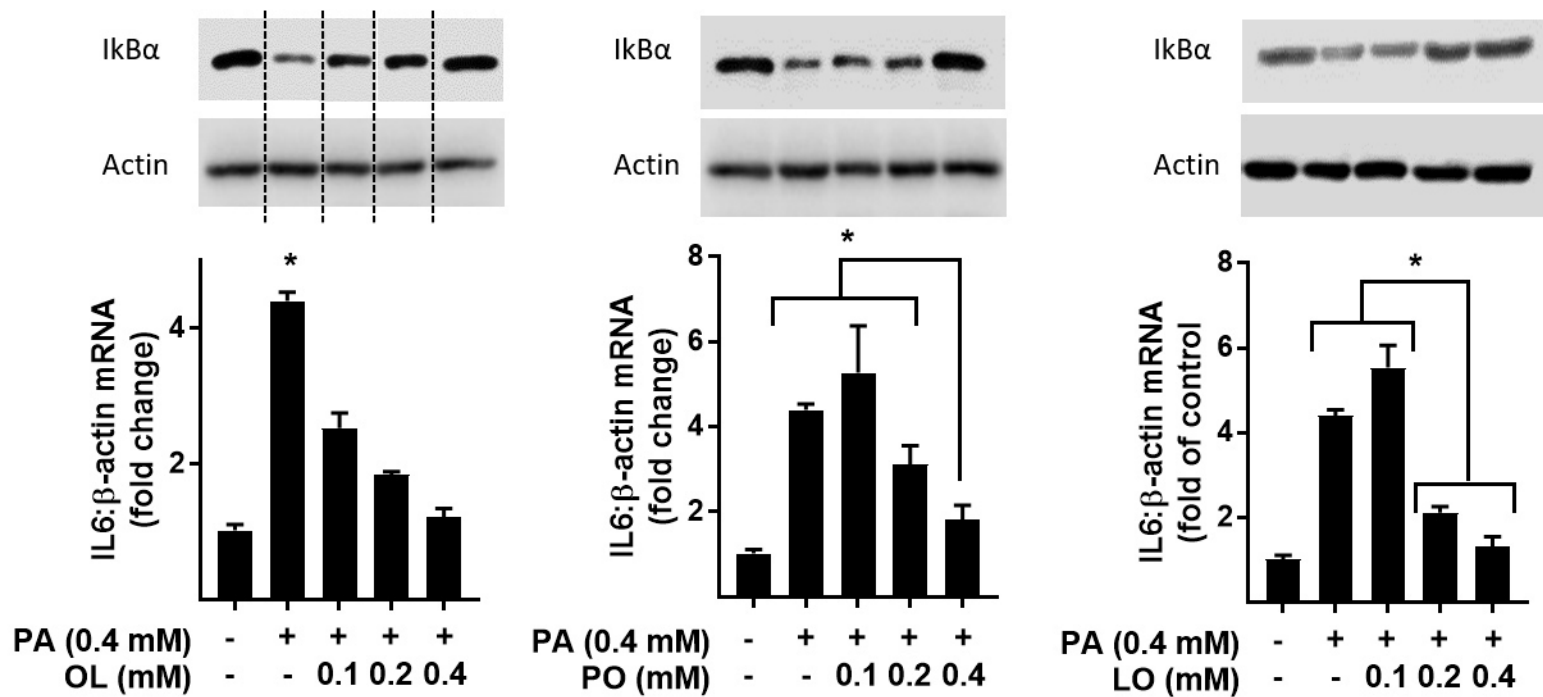


Supplementary Material

Mono- and Polyunsaturated Fatty Acids Counter Palmitate-Induced Mitochondrial Dysfunction in Rat Skeletal Muscle Cells

