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Examination of methylglyoxal levels in an *in vitro* model of steatosis and serum from patients with non-alcoholic fatty liver disease

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This abstract was presented as the Cellular and Molecular Nutrition Theme highlight.

Elevated levels of methylglyoxal (MG), a highly reactive glycating agent forming advanced glycation endproducts (AGEs), have been associated with diabetes, obesity and vascular disease[(1)]. However, its role in the hepatic manifestation of metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), is still a novel inquiry. The objective of these experiments was to assess MG levels in response to lipid loading in the liver.

Immortalised human hepatocytes (HepG2 cells) cultured in physiological levels of glucose (5 mM) were treated with either saturated (400 μM palmitic acid, PA) or mono-unsaturated (500 μM oleic acid, OA) fatty acids ($n = 3$). Fatty acid-induced lipid loading was confirmed by Nile red staining. MG content of cells and in culture medium was measured by stable isotopic dilution liquid chromatography-mass spectrometry (LC-MS/MS)[(2)]. The MG-derived major AGE, hydroimidazolone MG-H1, was assessed by competitive ELISA in serum samples from a cohort of biopsy-confirmed adult NAFLD patients ($n = 62$). Sample collection was under full NHS ethical approval and conducted in accordance with the Declaration of Helsinki. One-way ANOVA with Dunnett's test was used to analyse the *in vitro* data. Pearson or Spearman correlations were used to examine MG-H1 relationship to histological features and clinical biochemistries, followed by multiple linear regression analyses.

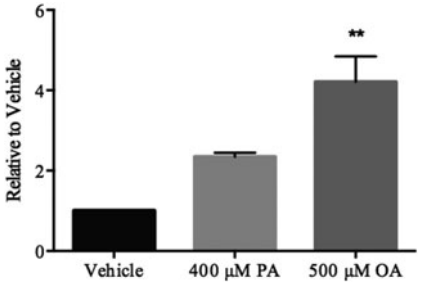

Fig. 1. Cellular Lipid

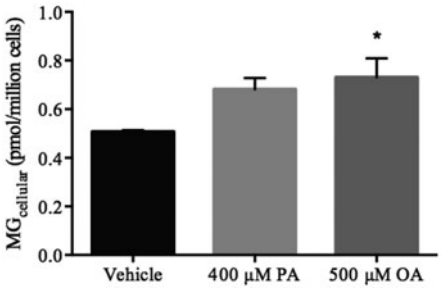

Fig. 2. Cellular Methylglyoxal

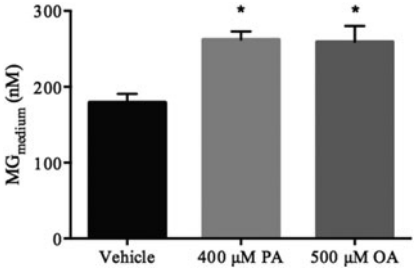

Fig. 3. Medium Methylglyoxal

Fatty acid treatment resulted in a 4-fold increase of intracellular lipid in the OA-treated cells (Fig. 1; $P < 0{\cdot}01$). MG increased by 44 % in OA-treated cells compared to vehicle (Fig. 2; $P < 0{\cdot}05$), while culture medium MG increased by 46 % and 45 % in PA- and OA-treated cells, respectively (Fig. 3; $P < 0{\cdot}05$). Serum MG-H1 ($n = 59$) was inversely correlated with alanine aminotransferase levels, lobular inflammation and hepatocyte ballooning ($P < 0{\cdot}001$). MG-H1 was positively correlated with body mass index (BMI) ($P < 0{\cdot}0001$) however, there was no correlation between MG-H1 and steatosis or fibrosis score and, surprisingly, in this cohort BMI was inversely correlated to inflammation and ballooning. The multiple regression analyses resulted in no conclusive relationships between MG-H1 and variables to predict NAFLD activity.

Accumulation of MG, as measured by LC-MS/MS, in fatty acid-treated cells and their associated medium suggests lipid accumulation increases MG formation and/or decreases MG metabolism. In contrast, serum MG-H1 from patients with fatty liver measured by ELISA did not correlate with extent of steatosis. If these findings can be corroborated by robust measurement of MG-H1 by LC-MS/MS, increased MG and AGEs formation in hepatic steatosis *in vivo* may be localised to the liver where proteolysis likely releases increased MG-H1 free adduct into plasma.

1. Rabbani N & Thornalley PJ (2011) *Sem Cell Dev Biol* **22**, 309–317.
2. Thornalley PJ & Rabbani N (2014) *Biochim Biophys Acta* **1840**, 818–819.