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Gut inflammation exacerbates hepatic injury in the high-fat diet induced NAFLD mouse: Attention to the gut-vascular barrier dysfunction

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ABSTRACT

Aims: Gut inflammation has been put forward to be associated with hepatic injury in the clinical practice. The dismantled intestinal barrier was highly concerned, however, largely unknown about the role of gut-vascular barrier (GVB) in this process. This study aimed to investigate if inflamed gut directly contributes to the progression of non-alcoholic steatohepatitis (NASH), especially attention to the GVB dysfunction.

Main methods: Male C57bl/6 mice were fed with a high-fat diet (HFD) and 1% DSS for 12 weeks. The colonic inflammatory injury as well as hepatic injury were evaluated. The GVB function was assessed via measuring the permeability to fluorescently-labeled dextran (70 kDa) and the expression of plasmalemma vesicle-associated protein-1 (PV1). Furthermore, the plasma endotoxin level and hepatic TLR4/TLR9 mRNA expression were detected.

Key findings: There were evident colitis in DSS-exposed mice, which trend to be more apparent in HFD ones. The HFD + DSS mice exhibited more serious hepatic steatosis, inflammation and fibrosis than HFD groups. The downregulated tight junction protein in HFD + DSS mice indicated loss of epithelial barrier. The GVB disruption were also confirmed with increased permeability to macromolecules and high expression of endothelial PV1 in HFD + DSS mice. Accordingly, potentially elevated plasma endotoxin levels and markedly increased TLR4/TLR9 mRNA expression were demonstrated in HFD + DSS mice rather than HFD groups.

Significance: Gut inflammation exacerbates liver injury and fibrosis in HFD mice, which may contribute to the development of NASH. Beyond the damaged intestinal epithelial barrier, GVB disruption with bacterial translocation into may play a key role in the pathogenesis of NASH.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) has gradually emerged as the most common chronic liver disease worldwide [1], which contains a series of disorders ranging from hepatic steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis. Generally, NASH is considered the key process in the potential development of fibrosis, cirrhosis, and hepatocellular carcinoma [2–4]. Although disordered lipid metabolism is a recognized cause of NAFLD, the mechanisms associated with the progression of NASH are largely unknown.

Gut-liver axis dysfunction has been put forward to be associated with the occurrence and development of NAFLD in recent years [5–7]. Indeed, NAFLD can be detected in up to 40% of inflammatory bowel disease (IBD) patients, which often in absence of metabolic risk factors [8]. Furthermore, intestinal bacterial overgrowth and imbalance is the hallmark of IBD subjects [9] which is also correlated to the pathogenesis of NAFLD [10]. Gut inflammation and dysbiosis could drive the hepatic injury by overloaded exposure to bacterial translocation, enteric antigens and toxins, as well as inflammatory mediators [5,11,12]. It indicated that dysfunction in the gut may induce and aggravate sustained immune activation in the liver, thereby accelerating NASH progression.

Intestinal barrier disruption is the key mechanism responsible for the inflammatory transport from the gut to the liver in the development of NAFLD/NASH [13,14]. In this process, the intestinal epithelial barrier was highly concerned which formed a strong wall preventing invasion of foreign substance from the lumen [14]. However, largely unknown about the role of gut-vascular barrier (GVB), a recently demonstrated endothelial wall consisted with endothelial cells, $GFAP^+$ enteric glial cells and α-SMA^+ $desmin^+$ pericytes [15]. /GVB acts as the last guard between the intestine and liver, as well as blood circulation, it defends against bacteria and macromolecules which escaped

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Table 1
Primers used for reverse transcription-quantitative polymerase chain reaction.

Primer	Forward primer	Reverse primer
GAPDH	ATGTTCCAGTATGACTCCACTCACG	GAAGACACCAGTAGACTCCACGACA
IL-1	AAGGGCTGCTTCCAAACCTTTGAC	TGCCTGAAGCTCTTGTTGATGTGC
IL-6	TCCTACCCCAATTTCCAATGCT	TGAATTGGATGGTCTTGGTCCTT
TNF-α	AGGGTCTGGGCCATAGAACT	CCACCACGCTCTTCTGTCTAC
MCP-1	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
TLR4	AGAAATTCCTGCAGTGGGTCA	TCTCTACAGGTGTTGCACATGTCA
TLR9	ACTTCGTCCACCTGTCCAA	AGGAAGGTTCTGGGCTCAAT
Collagen 1	TAGGCCATTGTGTATGCAGC	ACATGTTCAGCTTTGTGGACC
TGF-β	GTGGAAATCAACGGGATCAG	ACTTCCAACCCAGGTCCTTC
ACTA2	GTTCAGTGGTGCCTCTGTCA	ACTGGGACGACATGGAAAAG
TIMP-1	AGGTGGTCTCGTTGATTTCT	GTAAGGCCTGTAGCTGTGCC
PAI-1	GCCAGGGTTGCACTAAACAT	GCCTCCTCATCCTGCCTAA

