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Age-Related Structural Susceptibility of Rat Liver Parenchyma to HFCS-55-Induced Injury Under Distinct Energy Conditions: A Semi-Quantitative Histological Study

Farklı Enerji Koşulları Altında Sıçan Karaciğer Parenkiminin YFMŞ-55 Kaynaklı Hasara Karşı Yaşa Bağlı Yapısal Duyarlılığı: Yarı Kantitatif Histolojik Bir Çalışma

Sude Topkaraoğlu[1], Burcu Cevreli[2], Elif Sude Tiken[3], Tansel Sapmaz[1]

[1]*University of Health Sciences, Hamidiye Faculty of Medicine, Department of Histology and Embryology, İstanbul, Türkiye*

[2]*Üsküdar University Faculty of Medicine, Department of Physiology, İstanbul, Türkiye*

[3]*Biruni University Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, İstanbul, Türkiye*

ABSTRACT

Background: High-fructose corn syrup (HFCS) is widely used as a sweetener in processed foods and beverages, and excessive intake has been associated with histopathological alterations and hepatic injury. However, the age-dependent effects of chronic HFCS-55 consumption on hepatic fibrosis and structural liver alterations remain incompletely understood. This study aimed to investigate the age-dependent effects of HFCS-55 consumption on the development of hepatic fibrosis and histopathological alterations of the liver under different feeding regimens.

Materials and Methods: Young (2-month-old) and aged (15-month-old) male Sprague-Dawley rats were divided into three groups within each age category (n = 10 per group): control (standard chow and water ad libitum), HFCS plus restricted chow (10 g/day), and HFCS plus restricted chow (20 g/day). HFCS was administered as an 11% solution during the dark period. The feeding protocol lasted 16 weeks. At the end of the experiment, liver tissues were collected and evaluated using hematoxylin and eosin, Masson's trichrome, and Direct Red 80 staining to assess structural damage and collagen deposition.

Results: HFCS-fed groups, particularly the HFCS + restricted chow (20 g/day) groups in both young and aged rats, exhibited hepatocellular degeneration, sinusoidal dilatation, increased collagen deposition, and mild-to-moderate fibrosis compared to controls. A statistically significant increase in hepatic injury and fibrosis scores was observed only in the HFCS + restricted chow (20 g/day) groups compared to their respective controls ($p < 0.05$). These changes were more pronounced in aged rats.

Conclusion: Chronic consumption of HFCS-55-containing beverages was associated with age-dependent hepatic injury and fibrosis in rats. These findings suggest that both aging and high fructose intake may contribute to the progression of hepatic damage. However, because the present study was limited to histopathological evaluation, further studies incorporating molecular analyses (e.g., markers of oxidative stress, inflammation, and apoptosis) are warranted to elucidate the underlying mechanisms.

Keywords: Aging, high-fructose corn syrup (HFCS), hepatic fibrosis, Masson's trichrome, Direct Red 80

ÖZ

Amaç: Yüksek fruktozlu mısır şurubu (YFMŞ), işlenmiş gıdalarda ve içeceklerde yaygın olarak kullanılan bir tatlandırıcıdır ve aşırı alımı histopatolojik değişiklikler ile karaciğer hasarıyla ilişkilendirmiştir. Bununla birlikte, kronik YFMŞ-55 tüketiminin hepatik fibrozis ve karaciğerdeki yapısal değişiklikler üzerindeki yaşa bağlı etkileri henüz tam olarak anlaşılamamıştır. Bu çalışma, farklı beslenme rejimleri altında YFMŞ-55 tüketiminin hepatik fibrozis gelişimi ve histopatolojik karaciğer değişiklikleri üzerindeki yaşa bağlı etkilerini araştırmayı amaçlamıştır.

Address for Correspondence: Asst. Prof. Sude Topkaraoğlu, MD, University of Health Sciences, Hamidiye Faculty of Medicine, Department of Histology and Embryology, İstanbul, Türkiye

E-mail: sude.topkaraoglu@sbu.edu.tr **ORCID ID:** orcid.org/0000-0002-7680-7156

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ÖZ

Gereç ve Yöntemler: Genç (2 aylık) ve yaşlı (15 aylık) erkek Sprague–Dawley sıçanları, her yaş kategorisinde üç gruba ayrılmıştır (grup başına n = 10): Kontrol (standart yem + ad libitum su), YFMŞ + kısıtlı yem (10 g/gün) ve YFMŞ + kısıtlı yem (20 g/gün). YFMŞ, karanlık periyot boyunca %11'lik bir çözelti halinde uygulanmıştır. Beslenme protokolü 16 hafta sürmüştür. Deney sonunda karaciğer dokuları toplanmış; yapısal hasar ve kollajen birikimini değerlendirmek amacıyla hematoksilen ve eozin, Masson trikrom ve Direct Red 80 boyamaları kullanılarak incelenmiştir.

Bulgular: YFMŞ ile beslenen gruplarda, özellikle hem genç hem de yaşlı sıçanlardaki YFMŞ + kısıtlı yem (20 g/gün) gruplarında, kontrollere kıyasla hepatoselüler dejenerasyon, sinüzoidal dilatasyon, artmış kollajen birikimi ve hafif ila orta derecede fibrozis gözlenmiştir. Kendi kontrolleriyle kıyaslandığında, hepatik hasar ve fibrozis skorlarında istatistiksel olarak anlamlı artış yalnızca YFMŞ + kısıtlı yem (20 g/gün) gruplarında saptanmıştır ($p < 0,05$). Bu değişiklikler yaşlı sıçanlarda daha belirgin seyretmiştir.

