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Molecular, biochemical, and histopathological effects of long-term low and high-percentage fructose consumption on the liver in rats

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Abstract: The aim of this study was to investigate the lipogenic and inflammatory effects of low and high percentage fructose solutions in rats. Wistar albino rats were fed with fructose solutions for 10 weeks. The groups were as follows: Cont (Control), F15 (Fructose 15%), F30 (Fructose 30%), and F60 (Fructose 60%). Rats' body weights were measured weekly. Also, lipogenic and inflammatory gene expression levels, biochemical parameters, and histopathological changes in the liver were investigated. After 10 weeks, it was observed that the animals in the F60 were the heaviest, while the animals in the F30 were the lightest. In all experimental groups, triglycerides were significantly higher than those of controls ($P<0.05$). In F30 and F60, *TNFα*, *IL-6,* and *IL-1β* were upregulated in the liver compared to control ($P<0.05$). In addition, *SREBP-1c, ChREBP, FAS, ACACA,* and *SCD-1* were upregulated in all fructose feeding groups compared to Cont ($P<0.05$). The livers of rats in the F30 and F60 groups had degenerative changes and steatosis. The most detrimental effects of fructose were observed in F60. The concentration of fructose was found to be a very important factor for maintaining normal liver physiology at the molecular level.
Keywords: Fatty liver, fructose, inflammation, lipogenesis, NAFLD.

Ratlarda uzun süreli düşük ve yüksek doz fruktoz tüketiminin karaciğerde moleküler, biyokimyasal ve histopatolojik etkileri

Özet: Bu çalışmanın amacı ratlarda düşük ve yüksek konsantrasyondaki fruktoz solüsyonlarının lipojenik ve inflamatuar etkilerini araştırmaktır. Çalışmada Wistar albino ratlar 10 hafta boyunca fruktoz solüsyonları ile beslenmiştir. Gruplar: Kont (Kontrol), F15 (Fruktoz %15), F30 (Fruktoz %30), F60 (Fruktoz %60) şeklinde olmuştur. Ratların vücut ağırlıkları haftalık olarak ölçülmüştür. Ayrıca lipojenik ve inflamatuvar genlerin ekspresyon seviyeleri, biyokimyasal parametreler ve karaciğerdeki histopatolojik değişiklikler araştırılmıştır. F60 en hafif grupken, F30 en ağır grup olarak belirlenmiştir. Trigliserit seviyeleri tüm deneme gruplarında Kont grubundan önemli ölçüde yüksek olmuştur ($P<0,05$). F30 ve F60'da, *TNFα*, *IL-6* ve *IL-1β* gen ekspresyon seviyelerinin Kont grubuna kıyasla arttığı belirlenmiştir ($P<0,05$). Bununla birlikte tüm fruktoz gruplarında *SREBP-1c*, *ChREBP*, *FAS*, *ACACA* ve *SCD-1* Kont grubuna kıyasla artmıştır ($P<0,05$). F30 ve F60 gruplarında karaciğerde dejeneratif değişiklikler ve steatoz belirlenmiştir. Fruktozun en zararlı etkileri F60 grubunda gözlenmiştir. Fruktoz konsantrasyonunun, normal karaciğer fizyolojisini moleküler seviyelerde sürdürmek için çok önemli bir faktör olduğu belirlenmiştir.
Anahtar sözcükler: Fruktoz, inflamasyon, lipogenez, NAFLD, yağlı karaciğer.

Introduction

Regulation of energy metabolism is controlled by numerous complex mechanisms. Obesity and diabetes have become global problems, causing high costs in healthcare today, as they also trigger cancer and cardiovascular diseases (29, 33). Studies on the metabolism have reported that simple sugars, particularly fructose, found in diets are major risk factors in developing metabolic diseases (10). The most important source of fructose is high fructose corn syrup (HFCS), which is used in soft drinks and many packaged foods with different percentages of fructose. (31). Fructose is metabolized independently of insulin and most of the fructose is transferred to the liver through the portal vein (35). The fructose-rich diet increases the levels of proteins involved in de novo lipogenesis formation in the liver

compared to oil-rich diets (31, 33). Non-alcoholic Fatty Liver Disease (NAFLD) may be caused by eating too much fructose, according to a report (22).

