SUPPLEMENTARY DATA

Structural basis for the activation of PPARγ by oxidized fatty acids

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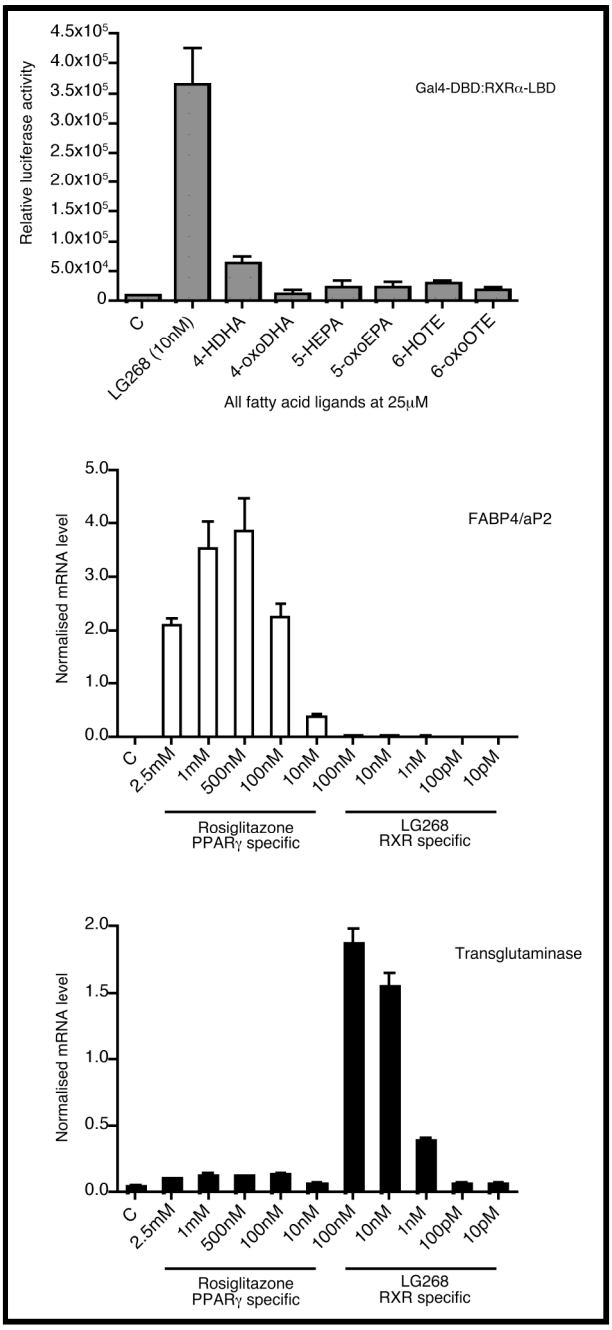
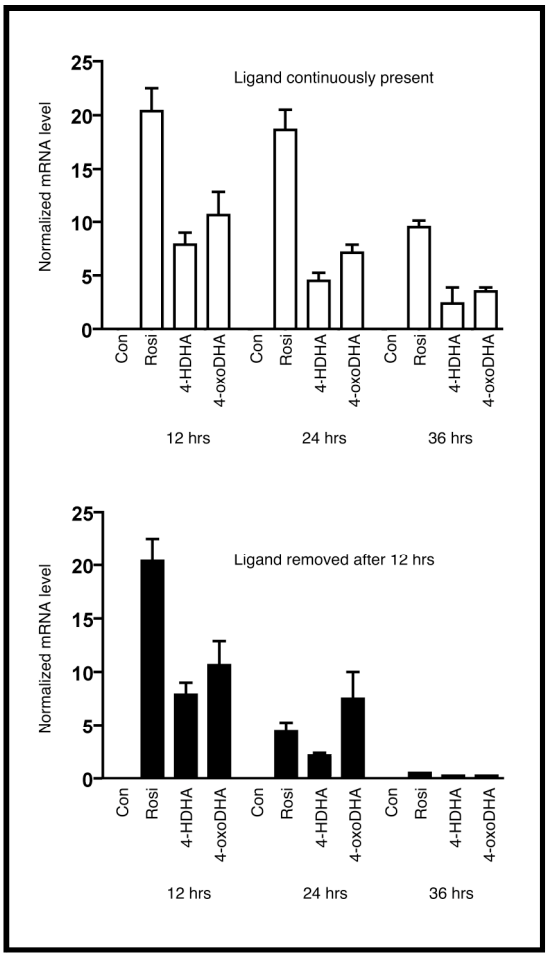


