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The PPARβ/δ ligand GW0742 inhibits oxidative metabolism target genes in the human endothelial EA.Hy.926 cell line.

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Activation of PPARβ/δ induces angiogenesis *in vitro* and *in vivo* (1). PPARβ/δ has also been implicated in the up-regulation of genes involved in lipid oxidative metabolism in tissues such as skeletal muscle (2). The role of PPARβ/δ in endothelial cell metabolism however is not known. Here we investigated activation of PPARβ/δ regulated genes involved in oxidative metabolism in EA.Hy 926 (EA.Hy) cells.

EA.Hy were cultured in DMEM supplemented with 10% FCS and 1% Penn/Strep and grown to 60-70% confluence. Experiments were performed in serum-free DMEM. Cells were incubated with the selective PPARβ/δ agonist GW0742 (1μM; (3)) or vehicle (DMSO; 0.01%) for 3h. The expression of the PPARβ/δ angiogenesis target gene angiopoietin like-4 protein (ANGPTL4) (3) along with 3 oxidative metabolism genes acyl-CoA dehydrogenase, very long chain (ACADVL), electron-transfer-flavoprotein, beta (ETFB) and lactate dehydrogenase B (LDHb) were measured by RT-PCR and normalised to β-actin.

ANGPTL4 was used as a positive control for PPARβ/δ activation (3), and GW0742 induced ANGPTL4 expression at 3h (Fig. 1). In contrast, GW0742 significantly reduced ACADVL, ETFB and LDHb mRNA expression (Fig. 2) by 3h. Data represents mean±S.E.M. fold change from vehicle control from n=4 independent experiments.* indicates $p<0.05$ between 3h and DMSO control (one sample t-test).

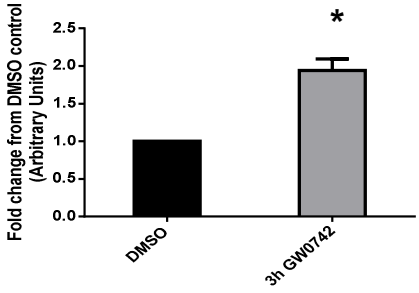

Figure 1: Change in mRNA expression for ANGPTL4 after 3 hour incubation with GW0742. * p = <0.05 vs. DMSO control.

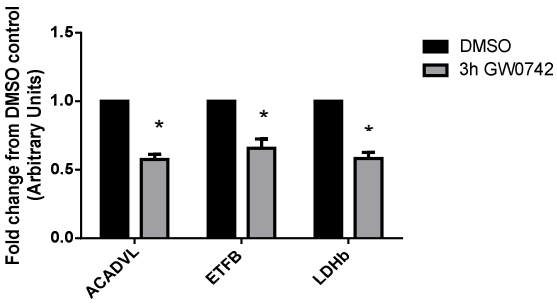

Figure 2: Change in mRNA expression for ACADVL, ETFB & LDHb after 3 hour incubation with GW0742.

*p = <0.05 vs. DMSO control.

Here we identify 3 novel endothelial PPARβ/δ ligand regulated genes involved in oxidative metabolism. Suppression of these pathways, particularly LDHb may favour local lactate production which has recently been implicated in hypoxia independent angiogenesis (4). The regulation of metabolism may therefore represent a novel pathway by which PPARβ/δ controls endothelial cell function.

1. Piqueras L, Reynolds AR, Hodivala-Dilke KM *et al.*, (2007) Arterioscler. Thromb. Vasc. Biol. 27:63-69
2. Kleiner S, Nguyen-Tran V, Baré O, *et al.*, (2009) J. Biol. Chem. 284:18624-18633
3. Capozzi M, McCollum GW, Savage SR, *et al.*, (2013) Invest. Ophthalmol. Vis. Sci. 54:4197-4207
4. Végran F, Boidot R, Michiels C, *et al.* (2011) Cancer Res. 71:2550-2560