ABLATION OF HMGB1 IN INTESTINAL EPITHELIAL CELLS CAUSES INTESTINAL LIPID ACCUMULATION AND REDUCES NON-ALCOHOLIC STEATOHEPATITIS IN MICE

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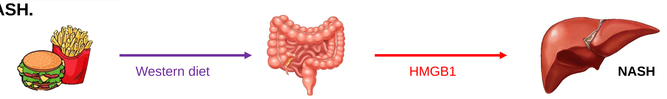
Introduction

Non-Alcoholic Steatohepatitis (**NASH**) affects ~25-30% of the US population [1], with 10-20% progressing to more severe disorders such as fibrosis [2], cirrhosis or hepatocellular carcinoma [3,4]. The mechanism underlying the progression of fatty liver disease is unclear, however the **gut-liver axis** plays a key role in liver homeostasis and may contribute [5].

High mobility group box-1 (**HMGB1**) is a damage associated molecular pattern involved in numerous liver disorders [6], where it is released from damaged hepatocytes [7] and activated immune cells [8]. Furthermore, it is secreted from intestinal epithelial cells (**IEC**) during local injury [9] where it contributes to gut inflammation [10]. However, the role of HMGB1 in NASH has yet to be investigated in detail.

We hypothesized that **HMGB1 from IEC** may be involved in the **onset and progression of NASH.**



Our aim was to determine the role of gut derived HMGB1 in the pathogenesis of diet-induced NASH

Methods

We used mice with conditional *Hmgb1* ablation in IEC (*Hmgb1*$^{\Delta IEC}$) and Cre^{+} littermates as controls. Mice were fed a high-fat, high-cholesterol and high-fructose (HFCF) diet or an equicaloric control diet for 1 or 24 wk. The Kleiner scoring system for NASH was used.

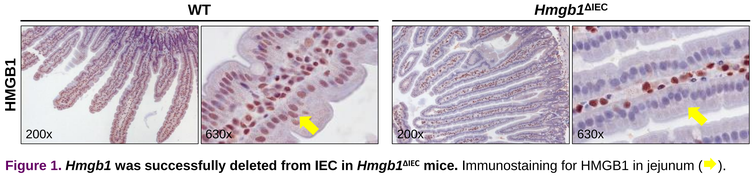
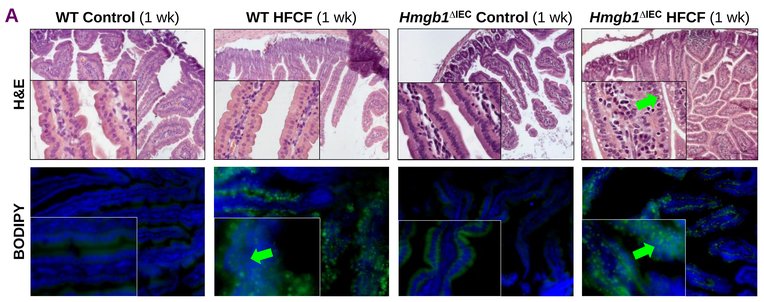


Figure 1. *Hmgb1* was successfully deleted from IEC in *Hmgb1*$^{\Delta IEC}$ mice. Immunostaining for HMGB1 in jejunum ( ).

Results



Parameter	WT Control (1 wk)	WT HFCF (1 wk)	*Hmgb1*$^{\Delta IEC}$ Control (1 wk)	*Hmgb1*$^{\Delta IEC}$ HFCF (1 wk)
Steatosis (% total area)	0.0 ± 0.0	1.7 ± 1.1	0.0 ± 0.0	**18.6 ± 2.7**
Villi length (µm)	380.2 ± 74.5	328.4 ± 34.5	337.7 ± 25.5	230.2 ± 13.1