Sonuç: YFMŞ-55 içeren içeceklerin kronik tüketimi, sıçanlarda yaşa bağlı hepatik hasar ve fibrozis ile ilişkilendirilmiştir. Bu bulgular hem yaşlanmanın hem de yüksek fruktoz alımının hepatik hasarın ilerlemesine katkıda bulunabileceğini düşündürmektedir. Bununla birlikte, mevcut çalışma histopatolojik değerlendirmeyle sınırlı olduğundan, altta yatan mekanizmaları aydınlatmak için moleküler analizleri (örneğin oksidatif stres, enflamatuvar ve apoptotik belirteçler) içeren daha ileri çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Yaşlanma, hepatik fibrozis, yüksek fruktozlu mısır şurubu (YFMŞ), Masson trikrom, Direct Red 80

Introduction

High-fructose corn syrup (HFCS) is a widely used sweetener composed primarily of fructose and glucose. Among its formulations, HFCS-55 (approximately 55% fructose and 45% glucose) is the most commonly used form in soft drinks due to its sweetness and stability (1). Excessive consumption of HFCS-containing beverages has been associated with obesity, insulin resistance, and non-alcoholic fatty liver disease (2,3).

Accumulating evidence suggests that these adverse hepatic alterations are largely attributable to the fructose component (4,5). Consistent with this, the literature indicates that high-fructose diets can induce systemic features of metabolic syndrome (such as insulin resistance and altered lipid profiles); at the tissue level, these processes manifest as profound structural changes. Indeed, differences in the metabolic processing of fructose and glucose by hepatocytes primarily contribute to the specific parenchymal and cellular alterations associated with fructose consumption (6).

Unlike glucose, which is tightly regulated by insulin, fructose metabolism occurs predominantly in the liver via fructokinase, thereby bypassing the key regulatory enzyme phosphofructokinase. This relatively unregulated metabolism has been associated with oxidative stress and may contribute to hepatic injury and histopathologically detectable fibrotic changes (7-9). Previous studies have reported that chronic fructose intake is associated with hepatocellular degeneration, and collagen accumulation which are features commonly observed in fibrosis (10,11). Moreover, aging may increase susceptibility to such changes due to reduced antioxidant capacity and impaired mitochondrial function (12,13).

Despite growing evidence linking fructose consumption to hepatic injury, the age-dependent patterns of HFCS-55-induced histopathological alterations and architectural collagen deposition under different dietary energy conditions have yet to be fully characterized morphologically. A clearer understanding of these effects is important for evaluating liver responses to dietary fructose across different stages of life.

Therefore, this study aimed to examine the histopathological and fibrotic effects of HFCS-55 in young and aged male Sprague–Dawley rats subjected to differential caloric intake via chow restriction. Hepatic changes were evaluated using hematoxylin and eosin (H&E), Masson's trichrome (MT), and Direct Red 80 (DR) staining to assess hepatocellular integrity and collagen deposition. We hypothesized that aging and differences in overall energy intake, despite similar HFCS exposure, would be associated with variations in hepatic injury and fibrotic changes.

Materials and Methods

Animals and Experimental Design

This investigation utilized male Sprague–Dawley rats, bred at the Experimental Research Unit of the Üsküdar University Neuropsychopharmacology Application and Research Center, representing two age groups: 2 months (young) and 15 months (aged). Two-month-old rats were selected to represent young animals, whereas 15-month-old rats were selected to represent aged animals. This age-based design was intended to determine whether age influences the histopathological findings observed under experimental HFCS-containing dietary conditions. The animals were housed under standard laboratory conditions at a temperature of 21 °C, a relative humidity of 50–60%, and a 12-h light/12-h dark photoperiod (lights on from 07:00 to 19:00). Wood shavings were used as bedding material, and two rats were housed in each conventional cage. Animals

were provided with a standard pellet diet appropriate for physiological requirements (57% starch, 20% protein, 4% fat, 4% cellulose, standard vitamin–mineral mixture; 3 kcal/g) and water ad libitum. Prior to study initiation, approval was obtained from the Üsküdar University Ethics Committee for Animal Experiments (protocol number: 2023-05, date: 20.01.2023).

Experimental Groups

A total of 60 male Sprague–Dawley rats were allocated to six experimental groups defined by two age cohorts (young: 2-month-old; aged: 15-month-old) and three dietary conditions. Each group contained the same number of animals, with two rats housed per cage.

Caloric intake was estimated based on chow (3 kcal/g) and HFCS solution (11% w/v; ~0.44 kcal/mL). Although fluid intake was not directly measured, previous studies indicate that rats consume approximately 30–50 mL/day. Accordingly, HFCS intake was estimated to contribute approximately 13–22 kcal/day. These values suggest that total caloric intake differed across experimental groups (14,15).