NAFLD is characterized by the storage of fat in the liver and is not dependent on intensive alcohol consumption. Excessive fructose consumption has been identified as the primary cause of this disorder (35).It has also been reported that chronic fructose consumption can trigger inflammation as well as fatty liver, leading to irreversible liver disorders (35). Chronic fructose consumption has been shown to induce lipid synthesis by triggering SREBP-1c (Sterol Regulatory Element Binding Transcription Factor 1c), ChREBP (Carbohydrate-Responsive Element-Binding Protein) transcription factors and thus target genes such as FAS (Fatty Acid Synthase), ACACA (Acetyl-CoA Carboxylase Alpha), and SCD-1 (Stearoyl-CoA Desaturase) (14).

Scientific debates on fructose consumption have been ongoing. However, molecular studies on the rate of fructose in diets are limited. In general, variable fructose solutions (mostly 10-30%) are used for the investigation of the metabolic effects of fructose on laboratory animals (2, 7). In this study, besides standard chow, consumption of 15%, 30%, and 60% concentration fructose solutions for 10 weeks was examined at the biochemical and histopathological levels. Expression levels of TNFα (Tumor Necrosis Factor Alpha), IL-1β (Interleukin 1 Beta) and IL-6 (Interleukin 6) genes in the inflammatory pathway and SREBP-1c, ChREBP, LXRα (Liver X Receptor alpha), FAS, ACACA, and SCD-1 genes in the lipogenesis pathway in the liver were also investigated.

Materials and Methods

Animals and experimental protocol: The study was ethically approved by the Animal Experiments Local Ethics Committee of Hatay Mustafa Kemal University (Decision number: 2018/2-8). Four groups were formed using 32 male Wistar albino rats. The rats were allocated in such a way that there was no statistical difference between the groups in terms of body weight averages (n = 8). The groups were as follows: Cont (Control group, standard chow), F15 (Fructose 15%, standard chow + 15% fructose solution), F30 (Fructose 30%, standard chow + 30% fructose solution), F60 (Fructose 60%, standard chow + 60% fructose solution). Rats in the Cont group consumed standard chow and water (31). On the other hand, standard chow and 15%, 30%, and 60% fructose solutions were presented to F15, F30, and F60 group rats, respectively. The feeding period was maintained for 10 weeks, and during the feeding period, the ambient light was set to be bright for 12 hours and dark for 12 hours (07:00–19:00 bright, 19:00–07:00 dark), and the humidity and ambient temperature were set at 55% and 21 ± 2 °C, respectively.

Euthanasia, blood, and tissue sample collection: At the end of the feeding period, the rats were fasted for 12 hours and euthanized by taking blood from their hearts under anesthesia (80 mg/kg Ketamine and 12 mg/kg Xylazine, IP). The liver tissues were divided into two pieces for molecular and histopathological analysis. Pieces taken for molecular analysis were frozen in liquid nitrogen and stored at - 86 °C. The other pieces were fixed with 10% formalin for histopathological analysis. Plasma analysis: Blood samples were centrifuged for 15 min at + 4 °C and plasma samples were stored at - 86 °C for analysis of glucose, insulin, HDL, LDL, total cholesterol, and triglycerides. While all biochemical parameters were detected with an auto-analyzer (Gesan Chem 200, Italy), insulin levels were determined with an ELISA reader (Multiskan GO Microplate Spectrophotometer, ThermoFisher Scientific, USA) using a rat-specific ELISA kit (SunRed Bio, China).

RNA isolation and cDNA synthesis: Total RNA isolation was performed according to the Trizol method (32). Following chloroform, isopropyl alcohol, and ethyl alcohol stages, RNA pellets were allowed to dry for about 10 min at room temperature as stated by the protocol of the TRI-Reagent kit (Sigma-Aldrich, USA, Cat. No: T9424). Pellets were diluted with 30-100 µL nuclease-free water (NFW). The purity and concentration of the samples were determined by the nucleic acid meter (Merinton, SMA-1000). Prior to cDNA conversion, samples were treated with DNase I (Thermo Fischer Scientific, USA) for possible genomic DNA contamination. For cDNA synthesis, the thermal cycler (Biorad T100, USA) protocol was 60 min at 42 °C, 5 min at 25 °C, and 5 min at 70 °C. The final volumes of cDNA samples were completed to a volume of 200 µL with NFW. RT-qPCR analysis: Expression levels of target genes were determined by qPCR (Rotorgene Qiagen, USA). The qPCR protocol was as follows: After 10 min for denaturation, 15 sec at 95 °C, 60 sec at 60 °C and 30 sec at 72 °C and 40 cycles. PPIA was used as an internal control, and all samples were studied in triplicate. A kit containing SYBR Green Dye was used for amplification of genes (Power SYBR Green PCR Master Mix, ThermoFisher Scientific, USA). The primers for target genes were checked at NCBI-Primer Blast and then used for amplification (Table 1).

