



Effect of vitamin D supplementation on the induction of pro-inflammatory cytokines (il-8) from hepatocytes

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Non-alcoholic fatty liver disease (NAFLD), typified by the accumulation of lipids in hepatocytes, is strongly associated with obesity and the metabolic syndrome with a hallmark of insulin resistance (IR)⁽¹⁾. Simple steatosis can progress to non-alcoholic steatohepatitis (NASH) which may lead to cirrhosis and hepatocellular carcinoma¹. It has been documented that inflammation, pro-inflammatory cytokines and oxidative stress play key roles in the pathogenesis of NAFLD^(1,2). Evidence from previous studies has shown an inverse relationship between serum 25(OH) D levels and IR, diabetes risk and metabolic syndrome⁽³⁾, suggesting a possible role for vitamin D in the pathogenesis of NAFLD. Previous studies have demonstrated that fatty acids: oleic acid (OA) and palmitic acid (PA) induce significant lipid accumulation in hepatocytes while PA induces significant neutrophil chemoattractant, IL-8 in hepatocytes⁽²⁾. The specific aim of this study was to investigate the effect of vitamin D supplementation / pre-treatment on the release of pro-inflammatory cytokines (IL-8).

Lipid accumulation was induced in hepatocytes (HEPG2 cells) by exposing cells to pathophysiological levels of oleic acid and palmitic acid. This was confirmed by fluorescent microscopy following Nile Red staining and Nile red assay for the quantification of lipid. HEP G2 cells were grown in Dulbecco Modified Eagle's Medium (DMEM) (low glucose) in the presence or absence of 50nM or 100nM 1, 25 (OH) D₃ (vit. D₃). At 70% confluency, cells were then treated with either Dimethylsulphoxide (DMSO), 400 mM PA or 500 mM OA for 24 hours. Cell-free culture supernatants were then collected and IL-8 levels were measured by enzyme-linked immunosorbent assay (ELISA). Cells were also treated with TNF – alpha which served as a positive control. Data were collected from four independent experiments.

Palmitic acid induced a significant level of IL-8 production in the hepatocytes ($P < 0.001$) while the induction of IL-8 production by OA was similar to DMSO (solvent control). There was a (non-significant) trend for a reduction in IL-8 levels after vitamin D supplementation ($P = 0.051$). Furthermore, there was a significant difference in the effect of 50nM compared to 100nM vit D₃ pre-treatment on the treated cells ($P = 0.033$).

This (to our knowledge) is the first study to report the effect of vitamin D supplementation on induction of IL-8 cytokines in hepatocytes. Overall, the study showed a trend for a reduction in IL-8 levels following vit. D₃ supplementation, but this just failed to reach statistical significance. Further research is certainly warranted as to the potential beneficial effects of vitamin D₃ on the induction of pro-inflammatory cytokines (IL-8).

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