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ANIMAL MODELS

Saturated and Unsaturated Dietary Fats Differentially Modulate Ethanol-Induced Changes in Gut Microbiome and Metabolome in a Mouse Model of Alcoholic Liver Disease

Irina A. Kirpich,[*†] Joseph Petrosino,[‡§] Nadim Ajami,[‡§] Wenke Feng,[*†] Yuhua Wang,[*†¶] Yanlong Liu,[*†‖] Juliane I. Beier,[†] Shirish S. Barve,[*†] Xinmin Yin,[**] Xiaoli Wei,[**] Xiang Zhang,[†**] and Craig J. McClain[*†,††]

From the Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, and the Departments of Pharmacology and Toxicology[†] and Chemistry,** University of Louisville School of Medicine, Louisville, Kentucky; the Baylor Department of Molecular Virology and Microbiology[‡] and the Alkek Center for Metagenomics and Microbiome Research,[§] Baylor College of Medicine, Houston, Texas; the College of Food Science and Engineering,[¶] Jilin Agricultural University, Changchun, China; the School of Pharmaceutical Sciences,[‖] Wenzhou Medical University, Wenzhou, China; and the Robley Rex Veterans Medical Center,[††] Louisville, Kentucky*

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Address correspondence to Irina A. Kirpich, M.P.H., Ph.D., Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, University of Louisville, Louisville, KY 40202. E-mail: i0kirp01@louisville.edu.

Alcoholic liver disease (ALD) ranks among major causes of morbidity and mortality. Diet and crosstalk between the gut and liver are important determinants of ALD. We evaluated the effects of different types of dietary fat and ethanol on the gut microbiota composition and metabolic activity and the effect of these changes on liver injury in ALD. Compared with ethanol and a saturated fat diet (medium chain triglycerides enriched), an unsaturated fat diet (corn oil enriched) exacerbated ethanol-induced endotoxemia, liver steatosis, and injury. Major alterations in gut microbiota, including a reduction in Bacteroidetes and an increase in Proteobacteria and Actinobacteria, were seen in animals fed an unsaturated fat diet and ethanol but not a saturated fat diet and ethanol. Compared with a saturated fat diet and ethanol, an unsaturated fat diet and ethanol caused major fecal metabolomic changes. Moreover, a decrease in certain fecal amino acids was noted in both alcohol-fed groups. These data support an important role of dietary lipids in ALD pathogenesis and provide insight into mechanisms of ALD development. A diet enriched in unsaturated fats enhanced alcohol-induced liver injury and caused major fecal metagenomic and metabolomic changes that may play an etiologic role in observed liver injury. Dietary lipids can potentially serve as inexpensive interventions for the prevention and treatment of ALD. *(Am J Pathol 2016, 186: 765—776; http://dx.doi.org/10.1016/j.ajpath.2015.11.017)*

Alcoholic liver disease (ALD) ranks among major causes of morbidity and mortality in the United States and worldwide. ALD includes a spectrum of conditions from simple steatosis to alcoholic steatohepatitis characterized by inflammation, with potential progression to fibrosis and cirrhosis over time. Alcoholic hepatitis occurs in approximately 10% to 35% of chronic drinkers, and severe alcoholic hepatitis accounts for significant morbidity and mortality approaching 35% to 45%.[1] Approximately, 10% to 20% of heavy drinkers will develop cirrhosis.[2,3]

The crosstalk between the gut and liver is an important determinant of alcohol-induced liver disease.[4—6] Several studies have found that alcohol consumption (both acute and chronic) increases bacteria-derived products, specifically lipopolysaccharide (LPS) and bacterial DNA, in the portal and systemic circulation.[7,8] Significantly increased levels of LPS, which induces a proinflammatory response in the liver, were found in patients with different stages of ALD (fatty liver, hepatitis, and cirrhosis)[9,10] and in experimental animal models of ALD.[11—13] As found in a

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number of recent clinical[14–18] and preclinical studies,[12,19,20] alcohol intake and alcohol-induced liver injury are associated with qualitative (dysbiosis) and quantitative (bacterial overgrowth) alterations of gut microbiota. The molecular mechanisms by which the altered gut microbiota contribute to ALD are not well understood. Alcohol-mediated changes in the gut microbiota facilitate disruption of gut barrier integrity,[12,19,21] resulting in increased intestinal permeability to bacteria-derived products, which eventually contributes to the development of ALD. Bacteria overgrowth may enhance ethanol production and metabolism with subsequent high concentrations of acetaldehyde[22] (product of ethanol degradation), which is known to affect intestinal intercellular junctions.[23] Mice that were protected from intestinal bacterial overgrowth and dysbiosis had decreased alcohol-induced endotoxemia and liver disease.[24] Antibiotic treatment to sterilize the gut,[25,26] as well as prebiotics or probiotics administration to normalize the gut microbiota community,[19,27,28] attenuates alcohol-induced intestinal barrier leakage and decreases endotoxemia and hepatic injury in rodents. The link between the dietary fat and alcohol in ALD is increasingly recognized. Several studies, including those from our laboratory, have found that dietary unsaturated fat exacerbates alcohol-mediated intestinal permeability, liver steatosis, inflammation, and injury.[11,29–32] These pathologic effects were prevented or blunted by dietary saturated fat, suggesting a significant contribution of specific dietary lipids in ALD development and progression. However, the exact mechanism(s) underlying these effects remains to be established. In the present study, we evaluated the response of the gut microbiota (in terms of composition and metabolic activity) to ethanol and different types of dietary fat in an experimental animal model of ALD. The results of the study contribute to understanding the complexity of the interplay among the diet, gut microbiota, and ethanol-induced fatty liver disease.