Histopathological analysis: The fixed tissues were washed overnight to remove formalin under tap water. The routine tissue process was followed. Then, tissues were embedded in paraffin, sectioned at 5µm thickness from each block and deparaffinized in xylol, then passed through a series of 100%, 96%, 80%, and 70% alcohol, respectively. After the tissues were stained with Hematoxylin and Eosin (H&E), microphotographs (Olympus DP12) of the tissues were obtained under a light microscope (Olympus CX31) (23).

Table 1. Forward and reverse sequences of genes primers for qPCR.

Genes	Forward and Reverse Primer Sequences	Reference
PPIA	F: 5'-CAGACAAAGTTCCAAAGACAGCA-3' R: 5'-CACCCTGGCACATGAATCCT-3'	(8)
FAS	F: 5'-GCTGCTACAAACAGGACCATC-3' R: 5'-TCCACTGACTCTTCACAGACCA-3'	(26)
ACACA	F: 5'-CAATCCTCGGCACATGGAGA-3' R:5'-GCTCAGCCAAGCGGATGTAGA-3'	(11)
SCD-1	F: 5'-CCTTAACCCTGAGATCCCGTAGA-3' R: 5'-AGCCCATAAAAGATTTCTGCAAA-3'	(37)
ChREBP	F: 5'-CGGGACATGTTTGATGACTATGTC-3' R: 5'- AATAAAGGTCGGATGAGGATGC-3'	(13)
SREBP-1c	F: 5'-GCAACACTGGCAGAGATCTACGT-3' R: 5'-TGGCGGGCACTACTTAGGAA-3'	(13)
LXRα	F: 5'-CCTGATGTTTCTCCTGACTC-3' R: 5'-TGACTCCAACCCTATCCTTA-3'	(27)
TNFα	F: 5′-GGCATGGATCTCAAAGACAACC-3′ R: 5′-CAAATCGGCTGACGGTGTG-3′	(4)
IL-1β	F:5′-ACAAGGAGAGACAAGCAACGAC-3′ R: 5′-TCTTCTTTGGGTATTGTTTGGG-3′	(4)
IL-6	F: 5'-TGATGGATGCTTCCAAACTG-3' R: 5'-GAGCATTGGAAGTTGGGGTA-3'	(19)

Histopathological findings were evaluated according to the following criteria: Grade 0: Histopathological changes below 5%; Grade 1: Slight histopathological changes in between 5% and 33% of the total area; Grade 2: Moderate histopathological changes in 33% to 66% of the total area; Grade 3: Severe histopathological changes in more than 66% of the total area (12).

Statistical analysis: For statistical calculations, the SPSS package program (Version 22.0) was used. Before performing the statistical analysis, the variables were examined using the Shapiro-Wilk test for normality and the Levene test for homogeneity of variance as parametric test assumptions. Differences between groups were determined by One-Way ANOVA and the Tukey test. $P<0.05$ was used as the cutoff for significance. For gene expression calculations, the method of $2^{-\Delta\Delta Ct}$ was used and the results were given as fold change (21). Peak values in histopathological findings were taken as score averages in groups.

Results

The consumption of chow, water, and fructose solution and amounts of consumed energy were given in Table 2. Compared to F60, body weight averages (BW) in F15 and F30 increased at the end of the first week ($P<0.05$). The third week was the first week in which a significant difference occurred between F60 and the other groups in terms of BW. This significant difference continued until the 8th week. The BW in the F15 and F60 were similar from the 8th week to the end of the study. However, the F30 was found to have the heaviest animals, while the rats in the F60 were the lightest animals (Table 2, Figure 1a-d).

The highest liver weight average was found in F30 ($P<0.05$). LDL was highest with the approximately 20 mg/dL levels in F60 ($P<0.01$). While triglyceride was approximately 50 mg/dL in the Cont, it was two folds more in the fructose fed groups ($P<0.05$). Glucose, insulin, HDL and total cholesterol levels were similar in all groups.