Supplementary Figure 1: Oxo fatty acids signal through PPARγ. The top panel shows the activation of a Gal4-DBD:RXRα-LBD chimera by the various oxidized fatty acids. The oxidized fatty acids at 25mM show negligible activation of RXR compared with activation by the RXR specific ligand LG268.
The middle and lower panels show that in dendritic cells, Rosiglitazone, but not LG268, results in activation of the FABP4/aP2, whereas LG268, but not Rosiglitazone, activates the RXR-specific target gene transglutaminase. These experiments support the interpretation that PPARγ is the mediator of activation by the various oxidized fatty acids. Error bars indicate s.d.



Supplementary Figure 2: Time course of activation by oxidized fatty acids. The top panel shows the continuous activation of FABP/aP2 in dendritic cells by Rosiglitazone and oxidized fatty acids. The lower panel shows essentially the same experiment but with the ligand being washed out after 12 hours treatment. Rosiglitazone was used at 1μM and both 4-HDHA and 4-oxoDHA were used at 50μM. Error bars indicate s.d. This experiment suggests that 4-oxoDHA may give a somewhat prolonged response compared with 4-HDHA and Rosiglitazone. Note that these data derive from one experiment. Other experiments did not show a significant difference between covalent and non-covalent ligands. This phenomenon therefore requires further investigation before firm conclusions can be reached.

Supplementary Methods

Protein expression and purification. The human PPARγ LBD (aa 204-477) was expressed using a modified pET30a vector with an N-terminal 6xHis tag cleavable by TEV protease. E.*coli* Rosetta (DE3), was freshly transformed with the plasmid and grown in two flasks containing 0.75L of 2xTY medium with 50 μg ml^{-1} kanamycin and 30 μg/ml chloramphenicol at 37 °C to an OD of 0.1. The cultures were then induced with 40 mM isopropyl-β-D-thiogalactopyranoside and further incubated at 20°C for 18h. Cells were harvested and resuspended in 30ml lysis buffer (50 mM Tris pH8, 500 mM NaCl, 1% v/v Triton X-100, 0.1% v/v mercaptoethanol and 1 tablet of Roche complete EDTA free). Cells were lysed by sonication, and the soluble fraction was isolated by centrifugation (18,000 x g for 20 min). The supernatant was applied to Ni-NTA agarose (Qiagen) and the resin thoroughly washed in lysis buffer with an addition 20mM imidazole. The PPARγ LBD was eluted with elution buffer (50 mM Tris pH8, 500 mM NaCl, 300 mM imidazole, 10% v/v glycerol and 0.1% v/v mercaptoethanol). 25 mg of TEV protease was added to the elution and the reaction incubated at RT for 18 h. The protein was dialysed overnight against dialysis buffer (30 mM Tris/Cl pH8, 50 mM NaCl, 5% v/v Glycerol, 1 mM Mercaptoethanol and 0.5 mM EDTA) and then loaded onto a Resource Q (6 ml) column (GE Healthcare) equilibrated with a 20 mM Tris-HCl buffer (pH 7.4) containing 1 mM TCEP and 1 mM AEBSF. The elution was performed by NaCl gradients in the starting buffer 0 to 0.5 M (60mL). The elution fractions were concentrated and loaded onto Superdex 200 gel-filtration column (GE Healthcare) equilibrated with 30 mM Tris/Cl pH8, 50 mM NaCl, 5% v/v Glycerol, 1 mM Mercaptoethanol and 0.5 mM EDTA.. The purified protein was concentrated to 8 mg ml^{-1}, which was estimated by measuring UV absorbance at 280 nm

Protein crystallization. All crystals were obtained through co-crystallization with the relevant ligand. Co-crystallization was performed by vapor diffusion at 22 °C using sitting drop made by mixing 1 mL of protein solution (8 mg ml^{-1}, in 20 mM Tris, 1 mM TCEP, 0.5mM EDTA, pH 8.0) with 1 mM ligand with 1 ml of reservoir solution (0.7 M sodium citrate, 0.1 M Tris, pH 8.0 or 7.4) under nitrogen atmosphere. The mixture was stored in the dark and prismatic crystals (100 to 200 μm) appeared after a few days. Crystals were flash-cooled in liquid nitrogen after a fast soaking in a cryoprotectant buffer (reservoir solution with glycerol 17% v/v).