Young Rat Experimental Groups (2-Month-Old)

To evaluate the effects of age on histopathological alterations, young rats were divided into three groups (G):

G1: Young Control (YC): Standard chow + water ad libitum

G2: Young HFCS + restricted chow (10 g/day): Restricted diet of 10 g/day chow + 11% HFCS during the dark period

G3: Young HFCS + restricted chow (20 g/day): Restricted diet of 20 g/day chow + 11% HFCS during the dark period

Aged Rat Experimental Groups (15-Month-Old)

To investigate the interaction between aging and HFCS exposure, aged rats were similarly divided into three groups:

G4: Aged Control (AC): Standard chow + water ad libitum

G5: Aged HFCS + restricted chow (10 g/day): Restricted diet of 10 g/day chow + 11% HFCS during the dark period

G6: Aged HFCS + restricted chow(20 g/day): Restricted diet of 20 g/day chow + 11% HFCS during the dark period

Feeding Protocol and HFCS Administration

The general health condition of the animals was routinely assessed throughout the experimental period. Daily chow intake was standardized according to group designation: animals in the Control and HFCS + 20 g groups received 20 g/day of chow per animal, whereas those in the HFCS + 10 g groups received 10 g/day of chow per animal (Table 1). Water was provided during the light period at a rate of 5–10 mL per 100 g of body weight.

HFCS was prepared as an 11% solution and administered exclusively during the dark period. The feeding protocol was maintained for a total of 16 weeks across all experimental groups. The body weights of all animals were recorded regularly during the experimental period to monitor the physiological effects of the dietary interventions.

Values represent estimated caloric intake based on standard chow (3 kcal/g) and the HFCS solution. These calculations indicate that total caloric intake differed across groups, and variations in feed and water consumption may influence experimental outcomes (18).

Euthanasia Protocol and Tissue Collection

At the end of the experimental protocol, all animals were euthanized in accordance with the AVMA Guidelines for the Euthanasia of Animals (2020) and with institutional ethical approval. Euthanasia was performed under deep anesthesia.

Rats were anesthetized via intraperitoneal administration of ketamine (80 mg/kg) and xylazine (10 mg/kg), and the depth of anesthesia was confirmed by the absence of the pedal reflex. Euthanasia was subsequently performed by decapitation. Following euthanasia, liver tissues were carefully harvested for histopathological examination. A midline abdominal incision was made with sterile surgical scissors, and the liver lobes were carefully dissected to preserve anatomical integrity and minimize tissue damage. Tissue specimens were prepared as sections measuring approximately 0.5–1 cm in thickness.

Table 1. Estimated (not directly measured) daily caloric intake across experimental groups.

Group	Chow intake (g/day)	Chow energy (kcal/day)	Estimated fluid intake (mL/day)	HFCS energy (kcal/day)*	Total energy (kcal/day)
Control	~20	60	~40	0	~60
HFCS + restricted chow (10 g/day)	10	30	~40	~17.6	~47.6
HFCS + restricted chow (20 g/day)	20	60	~40	~17.6	~77.6

*The energy content of HFCS was calculated from an 11% (w/v) solution (~0.44 kcal/mL). Fluid intake was not directly measured and was therefore estimated from the literature (30–50 mL/day; mean ~40 mL) (16,17). Estimated total daily caloric intake differed across experimental groups; HFCS-treated groups showed divergent patterns of energy intake due to chow restriction combined with the additional caloric contribution of the HFCS solution. HFCS, high-fructose corn syrup.

Immediately after collection, liver tissues were placed in 10% neutral buffered formalin (NBF) and fixed for 48–72 h. An adequate tissue-to-fixative ratio was maintained by immersing each sample in a volume of formalin at least ten times its volume. After fixation, tissues were transferred for routine histological processing.

Histopathological Assessment

Histopathological analyses were performed exclusively on liver tissue samples. Formalin-fixed, paraffin-embedded liver sections (4–5 μm thick) were stained with H&E, MT, and DR (Sirius Red). Hepatic injury and fibrosis were evaluated separately. The histopathological scoring system for hepatic alterations was adapted from previously established semi-quantitative models (19-21), specifically optimized to evaluate progressive architectural and vascular changes induced by dietary challenges. This system was selected because it provides a reliable and reproducible framework for grading cumulative tissue injury from early sinusoidal changes to marked collagen accumulation. Alterations were graded on a scale from 0 to 3 based on specific morphological criteria: Grade 0 represented normal tissue architecture with baseline collagen distribution and no observable injury; Grade 1 indicated mild, localized hepatocellular degeneration and early perisinusoidal collagen deposition; Grade 2 denoted moderate, diffuse hepatocellular degeneration accompanied by noticeable sinusoidal dilatation and expanded pericellular/periportal collagen accumulation; and Grade 3 represented severe, widespread hepatocellular degeneration with extensive sinusoidal distortion and prominent, dense collagen deposition throughout the parenchyma. Hepatic injury was assessed in H&E-stained sections based on sinusoidal dilatation and hepatocellular degeneration, and was scored as follows: 0, no injury; 1, mild injury; 2, moderate injury; 3, marked injury. Hepatic fibrosis was evaluated semiquantitatively based on the extent and distribution of collagen deposition observed in MT- and DR (Sirius Red)-stained sections, and scored as follows: 0, no fibrosis; 1, mild perisinusoidal or periportal collagen deposition; 2, moderate fibrosis; 3, marked fibrosis with septa formation. Histopathological evaluation was performed in a blinded manner by examining at least 5 randomly selected fields per section.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). Because hepatic injury and fibrosis scores were semi-quantitative ordinal variables, the data were expressed as medians (interquartile range). Differences among the six experimental groups were evaluated using the Kruskal–Wallis test, followed by Dunn's post-hoc test for multiple comparisons when a significant overall effect was observed. The results were interpreted in relation to the factorial structure of the experimental design, including age group (young vs. aged) and dietary condition (control, HFCS + restricted chow 10 g/day, and HFCS + restricted chow 20 g/day). To evaluate the effects of age, dietary treatment, and their interaction, a non-parametric two-way analysis of variance (the Scheirer–Ray–Hare test) was performed on the ranked hepatic injury and fibrosis scores. Main effects of age, dietary treatment, and their interaction terms were reported as the H statistic (equivalent to the chi-square statistic), the degrees of freedom (df), and exact p values. Statistical significance was defined as $p < 0.05$.