Isolated total RNA purity (A260/A280: 1.94 ± 0.02) and concentration (322.57 ± 26.02 ng/µL) were appropriate quality. *TNFα*, *IL-6, and IL- 1β* genes expression levels were upregulated in F30 and F60 ($P<0.05$) (Figure 2a, Figure 2b, Figure 2c, respectively). While *LXRα* was similar (Figure 2f), *SREBP-1c* and *ChREBP* were upregulated all fructose feeding groups (Figure 2d, Figure 2e) ($P<0.05$). In addition, *FAS, ACACA* and *SCD-1* levels were found to increase at varying levels (Figure 2g, Figure 2h, Figure 2i, respectively).

Macroscopic examination showed that the livers of rats in the F30 and F60, which were more severe in the F60, were swollen, dark red in color and the edges were blunt. Moreover, there was blood on the cross section of the liver.

The histopathologic injury scores of the liver was given in Table 3. Hepatocytes, Remark cords, and hepatic central vein of Cont and F15 were in the normal histological structure (Figure 3a, 3b and 3c). In F30, Remark cords in some areas were detected to lose normal regularity. There were fatty changes which characterized by large empty vacuoles of sharp borders and degenerative

changes from parenchyma to hydropic in the cytoplasm of hepatocytes in focal areas (Figure 3d). In F30, portal veins and sinusoidal regions enlarged due to erythrocyte deposition (passive hyperemia). Moreover, mononuclear cell infiltrations were observed as small foci, especially in periportal areas (Figure 3e). In F60, dissociation in Remark cords was observed. Comparing to F30, larger fat vacuoles with diffuse distribution (steatosis) were detected (Figure 3f). Similarly, more severe passive hyperemia and mononuclear cell infiltration were observed especially in perivascular regions compared to F30 (Figure 3g). Hydropic degeneration in hepatocytes was present especially in periportal regions (Figure 3f, 3h). Focal necrosis of 2 rats was noted in F60, as well as necrotic changes in some hepatocytes (Figure 3i).

Table 2. Average of body weights (g), consumption of chow (g/rat) and water/solution (mL/rat) in groups.

Wks	Tra	Cont	F15	F30	F60	P
0	**BW**	209.38±7.50	208.50±7.89	206.88±9.82	206.75±9.75	-
	BW	254.04±11.16^{ab}	261.31±7.99^{a}	260.05±10.67^{a}	220.36±9.95^{b}	*
1	**Ch**	253.13	160.72	144.38	80.00	
	W/S	426.25	375.56	277.22	177.22	
	BW	296.12±14.22	297.16±8.52	297.78±14.14	255.55±13.88	-
2	**Ch**	248.50	156.78	143.22	86.67	
	W/S	471.25	405.56	298.33	202.78	
	BW	338.63±14.40^{a}	352.21±6.63^{a}	348.24±14.22^{a}	276.40±15.95^{b}	**
3	**Ch**	256.38	171.11	152.22	89.56	
	W/S	463.13	528.89	345.00	196.11	
	BW	369.15±16.00^{ab}	380.10±7.68^{a}	387.11±14.55^{a}	320.51±13.60^{b}	**
4	**Ch**	258.90	150.21	146.62	99.56	
	W/S	471.25	541.11	327.22	216.67	
	BW	394.19±18.57^{ab}	401.18±5.28^{a}	417.19±14.42^{a}	342.13±14.28^{b}	**
5	**Ch**	270.63	153.33	137.38	93.16	
	W/S	481.25	443.89	348.89	212.22	
	BW	418.61±19.94^{a}	425.58±6.35^{a}	436.85±15.74^{a}	359.89±15.38^{b}	**
6	**Ch**	260.29	138.72	134.67	87.56	
	W/S	438.13	685.00	335.00	221.67	
	BW	425.03±19.04^{ab}	444.93±5.42^{a}	457.00±16.14^{a}	376.30±15.89^{b}	**
7	**Ch**	265.06	152.80	128.44	90.71	
	W/S	482.50	598.33	290.56	211.11	
	BW	439.91±21.31^{ab}	454.98±5.80^{ab}	463.73±16.68^{a}	393.61±16.92^{b}	*
8	**Ch**	251.13	134.56	125.56	88.56	
	W/S	480.63	524.44	350.00	211.67	
	BW	458.15±23.41	468.79±6.65	485.15±17.33	423.44±15.43	-
9	**Ch**	247.71	139.10	115.91	78.30	
	W/S	465.63	638.33	327.78	215.00	
	BW	460.56±23.15^{ab}	475.93±4.98^{ab}	494.00±17.51^{a}	424.15±15.72^{b}	*
10	**Ch**	273.44	145.61	127.31	85.62	
	W/S	500.00	805.56	391.67	252.78	

