DIRECT MEASURE OF TRANSPORT OF ^{13}C LABELED FATTY ACIDS INTO ADIPOCYTES WITH MULTI-ISOTOPE IMAGING MASS SPECTROMETRY (MIMS)

Alan Kleinfeld (akleinfeld@tpims.org)[1], Patrick Kampf[1], Claude Lechene[2]

[1]Torrey Pines Institute, 3550 General Atomics Crt, Membrane Biology Department, San Diego, CA, 92121, USA

[2]Harvard Medical School and Brigham and Women's Hospital, 65 Landsdowne Street, Room 535, Cambridge, MA, 02139, USA

The mechanisms of storage, mobilization and utilization of fat by the human body are important determinants of health, obesity and numerous diseases. One essential parameter is fatty acid metabolism. The long-term goal of this study is to help determine the mechanism(s) by which free fatty acids (FFA) are transported across cell membranes. In the initial phase of this work we are determining, in 3T3F442A adipocytes, the time course of ^{13}C labeled FFA influx and efflux. To this end, we have re-cloned 3T3F442A adipocytes to generate lines that reveal large lipid droplets at high frequencies and that appear to grow well on the silicon chips used in the MIMS instrument. We have plated silicon chips with these cells and treated them with FFA/BSA complexes, and we are using MIMS to reveal and to measure certain discrete steps along the influx and efflux trajectories. Specifically, we generated silicon chip-plated cells in 3 states. First were control cells that were not exposed to FFA. Second were cells that were incubated with all ^{13}C oleate:BSA complexes (for which the unbound oleate concentration was 400 nM as measured using ADIFAB fluorescence). Third were cells that were washed with fatty acid-free BSA after incubation with ^{13}C oleate:BSA. All cells were incubated for 30 minutes at 37C, sufficient time for intracellular unbound oleate to achieve steady state levels. The samples were analyzed with MIMS, recording -in parallel- quantitative images at masses ^{12}C, ^{13}C, $^{12}C^{14}N$ and $^{13}C^{14}N$. The ^{13}C over ^{12}C ratios were calculated both from the carbon ions ratios $^{13}C^-/^{12}C^-$ and from the cyanide ions ratios $^{13}C^{14}N^-/^{12}C^{14}N^-$. The results indicate that the concentrations of ^{13}C oleate in the intracellular lipid droplets may exceed by several fold those estimated from the lipid/water partition coefficient and the extracellular unbound oleate concentration. An internal control provides us with a robust validation of the results. Parallel imaging at masses ^{12}C, ^{13}C, $^{12}C^{14}N$ and $^{12}C^{15}N$ allowed us to determine that while there was excess accumulation of ^{13}C in the intracellular lipid droplets, the $^{12}C^{15}N/^{12}C^{14}N$ ratio was uniform across the adipocyte and equivalent to the natural 15N/14N ratio.
These results raise the exciting possibility that FFA may be pumped into adipocytes against their electro-chemical potential, and thus by an energy-dependent process.

Supported in part by research resource grant 9 P41 EB001074-04 and NIH grant DK05872