Results

Body Weight Alterations

To evaluate the systemic metabolic effects of HFCS-55 consumption under different energy conditions, body weights of young and aged rats were monitored over the 16-week experimental period. At the end of the study, a significant difference in body weight was observed among the experimental groups. As shown in Figure 1, aged rats generally maintained a higher body weight profile than the young cohorts. Distinct variations in weight gain were observed among the dietary intervention groups, depending on the amount of restricted chow provided alongside the 11% HFCS solution. Statistical analysis demonstrated a significant increase in body weight at Week 16 compared with baseline (week 1) in all young rat groups (G1, G2, and G3; adjusted $p = 0.0469$ for all groups). In contrast, no statistically significant within-group changes in body weight were observed between week 1 and week 16 in the aged rat groups (G4, G5, and G6).

Hepatic Injury and Fibrosis

Histopathological evaluation (Figure 2) demonstrated that hepatic injury scores were higher in HFCS-treated groups compared with controls; however, statistical significance was observed only in the HFCS + restricted-chow (20 g/day) groups ($p < 0.05$).The highest injury scores were observed in the HFCS + restricted chow (20 g/day) groups across both age categories, with greater severity in aged animals. F ibrosis scores (Figure 3) increased in HFCS-treated groups; however, compared with controls, the increase was statistically significant only in the HFCS + restricted chow (20 g/day) groups ($p < 0.05$). More advanced fibrotic alterations, including septa formation, were particularly evident in aged HFCS-treated groups. Overall, both hepatic injury and fibrosis scores were significantly higher in aged

HFCS-treated rats than in their young counterparts ($p < 0.05$). Hepatic injury and fibrosis scores for all experimental groups are summarized in Figure 4.

The Kruskal-Wallis tests demonstrated statistically significant differences in hepatic fibrosis scores (Kruskal-Wallis statistic H = 21.76, p = 0.0006) (Table 2) and hepatic injury scores (Kruskal-Wallis statistic H = 22.64, p = 0.0004) (Table 3) among the experimental groups.

Two-factor non-parametric analysis of variance revealed a highly significant main effect of dietary treatment on hepatic fibrosis scores (H = 21.42, df = 2, $p < 0.0001$). The main effect of age was also statistically significant (H = 4.88, df = 1, $p < 0.0271$), confirming that aged rats exhibited higher baseline susceptibility and greater treatment-induced susceptibility to collagen deposition than young counterparts. However, the interaction between age and dietary treatment did not reach statistical significance (H = 0.35, df = 2, $p < 0.8395$), indicating that HFCS exposure, combined with feed restriction, drove the progression of fibrotic alterations consistently across both age cohorts. Post-hoc comparisons confirmed that significant fibrotic advancement was primarily observed in the HFCS + restricted chow (20 g/day) groups ($p < 0.05$).

In Young Rats

Normal liver tissue morphology was observed in the control group. Light microscopic examination of liver tissue samples from the control group revealed that the classic liver lobule structure, consisting of the central vein, portal triad, and hepatocyte cords, appeared normal.

Fibrosis was observed in all HFCS groups. Mild dilatation and degenerative changes were observed between the sinusoids. The Young HFCS + restricted chow (20 g/day) group exhibited more severe fibrosis and edema compared with the Young HFCS + restricted chow (10 g/day) group.

In Aged Rats

Compared with the normal liver architecture of the YC group, the control group exhibited deformed hepatic lobular structures. Fibrosis was observed in all HFCS groups. The Aged HFCS + restricted chow (20 g/day) group exhibited more severe deformed hepatic lobular structures compared to the Aged HFCS + restricted chow (10 g/day) group.

It should be noted that these fibrotic alterations were evaluated and graded strictly within the framework of a semi-quantitative scoring system based on spatial-architectural distribution, rather than by continuous digital pixel-area quantification.

Although HFCS-fed groups tended to show increased collagen deposition and hepatocellular damage compared with control groups, statistically significant differences were mainly observed in the HFCS + restricted chow (20 g/day) groups. These histopathological alterations were more pronounced in aged rats, indicating that aging may enhance hepatic vulnerability under HFCS-containing and non-isocaloric dietary conditions.