$^{a, b}$: Means with different letters in rows differ significantly, **Wk**: Weeks, **Tra**: Traits, **BW**: Body Weights, **W/S**: Water/Solution, **Ch**: Consumption of chow, -: P>0.05, *: P<0.05, **: P<0.01.

Table 3. Histopathological findings in liver.

Histopathological Changes	Cont	F15	F30	F60
Inflammation	0	0	1	1
Passive hyperemia	0	0	1	2
Fatty degeneration	0	0	1	2
Hidropic degeneration	0	0	0	1
Necrose	0	0	0	1

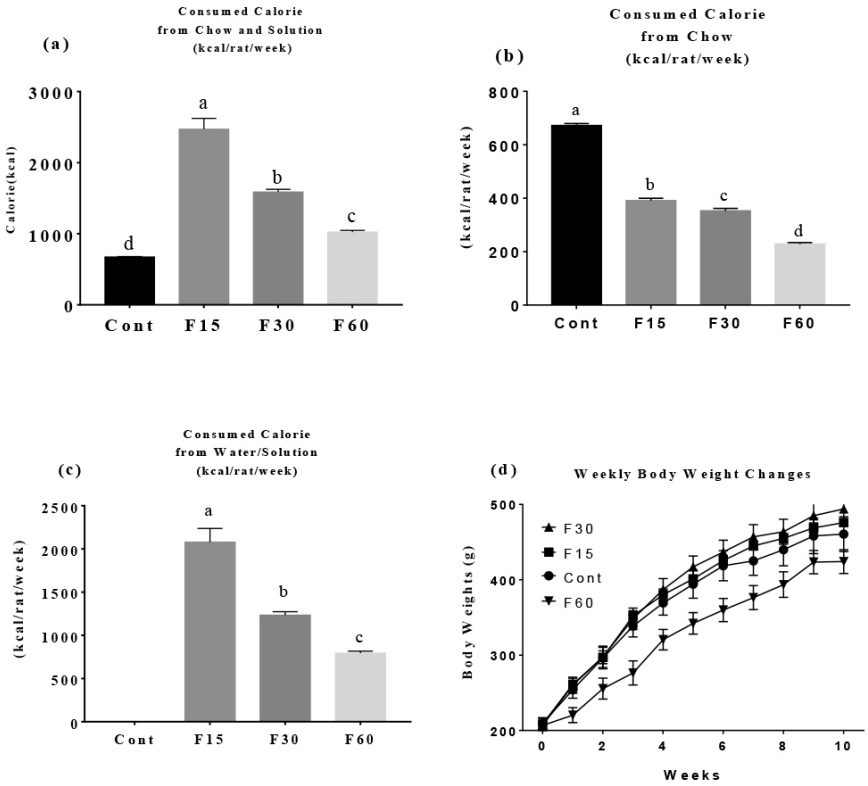

Figure 1. Average weekly consumed calorie in groups.
a) Weekly consumed total calorie (from chow + solution); **b)** Weekly consumed calorie from Chow; **c)** Weekly consumed calorie from Solution (In Cont group, there is no any fructose in water); **d)** Weekly body weight changes in groups.

