acetylcarnitine thus estimated from the ratio of m/z 204/189 yielded $8.4\pm1.1\times10^{-4}$ M. This corresponds to a weight concentration of 170±20 μg/g of tissue. Assuming similar ionization efficiencies for the acetylated and unacetylated analogues the concentrations of acetylcholine and carnitine were also estimated using the calibration curves obtained for d_9-choline and acetyl-d3-carnitine, respectively. The tissue concentration of acetylcholine thus determined was 190±70 μg/g of tissue, and that of carnitine was 450±30 μg/g of tissue.

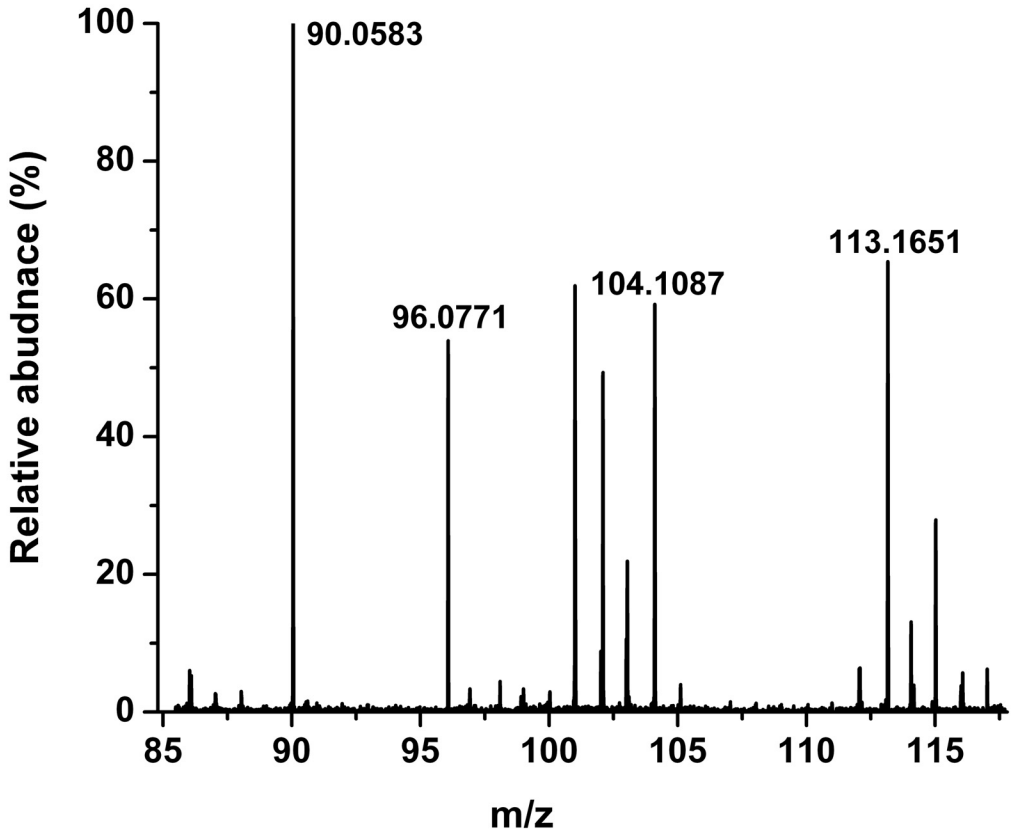

Figure S1. Expanded LAESI spectrum of tissue spiked with d_9-choline (470 μM) showing choline (m/z 104.1087) and d_9-choline (m/z 113.1651).