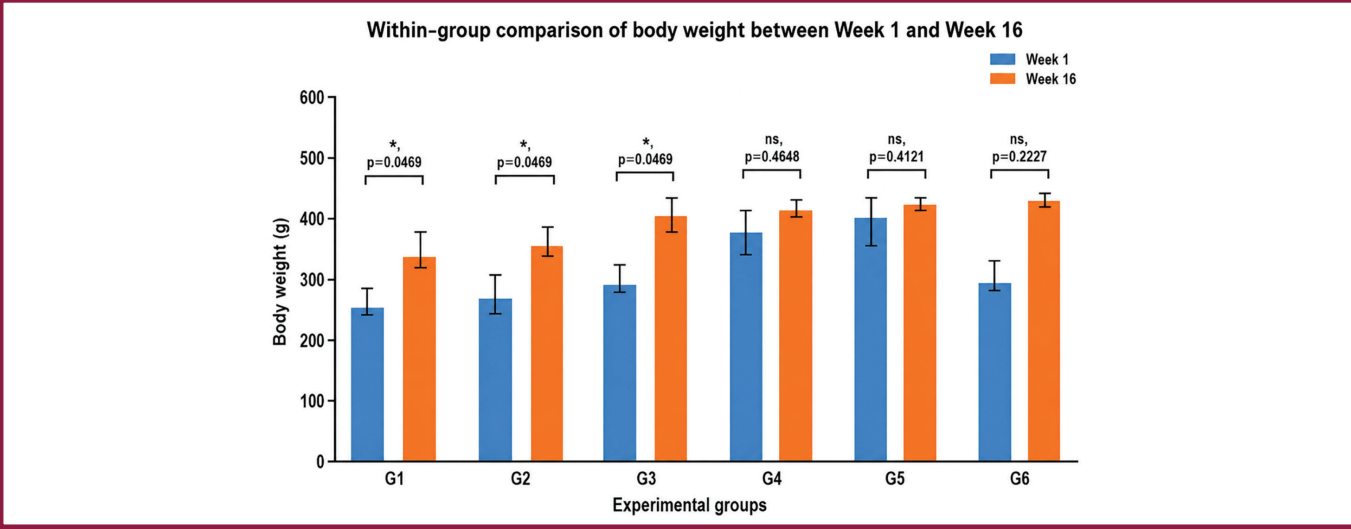


Figure 1. Within-group comparison of body weight between week 1 and week 16 in young and aged rats. Data are presented as median (interquartile range). Within-group longitudinal comparisons were performed using the two-sided Wilcoxon signed-rank test, with p values adjusted for multiple comparisons using the Holm–Bonferroni method. G1: Young control, G2: Young HFCS + restricted chow (10 g/day), G3: Young HFCS + restricted chow (20 g/day), G4: Aged control, G5: Aged HFCS + restricted chow (10 g/day), G6: Aged HFCS + restricted chow (20 g/day), $p < 0.05$; ns, not significant; HFCS, high-fructose corn syrup.