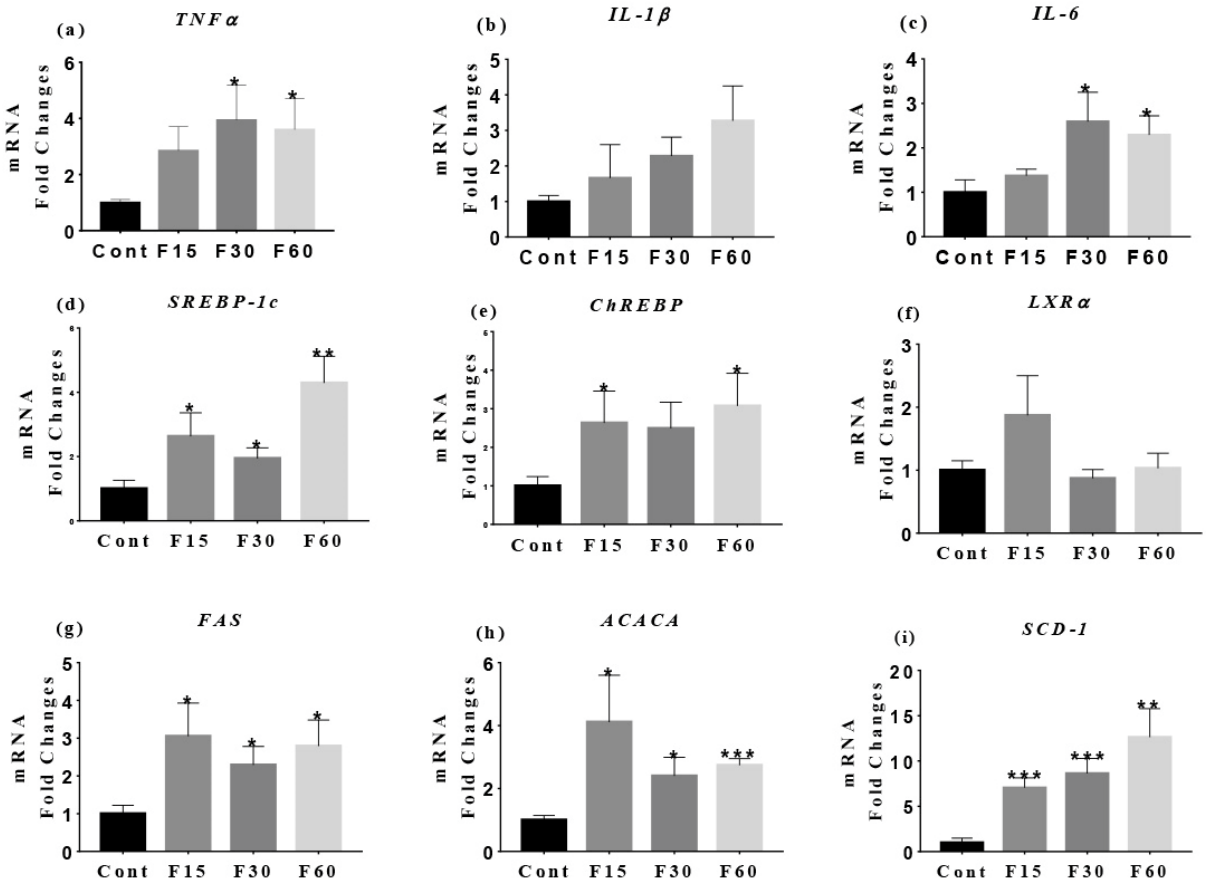

Figure 2. mRNA fold changes of genes in liver. *: P<0.05, **: P<0.01, ***: P<0.001.

