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Fatty Liver Promotes Fibrosis In Monkeys Consuming High Fructose

Michael A. Cydylo, **Ashley T. Davis**, and **Kylie Kavanagh**

Wake Forest School of Medicine Department of Pathology, Medical Center Blvd. Wake Forest University Health Sciences, Winston-Salem 27157 NC USA

Abstract

Objective—Non-alcoholic fatty liver diseases (NAFLD) are related to development of liver fibrosis which currently has few therapeutic options. Rodent models of NAFLD inadequately model the fibrotic aspects of the disease and fail to demonstrate the spectrum of cardiometabolic diseases without genetic manipulation. We aimed to document a monkey model of fatty liver and fibrosis, which naturally develop cardiometabolic disease pathophysiologies.

Methods—We studied 27 cynomologus monkeys (*Macaca fascicularis*) fed diets either low or high in simple carbohydrates, supplied as fructose, (CTL and HFr), on low fat, cholesterol-free background. The HFr was consumed for up to 7 years and liver tissue was histologically evaluated for fat and fibrosis extent.

Results—The HFr diet increased steatosis, and its extent was related to duration of fructose exposure. Lipid droplet size also increased with HFr duration, however compared to CTL the lipid droplets were smaller on average. Fibrosis extent was significantly greater with fructose feeding and was predicted by fructose exposure, extent of fatty liver, and age.

Conclusions—These data are the first to demonstrate that high carbohydrate diets alone can generate both liver fat and fibrosis and thus allows further study of mechanisms and therapeutic options in this translational animal model.

Keywords

fatty liver; liver fibrosis; fructose

Introduction

Non-alcoholic fatty liver diseases (NAFLD) are prevalent in developed and developing countries (1) and increase risk for cardiometabolic diseases (2). Fatty liver has been attributed to calories excess, and specifically to calories supplied as fructose (3). Controversy exists regarding the role fructose plays, as it is typically consumed within diets characterized by excess calories, high dietary fat and cholesterol. These combined dietary factors drive fatty liver and liver fibrosis in human populations, however they do not

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consistently produce representative disease in rodents or produce NAFLD through pathways that are implicated in human disease (4).

Rodent studies indicate fructose and high carbohydrate diets potently drive fatty liver, however rodents have far greater lipogenesis rates and thus dietary effects in this model may be exaggerated (5). Rodents also have dissimilar primary sites of lipogenesis, inflammatory responses, toll like receptor expression patterns and do not readily develop hepatic fibrosis, which is the disease feature that is the most difficult to induce experimentally and is without therapeutic options in people (4, 6–8). We have previously shown that high carbohydrate consumption as fructose rapidly causes hepatitis and over time drives fatty liver development in monkeys (9). The current study was undertaken to extend these findings by examining hepatic fibrosis and steatosis in fructose-fed monkeys.

Methods

All procedures were approved and followed the guidelines of the Institutional Animal Care and Use Committee of Wake Forest University Health Sciences. Primates, *Macaca fasciculus* (n=27), were fed one of two diets: a control diet (CTL; Diet 5038, LabDiet, Purina, St. Louis, MO, n=10) or a high fructose diet (HFr; n=17) which have been previously described in detail (9). The CTL diet was high in carbohydrates (69% of calories), with less than 3% glucose and 0.5% fructose supplying the calories. The HFr diet was equivalent in the percentage of calories supplied by carbohydrates however 24% of the total caloric intake was supplied from fructose. The HFr cohort was fed the diet for up to seven years (mean 2.75 years, range 0.27 – 6.6 years; Figure 1A), which approximates 1 to 20 human years, and were older and heavier than CTL monkeys, as previously described (9). Control monkeys had lifelong exposure to their diet condition.

Liver tissue collected at necropsy from monkeys was fixed and stained using H&E and Masson's trichrome (MTC). Sections were assessed for steatosis by lipid droplet number and size and for fibrosis (additional detail available in Supplementary Information). Data were analyzed by analysis of covariance using age and bodyweight as covariates, associations were assessed Pearson's correlation coefficients, and multiple regression modeling was employed using Statistica (StatSoft Inc., Tulsa, OK). A $p<0.05$ was used to denote significance. Data are reported as mean ± standard error.

