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Characterisation of an *in vitro* model for proteomic profiling of progressive steatosis in human hepatocytes

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Non-alcoholic fatty-liver disease (NAFLD) has recently emerged as the most common reason for referral to hepatology clinics[(1)]. A primary concern is to identify those patients who have or will develop progressive non-alcoholic steatohepatitis (NASH), which leads to fibrosis, cirrhosis and potentially death from end-stage liver disease. Although liver biopsy is the diagnostic 'gold standard' for NASH, because of its limitations and associated morbidities the need for a non-invasive biomarker to diagnose and stage fatty-liver disease remains[(2)].

The objectives of these experiments were to characterise an *in vitro* model of progressive hepatic steatosis and to begin developing a robust quantitative proteomic profiling strategy for biomarker discovery utilizing this model. The culturing of HuH7 human hepatocytes with increasing concentrations of palmitate produced a dose-dependent accumulation of intracellular lipid, as visualised by Oil Red O staining. Treating hepatocytes with 150 and 200 μM-palmitate caused a 4.3- and 5.5-fold increase in intracellular TAG levels that was highly reproducible in replicate (*n* 5) experiments (Figure; A). As palmitate is also recognised to have lipoapoptotic effects, its cytotoxicity was assessed over 48 h using the lactate dehydrogenase assay (Figure; B). By 24 h dose-dependent cytotoxicity was seen for 100, 150 and 200 μM-palmitate-treated cells, while cells treated with the physiological dose of palmitate were no different from those cultured with vehicle (dimethyl sulfoxide and fatty acid-free BSA) or serum-containing media (control).

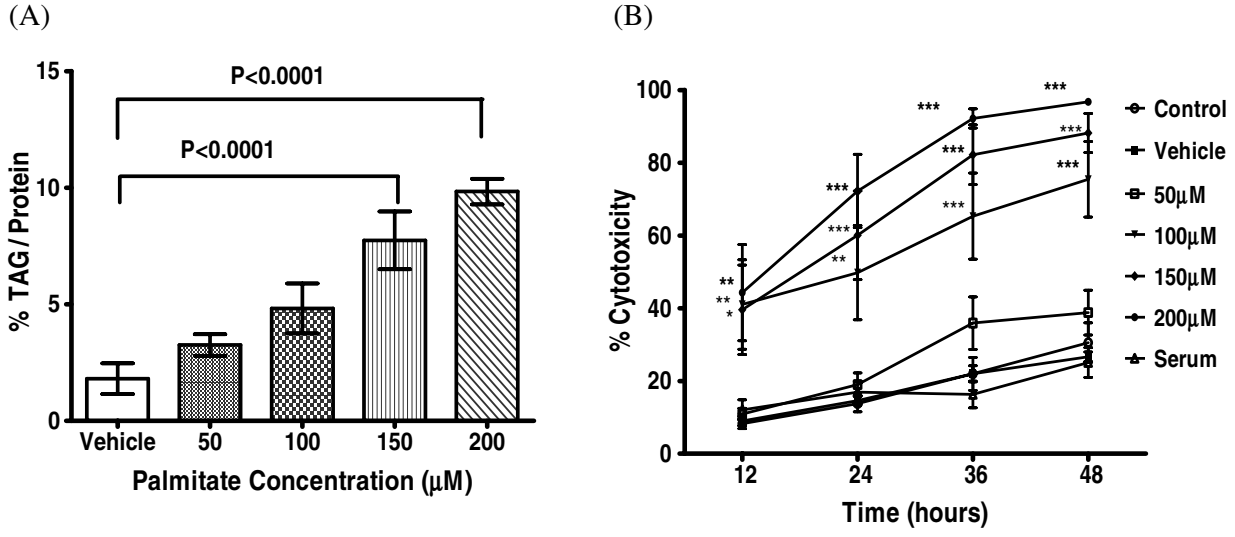

Figure. Palmitate concentration induces progressive lipid accumulation and cytotoxicity in HuH7 human hepatocytes. (A) Hepatocyte intracellular TAG levels after palmitate treatment. Values are means with their standard errors represented by vertical bars for five experiments. Statistical analysis was by one-way ANOVA and Dunnett's multiple comparison. (B) Palmitate-induced cytotoxicity. Values are means with their standard errors represented by vertical bars for four experiments. Means for 100, 150 and 200 μM were significantly different from those for the vehicle (two-way repeated measures ANOVA): $*P<0.05$, $**P<0.01$, $***P<0.001$.

Palmitate treatment of HuH7 human hepatocytes induced progressive lipid accumulation observed both by Oil Red-O staining and TAG assay. Additionally, assessment of palmitate-induced cytotoxicity using the lactate dehydrogenase assay indicated a dose response. Quantitative proteomics methodologies are now being optimised for biomarker discovery using this model system before running precious clinical samples. Isobaric tags for relative and absolute quantification are used in a multiplexing approach along with two-dimensional liquid chromatography–MS–MS in order to identify potential protein changes associated with increasing levels of steatosis.

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