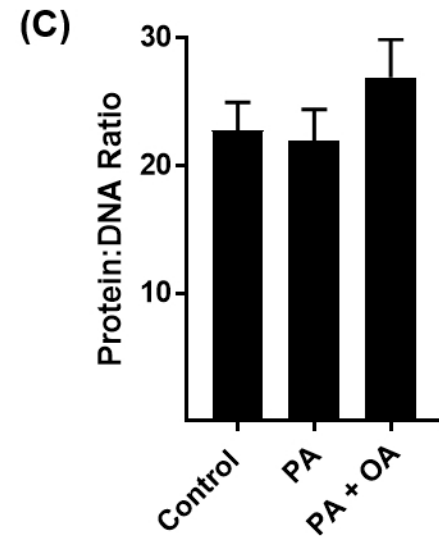
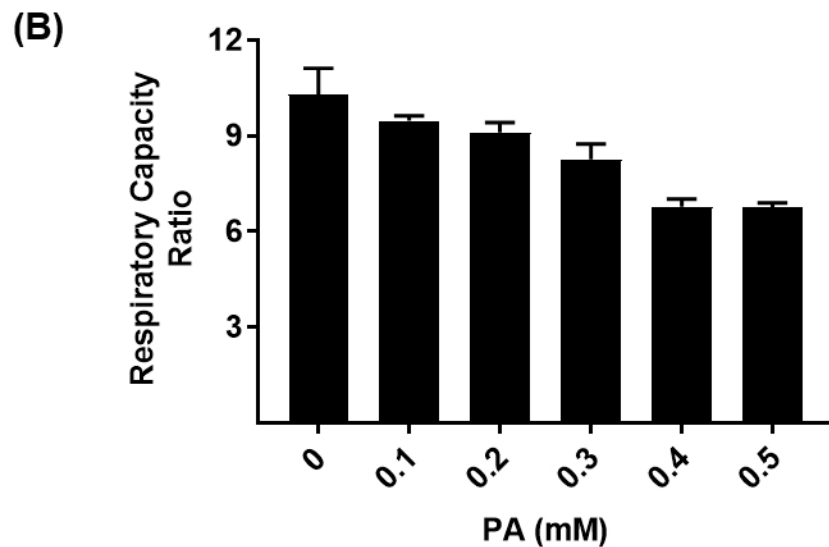
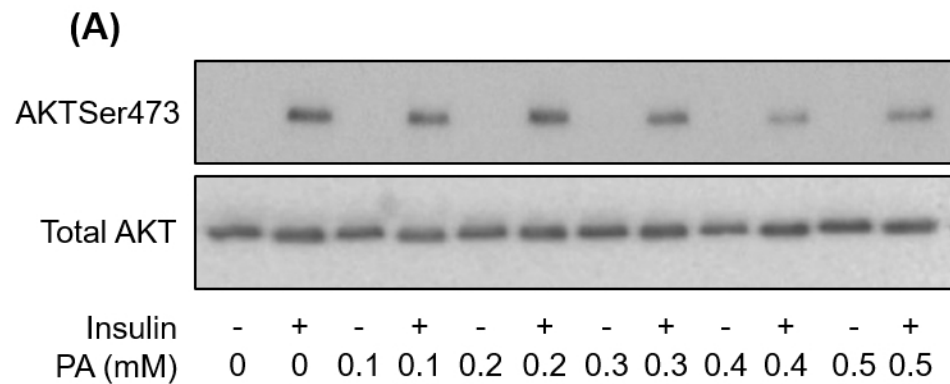
Raid B. Nisar Dinesh S. Shah Harinder S. Hundal

Division of Cell Signalling and Immunology, Sir James Black Centre, School of Life Sciences, University of Dundee, Dundee, UK



Supplementary Figure S1

Figure S1: Effect of varying the concentration of unsaturated fatty acids OL, LA or PO on NFκB-induced inflammatory signalling by palmitate. L6 myotubes were incubated for 16 h in presence of glucose with or without 0.4 mM palmitate in the absence or presence of OL, LA and PO at the indicated concentrations. IκBα cellular abundance was analysed by immunoblotting and the abundance of IL6 / β-actin mRNA measured by qPCR.



Supplementary Figure S2

Figure S2: Effect of palmitate (PA) dose on Akt activation/phosphorylation and mitochondrial function in L6 myotubes. Differentiated myotubes were incubated for 16 h in media containing glucose in the absence and presence of palmitate at the indicated doses (0.1-0.5 mM). At the end of this incubation period myotubes were incubated in the absence or presence of insulin (20 nM) for 10 min before being lysed and used for analysis of (A) Akt-Ser⁴⁷³ phosphorylation or total Akt analysis by immunoblotting or (B) non-insulin stimulated myotubes were used for assessing effects of PA on mitochondrial respiratory capacity by Seahorse assay at the concentrations indicated. Panel C shows the effect of incubating L6 myotubes in media containing 5 mM D-glucose supplemented with or without PA (0.4 mM), oleate (OL, 0.4 mM) for 16 h prior to analysis of total protein (by the Bradford method) or DNA (extracted using the Trizol method as per manufacturer's (Invitrogen) instructions) measured using a NanoDrop spectrophotometer.