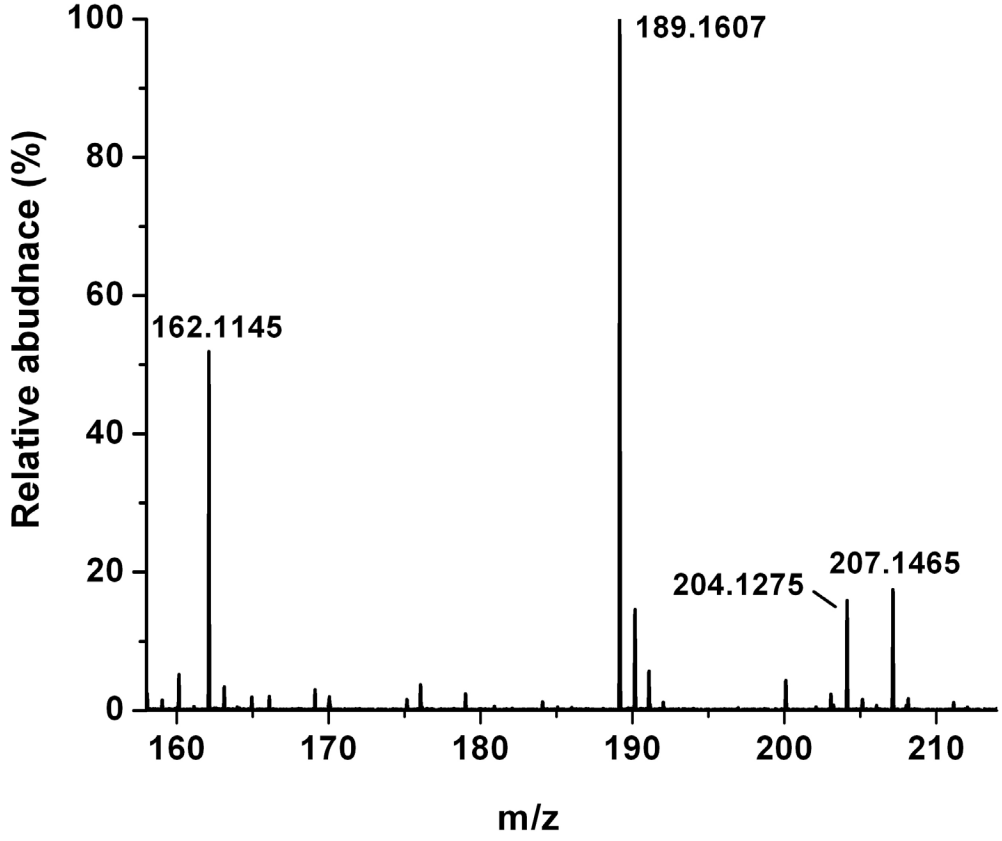

Figure S2. Expanded LAESI spectrum of tissue spiked with acetyl-d_3-carnitine (1 mM) showing acetylcarnitine (m/z 204.1275) and acetyl-d_3-carnitine (m/z 207.1465).

