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Dietary fructose augments ethanol-induced liver pathology☆,☆☆

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Abstract

Certain dietary components when combined with alcohol exacerbate alcohol-induced liver injury (ALI). Here, we tested whether fructose, a major ingredient of the western diet, enhances the severity of ALI. We fed mice ethanol for 8 weeks in the following Lieber-DeCarli diets: (a) Regular (contains olive oil); (b) corn oil (contains corn oil); (c) fructose (contains fructose and olive oil) and (d) corn + fructose (contains fructose and corn oil). We compared indices of metabolic function and liver pathology among the different groups. Mice fed fructose-free and fructose-containing ethanol diets exhibited similar levels of blood alcohol, blood glucose and signs of disrupted hepatic insulin signaling. However, only mice given fructose–ethanol diets showed lower insulin levels than their respective controls. Compared with their respective pair-fed controls, all ethanol-fed mice exhibited elevated levels of serum ALT; the inflammatory cytokines TNF-α, MCP-1 and MIP-2; hepatic lipid peroxides and triglycerides. All the latter parameters were significantly higher in mice given fructose-ethanol diets than those fed fructose-free ethanol diets. Mice given fructose-free or fructose-containing ethanol diets each had higher levels of hepatic lipogenic enzymes than controls. However, the level of the lipogenic enzyme fatty acid synthase (FAS) was significantly higher in livers of mice given fructose control and fructose–ethanol diets than in all other groups. Our findings indicate that dietary fructose exacerbates ethanol-induced steatosis, oxidant stress, inflammation and liver injury, irrespective of the dietary fat source, to suggest that inclusion of fructose in or along with alcoholic beverages increases the risk of more severe ALI in heavy drinkers.

Keywords: Fatty liver; Fructose; Alcohol; Insulin; Inflammation; Oxidant stress

1. Introduction

Alcoholic liver disease (ALD) exhibits a spectrum of liver injury beginning with simple fatty liver (steatosis) and progressing to more severe forms of liver injury, including steatohepatitis, fibrosis/cirrhosis and liver cancer [1]. Simple steatosis develops in more than 90% of heavy drinkers, about 30% of whom develop chronic liver disease, such as fibrosis and cirrhosis [1]. Many factors, including genetic polymorphisms in alcohol-metabolizing enzymes, comorbid hepatitis B or C infections, either accelerate or slow the development of ALD [1,2]. Findings from animal studies suggest that inclusion of dietary fats, especially unsaturated fatty acids, worsens alcohol-induced liver pathology [3–6], underscoring the notion that dietary factors significantly affect the development of ALD.

The role of dietary fat in promoting alcohol-induced liver injury (ALI) has been extensively investigated [6]. However, there is growing evidence that simple sugars, especially dietary fructose, are major causative agents of the metabolic syndrome and may aggravate alcohol-induced liver pathology. Fructose is a ketose monosaccharide and an isomer of glucose, an aldose monosaccharide. Fructose is found naturally in fruits and vegetables [7]. However, today, the principal dietary source of fructose in the Western diet comes through consumption of processed foods and beverages that are rich in fructose [7]. Fructose is transported into cells *via* the GLUT5 and GLUT2, transporters, specific for both glucose and fructose and which are highly expressed in liver, small intestine and pancreas [8]. Liver epithelial cells (*i.e.*, hepatocytes) have a high fructose extraction rate; thus, virtually an entire ingested fructose load can be cleared by the liver [7]. In the hepatocyte, fructose is phosphorylated to fructose-1-

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; ADH, alcohol dehydrogenase; CYP2E1, cytochrome P450 2E1; SDS-PAGE, sodium dodecyl sulfate; ELISA, enzyme-linked immunosorbent assay; TBARS, thiobarbituric acid reactive substances; mRNA, messenger RNA; qRT-PCR, quantitative reverse transcription polymerase chain reaction; LC3, microtubule-associated protein 1 light chain 3; AceCS1, acetyl-CoA synthetase; SCD1, Stearoyl-CoA desaturase-1; SQSTM1/p62, Sequestosome 1; PPAR-α, peroxisome proliferator-activated receptor-α; mTOR, mechanistic target of rapamycin complex 1; MDA, malondialdehyde.

