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# Nonalcoholic Steatohepatitis and the Intestinal Microbiota

SEE ARTICLE ON PAGE 451

Nonalcoholic steatohepatitis (NASH), characterized by fatty infiltration of the liver, hepatocellular injury, and hepatic inflammation, is a major cause of liver failure. The pathogenesis of NASH is incompletely understood, but generally occurs in individuals with preexisting nonalcoholic fatty liver disease (NAFLD) or uncomplicated steatosis. Whereas most individuals with NAFLD will not develop liver disease, at least 10% progress to NASH and a large portion of these individuals develop cirrhosis and, ultimately, liver failure. Understanding which factors drive progression from simple steatosis to NASH is profoundly important. In this regard, the intestinal microbial community has become a major focus of investigation for its potential role in inciting liver inflammation and injury.

In humans, the intestine plays hosts to a dense microbial community (the microbiota) comprised of thousands of species. The gut microbiota are collectively an intense biochemical reactor, generating byproducts that can cross the intestinal mucosa to the portal circulation and directly to the liver. Whereas some microbial metabolites are undoubtedly beneficial, others are suspected to stimulate inflammation or cause hepatocellular injury. Most notable in this regard is lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria and an exceptionally potent stimulus for inflammatory cytokines. LPS can be detected in plasma of obese patients where it has been correlated with the degree of liver injury, suggesting a link between microbiota-derived LPS and progression to NASH.[1] Several animal models of NASH support the contribution of LPS to liver inflammation.[2] Other metabolite levels are affected by microbiota biochemical activities and some of these and are felt to contribute to NASH, such as short-chain fatty acids, ethanol, and bile acids. Consistent with a role for the gut microbiota in liver disease, animal models reveal that antibiotic treatment,[3] probiotics,[4] and prebiotics[5] can delay disease progression. Treatment of patients with the antibiotic, rifaximin, decreased markers of hepatic injury in patients with NASH.[1]

Despite these intriguing findings, the precise contribution of intestinal microbes to progression from NAFLD to NASH remains unclear. Studies examining microbial species in the human microbiota have been inconsistent, in some cases reporting completely opposite associations between NASH and specific intestinal microbes. Previous studies have found that *Bacteroides* and Proteobacteria, which includes *Escherichia coli* and other *Enterobacteraciae*, are most commonly different between patients with NASH compared to those without, but the direction of the associations has not been consistent. For example, abundance of *Bacteroides* was either significantly increased,[6] significantly decreased,[7] or not significantly different[8] between patients with NASH compared to healthy controls or those with NAFLD.

The study by Del Chierico et al., in the current issue of HEPATOLOGY,[9] provides a rich new data set, which, from associations between intestinal microbes, fecal metabolites, and liver disease, are explored anew. The investigators studied both the gut microbiota and the metabolite composition of feces from patients with obesity (n = 8), NAFLD (n = 27), or NASH (n = 26) and compared these to healthy age-matched controls (n = 54). As has been observed by other

*Abbreviations: LPS, lipopolysaccharide; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PLS-DA, partial least squares discriminant analysis.*

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**ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:**

David B. Haslam, M.D.
Cincinnati Children's Hospital Medical Center
3333 Burnet Avenue
Cincinnati, OH 45229-3026
E-mail: david.haslam@cchmc.org
Tel: +1-513-803-1170

investigators, decreased microbial diversity generally correlated with obesity and NAFLD when compared to healthy controls. The investigators performed a number of group-wise comparisons, but our focus is on the comparisons between patients with NASH and those without. Here, the new findings do not recapitulate those of past investigations. Elevated abundance of *Lachnospiraceae*, *Ruminococcus*, and *Dorea* were observed in children with NASH, whereas decreased abundance of *Oscillospira* were observed in children with steatosis. All of these organisms belong to the Firmicutes, and, unlike previous studies, neither Proteobacteria nor *Bacteroides* were associated with the NASH disease phenotype. Volatile organic compounds were measured by gas chromatography mass spectrometry. Statistically significant differences were found for two metabolites (2-butanone and 4-methyl-2-pentanone) when comparing patients with NASH to controls. Finally, the investigators performed partial least squares discriminant analysis (PLS-DA), a multivariate analysis, to identify which factors among the combined microbiota and metabolome data were able to distinguish patients from controls. Several outliers and all obese patients were excluded in the final model, which was able to distinguish healthy controls from those with NAFLD or NASH, but was not able to distinguish these two. A number of bacterial species and organic compounds were included in the final model, which the investigators propose may be a useful biomarker for the identification of NAFLD patients.

One of the perils of studying microbial community structures and metabolomic signatures is the exceptionally high dimensionality of the data. The number of variables (abundance of microbial species and fecal metabolites) vastly outnumbers the number of samples analyzed, which can lead to spurious associations. The analysis by Del Chierico et al. largely addresses these concerns by performing leave-one-out cross-validation and post-hoc correction for multiple testing during multivariate and univariate analysis, respectively. Nevertheless, the predictive model built from PLS-DA analysis is highly fitted to the investigators' patient population, and validation of the model's utility as a biomarker awaits confirmation in different patient populations. In summary, the study presented by Del Chierico et al. adds a massive amount of new data with which to probe the association between intestinal microbiota and progression of liver disease, but, unfortunately, the specific organisms found to correlate with NASH do not recapitulate those found in previous studies.

What to make of the inconsistent animal and human associations published thus far? On the one hand, animals with altered intestinal microbiota and those with defects in the ability to sense microbial products suggest a major role for microbes in development of NASH. However, the inability to find a distinct microbial signature that correlates with disease may suggest that no single group of organisms drives NASH development. The ability to draw conclusions from studies performed thus far is complicated by the studies' differing patient populations, different sequencing methodologies, potential medication effects, the possibility that different microbial communities may produce similar NASH-inducing metabolites, or the possibility that a single snapshot of the intestinal microbiome fails to capture key differences that occur over time. By including analysis of both the gut microbiota and the metabolome, the study by Del Chierico et al. adds an important new perspective. Additional carefully designed studies with well-standardized analytical methods will help to clarify whether targeted modification of microbes in the gut might yet be a useful means to prevent development of NASH.

**David B. Haslam, M.D.**
**Cincinnati Children's Hospital Medical Center**
**Cincinnati, OH**

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Author names in bold designate shared co-first authorship.