Results

Fructose-fed monkeys were heavier than CTL animals (3.03±0.09kg vs. 5.42±0.83kg; $p=0.04$). Fructose diets increased the number of lipid droplets, or steatosis score ($p<0.001$; Figure 1B, graph modified from (9)) but the droplets were of smaller average diameter ($p=0.002$) (Figure 1C). Fructose consumption significantly increased liver collagen content. Figures 1D and E show that the area stained for collagen in hepatic tissue was increased with fructose consumption when measured as either a percentage of the entire histological section ($p=0.03$) or the liver parenchyma that was distributed between the portal triads ($p=0.007$). Representative sections (Figure 1 F–H) illustrate pathological features resembling those seen in human NAFLD. Association analyses from data obtained from the fructose-consuming

animals only (n=17; Figure 2) show that the number and diameter of the lipid droplets increased with longer consumption of fructose-containing diet. The steatosis score, or number of droplets, also had a significant positive relationship with hepatic fibrosis. CD3 cell counts were non-significantly higher in HFr monkeys (p=0.36) and not related to liver fat or fibrosis.

To examine the relative contributions of steatosis score, age, weight, duration of consumption, sex, and the experimental diets fed on liver fibrosis, a multiple regression analysis was conducted, with percent area of the liver staining positive for collagen as the outcome variable. The overall model was highly significant (Table 1; $p<0.001$) only with diet type (HFr vs. CTL), the extent of steatosis and age and being retained as significant predictors. Fructose exposure had the greatest magnitude of effect as compared to steatosis score, with age having a nominal effect on fibrosis.

Discussion

This report is the first to demonstrate, using a non-human primate animal model, that a high carbohydrate diet not augmented with dietary fat or cholesterol drives the important features of NAFLD including fibrosis (4, 9, 10). The demonstration of these pathologies in a non-human primate is relevant to the human disease as they have both comparable gastrointestinal and hepatic anatomies and functions, and immunological responses (11).

While there has been controversy on the exact cause of steatosis, whether excess calories, fat, fructose, or a combination of the three, this study produced clear evidence that just carbohydrate consumption as fructose induces fatty liver and also promotes fibrotic processes. These results further confirm that non-human primates, unlike rodents, develop fibrosis as part of their NAFLD expression in response to a carbohydrate diet challenge. Only one other primate model, *Macaca radiata*, describes fibrosis accompanying steatosis (12). In this monkey model, older age was also identified as a risk factor for disease however fibrosis extent was described categorically and the interactions of age and fat were not explored. In our larger cohort of monkeys, the fibrosis extent measured was variable among individuals, as reflected by lack of a direct significant relationship between the duration of diet consumption and liver fibrosis, while steatosis development with the fructose diet was more consistent. This represents an opportunity to further investigate the mechanisms of fibrotic processes and why certain individuals are more vulnerable to this negative consequence of consuming a high simple carbohydrate or fructose-containing diet.

Confidence in our histological results is high as the steatosis and fibrosis outcomes were measured by two different methods and produced consistent results: increased fat and fibrosis in the fructose-fed monkeys. However, these results do not implicate fructose specifically in fibrosis induction, as this study did not include a group of monkeys consuming comparable amounts of glucose, and it is possible that dextrose may also produce similar results. In rodent and human studies, fructose is more potent in inducing fatty liver (13, 14) which should drive fibrosis in a vulnerable clinical population. This hypothesis is supported by clinical reports of fructose being related to liver fibrosis in patients with nonalcoholic hepatosteatitis, even when controlling for caloric intake in

statistical models (15). Our monkey cohort was predominantly female, and studies have shown that NAFLD prevalence is higher in males than females (16). Thus, our findings may underestimate the effects of a high carbohydrate diet on fat and fibrosis in the liver of a larger population. The predominant strength for this study was the use of cynologus monkeys as the animal model, and the long duration of dietary manipulation which to date has not yet been reported. Ectopic fat deposition in the liver is linked to the development of metabolic diseases (17). In this regard, monkeys are uniquely translational as rodents do not spontaneously develop diabetes or cardiovascular disease in the absence of genetic manipulation, while monkeys will develop both without experimental intervention (18, 19). Human clinical trials would be ideal, however long-term controlled dietary studies are difficult and liver samples cannot be routinely collected. We conclude that the monkey is the optimal model to study NAFLD etiologies and progression, and to evaluate potential therapeutic options for reversal of fat and fibrosis deposition with amelioration of related cardiometabolic diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Study importance

- Diets high in fructose-containing sugars cause fatty liver in animal models but evidence for fibrosis development in animals and human experiments is lacking in the former or confounded by other dietary factors in the latter species.
- We demonstrate for the first time that fructose consumption alone can drive increased collagen deposition, indicative of liver fibrosis, in a relevant primate model over a multi-year period of fructose exposure.