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phosphate, initiating the breakdown of fructose (fructolysis), eventually forming pyruvate and subsequently acetyl-CoA, a primary substrate for lipogenesis [9]. While glucose and fructose metabolism both yield acetyl-CoA, glucose-derived acetyl-CoA is tightly regulated by insulin [7] and by feedback inhibition of glycolytic flux by ATP generated from glycolysis [9]. In contrast, acetyl-CoA generation *via* fructolysis continues uninterrupted and is not ATP sensitive [9]. Thus, unabated fructolysis leads to unabated lipogenesis [8,9]. Therefore, in addition to fat, fructose consumed in excess is another specific food component that promotes metabolic disruptions, including dyslipidemia, nonalcoholic fatty liver (NAFL), obesity and diabetes [7–9]. These precede the development of advanced forms of liver disease, including fibrosis [10].

Fructose is largely consumed as a major ingredient of carbonated beverages (*e.g.*, Colas) [11], which are often co-ingested with fat-enriched fast foods [12]. After being ingested chronically, both ingredients promote the development of fatty liver [6,7,13], leading to hepatic inflammation, an important risk factor for exacerbating ALI. Here, we hypothesized that a diet rich in unsaturated fat and fructose will accelerate alcohol-induced liver pathology. Therefore, to determine the dietary fat source and fructose combination that exacerbates ALI, we fed mice continuously with modified Lieber-DeCarli control or ethanol diets containing fructose and dietary fat derived from olive oil, rich in oleic acid or corn oil, in which linoleic acid is predominant (Table 1). Our findings revealed that fructose supplementation augmented ethanol-induced liver injury, irrespective of the dietary fat source used.

2. Materials and methods

2.1. Reagents

Antibodies to ADH, CYP2E1, ACeCS1, SCD-1, FAS, ACC, PPAR-α and LC3B were from Cell Signaling Technology Inc. (Danvers, MA, USA). Anti-P62/SQSTM1 was purchased from Medical and Biological Laboratories LTD (Japan). We obtained anti-GAPDH from Santa Cruz (Dallas, TX, USA).

2.2. Diets

Regular Lieber-DeCarli (LDC) control and ethanol diets were altered to contain dietary fat derived from either olive oil or corn oil and carbohydrate from fructose (Table 1). Dyets Inc. (Bethlehem, PA) formulated these diets. The individual components of these diets are presented in Table 1.

2.3. Animals

The animal studies subcommittee (IACUC) of Carolinas Medical Center approved the animal protocol. We followed the Guidelines for the *Use and Care of Laboratory Animals*, published by the National Institutes of Health. For these studies, 8-week-old male C57BL/6 mice obtained from Jackson Laboratory (Bar Harbor, ME, USA) were used. Mice were acclimated to Purina chow and then randomly assigned to four groups, acclimating all animals to their respective liquid control diets for 3 days. Animals within each of the four control diet-fed groups were weight matched and pair fed their respective control or ethanol-containing diets as previously described [14]. The ethanol concentration was gradually increased in the diet as described [15], and animals were administered full-strength ethanol (29.2% of total calories; 5.16% ethanol by volume) for 8 weeks. At sacrifice, mice were anesthetized with isoflurane, and blood, liver, brown adipose tissue (BAT) and white adipose tissue (WAT) were collected from each animal.

Table 1
Dietary components of the control and ethanol liquid diets

Ingredients	Regular		Corn oil		Fructose		Corn–Fructose	
	Con	EtOH	CO-Con	CO-EtOH	FR-Con	FR-EtOH	COFR-Con	COFR-EtOH
Malto dextrin	47	18	47	18	29	—	29	—
Olive oil	35	35	0	0	30	30	—	—
Corn oil (CO)	—	—	35	35	—	—	30	30
Fructose (FR)	—	—	—	—	23	23	23	23
Ethanol	—	29	—	29	—	29	—	29
Protein	18	18	18	18	18	18	18	18

Values presented are the percent of total calories in each diet.