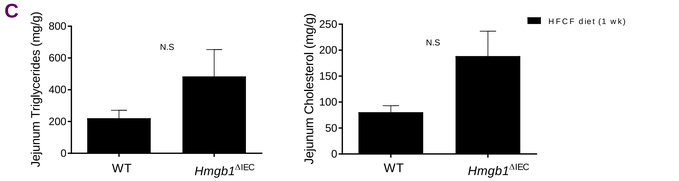
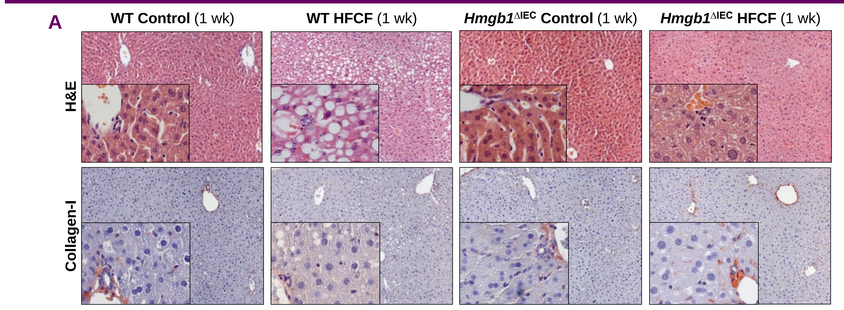


Figure 2. *Hmgb1*$^{\Delta IEC}$ mice display lipid accumulation in IEC after only 1 wk on HFCF diet. WT or *Hmgb1*$^{\Delta IEC}$ mice fed HFCF or control diet for 1 wk. Jejunum H&E and BODIPY lipid stain (green,) 200x and 630x (inset) images **(A)**, and corresponding pathology scores **(B)** show steatosis in IEC in *Hmgb1*$^{\Delta IEC}$ mice. Triglyceride and cholesterol content of jejunum increased in *Hmgb1*$^{\Delta IEC}$ mice **(C)**. Data are expressed as mean ± SEM (n=6).



Parameter	WT Control (1 wk)	WT HFCF (1 wk)	*Hmgb1*$^{\Delta IEC}$ Control (1 wk)	*Hmgb1*$^{\Delta IEC}$ HFCF (1 wk)
Steatosis (% total area)	1.66 ± 1.22	**22.76 ± 7.73** $^{+++}$	3.0 ± 1.57	**2.68 ± 0.93** ***
Inflammation (0-3)	0.11 ± 0.05	**0.97 ± 0.67** $^{+}$	0.36 ± 0.08	**0.04 ± 0.02** *
Ballooning (0-3)	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Fibrosis (0/1/A/B/C/2/3/4)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NASH score	0.45 ± 0.28	**1.17 ± 0.86**	0.3 ± 0.08	**0.05 ± 0.02**

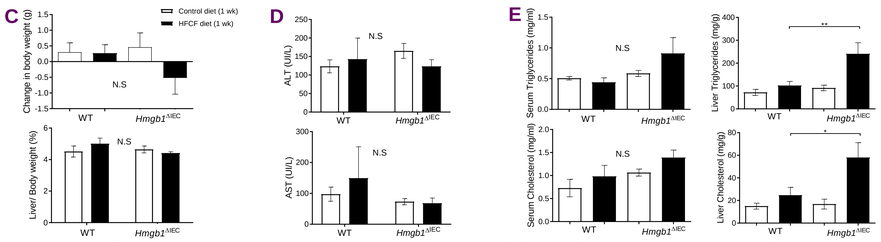
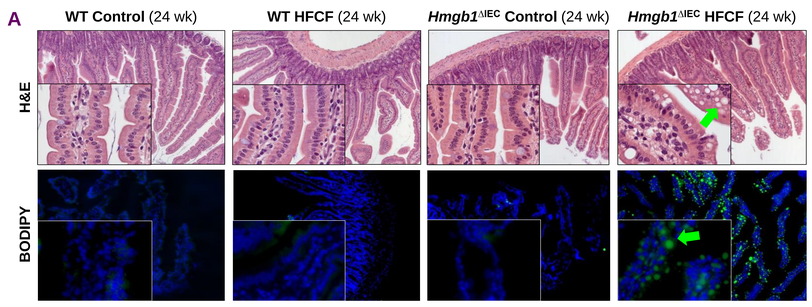


