Supplementary information

Methods:

Adipogenic Differentiation

Primary human skin fibroblasts (Coriell Institute, Camden, NJ) were obtained at passage 2 and seeded into 35mm round glass bottom culture dishes (MatTek, Ashland, MA) at 15,000 cells per dish. The fibroblasts were cultured in Dulbecco's modified eagle's medium (Sigma Aldrich, St. Louis, MO) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (both from Sigma Aldrich, St. Louis, MO). Upon reaching 100% confluency (~2 weeks), growth media containing 1 μM dexamethasone, 10 μg/mL insulin, 0.5mM isobutylmethylxanthin (IBMX), and 0.2mM indomethacin (all from Sigma Aldrich, St. Louis, MO) was added to induce adipogenic differentiation following the method from Lysy et al[24]. Growth media was replaced in culture dishes twice weekly. After 14 days, lipid droplets of varying sizes were visualized in culture plates with a TE2000 inverted microscope (Nikon, Melville, NJ) and prepared for nanoextraction with an L-200 nanomanipulator (DCG Systems, Inc. Fremont, CA) operating in coarse mode (100 nm resolution).

Nanomanipulation

Upon observation of lipid droplets, the differentiating media was removed and replaced with 1mL of PBS (Fisher Scientific, Fair Lawn, NJ) for extraction. One joystick-controlled piezoelectric nanopositioner holding a palladium-gold coated nanospray emitter with 1 ± .2 μm inner diameter (Econo12, New Objective, Woburn, MA) containing 10μL of extraction solvent consisting of 2:1 chloroform-methanol (v/v) with 0.1% ammonium acetate (all HPLC grade from Sigma Aldrich, St. Louis, MO),) was positioned above the adipocyte for whole cell extraction. This consisted of

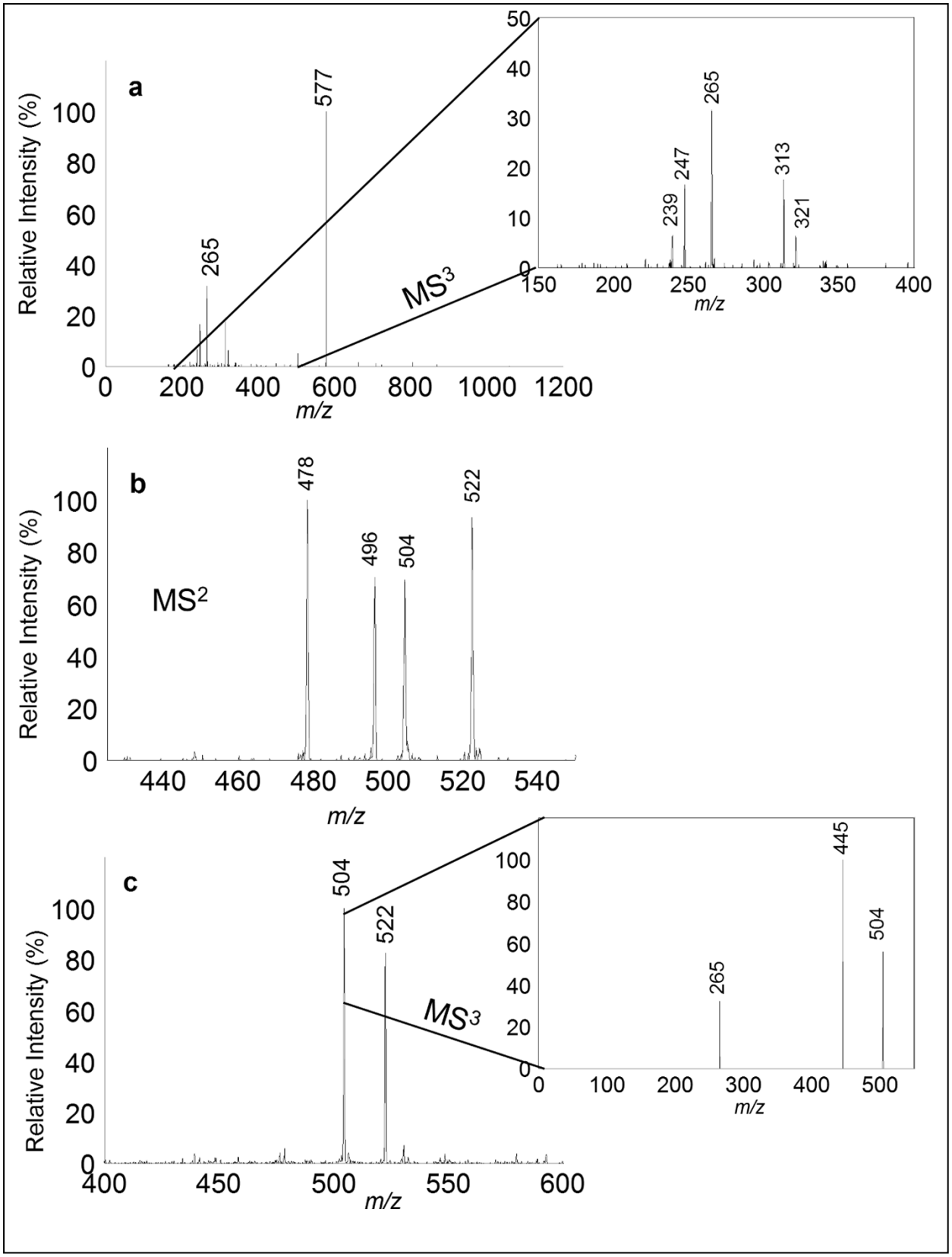
injecting the extraction solvent with 3psi pressure from a four-channel pressure injector (MicroData Instrument Inc., S. Plainfield, NJ) for 0.1-0.5 seconds to cover the entire adipocyte in a solvent bubble. After visual confirmation of solvation of lipid droplets, the extraction solvent was aspirated back into the nanospray emitter using 18 psi pressure. The emitter was transitioned out of the PBS with the nanopositioner and placed into the nanoelectrospray-ionization source (Proxeon Biosystems, Odense, Denmark) for rapid mass analysis. The total process for single adipocyte extraction and mass analysis for each cell were performed in an average total time of ten minutes. Following extraction, growth medium can be replaced into the culture dish so cells continue to grow in incubation and used for further experiments.