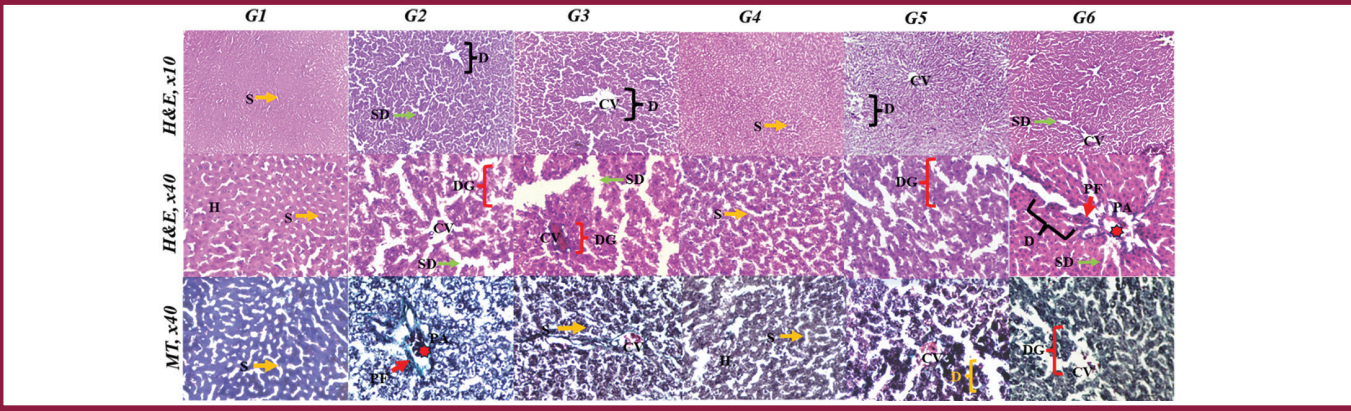


Figure 2. Hematoxylin and eosin (H&E) and Masson's trichrome (MT) staining were performed to evaluate hepatic histopathology and fibrosis. H&E and MT staining demonstrated preserved hepatic architecture in the young control group. HFCS administration to young animals resulted in sinusoidal dilatation and hepatocellular degeneration; portal fibrosis was observed in the high-fructose corn syrup (HFCS) +10 g group. In aged animals, HFCS exposure was associated with more pronounced histopathological alterations, including hepatocellular degeneration, vascular changes, and dilatation. The most prominent findings were observed in the HFCS +20 g group, which was characterized by portal expansion and perisinusoidal fibrosis. CV, central vein; D, dilatation (black bracket); DG, degeneration (red bracket); H, hepatocytes; PA, portal area (red star); PF, portal fibrosis (red arrow), S, sinusoids (yellow arrow), SD, sinusoidal dilatation (green arrow). G1: Young control, G2: Young HFCS + restricted chow (10 g/day), G3: Young HFCS + restricted chow (20 g/day), G4: Aged control, G5: Aged HFCS + restricted chow (10 g/day), G6: Aged HFCS + restricted chow (20 g/day), Magnifications: ×10, ×40 (H&E) and ×40 (MT), respectively.

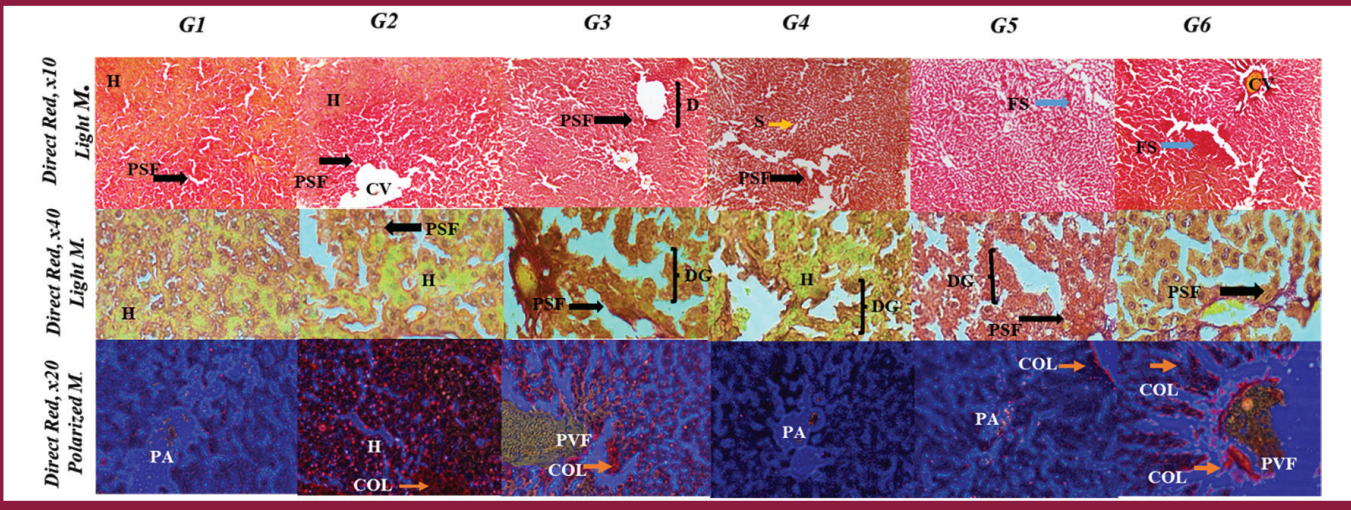


Figure 3. Direct Red (DR) staining showed minimal collagen deposition in the control groups, whereas high-fructose corn syrup (HFCS)-treated groups exhibited increased perisinusoidal and perivascular collagen accumulation, particularly in the HFCS + restricted chow (20 g/day) groups (G3 and G6). In aged animals, fibrotic changes were more pronounced following HFCS exposure, including perisinusoidal fibrosis and fibrotic septa formation. Polarized microscopy demonstrated increased collagen deposition, especially in the G6, where perivascular fibrosis was also evident. C, collagen (orange arrow); CV, central vein; D, dilatation (black bracket); DG, degeneration (black bracket); FS, fibrotic septa (blue arrow); H, hepatocytes; PA, portal area; PSF, perisinusoidal fibrosis (black arrow); PVF, perivascular fibrosis; S, sinusoids (yellow arrow). G1: Young control, G2: Young HFCS + restricted chow (10 g/day), G3: Young HFCS + restricted chow (20 g/day), G4: Aged control, G5: Aged HFCS + restricted chow (10 g/day), G6: Aged HFCS + restricted chow (20 g/day), Magnifications: ×10, ×40 (Direct Red-Light Microscope) and ×20 (Direct Red-Polarized Microscope), respectively.

Discussion

The present study investigated the age-dependent effects of HFCS-55 consumption on hepatic fibrosis in rats and demonstrated that both aging and HFCS exposure under different dietary conditions were associated with more pronounced histopathological liver injury. These findings are consistent with previous reports indicating that chronic fructose exposure contributes to liver injury (22). Notably, the severity of fibrosis was greater in aged animals, suggesting that age-related structural vulnerability and baseline parenchymal changes may enhance the susceptibility of the liver to fructose-induced histopathological damage. However, given the differences in estimated total caloric intake among groups, these effects should be interpreted as the combined influence of fructose exposure and overall energy intake rather than as a strictly dose-dependent HFCS effect.

