Deficiency of Antimicrobial Peptide Cathelicidin Attenuated High Fat Diet plus Alcohol-induced Liver Injury through Regulation of FGF21/Adiponectin Signaling and Adipose Tissue Lipolysis

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Introduction

Alcohol consumption and obesity are known risk factors of steatohepatitis, which can lead to liver cirrhosis and eventually hepatocellular carcinoma (HCC). The combination of a high fat diet (HFD) and acute alcohol intake synergistically induce fat deposition and inflammation in the liver. CRAMP (Cathelicidin-related antimicrobial peptide), the murine ortholog of LL-37, the only known member of human cathelicidin antimicrobial peptide family, possesses both anti- and pro-inflammatory activity. Our previous studies showed that CRAMP deficiency exacerbated binge-on-chronic alcohol-induced liver injury. Here we aimed to investigate the role of CRAMP in HFD plus acute alcohol-induced liver injury using CRAMP knockout mice, and to explore the underlying mechanisms. Our results showed that CRAMP deficiency protected mice from HFD plus acute alcohol-induced fatty liver and liver injury through FGF21/adiponectin signaling and adipose tissue lipolysis.

Methods

CRAMP KO mice (*Camp*$^{-/-}$) and wild type (WT) mice were fed a control diet (CD) or a HFD for 10 weeks, after which a bolus of alcohol was gavaged 9 hours before sample harvesting. The mice were divided into 4 groups: WT CD+E, WT HFD+E, *Camp*$^{-/-}$ CD+E and *Camp*$^{-/-}$ HFD+E, with 5 mice in each group. Body weights were recorded weekly and before harvesting. The liver weight and the epididymal white adipose tissue (eWAT) weight were also recorded. Liver injury was examined via serum transaminase measurements. Liver and eWAT tissues were embedded with paraffin and sliced for histological analysis. mRNA and protein levels in liver, eWAT and brown adipose tissue (BAT) were determined by q-PCR and western blot. Serum adiponectin levels were determined by ELISA. The data was analyzed using one way ANOVA or student *t*-test where appropriate.

Results

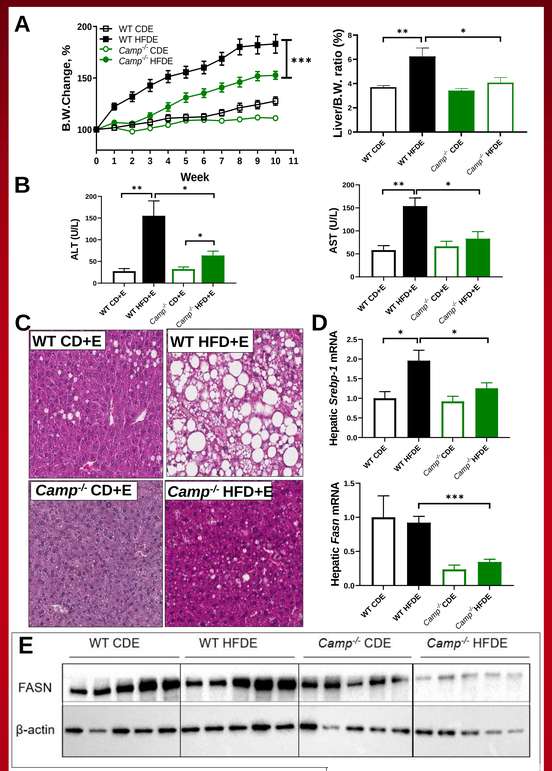

Fig. 1. *Camp* depletion inhibited liver steatosis and injury, as well as adipose hypertrophy by HFD+E. (A) Body weights and liver/body weight ratio. (B) Serum ALT and AST levels. (C) Representative microphotographs of Haemotoxylin and Eosin (H & E) stained mouse liver sections. (D) Hepatic mRNA expression of *Srebp-1* and *Fasn*. (E) Liver FASN protein levels. (F) H & E staining of eWAT tissue. (G) eWAT weights. Data are expressed as mean ± SEM, (n=5). *p<0.05, **p<0.01, ***p<0.001.