2.4. Plasma analyses

Plasma ethanol levels were measured using an ethanol assay kit (BioVision, Mountain view, CA, USA). Aspartate aminotransferase (AST) activity was colorimetrically measured using an Infinity AST Reagent (Thermo Scientific, Waltham, MA, USA). Alanine aminotransferase (ALT) activity and glucose levels were measured using colorimetric assay kits from Cayman Chemical (Ann Arbor, MI, USA). Insulin levels were measured using an ultrasensitive mouse insulin ELISA kit from Crystal Chem (Downers Grove, IL, USA).

2.5. Hepatic triglycerides and lipid peroxidation

Frozen livers were homogenized and assayed for triglyceride content and lipid peroxidation (TBARS), as described by the manufacturer (Cayman, Ann Arbor, MI, USA).

2.6. RNA isolation and real-time PCR

Total RNA was isolated from liver pieces (~50–70 mg each) using Trizol Reagent (Invitrogen, Inc., Carlsbad, CA, USA). RNA (100 ng) was reverse transcribed using Iscript cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA) to generate cDNAs. Messenger RNA levels were quantified from amplified cDNA samples using PCR master mix, specific primers and SYBR green as the fluorescent marker. Primers used for PCR are listed in Table 2. Relative mRNA levels were normalized to 18S ribosomal RNA.

2.7. Detection of proteins by Western blots

Proteins from tissue homogenates were separated under denaturing conditions on SDS-polyacrylamide mini-gels and transferred onto nitrocellulose membranes. The membranes were incubated with primary antibodies overnight at 4 °C. After washing, membranes were incubated with secondary antibodies conjugated to horseradish peroxidase for 1 h. Proteins were detected using enhanced chemiluminescence (ECL) reagent (Amersham, PA). Protein band densities were calculated using Quantity One software (Bio-Rad, Hercules, CA, USA). Protein loading was normalized by calculating the densitometric ratio of the protein of interest to that of GAPDH.

2.8. Liver pathology

Pathological changes were evaluated in hematoxylin and eosin-stained liver sections by a board-certified pathologist (C.J.). Total liver pathology was defined as the sum of the steatosis, inflammation and hepatocyte ballooning scores.

2.9. Statistical analysis

Statistical significance among different diets, controls and ethanol-fed groups was evaluated by a two-way analysis of variance (ANOVA) followed by a Student's *t* test. Data are expressed as mean values ± S.E.M. A probability (P) value≤0.05 was considered statistically significant.

Table 2
Primer sets used to detect mRNAs encoding proinflammatory cytokines, chemokines and TLR signaling

Gene	Forward primer 5′ – 3’	Reverse primer 5′ – 3’
TNF-α	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
MCP-1	AGGTCCCTGTCATGCTTCTG	TCTGGACCCATTCCTTCTTG
CCR2	CAGGACTGAGTGAGAAGGAG	AGCCTCCAAAATTAAACACA
MIP-1	CAGTTAGCAAGAGGCAGAAT	TGGTTCAAGAAGTCATACCC
MIP-2	GCGCCCAGACAGAAGTCATAG	AGCCTTGCCTTTGTTCAGTATC
IL-8	CACCTCAAGAACATCCAGAGCT	CAAGCAGAACTGAACTACCATCG
18S	ACGGAAGGGCACCACCAGGA	CACCACCACCCACGGAATCG
MyD88	TGTCTCCAGGTGTCCAACAG	CTTGGTGCAAGGGTTGGTAT
TLR1	TTGCCAATATGAACATCCAAA	TGCAGAAATGGGCTAACTTG
TLR2	CCTACATTGGCCATGGTGAC	CCTCTATTGTATTGATTCTGCTGGA
TRAF6	TTGCTGGAACCCATTCTACAG	ATTCCAAGTAAGTCCGCTGTG
TLR4	CAGTGGTCAGTGTGATTGTGG	TTCCTGGATGATGTTGGCAGC