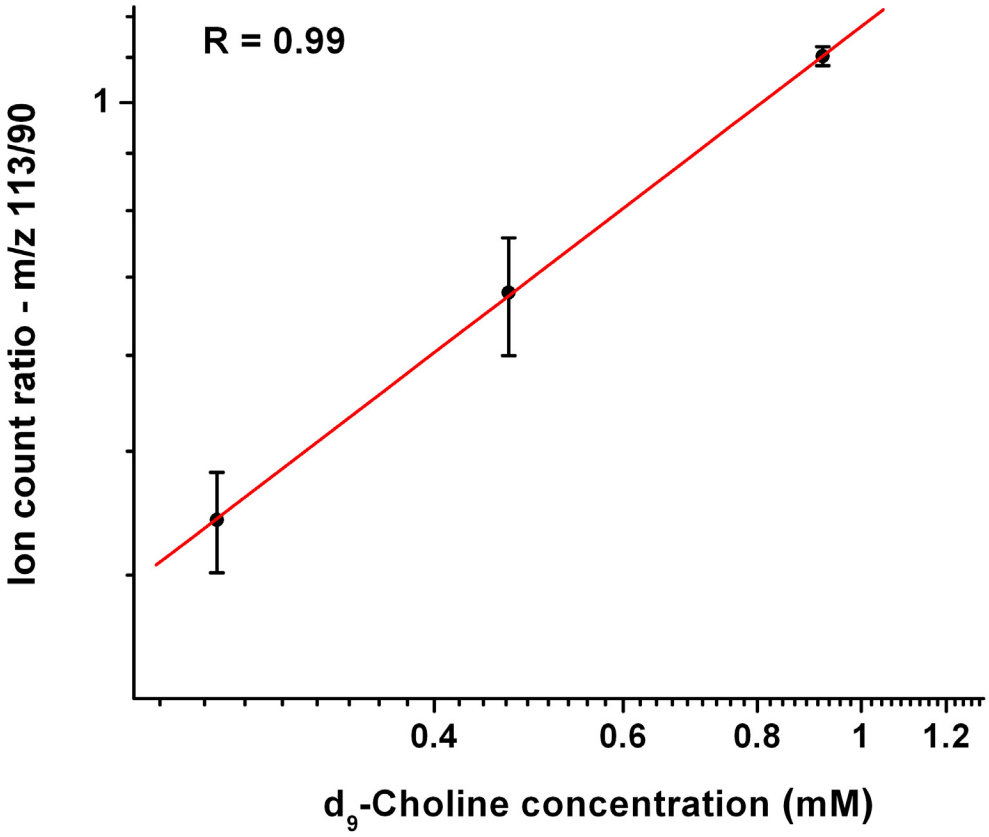

Figure S3. Ion count ratio for m/z 113/90 vs. spiked d_9-choline concentration in the electric organ tissue.

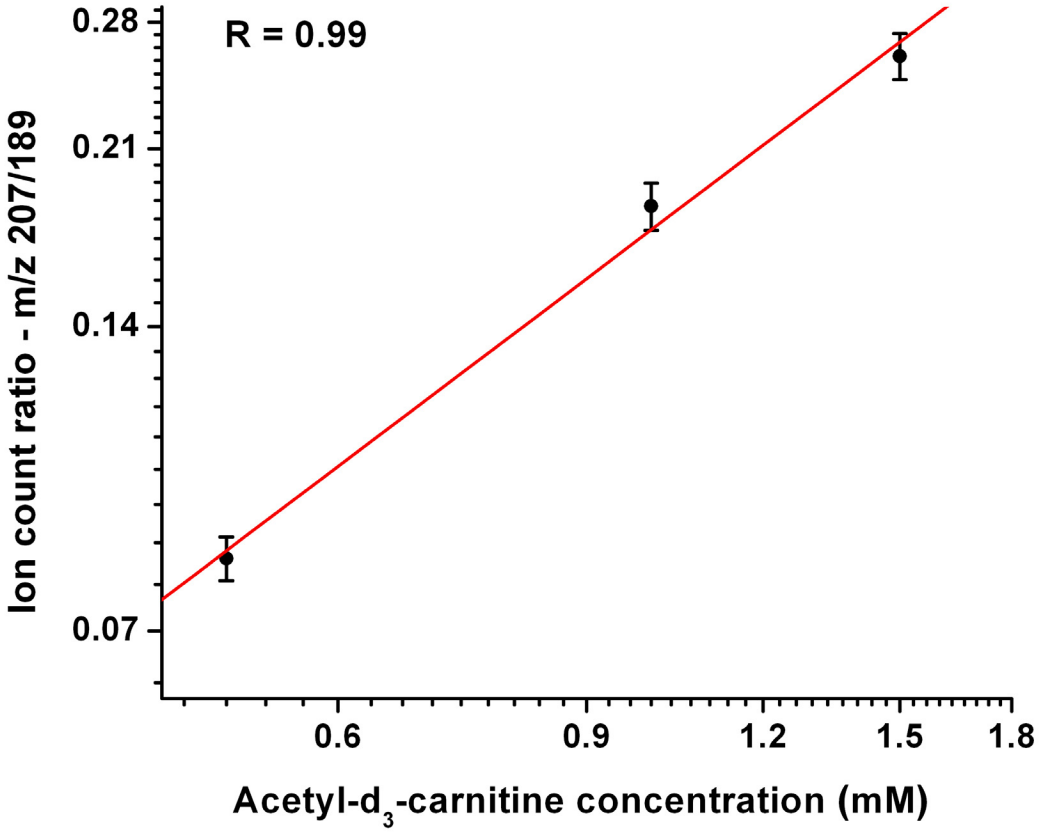

Figure S4. Ion count ratio for m/z 207/189 vs. spiked acetyl-d_3-carnitine concentration in the electric organ tissue.