Oxidative stress rather than triglyceride accumulation perturbs glutathione metabolism in an in vitro model of cellular steatosis

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Oxidative stress rather than triglyceride accumulation perturbs glutathione metabolism in an in vitro model of cellular steatosis

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Introduction: Oxidative stress is the central to molecular events leading to the progression of simple steatosis to steatohepatitis in nonalcoholic fatty liver disease (NAFLD). We have previously shown that an in vitro cellular steatosis model using C3A cells treated with energy substrates; lactate (L), pyruvate (P), octanoate (O) and ammonia (N), recapitulates the sequence of events in dietary-induced NAFLD; namely enhanced acute respiration and reactive oxygen species (ROS) formation leading to mitochondrial impairment. In contrast, treatment with oleate results in similar triglyceride accumulation but with relatively low ROS. Using a combined microarray, proteomic and metabolomic approach, we aimed to explore how triglyceride accumulation and enhanced ROS affect glutathione metabolism in our in vitro cell model.

Methods: C3A cells were treated with either LPON or oleate for 72 hours. Microarray RNA expression was measured using Illumina® Whole Human Genome BeadChip H12 Microarray. For proteomics, peptides were analysed by liquid chromatography (LC) coupled mass spectrometry (MS) (Agilent HPLC/OrbitrapXL). Data were quantified label-free using Progenesis LC-MS and MASCOT. For metabolomics, LC separation was performed using hydrophilic interaction chromatography with a ZIC–HILIC. MS was performed using Orbitrap Exactive with HESI 2 probe. Raw LC/MS data were processed with XCMS Centwave and mzMatch.

Results: LPON led to 2-fold downregulation of GCLC (encodes glutamate-cysteine ligase catalytic subunit, the rate limiting enzyme for glutathione synthesis) and upregulation of GPX1 and TXNDC12. Expression of GCLC and TXNDC12 was unchanged with oleate. Metabolomics confirmed that oxidised glutathione, glutathione disulfide, was higher in LPON- than oleate-treated cells. Among glutathione S-transferase genes, GSTA1 was unchanged with oleate but was upregulated by LPON (2.4-fold). Similarly, GSTT1, GSTK1 and GSTO1 were significantly increased by LPON. In contrast, MGST2 expression was higher in oleate than LPON-treated cells. Finally, proteomics showed that microsomal glutathione S-transferase 2 was downregulated by 2.5-fold by LPON.

Conclusion: Our data show that increased ROS formation rather than triglyceride accumulation alters glutathione metabolism. Such alterations may influence susceptibility to further insults, particularly those accelerating glutathione depletion, for example, paracetamol overdose.

Disclosure of Interest: None Declared