Materials and Methods

Animals and Treatments

C57BL/6N male mice obtained from Harlan Laboratories, Inc. (now Envigo RMS, Indianapolis, IN) were pair-fed control or ethanol-containing (5% ethanol v/v) diets *ad libitum* for 8 weeks (Figure 1A).[11] Mice were fed a modified Lieber-DeCarli liquid diet (Research Diet, New Brunswick, NJ) that contained saturated fat (SF) or unsaturated fat (USF). The SF or USF diets were enriched in medium chain triglycerides (MCTs) and beef tallow (18:82 ratio) or corn oil, respectively (Figure 1B). The detailed dietary fatty acid composition has been described previously.[32] Control mice were pair-fed SF or USF maltose-dextrin diets that were isocaloric with the ethanol diets. In the control group diets, the levels of protein, carbohydrate, and fat were held constant at 17%, 43%, and 40% of total energy, respectively. In the alcohol diets, ethanol (35% of total calories) was substituted for carbohydrate energy. At the end of the study period, the mice were anesthetized, and blood, liver, and intestinal tissue samples were collected. Stool samples were collected weekly throughout the study. Animals were housed in a pathogen-free barrier facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and the study protocol was approved by the University of Louisville Institutional Animal Care and Use Committee.

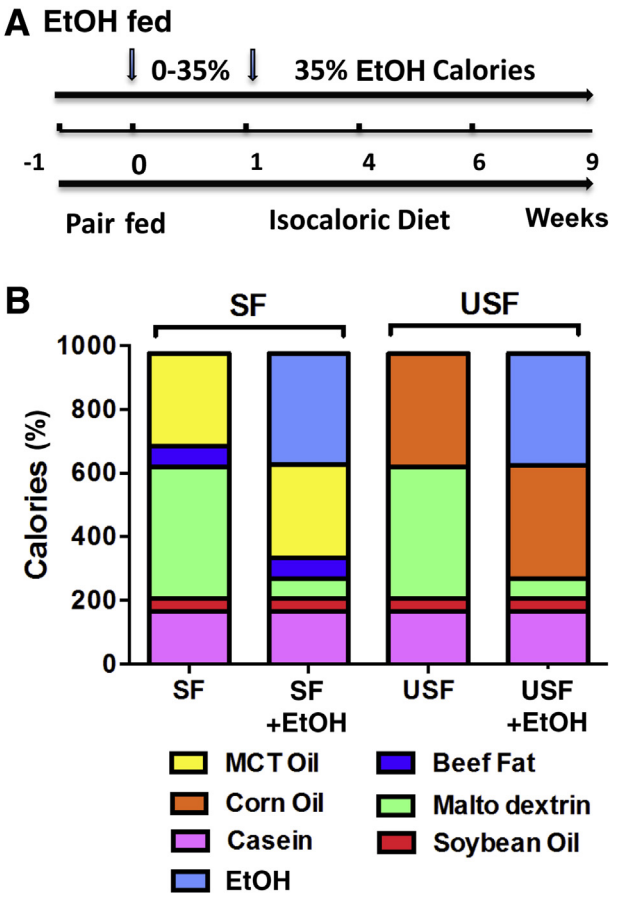

Figure 1 The experimental animal model of alcoholic liver disease. **A:** The schematic presentation of chronic ethanol (EtOH) or control diet feeding protocol. Initially, all mice were given the control liquid maltose dextrin diets [saturated fat (SF) or unsaturated fat (USF), no ethanol] *ad libitum* for 1 week. Afterward, mice were fed either the liquid ethanol-containing diets or the control liquid maltose-dextrin diets. Ethanol was gradually increased to 35% of total calories (5.0% v/v). The mice were fed the ethanol diet (5% ethanol v/v) *ad libitum* for 8 weeks. **B:** The composition of the experimental liquid diets. The SF diet was enriched with medium chain triglyceride oil and beef tallow fat (82:18 ratio). The USF diet was enriched with corn oil. Soybean oil was used in both diets to provide essential free fatty acids. The control (SF and USF) diets contained 43% of calories from carbohydrate, 17% from protein, and 40% from fat. The SF and ethanol and USF and ethanol diets contained 35% of calories from ethanol to replace the calories from carbohydrate.

Blood and Liver Biochemical Analysis

Plasma endotoxin levels were measured with the limulus amebocyte lysate kit (Lonza, Walkersville, MD) according to