SUPPLEMENTAL FIGURE LEGENDS

Supplemental FIG. 1: Induction of hypertrophy in adipocytes by excess glucose and FFAs

3T3-L1 and human SGBS adipocytes were differentiated in 5 mM or 25 mM glucose and cultured for 7 days in the same medium with the various FFAs (250 μM) indicated. Hypertrophy was analyzed by measuring area (μm^2) of lipid droplets (*A*) after staining neutral lipid with HCS LipidTOX (Invitrogen) in 3T3-L1 adipocytes. Cellular lipids were extracted with chloroform: methanol, and triglyceride content *(B)* was measured with a colorimetric assay kit in 3T3-L1 adipocytes. Human SGBS adipocytes were also photographed (*C,* original magnification x400) (Control = fatty acid free albumin. **P*< 0.001 vs. 5 mM glucose, ***P*< 0.001 vs. 25 mM glucose.

Supplemental FIG. 2: DHA inhibits SFA and glucose-stimulated SAA and MCP-1 expression, and glucose-stimulated HAS2 gene expression in differentiated human SGBS and murine embryonic fibroblasts (MEFs)

SGBS (*A*-*D*) and MEF (*E*-*G*) adipocytes were differentiated in 5 mM or 25 mM glucose and cultured for 7 days in the same medium with various FFAs (250 μM). Total RNA was isolated and analyzed by multiplex real time RT-PCR using SAA1 (*A*), SAA3 (*E*) or MCP-1 (*B, F*) HAS2 (*C, G*) specific primers and probes, and normalized to GAPDH. Conditioned media were analyzed by immunoblot using an anti-human SAA1 antibody (*D*). **P*< 0.001 vs. 5 mM glucose control, ***P*< 0.001 vs. 25 mM glucose control.

Supplemental FIG. 3: Palmitate, stearate and DHA do not increase apoptosis of differentiated 3T3-L1 adipocytes.

Differentiated 3T3-L1 adipocytes were exposed to the indicated concentrations of glucose (left) or 250 μM of palmitate, stearate and DHA (top) for 7 days with daily media changes. At the end of 7 days, the cells were detached with trypsin and subjected to FACS analysis. The horizontal axis represents annexin V staining, a measure of apoptosis. The vertical axis is propidium iodide staining, a measure of cell necrosis. The upper right panel shows cells exposed to H_2O_2 (100 μM), which was used as a positive control, indicated by detecting annexin V positive cell in the lower right hand quadrant.

Supplemental FIG. 4: Adiponectin expression is increased by arachidonate, EPA and DHA.

3T3-L1 adipocytes were differentiated in 5 mM or 25 mM glucose and cultured for 7 days in the same medium with various FFAs (250 μM). Total RNA was isolated and analyzed by multiplex real time RT-PCR using adiponectin specific primers and probes, and normalized to GAPDH. **P*< 0.001 vs. 5 mM glucose control.

Supplemental FIG. 5: Antioxidants prevent excess glucose and palmitate from stimulating NFκB, and increasing the production of SAA3, MCP-1 and HA in 3T3-L1 adipocytes.

3T3-L1 adipocytes differentiated in 5 mM or 25 mM glucose were cultured in the same media with or without 250 μM of palmitate for 7 days. Some adipocytes were also replenished with the antioxidants, NAC (10 mM), catalase (100 units/ml) or SOD (100 units/ml). Adipocytes were fixed and stained using an anti-p65 NFκB antibody, followed by the addition of a FITC-secondary antibody (*A*, original magnification x400). Total RNA was collected for analysis of SAA3 and MCP-1 mRNA expression by real time RT-PCR using SAA3 and MCP-1 specific primers and probes, and normalized to GAPDH (*B and C*). Conditioned media from adipocytes were analyzed by immunoblot using a SAA3 antibody (*D*) and monocyte chemotactic activity of these conditioned media were analyzed using the 96-well Boyden chemotaxis described in Fig. 3 (*E*). Cell lysates were also harvested for analysis of HA content by ELISA (*F*). **P*< 0.001 vs. 25 mM glucose, ***P*< 0.001 vs. 25 mM glucose + palmitate.