Figure 3. *Hmgb1*$^{\Delta IEC}$ mice are protected from hepatic steatosis after 1 wk on HFCF diet. WT and *Hmgb1*$^{\Delta IEC}$ mice fed HFCF or control diet for 1 wk. Liver H&E and collagen-I immunostaining 200x and 630x (insert) images **(A)**, and the corresponding pathology scores **(B)** show decreased steatosis and inflammation in *Hmgb1*$^{\Delta IEC}$ mice. Body weight change and liver to body weight ratio **(C)** did not increase in *Hmgb1*$^{\Delta IEC}$ mice. ALT and AST activities were not significantly changed **(D)**. Serum and liver triglycerides and cholesterol **(E)**. Data are expressed as mean ± SEM (n=6). $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 compared to WT Control 1 wk. *p<0.05, **p<0.01, ***p<0.001 compared to WT HFCF 1 wk.



Parameter	WT Control (24 wk)	WT HFCF (24 wk)	*Hmgb1*$^{\Delta IEC}$ Control (24 wk)	*Hmgb1*$^{\Delta IEC}$ HFCF (24 wk)
Steatosis (% total area)	0.0 ± 0.0	10.0 ± 10.0	0.0 ± 0.0	**21.4 ± 4.8**
Villi length (µm)	353.5 ± 8.2	347.2 ± 27.4	370.8 ± 16.5	404.2 ± 12.8

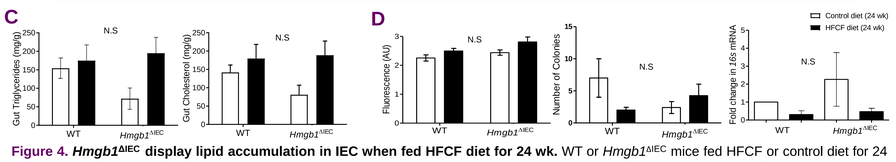
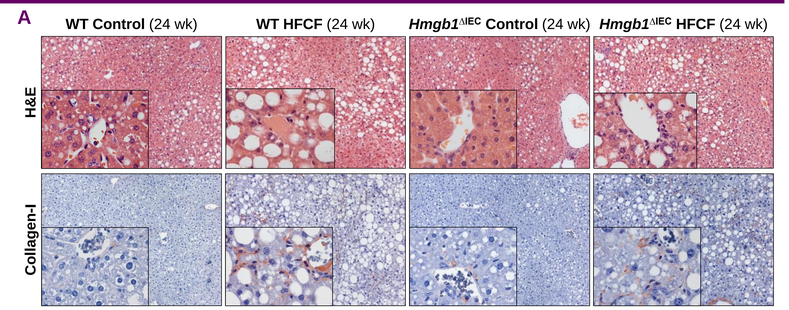


Figure 4. *Hmgb1*$^{\Delta IEC}$ display lipid accumulation in IEC when fed HFCF diet for 24 wk. WT or *Hmgb1*$^{\Delta IEC}$ mice fed HFCF or control diet for 24 wk. Jejunum H&E and BODIPY lipid stain (green) 200x and 630x (inset) images **(A)**, corresponding pathology scores **(B)** show steatosis in *Hmgb1*$^{\Delta IEC}$ mice. Gut triglyceride and cholesterol values **(C)**. Gut permeability analyzed by FITC-dextran florescence in portal serum, number of bacterial colonies in the liver and fold change in liver bacterial 16S mRNA **(D)** show no difference between genotypes. Data are expressed as mean ± SEM (n=10).