Mass Analysis

An LCQ DECA XP Plus 3D ion trap mass spectrometer (Thermo Finnigan, San Jose, CA) was utilized for NSI-MS and an LTQ XL Linear Ion Trap Mass Spectrometer ((Thermo Finnigan, San Jose, CA) for ESI-MS. Typical analysis lasted on average one to two minutes for nanoelectrospray ionization, using settings of 2.5 kV source voltage, 250° C capillary temperature, 2.5 μA source current, and 3.35 V capillary voltage. A source voltage of 4.5 kV, source current of 1.5 μA, and capillary voltage of 44 V was used in electrospray-ionization for typical whole flask extraction spanning over 30 minutes of time. The method from Folch et al.[20] was followed to perform the whole flask digestion, dilution, and extraction for ESI-MS. The mass range used was from *m/z* 100 to 1,100 to observe multiple lipid classes that are contained within adipocytes. Extractions were performed in replicates of 7 for both large lipid droplet adipocytes and small lipid droplet adipocytes to ensure reproducibility of lipid presence. Large and small lipid droplet adipocytes consistently displayed the lipid profile reported in Figure 2. Data acquired was processed with Xcaliber software

(Thermo Finnigan, San Jose, CA) and put into graphical form with PSI-Plot (Poly Software International, Pearl River, NY).

CID was performed to identify lipid species present and confirmed with known lipid fragmentation patterns previously published[6, 21-23] and LIPID MAPS online tools for lipid research (http://www.lipidmaps.org/). Examples of spectra obtained from CID are shown in Supplementary Figure 1. MS^2 was utilized for TAG ion *m/z* 876 which yielded an initial daughter ion at *m/z* 577 indicating a loss of 18:1 oleic fatty acid substituent. MS^3 was then used on daughter ion *m/z* 577 to yield 18:1 and 16:0 fatty acid substituent ion losses at *m/z* 265 and 239 respectively. In addition, 18:1-H_2O appeared at *m/z* 247 and 16:0 + glycerol backbone (+74) at *m/z* 313. This spectrum is consisted with TAG fragmentation patterns previously published.[25] MS^2 of *m/z* 760 was performed which appeared in both the small lipid droplet adipocyte and whole flask extraction. The major daughter ion that appeared was *m/z* 184 indicating a protonated phosphocholine (PC) head group[23] in addition to known fatty acid losses shown from *m/z* 420-540. Characteristic palmitic 16:0 and oleic 18:1 + headgroup ions were obtained (*m/z* 478, 496 and 504) and consistent with previously published phosphocholine fragment patterns.[22, 23] MS^2 of *m/z* 786 also displayed *m/z* 184 protonated phosphocholine headgroup and a major daughter ion at *m/z* 504, indicating a loss of fatty acid 18:1. MS^3 was then used on daughter ion *m/z* 504 which indicated an 18:1 fatty acid presence at *m/z* 265, in addition to a loss of $N(CH_3)_3$ at *m/z* 445 also concurrent with phosphocholine lipid fragmentation.[22, 23, 26]



Supplementary Figure 1. a.) MS^3 spectrum of diacylglycerol daughter ion *m/z* 577 from initial CID of TAG ion *m/z* 876 with inset displaying *m/z* 150-400. b.) CID spectrum of glycerophosphocholine m/z 760. c.) CID spectrum of

glycerophosphocholine *m/z* 786 with inset MS^3 spectrum of primary daughter ion *m/z* 504.

Supplementary References

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