Statement of Purpose: Chronic inflammation within adipose tissue ties obesity to numerous life-threatening diseases. In order to develop effective treatments, obesity must be better understood at the cellular level with respect to metabolic state and environmental stress. We have previously demonstrated our 3-D spheroid model created atop a biopolymer conjugate, elastin-like polypeptide-polyethyleneimine (ELP-PEI), using 3T3-L1 adipocytes.[1] This work examines the effect on cellular phenotype when subjected to various nutritionally relevant free fatty acids and an inflammatory cytokine TNF-α.

Methods: Cell Culture. ELP-PEI (5 mol%) was coated onto 24-well TCPS plates (0.5 mg/cm²). Undifferentiated 3T3-L1 cells (26,000 cells/cm²) were cultured on ELP-PEI coated surfaces to form the 3-D spheroids and on uncoated TCPS controls to form 2-D monolayer. After 3 days of acclimation and 3 days of exposure to differentiation cocktail, cells were exposed to media containing oleic acid (OA; C18:1 ω-9), linoleic acid (LA; C18:2 ω-6), and stearic acid (SA; C:18:0) for 5 days. Then the cells were exposed to 125 ng/mL TNF-α or control media (DMEM + 10% FBS) for 24 hours.

Biochemical Characterization. Intracellular protein and triglyceride content were derived using commercially available BCA Total Protein and Serum Triglyceride kits. ELISA assays specific to mouse CD36 and CD40 proteins were performed. Results reported as mean ± 95% confidence intervals (CI). Data analyzed by one-way ANOVA with Games-Howell post-hoc test for unequal variance using SPSS statistics package.

RT-PCR: RNA was collected from 3T3-L1 cultures using Qiagen RNeasy Plus Mini kit. mRNA expression levels were determined relative to control GAPDH gene (ΔΔCT) and expressed as mean fold change ($2^{ΔΔCT}$) ± 95% CI.

Results: Assay of cell lysates collected from 3T3-L1 cultures (Fig. 1) indicated varying triglyceride content with respect to culture surface (*), fatty acid class (#), though all fatty acid treatments indicated higher triglyceride accumulation than controls (*). The intracellular content was not reduced by TNF exposure.

Figure 1: Triglyceride content of 3T3-L1 adipocytes.

Conclusions: Our 3-D spheroid model has shown upregulation of markers specific to an adipogenic phenotype, including triglyceride accumulation, CD36 fatty acid transporter, and CD40 cytokine receptor. These results suggest improved adipogenesis and differentiated phenotype in 3-D cultures compared to 2-D monolayer. This 3-D model also appears to display an increased sensitivity to exogenous fatty acids and cytokines.

References: