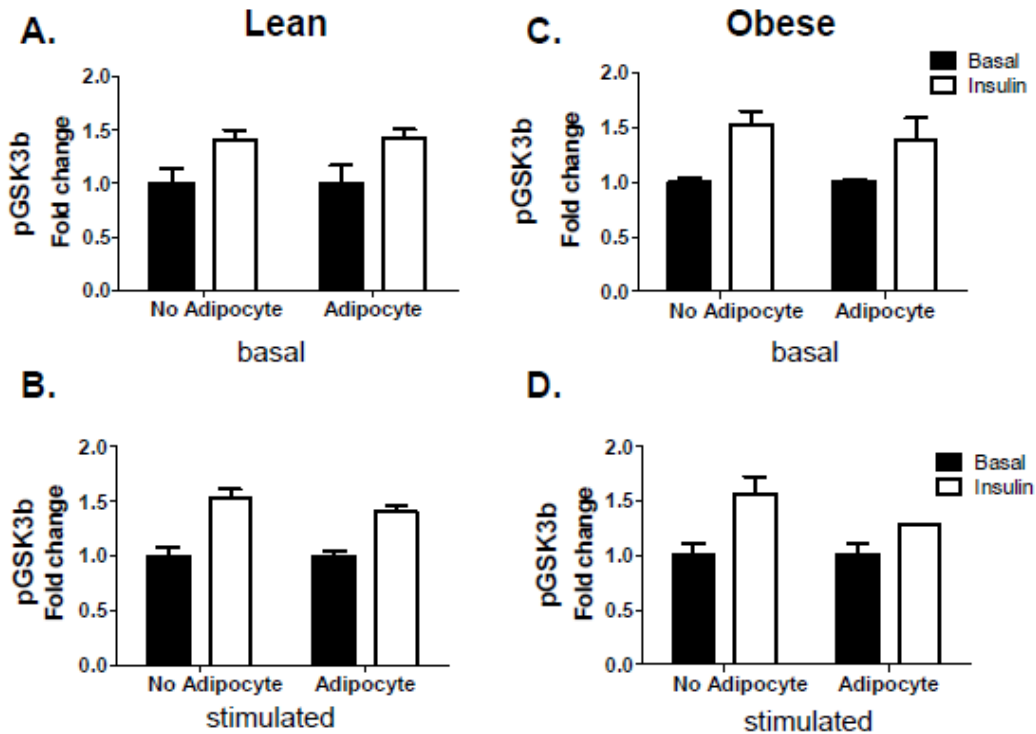


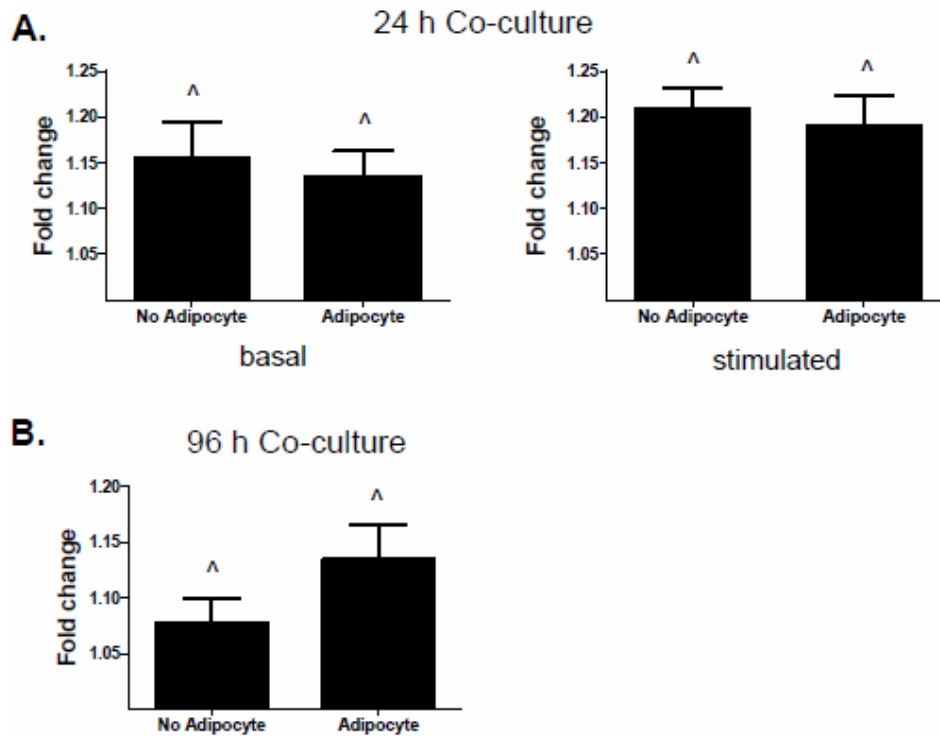
SUPPLEMENTARY DATA

Supplementary Figure 1. Effect of twenty four hour co-culture exposure on myotube GSK-3 β phosphorylation. Primary human skeletal myotubes from lean (A and B) and severely obese (C and D) women were maintained 24 h in the absence or presence of human adipocytes under basal or stimulated (100 μ M IBMX) conditions. Adipocytes were removed and myotubes were washed and incubated with alpha-MEM \pm 100 nM insulin for 10 min. Protein extracts were used for immunoblot analysis of GSK-3b Serine⁹ phosphorylation, corrected for total protein loading using anti-tubulin staining. Measurement of total GSK-3b protein showed no treatment effects. Results are expressed as mean \pm SEM.



SUPPLEMENTARY DATA

Supplementary Figure 2. Effect of adipocyte exposure on insulin-stimulated glucose uptake in primary human myotubes. Primary human skeletal myotubes from lean women were maintained in the absence or presence of human adipocytes for 24h under basal or stimulated (100 μ M IBMX) conditions (A) or 96 h under stimulated conditions (B). At end of the co-culture period, myotubes and adipocytes were washed and incubated for 3 h in serum-free DFM. Adipocytes were then removed and myotubes were pre-treated with media \pm 200nM insulin or 10 μ M cytochalasin B for 30 minutes followed by incubation with media containing 1 μ Ci/mL [3 H]2-D-deoxyglucose for 1 hour. Cells were then washed and lysates prepared using 0.1% SDS. Glucose uptake rates were determined by measuring label incorporation into cellular lysates. Results are expressed as mean \pm SEM and are representative of at least three experiments performed in triplicate. \wedge Significant ($p < 0.05$) effect of insulin exposure analyzed by *Student's t-test*.



SUPPLEMENTARY DATA

Supplementary Figure 3. Effect of adipocyte exposure on lipid levels in primary human myotubes. Primary human skeletal myotubes from lean women were maintained in the absence or presence of human adipocytes for 24h (A and C) or 96 h (B, D and E) under stimulated (100 μ M IBMX) conditions. At the end of the co-culture period cell extracts were used to measure to total glycerolipid and the fatty acid composition of triacylglycerol (A and B) and diacylglycerol (C and D), as well as individual ceramide species (E). Results are expressed as mean \pm SEM and are representative of two independent experiments performed in quadruplicate. * Significant ($p < 0.05$) effect of adipocyte exposure analyzed by *Student's t*-test.