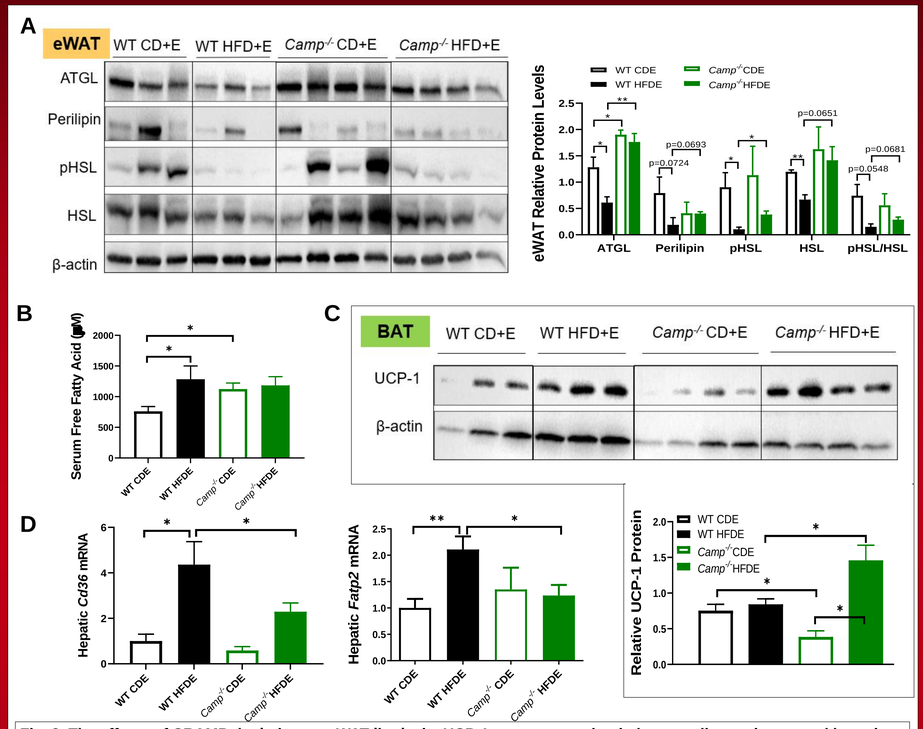

Fig. 2. The effects of CRAMP depletion on eWAT lipolysis, UCP-1 gene expression in brown adipose tissue, and hepatic lipid transporter gene expression in mice fed with HFD plus ethanol. (A) Protein levels of lipolytic enzymes and molecules in eWAT tissues. (B) Serum free fatty acid (FFA) levels. (C) BAT UCP-1 protein level. (D) Hepatic mRNA expression of *Cd36* and *Fatp2*. Data are expressed as mean ± SEM, (n=3-4). *p<0.05, **p<0.01.

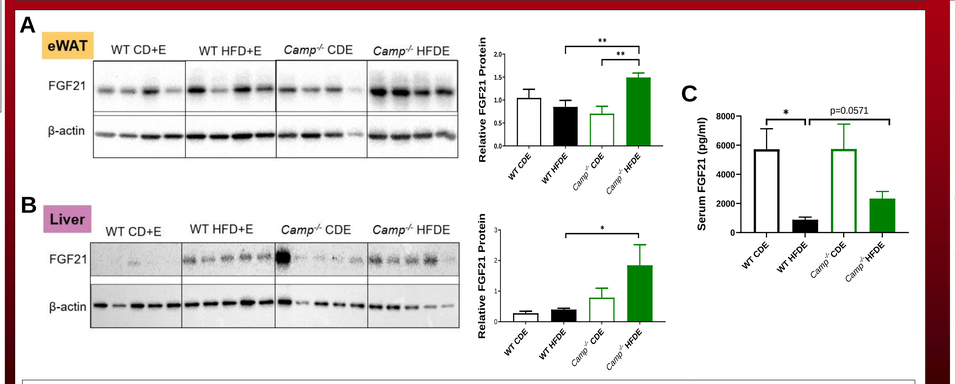

Fig. 3. HFD feeding plus ethanol increases FGF21 protein levels in eWAT and liver of *Camp*$^{-/-}$ mice. (A) eWAT and (B) hepatic FGF21 protein levels. (C) Serum FGF21 levels. Data are expressed as mean ± SEM, (n=4-5). *p<0.05, **p<0.01.

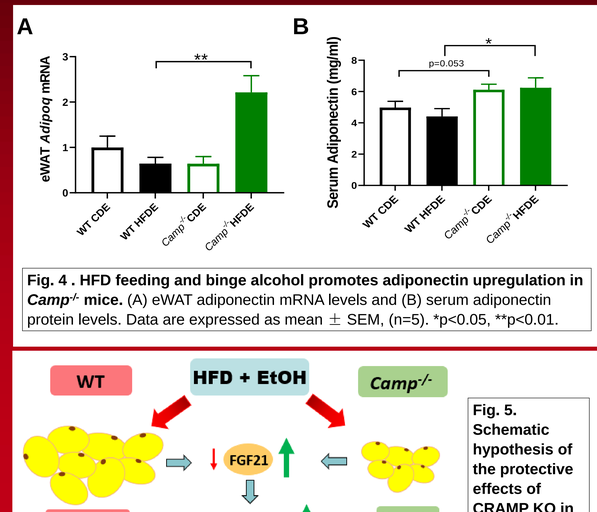

Fig. 4 . HFD feeding and binge alcohol promotes adiponectin upregulation in *Camp*$^{-/-}$ mice. (A) eWAT adiponectin mRNA levels and (B) serum adiponectin protein levels. Data are expressed as mean ± SEM, (n=5). *p<0.05, **p<0.01.

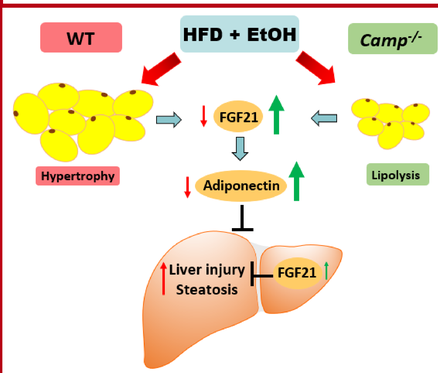

Fig. 5. Schematic hypothesis of the protective effects of CRAMP KO in upregulating adiponectin and preventing liver injury and steatosis in mice fed with HFD and alcohol.

Summary

1. ***Camp*$^{-/-}$ mice gained significantly less weight and had lower liver/body weight ratio and eWAT weight than WT mice after HFD plus alcohol feeding.**
2. **HFD feeding primed the liver to binge alcohol induced-liver injury and steatosis in WT mice but not in *Camp*$^{-/-}$ mice.**
3. **Deficiency of *Camp* partially prevented adipocyte hypertrophy induced by HFD plus alcohol treatment through increased lipolysis.**
4. **Deficiency of *Camp* increased adipose FGF21 expression leading to increased adiponectin production.**
5. ***Camp*$^{-/-}$ mice are protective against HFD plus alcohol-induced liver fat accumulation and liver injury through adipose lipolysis and FGF21/adiponectin production.**
6. **Targeting CRAMP could be an effective approach for prevention/treatment of high fat diet plus alcohol consumption-induced steatohepatitis.**

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