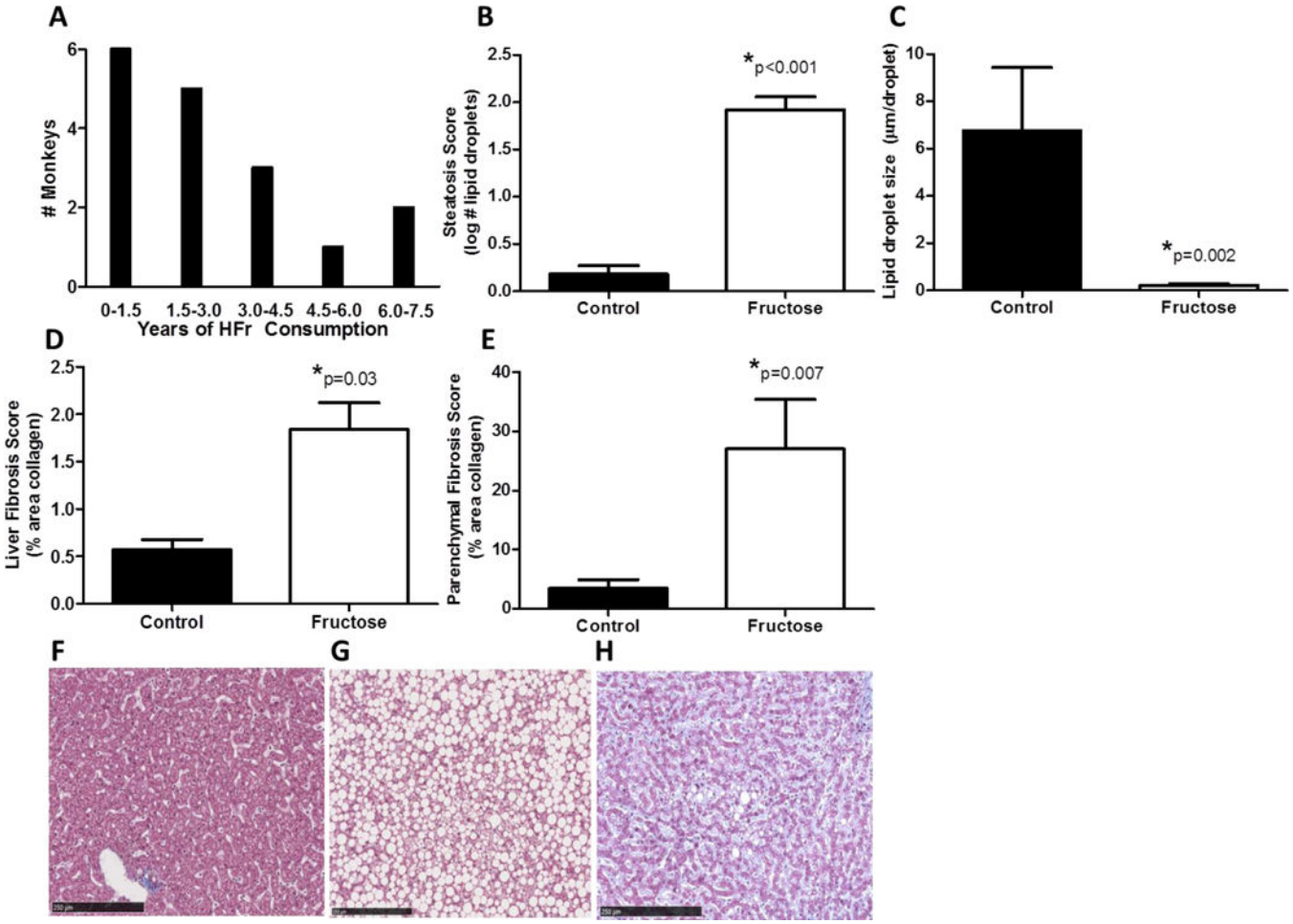

Figure 1.

Liver tissue was evaluated from 17 monkeys consuming fructose for 0.27 – 6.6 years, with the number assessed depicted in A. Fructose consumption increased the number of lipid droplets (B) however reduced average lipid droplet size (C) in the liver as compared to monkeys eating a fructose-free control diet. Fibrosis was increased in fructose fed monkeys when measured from the entire histological section (D) and from triplicate parenchymal regions of interest (E). Representative histological examples of liver stained for collagen are shown from a control monkey (F, a severely steatotic monkey fed fructose (G), and a monkey with high fibrosis and moderate steatosis (H).