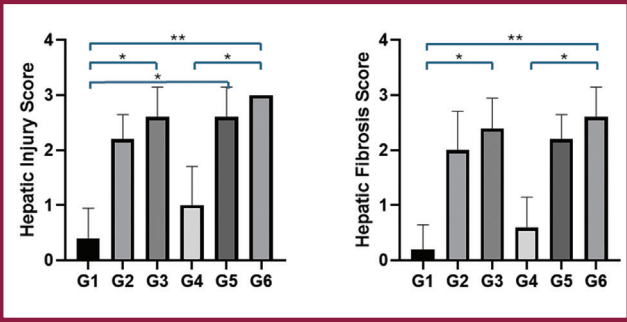


Figure 4. Hepatic injury and fibrosis analyses across experimental groups. Hepatic histopathology and fibrosis were evaluated using hematoxylin and eosin and Masson's trichrome staining. Bar graphs show liver injury and fibrosis scores across all groups. Data are presented as median (IQR) (n = 10). Statistical analysis was performed using the Kruskal–Wallis test followed by Dunn's post-hoc test. Statistical significance between groups is indicated by connecting lines (*, $p < 0.05$, **, $p < 0.01$).

Histopathological findings, including hepatocellular degeneration, disruption of lobular architecture, and increased collagen deposition, as demonstrated by MT and DR staining, support the presence of fibrosis in HFCS-treated groups. DR staining is particularly sensitive for detecting early perisinusoidal collagen deposition (21). In line with our findings, Seki et al. (11) reported that chronic fructose feeding exacerbates hepatic fibrosis. Moreover, aging appears to exacerbate these changes, as evidenced by more advanced fibrotic alterations in aged HFCS-treated animals. Interestingly, even in the absence of HFCS exposure, AC animals exhibited sinusoidal dilatation and hepatocellular degeneration, further supporting the concept that aging itself contributes to hepatic vulnerability (23). T he minor perisinusoidal staining observed in some control animals was evaluated as a physiological baseline presentation or as a reflection of a standard tissue processing artifact, rather than as evidence of active pathological fibrosing pathways. True progressive accumulation of collagen was exclusive to the experimental treatment groups.

These observed histopathological alterations and parenchymal damage are highly consistent with the

Table 2. Post-hoc Dunn's multiple comparison results for hepatic fibrosis.

Dunn's multiple comparisons test	Mean rank diff.	Significant	Summary	Adjusted p value
G1 vs. G2	-13.10	No	ns	0.2060
G1 vs. G3	-16.70	Yes	*	0.0252
G1 vs. G4	-2.200	No	ns	>0.9999
G1 vs. G5	-14.80	No	ns	0.0806
G1 vs. G6	-18.60	Yes	**	0.0070
G2 vs. G3	-3.600	No	ns	>0.9999
G2 vs. G4	10.90	No	ns	0.6050
G2 vs. G5	-1.700	No	ns	>0.9999
G2 vs. G6	-5.500	No	ns	>0.9999
G3 vs. G4	14.50	No	ns	0.0957
G3 vs. G5	1.900	No	ns	>0.9999
G3 vs. G6	-1.900	No	ns	>0.9999
G4 vs. G5	-12.60	No	ns	0.2667
G4 vs. G6	-16.40	Yes	*	0.0305
G5 vs. G6	-3.800	No	ns	>0.9999

Table 3. Post-hoc Dunn's multiple comparison results for hepatic injury.

Dunn's multiple comparisons test	Mean rank diff.	Significant	Summary	Adjusted p value
G1 vs. G2	-11.80	No	ns	0.3859
G1 vs. G3	-16.00	Yes	*	0.0374
G1 vs. G4	-3.200	No	ns	>0.9999
G1 vs. G5	-16.00	Yes	*	0.0374
G1 vs. G6	-20.20	Yes	**	0.0020
G2 vs. G3	-4.200	No	ns	>0.9999
G2 vs. G4	8.600	No	ns	>0.9999
G2 vs. G5	-4.200	No	ns	>0.9999
G2 vs. G6	-8.400	No	ns	>0.9999
G3 vs. G4	12.80	No	ns	0.2333
G3 vs. G5	0.000	No	ns	>0.9999
G3 vs. G6	-4.200	No	ns	>0.9999
G4 vs. G5	-12.80	No	ns	0.2333
G4 vs. G6	-17.00	Yes	*	0.0197
G5 vs. G6	-4.200	No	ns	>0.9999

conceptual framework of the gut-liver axis in fructose pathology. As comprehensively reviewed by Febbraio and Karin (24), chronic HFCS consumption acts as a metabolic stressor that compromises epithelial barrier integrity, thereby promoting downstream hepatic injury and structural remodeling through this axis. This axis remains a critical frontier in understanding organismal-level fructose pathologies, especially given the profound scarcity of focused clinical data tracking direct hepatic outcomes in humans (25). This mechanistic link supports our morphological findings, indicating that the pronounced fibrotic changes and architectural disruption in the liver tissue represent the end-stage structural manifestations of chronic dietary fructose toxicity.