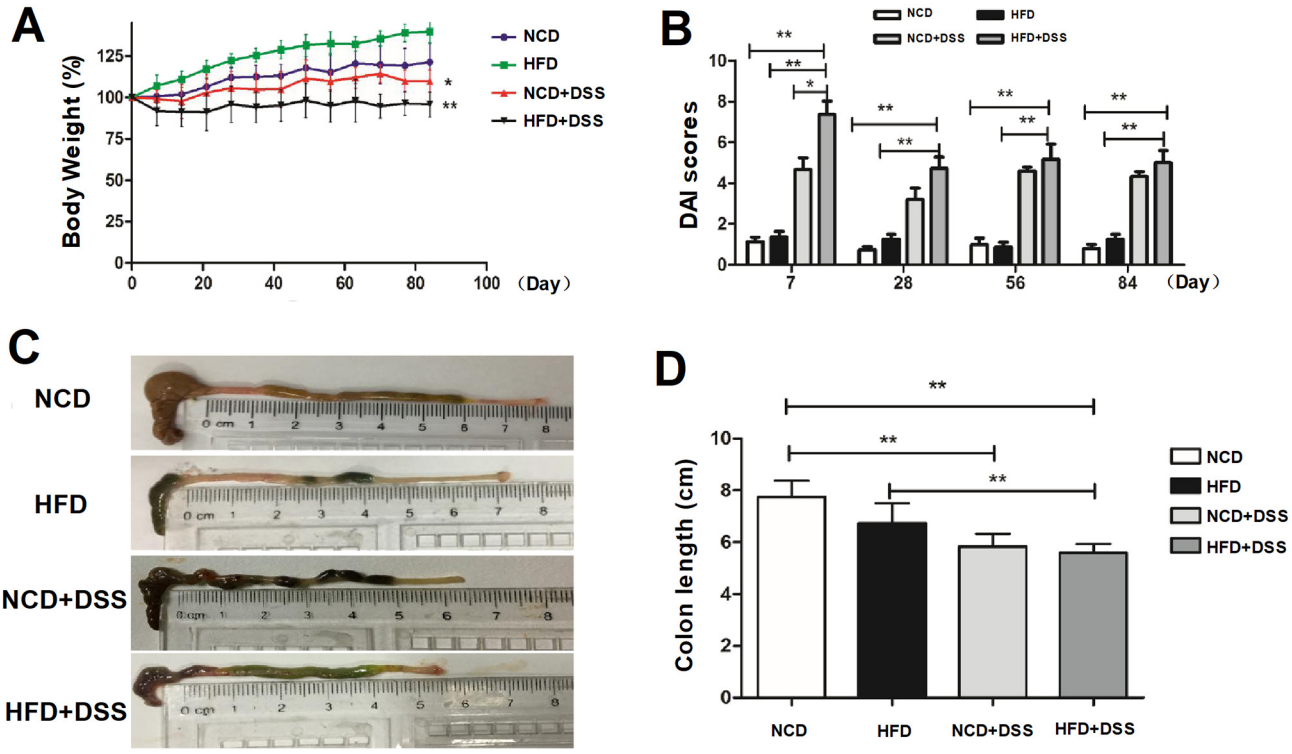

Fig. 1. DSS induced marked changes in the weight gain, stool property and colonic length in HFD mice. DSS treatment induced failure to thrive (A), significantly increased DAI scores (B) and decreased length of colon (C) in both NCD and HFD mice relative to those without DSS-exposure. $*p < 0.05$, $**p < 0.01$, $N = 6$ to 8 for each group.

from the epithelial barrier [15]. GVB impairment has been detected in some patients with celiac disease, hepatocirrhosis and ankylosing spondylitis [15,16]. Whether GVB plays a role in the pathogenesis of NAFLD/NASH remains to be further confirmed.

In the current study, we utilized HFD mice with dextran sulfate sodium (DSS)-induced colitis to investigate if inflamed gut directly contributes to the progression of non-alcoholic steatohepatitis (NASH), especially attention to the GVB dysfunction. Our present work suggests gut inflammation exacerbates hepatic injury in the high-fat diet induced NAFLD mouse due to damaged intestinal mucosal barrier, GVB functional impairment and bacterial translocation. More importantly, these findings demonstrate a critical role for GVB in NASH pathogenesis and further underscore the complex interplay between diet, intestinal barriers and gut microbiota in driving NAFLD progression. It may provide new insights and potential targets for the prevention and treatment of NASH.

2. Materials and methods

2.1. Animal and treatment

Male C57bl/6 mice (aged 6 weeks, weighed 18–22 g; Huafukang Bioscience, Beijing, China) were used. Mice were housed in specific pathogen-free conditions maintained at 23 °C with 12/12-h light/dark cycle. Accordingly, mice were provided with normal chow diet (NCD) or high-fat diet (HFD; 20 kcal% protein, 20 kcal% carbohydrates and 60 kcal% fat; D12492, New Brunswick, NJ, USA), with or without 1% dextran sulfate sodium (DSS; MP Biomedicals, LLC, IIIkirch, France) in drinking water for 12 weeks. DSS was applied repeatedly in cycles (a 7-day DSS treatment followed by a 10-day interval with normal drinking water). Thus, the mice were randomly divided into 4 groups: NCD group, HFD group, NCD + DSS group and HFD + DSS group. Finally, all mice were anesthetized and sacrificed, then the portal vein blood were harvested and plasma were isolated, the colonic and liver tissues were collected and fixed in 4% paraformaldehyde for histopathology or