* indicates significance, with the adjusted p-values (age and bodyweight used as covariates) displayed

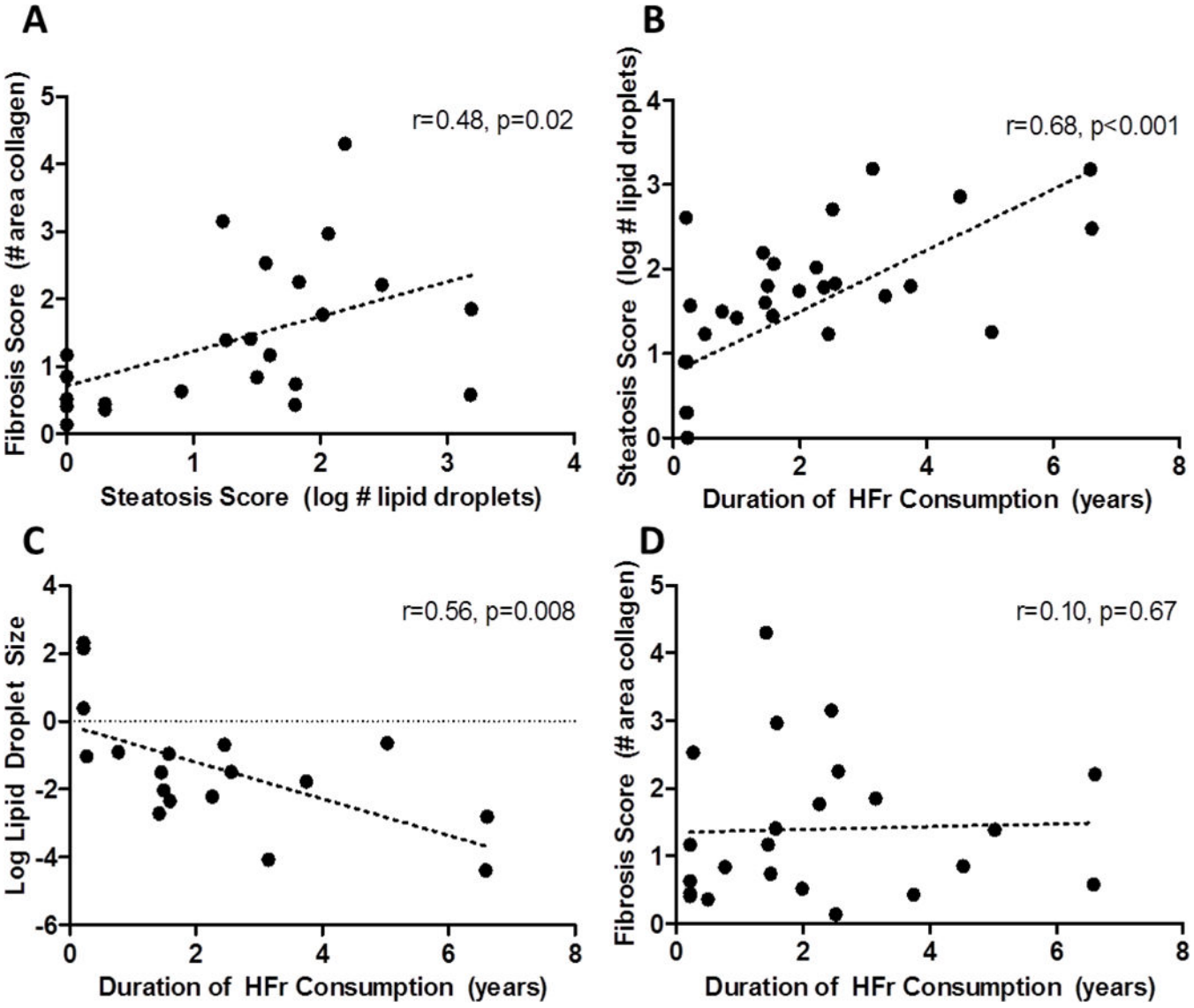

Figure 2.

Associations between fibrosis and steatosis (A), steatosis (B) lipid droplet size (C), and fibrosis (D) and duration of high fructose consumption in monkeys. Pearson's correlation coefficients and p-values are shown on each graph.

Table 1

Multivariate regression modeling results for liver fibrosis show that fructose exposure, the extent of liver fat and age all significantly influence collagen deposition.

Model Variable	R^2	ß-coefficient	p-value
Fructose Diet	0.33	0.64	0.004
Steatosis Score	0.23	0.52	0.02
Age	0.44	0.088	0.0006