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Fructose Promotion of Intestinal and Liver Injury: A Sugar by Any Other Name That Isn't So Sweet

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Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are of growing concern with an estimated 75 – 100 million people diagnosed with NAFLD in the United States. Excessive fructose consumption may be associated with human NAFLD development and increased prevalence (1, 2). Indeed, increased fructose intake promotes fatty liver, obesity and inflammation in animal models (1). Fructose interacts with the gut microbiota to aid in NAFLD/NASH progression (3). This is important since dietary composition is able to influence gut microbial genera (4), and these changes may influence gut permeability. However, the mechanism by which fructose promotes gut permeability is unknown.

Cytochrome P450 2E1 (CYP2E1) plays a role in metabolic reactions. CYP2E1 promotes intestinal permeability in models of alcoholic-induced injury, but little is known about its role in NAFLD/NASH. Similarly, CYP2E1 hepatic expression increases in models of alcohol-induced injury. Nitric oxide synthase (iNOS) produces nitric oxide from L-arginine, and increases gut permeability during intestinal obstruction (5). Additionally, downregulation of iNOS is protective against liver injury in a rat model of NAFLD subjected to ischemia-reperfusion (6). Cho *et al*. (7) evaluated the role of intestinal and hepatic CYP2E1 on the promotion of gut leakiness and subsequent liver inflammation in fructose-exposed rodents. The authors evaluated parameters indicative of gut leakiness in samples obtained from humans with NASH to verify relevance to human disease. The authors found that NASH patients had increased levels of lipopolysaccharide (LPS) alongside decreased intestinal tight junction (TJ) and adherens junction (AJ) proteins, indicative of gut permeability. Furthermore, patients had increased CYP2E1, iNOS expression and nitrated proteins in their ileum.

To understand the impact of fructose feeding on the intestine and liver, the authors fed rodents 30% (w/v) fructose in drinking water for 8 wks. Fructose-exposed rodents had increased intestinal inflammation, alongside enhanced plasma endotoxin and liver *E. coli* levels compared to controls. Interestingly, fructose feeding altered the gut microbiota profile compared to control feeding, which is significant considering changes in gut flora have been noted in NASH patients (8). Similar to the human NASH samples, fructose-exposed rodents had increased intestinal levels of CYP2E1, iNOS and markers of oxidative and nitrative stress compared to controls. Loss of TJ and AJ proteins is a hallmark of intestinal leakiness, and fructose fed rodents had a decrease in these parameters, with increased enterocyte apoptosis, indicating that fructose mediates gut leakiness.

The role of CYP2E1 in the context of NAFLD/NASH has yet to be elucidated. Wild-type (WT) or *Cyp2e1*-null mice were given fructose similar to the rodent model. *Cyp2e1*-null mice had a decrease in fructose-induced plasma endotoxin and hepatic *E. coli* levels, along with restored TJ and AJ expression and decreased enterocyte apoptosis. These results were further confirmed *in vitro* with cultured colon cells treated with a CYP2E1 inhibitor prior to fructose exposure. These findings support the concept that CYP2E1 promotes gut leakiness during fructose consumption.

The gut-liver axis plays a significant role in NAFLD progression to NASH. Fructose-exposed rodents showed increased hepatic steatosis, inflammation, CYP2E1 expression and iNOS levels compared to their controls, suggesting that fructose alters hepatic nitroxidative stress in a CYP2E1-dependent manner. Further, fructose exposure promoted liver fibrosis in rats, but not mice, via Sirt1 signaling. The authors confirmed that LPS induces hepatic stellate cell (HSC) activation *in vitro*, but this was not noted with fructose treatment alone.

This data implies that, following fructose exposure, HSC activation is mediated by LPS presumably derived from fructose-induced leaky gut, but not from fructose alone.

Finally, the authors evaluated if loss of CYP2E1 altered fructose-induced liver injury. Fructose-exposed *Cyp2e1*-null mice had a reduction in liver inflammation compared to WT. Further, plasma levels of liver enzymes were increased in fructose fed WT mice compared to controls, but reduced in fructose fed *Cyp2e1*-null mice compared to WT. The authors conclude that fructose intake promotes liver inflammation indicative of NAFLD/NASH in a CYP2E1-dependent manner.

Conclusions

While it has been suggested that excess fructose consumption contributes to the acquisition and progression of NAFLD/NASH, the exact mechanisms are still being investigated. This manuscript suggests that changes in AJ and TJ formation in the intestines is influenced by fructose-dependent changes in CYP2E1 signaling; however, the authors utilized a mouse model globally lacking *Cyp2e1*. Since CYP2E1 was shown to be upregulated in both the intestines and liver following fructose consumption in mice and rats, it is difficult to tease out whether changes in hepatic CYP2E1 mediated NAFLD/NASH or if the increased CYP2E1 signaling in the intestines indirectly caused liver injury. Further studies using organ or cell specific knockout of *Cyp2e1* are warranted.

