Abstract
Activated hepatic stellate cells (HSCs) are a key contributor to liver fibrosis and drive the progression to advanced disease for many liver conditions, including non-alcoholic fatty liver disease. Previous studies suggest vitamin D may reduce inflammatory and profibrogenic activity of HSCs in vitro. However, the mechanisms underpinning the effects of vitamin D in HSCs are not fully understood. The overall aim of these experiments was to mimic a lipid loading model on immortalised HSCs to test their responses to 1α,25-dihydroxyvitamin D3 (1α,25(OH)2D3). Two different human immortalised cell lines: HepG2, hepatocellular carcinoma cells, and LX-2, hepatic stellate cells; were cultured using standard methods. Cell viability in different treatment vehicles (2% DMSO and/or 0.1% ethanol) under serum free conditions was measured by MTT assay after 6 and 24 h. Cells were cultured with increasing concentrations of fatty acids (0–500 μM, 1:1 oleic acid: palmitic acid) or vitamin D. Nile red, a neutral lipophilic fluorescent dye, was used to measure total intracellular lipid and quantified relative to vehicle. CYP24A1 mRNA expression was measured by qPCR in response to 1000nM 1α,25(OH)2D3 treatment in both cell lines for 24 h using TaqMan® gene expression assays and normalised to 18S rRNA. Cell viability in response to vehicle was examined at 6 h and 24 h to determine the optimal experimental time points. Whereas, HepG2 cells remained unaffected at 24 h in response to either or both vehicles combined (n = 4; combined vehicles, P = 0.3187), LX-2 cells showed reduced viability even at 6 h (n = 5; combined vehicles, P = 0.0050). Fatty acid treatment led to intracellular lipid accumulation in both cell lines. In response to 500μM fatty acid treatment, intracellular lipid increased by 1.7-fold in LX-2 cells at 6 h (n = 5, P = 0.00174) and 3.9-fold in HepG2 cells after 24 h (n = 4, P = 0.00184). Notably, CYP24A1 mRNA expression was markedly induced by vitamin D treatment in LX-2 cells (136 ± 7.64-fold, n = 3, P = 0.0010) in comparison to HepG2 cells (22 ± 0.78-fold, n = 3, P < 0.0001). In summary, the cell viability data suggested optimal time points for both fatty acid and vitamin D treatments may be 6 h for LX-2 cells, and 24 h for HepG2 cells. While intracellular lipid accumulation differed between the cell lines in response to fatty acid treatment, both cell lines produced a dose-dependent increase in intracellular lipid. Lastly, CYP24A1 mRNA expression confirmed the responsiveness of both cell types to vitamin D treatment. Ongoing experiments are examining microRNA expression in HSCs in response to both vitamin D and lipid loading.

Conflict of Interest
There is no conflict of interest