Parameter	WT Control (24 wk)	WT HFCF (24 wk)	*Hmgb1*$^{\Delta IEC}$ Control (24 wk)	*Hmgb1*$^{\Delta IEC}$ HFCF (24 wk)
Steatosis (% total area)	14.42 ± 5.4	**75.23 ± 6.6** $^{++++}$	47.23 ± 6.7	65.1 ± 15.7
Inflammation (0-3)	1.03 ± 0.27	**1.88 ± 0.26**	0.61 ± 0.11	2.07 ± 0.50
Ballooning (0-3)	0.00 ± 0.00	**0.72 ± 0.17** $^{++++}$	0.01 ± 0.01	0.73 ± 0.18
Fibrosis (0/1/A/B/C/2/3/4)	0.41 ± 0.27	**1.08 ± 0.34**	0.14 ± 0.07	0.71 ± 0.17
NASH score	2.07 ± 0.52	**6.87 ± 1.9** $^{++++}$	2.75 ± 0.33	5.86 ± 1.42

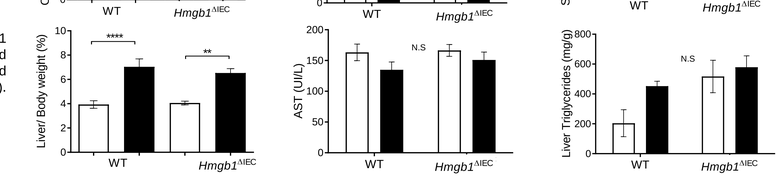


Figure 5. *Hmgb1*$^{\Delta IEC}$ mice are not protected from NASH after 24 wk on a HFCF diet. WT or *Hmgb1*$^{\Delta IEC}$ mice were fed HFCF or control diet for 24 wk. Liver H&E and collagen-I immunostaining 200x and 630x (insets) images **(A)** and corresponding pathology scores **(B)** show that mice developed similar NASH features in both genotypes. Body weight change and liver to body weight ratio **(C)**, liver injury indicated by ALT and AST activities **(D)**, and serum and liver triglycerides **(E)**, were not significantly different between genotypes. Data are expressed as mean ± SEM (n=10). $^{++++}$p<0.0001 compared to WT Control 24 wk. **p<0.01, ****p<0.0001 compared to the same genotype on control diet 24 wk.

Conclusions

We identified a novel role of **HMGB1** in **lipid accumulation in the gut in the** early stages of **NASH**. *Hmgb1*$^{\Delta IEC}$ mice fed **HFCF** diet show **accumulation of lipids** in the **IEC of the jejunum**, increased serum triglycerides and **protection from hepatic steatosis** after 1 wk. This suggests that HMGB1 plays a role in lipid transport and packaging, modulating the amount of fat transferred from the intestine to the liver and subsequent hepatic steatosis. HMGB1 may well serve as a target to treat lipid storage disorders and NASH.

References

1. Basaranoglu M et al: Understanding mechanisms of the pathogenesis of nonalcoholic fatty liver disease. WJG 2010
2. Charlton M: Nonalcoholic fatty liver disease: a review of current understanding and future impact. *Clin Gastroent Hepatol* 2004
3. Angulo P: Nonalcoholic fatty liver disease. *N Engl J Med* 2002
4. Clark JM: The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006
5. Vanni et al: The gut-liver axis in nonalcoholic fatty liver disease: Another pathway to insulin resistance? Hepatology 2009
6. Gaskell H et al.: High-Mobility Group Box-1 and Liver Disease. Hepatol Commun 2018
7. Arriazu E et al: Signalling via the osteopontin and high mobility group box-1 axis drives the fibrogenic response to liver injury. *Gut* 2017
8. Lu B et al: Regulation of HMGB1 release by inflammasomes. Protein Cell 2013,
9. Liu S et al: HMGB1 is secreted by immunostimulated enterocytes and contributes to cytomix-induced hyperpermeability of Caco-2 monolayers. Am J Physiol Cell Physiol 2006
10. Chandrashekaran V et al: HMGB1-RAGE pathway drives peroxynitrite signaling-induced IBD-like inflammation in murine nonalcoholic fatty liver disease. Redox Biol 2017