These findings are particularly relevant given the widespread consumption of HFCS-containing beverages. Epidemiological evidence suggests that excessive intake of fructose-rich diets is associated with hepatic steatosis and progressive structural liver alterations (1-3). The present results reinforce these observations at the tissue level, indicating that aged liver parenchyma exhibits greater structural susceptibility to adverse alterations induced by chronic exposure to HFCS. Therefore, dietary strategies aimed at reducing fructose intake may be especially important in older individuals to mitigate the risk of liver injury and fibrosis. Although our findings clearly demonstrate a progressive increase in collagen accumulation, as assessed by validated histopathological scoring, these descriptive data reflect structural localization and severity patterns rather than absolute volumetric or morphometric percentages.

Although the non-isocaloric nature of the experimental groups and the accompanying feed-restriction protocol could act as confounding factors, these conditions were specifically designed to simulate distinct energetic states. D espite these metabolic variables, the pronounced differences observed between the groups strongly suggest that the structural alterations are predominantly driven by HFCS exposure, rather than representing a non-specific consequence of dietary restriction alone.

In the present study, 2-month-old rats were specifically selected to represent the onset of the young adult phase, a methodological choice directly supported by established chronological frameworks showing that postnatal day 63 (P63) marks the definitive biological transition into early adulthood in rodents (26). Conversely, 15-month-old rats were chosen to capture an advanced stage of biological aging in which cellular senescence and structural remodeling become prominent. This age-based design aimed to determine whether age-dependent factors influence the histopathological findings observed under experimental HFCS-containing dietary conditions, because these specific time points correspond to distinct biological windows in which structural and metabolic susceptibilities significantly diverge. This divergence aligns with the foundational findings of Yu et al. (27), who established that baseline hepatic metabolic characteristics and lipid profiles undergo critical age-related shifts that are highly susceptible to dietary modulation. Assessing these age-specific responses is essential, as the senescent parenchyma exhibits an

altered baseline vulnerability to dietary and metabolic stressors, rendering advanced age a potential accelerator for the structural manifestations of chronic fructose toxicity.

The necessity of detailed microstructural and histopathological scoring in animal models is further underscored by the profound scarcity of robust clinical data from human studies. A recent systematic review by Yu et al. (28) underscored this gap, revealing that out of thousands of screened records, only a single clinical study met the criteria for evaluating the direct links between high HFCS consumption, liver injury, and glucose metabolism in adults. The stark deficit of focused human investigations reinforces the critical value of controlled translational rodent models, such as the one used in our study, as tools for carefully dissecting the specific, age-dependent parenchymal and structural alterations directly attributable to chronic HFCS exposure.

Study Limitations

This study has several limitations. First, although histopathological findings clearly demonstrate hepatic injury and fibrosis, the underlying molecular mechanisms were not investigated. Second, fluid intake, HFCS consumption, and total caloric intake were not directly measured, which may have influenced the observed outcomes. In addition, liver weight, liver-to-body weight ratio, longitudinal changes in body weight, and separate semi-quantitative scores for steatosis and inflammation were not available. Therefore, the histopathological findings could not be correlated with morphometric, metabolic, or inflammatory indices, which limits the interpretation of the hepatic alterations observed in this study. Furthermore, serum alanine aminotransferase and aspartate aminotransferase levels, triglyceride levels, glucose levels, and insulin levels were not measured in the present study. Therefore, the histopathological findings could not be correlated with morphometric, metabolic, inflammatory, or biochemical markers of hepatic injury, lipid or glucose metabolism, or insulin status, thereby limiting the interpretation of the hepatic alterations observed in this study. Based on estimated intake calculations, the HFCS + restricted chow (20 g/day) group likely had a higher total caloric intake than both the control group and HFCS + restricted chow (10 g/day) group. Thus, differences between groups may reflect variations in total energy intake in addition to fructose exposure. Furthermore, the use of an animal model limits direct extrapolation to humans due to species-specific metabolic differences. The relatively small sample size and the lack of evaluation of potential confounding factors, such as physical activity and other dietary components, are additional limitations. Future studies incorporating molecular analyses, using isocaloric feeding designs, including female animals, and employing longer exposure periods are needed to further clarify the age-dependent hepatic effects of HFCS-55.

Conclusion

Chronic consumption of HFCS-55-containing beverages was associated with age-dependent hepatic injury and fibrotic changes in rats, which were more pronounced in aged rats. Histopathological evaluation with H&E, MT, and DR staining demonstrated increased collagen deposition and disruption of hepatic architecture, supporting an enhanced susceptibility to HFCS-related liver injury with aging.

However, given the estimated differences in total caloric intake among groups, these findings should be interpreted as the combined influence of fructose exposure and overall energy intake, rather than as a strictly dose-dependent effect of HFCS. Furthermore, as the analysis was limited to histopathological observations, additional studies incorporating molecular approaches and controlled feeding designs are needed to better elucidate the underlying mechanisms.

Ethics

Ethics Committee Approval: Prior to study initiation, approval was obtained from the Üsküdar University Ethics Committee for Animal Experiments (protocol number: 2023-05, date: 20.01.2023).

Informed Consent: Not applicable, as this study was conducted exclusively on laboratory animals and did not involve human participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.T., B.C., Concept: S.T., B.C., T.S., Design: S.T., B.C., T.S., Data Collection or Processing: S.T., E.S.T., Analysis or Interpretation: S.T., E.S.T., T.S., Literature Search: S.T., B.C., E.S.T., Writing: S.T., E.S.T.,

Conflict of Interest: No conflict of interest was declared by the authors.

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