**Anti-inflammatory effects of epoxyeicosatrienoic acids (EETs) and their corresponding diols against pro-inflammatory cytokine-toxicity in BRIN-BD11 cells.**

**Introduction:** CYP450-derived EETs display anti-inflammatory activity in cardiac models of inflammation, in part through activation of PPARγ and inhibition of NF-κB activation. Therefore we aimed to investigate the cytoprotective effects of EET regioisomers and their corresponding vicinal diols in a beta cell model of pro-inflammatory cytokine-toxicity.

**Methods:** BRIN-BD11 cells were treated with 100U/mL IL-1β, 20U/mL IFNγ and 500U/mL TNFα in co-incubation with either 10µM 8(9)-EET, 11(12)-EET, 14(15)-EET, or their dihydroxyeicosatrienoic acidderivatives (DHETs) for 24 hours. Cell viability was assessed by vital dye exclusion (Trypan Blue; expressed as viable cells/mL) or multicaspase-activity assay and NF-κB activity was measured using a NanoLuc® luciferase reporter assay.

**Results:** All EETs protected against cytokine-induced cell death, such that cytokines decreased viable cell number from 0.79x106 to 0.33x106 and in co-incubation with 8(9)-EET, 11(12)-EET and 14(15)-EET, this increased to 0.64x106, 0.61x106 and 0.59x106,respectively (p<0.05). Similarly, cytokine treatment increased caspase activity to 35%(+/-5.7), decreasing to 18%(+/-1.9), 19%(+/-2) and 19%(+/-1.9) in the presence of these EETs (p<0.05), accompanied by a 32% decrease in NF-κB activation. Of the corresponding diols only 8(9)-DHET attenuated cytokine-toxicity, reducing caspase activity from 41%(+/-4.5) to 21%(+/-2.4) (p<0.05).

**Conclusion:** EETs protected against cytokine-toxicity in BRIN-BD11 cells, in part via reduced activation of NF-κB. We also consider the novel observation that 8(9)-DHET, unlike other EET-derived DHETs, similarly protected against cytokine-induced apoptosis. This data highlights a potential role of EETs in attenuating cytokine-toxicity in type 1 diabetes and our ongoing work is exploring the production of endogenous EETs by CYP450 isoforms and actions of EET analogues.

**Exploring cytoprotective mechanisms of epoxyeicosatrienoic acids (EETs) and their attenuation of palmitate-induced lipotoxicity in BRIN-BD11 cells**

**Introduction:** Palmitate is a well-established contributor to beta cell death in type 2 DM, with effects ameliorated by polyunsaturated fatty acids, including arachidonic acid. We have also observed that EETs but not their corresponding diols attenuate palmitate toxicity in BRIN-BD11 cells. Our current work is exploring mechanisms of EET action through pharmacological inhibition of enzymes involved in fatty acid metabolism, antagonists of PPAR and RXR isoforms and with non-metabolisable analogues of fatty acids and EETs.

**Methods:** BRIN-BD11 cells were treated with 250µM palmitate in co-incubation with 8(9)-EET 11(12)-EET or 14(15)-EET in the presence or absence of a range of pharmacological inhibitors. Effects on the protective action of EETs against lipotoxicity were screened using the MTT assay, with confirmation of effects by vital dye exclusion (Trypan Blue) and multicaspase-activity assays with 8(9)-EET.

**Results:** Whilst EETs protected against palmitate-induced cell death, this was not attenuated either by inhibition of PPAR isoforms and the RXR transcription factor. Whether EETs mediate their activity as a result of increased rates of palmitate β-oxidation or incorporation into triglycerides were also explored, whereby Etomoxir (50µM; carnitine palmitoyl transferase-1 inhibitor) or Xanthohumol (5µM; diglyceride acyltransferase inhibitor) when used alone or in combination, failed to attenuate EET action.

**Conclusion:** Given that pharmacological inhibition of known EET-activated transcription factors and enzymes involved in fatty acid metabolism failed to alter the protective action of EETs against palmitate toxicity highlights the potential importance of these AA-derived CYP450 metabolites in beta cell pathophysiology. Our ongoing work is further exploring mechanisms of EET action via the use of non-metabolisable fatty acid analogues and antagonists of the putative EET receptor, as well as