Fructose exposed mice and rats had changes in their gut microbial profile. Gut dysbiosis leads to intestinal permeability and may impact NAFLD/NASH progression (3); therefore, this alteration may promote gut leakiness. While fructose-exposed mice and rats exhibited changes in gut flora, it is not shown how intestinal permeability and CYP2E1 signaling are molecularly linked to these changes. Fructose may be directly causing gut

dysbiosis, which in turn mediates gut leakiness, or it may be affecting CYP2E1 signaling that is causing these changes. Future work is needed to understand the direct target of fructose.

Interestingly, fructose-induced CYP2E1 levels were coupled with increased intestinal CLOCK and PER-2 expression in mice and rats. Another manuscript found that alcohol exposure increases CYP2E1 expression that regulates CLOCK and PER-2 in colon cells (9). While this data does not necessarily align with the aim of the manuscript, it does raise an interesting question on the role of circadian rhythm in the promotion of NAFLD/NASH. Little work has been generated on the role of circadian rhythm during NAFLD/NASH, and would be an interesting avenue to pursue.

Daily fructose intake has been associated with increased hepatic fibrosis in NAFLD/NASH. The study discussed in this editorial found increased hepatic fibrosis in fructose-exposed rats. As discussed, changes in the gut flora were noted in fructose-exposed rodents in this study suggesting the proposed signaling mechanism may play a role in the gut dysbiosis seen in overweight and obese humans.

The authors used a feeding model consisting of 30% (w/v) fructose in drinking water; however, recent questions have arisen as to the applicability of fructose and other sugar feeding models to mimic human intake. Rodent models fed fructose at supraphysiologic doses, that may induce steatohepatitis, may not reflect actual human consumption. Indeed, one study found no effect of fructose consumption on clinical markers of NAFLD in isocaloric conditions in humans (10). Additionally, studies evaluating fructose or glucose intake alone do not reflect typical human nutrition, since intake of both in the form of sucrose (50% glucose, 50% fructose) or HFCS (45% glucose, 55% fructose) is more applicable.

The authors conclude that fructose intake causes gut leakiness and subsequent steatohepatitis in a CYP2E1-dependent manner. The data indicates that high intake of fructose leads to gut dysbiosis, intestinal permeability and NAFLD/NASH phenotypes. While this work supports other publications stating that fructose promotes NAFLD/NASH, direct targets are lacking. While future work is necessary, it is clear that this sweetener isn't so sweet.

References:

1. Lim JS, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. Nat Rev Gastroenterol Hepatol 2010;7:251-264.

2. Ouyang X, Cirillo P, Sautin Y, McCall S, Bruchette JL, Diehl AM, Johnson RJ, et al. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. J Hepatol 2008;48:993-999.

3. Lambertz J, Weiskirchen S, Landert S, Weiskirchen R. Fructose: A Dietary Sugar in Crosstalk with Microbiota Contributing to the Development and Progression of Non-Alcoholic Liver Disease. Front Immunol 2017;8:1159.

4. Singh RK, Chang HW, Yan D, Lee KM, Ucmak D, Wong K, Abrouk M, et al. Influence of diet on the gut microbiome and implications for human health. J Transl Med 2017;15:73.

5. Quirino IE, Cardoso VN, Santos R, Evangelista WP, Arantes RM, Fiuza JA, Gloria MB, et al. The role of L-arginine and inducible nitric oxide synthase in intestinal permeability and bacterial translocation. JPEN J Parenter Enteral Nutr 2013;37:392-400.

6. Duan YF, Sun DL, Chen J, Zhu F, An Y. MicroRNA-29a/b/c targets iNOS and is involved in protective remote ischemic preconditioning in an ischemia-reperfusion rat model of non-alcoholic fatty liver disease. Oncol Lett 2017;13:1775-1782.

7. Cho YE, Kim DK, Seo W, Gao B, Yoo SH, Song BJ. Fructose Promotes Leaky Gut, Endotoxemia and Liver Fibrosis through CYP2E1-Mediated Oxidative and Nitrative Stress. Hepatology, in press 2019.

8. Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, Gill SR. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology 2013;57:601-609.

9. Forsyth CB, Voigt RM, Keshavarzian A. Intestinal CYP2E1: A mediator of alcohol-induced gut leakiness. Redox Biol 2014;3:40-46.

10. Chiu S, Sievenpiper JL, de Souza RJ, Cozma AI, Mirrahimi A, Carleton AJ, Ha V, et al. Effect of fructose on markers of non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of controlled feeding trials. Eur J Clin Nutr 2014;68:416-423.