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EDITORIAL

The Gut Microbiota, Intestinal Permeability, Bacterial Translocation, and Nonalcoholic Fatty Liver Disease: What Comes First?

Nonalcoholic fatty liver disease (NAFLD) is one of the leading causes of chronic liver disease in the United States, and its prevalence is rising in the developed as well as in the developing world.[1] Although tremendous headway has been made in understanding the pathogenesis of NAFLD, the role of the gut microbiota and intestinal permeability in augmenting disease progression from nonalcoholic fatty liver (NAFL), the nonprogressive form of NAFLD, to nonalcoholic steatohepatitis (NASH), the progressive form of NAFLD, remains incompletely understood.

In the current issue of *Cellular and Molecular Gastroenterology and Hepatology*, Luther et al[2] evaluate the association between NAFLD and increased intestinal permeability. A higher prevalence of increased intestinal permeability has been seen in patients with obesity or NAFLD, with some studies suggesting a role in the pathogenesis of NAFLD.[3–6] Through a meta-analysis of five such studies, Luther et al found that almost 40% of patients with NAFLD had increased intestinal permeability. In contrast, only 6.8% of healthy controls demonstrated these changes. A subgroup analysis of NASH patients showed a stronger association with altered intestinal permeability, with nearly 50% affected, suggesting that the necroinflammatory changes seen in NASH may be more closely associated with increased gut permeability.

Previous studies have tried to explain the association between intestinal permeability and NAFLD. In an experimental model of NASH, Gäbele et al[7] were able to propagate liver injury and inflammation by inducing colitis. They hypothesized that injury to the intestinal epithelium led to increased permeability and passage of bacterial products to the portal vein, leading to enhanced liver injury. Others have shown that obesity itself may lead to altered intestinal permeability through an increase in tumor necrosis factor-α (TNFα) levels and increased intestinal inflammation.[8,9] Furthermore, it has been found that the intestinal microbiome is perturbed in both obesity and NAFLD, which may lead to changes in microbial products and a resultant leaky gut.[5,10,11]

In their study, Luther et al[2] have taken another approach to explain this association by hypothesizing that liver injury may precede the development of increased intestinal permeability. They suggest that an initial liver injury may lead to the release of inflammatory cytokines, which in turn affects the intestinal tight junctions and increases intestinal permeability passage of bacterial elements into the portal circulation propagating the preexisting liver injury. To evaluate this hypothesis, they chose a dietary model in which mice rapidly develop steatosis and inflammation when fed a methionine and choline deficient (MCD) diet. They sacrificed mice at different time points and measured both serum and hepatic levels of TNFα, interleukin-6 (IL-6), and IL-1β, as well as noted the timeline of development of liver steatosis and inflammation. They then made serial measures of intestinal permeability using a previously verified technique.

The data showed that the systemic and hepatic levels of TNFα and alanine aminotransferase followed a similar timeline, with an initial rise around day 6 and a subsequent peak at day 21. The histologic changes of NASH, quantified using a modified NAFLD activity score, paralleled these biochemical changes. Other indicators of inflammation, including levels of the cytokines IL-6 and IL-1β, did not show significant changes during this period. Interestingly, increases in intestinal permeability could not be detected until after initial damage to the liver had been identified, suggesting that, at least in this model, leaky gut could not be blamed for the initial insult to the liver. Immunofluorescence microscopy of the small intestines demonstrated disruption of zona occludens-1 on day 10, consistent with the idea that epithelial tight junction injury occurred after the initial liver injury.

Direct incubation of hepatocytes with MCD medium led to steatosis, inflammation, and an increase in TNFα, but incubation of intestinal cells did not lead to alteration of tight junctions or intestinal permeability in vitro. This suggests that the MCD diet was sufficient to cause liver injury but did not directly injure the intestines. Luther et al[2] hypothesized that intestinal injury in this model may be a consequence of liver injury, probably through release of systemic cytokines such as TNFα. To test this, they performed similar experiments in MLCK (myosin light chain kinase) mice, which are resistant to acute TNFα-induced intestinal permeability. Feeding these mice the MCD diet led to similar liver and intestinal changes, arguing against TNFα production as the primary mechanism of intestinal tight junction changes in this model.

Although the MCD murine model has been widely used to study NASH, it has some notable limitations that should be considered to put the results of Luther et al[2] in perspective. As opposed to what we see in human NAFLD, mice fed the MCD diet do not show features of metabolic syndrome such as obesity, insulin resistance, or dyslipidemia. Therefore, these results need to be validated in other animal models that more closely mimic human NAFLD such as the high-fat diet mouse model. Furthermore, the conclusion that increased intestinal permeability is not related to TNFα needs to be validated in humans. TNFα, IL-6, and

IL-1β may be involved in disease progression in human NAFLD through pathways not applicable to the mouse model. On the other hand, findings from the MCD mouse model raise the interesting possibility that the increased intestinal permeability seen in NAFLD may be, at least in part, independent of the obesity phenotype.

A strength of this study is the use of clinical meta-analytic methods as a means of developing a rationale to support the mechanistic experimental study using a mouse model. The evaluation of the published literature together with the insights gathered from the mouse work further strengthens the association between NAFLD and increased intestinal permeability, and the study by Luther et al[2] provides key data to further our understanding of the role of altered gut permeability in the pathogenesis of NAFLD. Prospective clinical studies using well-characterized patients are needed to examine whether hepatic steatosis is associated with further increases in gut permeability above and beyond that seen in obesity. Future research should examine whether alterations in intestinal permeability and the nature of the gut microbiota can predict disease progression from NAFL to NASH to cirrhosis.

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Conflicts of interest
The authors disclose no conflicts.

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