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Hepatocyte-specific high-mobility group box 1 (HC-HMGB1) protects against liver fat accumulation and cellular stress during high fat diet feeding

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Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver disorders worldwide. It is associated with obesity, insulin resistance, and type 2 diabetes, and covers a wide range of liver changes, from simple steatosis and non-alcoholic steatohepatitis (NASH) to liver cirrhosis and hepatocellular carcinoma. HMGB1 is a potent inflammatory mediator involved in several liver diseases, but its role in NAFLD and NASH is not fully understood. Here we investigated the role of hepatocyte-specific HMGB1 (HC-HMGB1) in regulating hepatic steatosis via regulation of hepatocyte metabolism, and intrinsic cellular stress pathways.

Methods: Wild type (WT) C57BL/6 and HC-HMGB1$^{-/-}$ mice were fed high-fat diet (HFD) (45%kcal from fat) or low-fat diet (LFD) (10%kcal from fat) for up to 16 weeks. Body weight was monitored weekly, and body fat composition, glucose tolerance, and energy expenditure were measured at the end of the experiment. Liver fat accumulation was determined by Oil Red staining of liver sections. Liver damage was assessed by H&E and serum ALT levels. Inflammation was determined by serum IL-6 concentrations. Liver expression of b-oxidation enzymes were determined by PCR, and ER-stress markers measured by WB of whole liver lysates. *In vitro* primary mouse hepatocytes from WT and HC-HMGB1$^{-/-}$ mice were exposed to 800μM palmitic acid (PA) for 6, 24, 36h, with lipid uptake, β-oxidation and ER stress markers analyzed. We also overexpressed HMGB1 in HC-HMGB1$^{-/-}$ hepatocytes and repeated *in vitro* experiments repeated.

Results: HC-HMGB1 deficiency promoted rapid HFD-induced weight gain and obesity by 2 weeks, with enhanced hepatic fat deposition that increased up to 16 weeks. There was no difference between WT and HC-HMGB1$^{-/-}$ mice in glucose tolerance or energy expenditure at any time point measured. HC-HMGB1 deficiency significantly reduced circulating HMGB1 (64.6% decrease vs WT HFD group; $p<0.05$), but did not influence liver damage (ALT) or systemic inflammation (IL-6). Hepatic gene expression related to FFA β-oxidation was significantly down-regulated in HC-HMGB1-/-mice compared with WT (CPT-1α, MCAD, LCAD and VLCAD gene expression decreased by 43.2%, 43.2%, 62.2% 46.2% and 55.4% respectively; $p<0.05$ for each gene). Interestingly, ER stress markers (e.g. CHOP and cleaved ATF6), were significantly increased in livers of HC-HMGB1$^{-/-}$ mice vs WT in HFD groups. In vitro, HMGB1-deficient hepatocytes had increased intracellular lipid accumulation, which was accompanied by ER stress and was associated with reduced mitochondrial oxidative phosphorylation (measured by Seahorse) and significant downregulation of FFA β-oxidation. In contrast to the WT mouse hepatocytes exposed to PA treatment, hepatocellular gene expression of CPT-1α, MCAD, LCAD and VLCAD decreased by 36.3%, 40.7%, 38.0% 46.2% and 30.3% respectively in HC-HMGB1$^{-/-}$ group ($p<0.05$ for each gene). Overexpression of HMGB1 in HC-HMGB1$^{-/-}$ mouse hepatocytes attenuated FFA-induced excessive lipid accumulation and ER stress.

Conclusion: These data suggest HC-HMGB1 protects against dysregulated lipid metabolism via maintenance of β-oxidation and prevention of ER stress. Our finding uncovers a novel mechanism for HMGB1-regulation of hepatocellular steatosis.