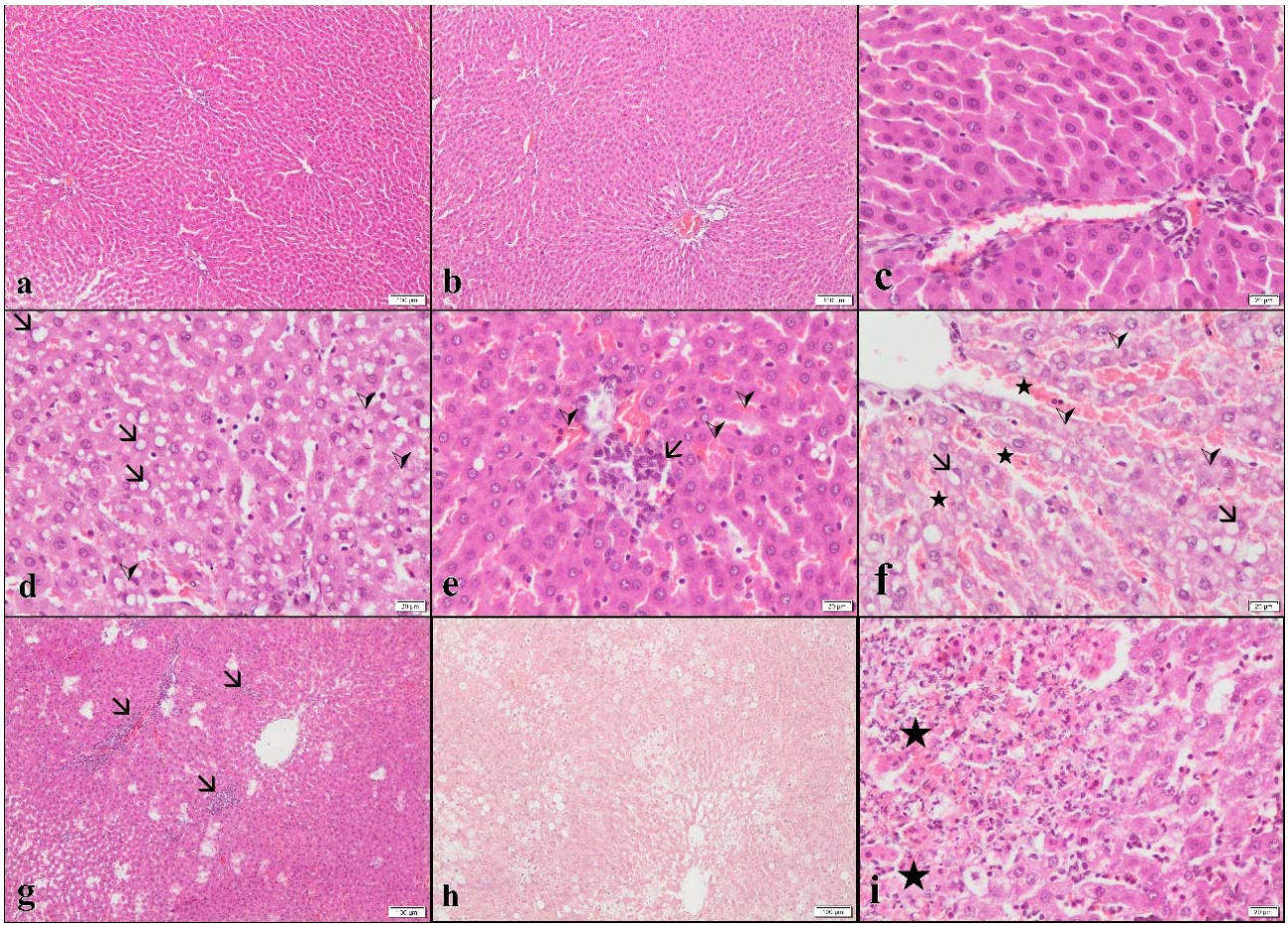

Figure 3. a: Normal histological structure of the liver in the Cont, H&E, ×100 µm. **b:** Normal histological structure of the liver in F15, H&E, ×100 µm. **c:** Normal histological structure of the liver in F15, H&E, ×20 µm. **d:** Fat vacuoles (arrows) and hydropic degeneration (arrowheads) in hepatocytes in F30, H&E, ×20 µm. **e:** Erythrocytes (arrowheads) and perivascular inflammatory cells (arrow) in sinusoids in F30, H&E, ×20 µm. **f:** Expansion with congestion in the sinusoidal spaces (stars), fat vacuoles (arrows) and hydropic degeneration (arrowheads) in F60, H&E, ×20 µm. **g:** Perivascular mononuclear cell infiltration in periportal areas (arrows) in F60, H&E, ×100 µm. **h:** Hydropic degeneration in hepatocytes in F60, H&E, ×100 µm. **i:** Focal necrosis in the liver (stars) in F60, H&E, ×20 µm.

Discussion and Conclusion

Studies for about 30 years have reported an increase in the incidence of obesity and complications in connection with the consumption of fructose (7). Although fructose is metabolized independently of insulin, as the concentration of fructose in solution increases, the amount of consumed chow decreases (35). In addition, the total amount of energy taken by diet varies as expected depending on fructose concentrations. The 60% fructose solution was consumed less by the F60 depending on the amount of energy in mL, and also chow consumption was suppressed in this group.

In a study of adult rats fed with 10% and 60% fructose solutions in addition to standard chow, it was reported that BW of both groups was the same as those of Control similar the results of our study (7). Although the F15 had the highest energy consumption, the BW were similar to those of the other groups. The BW of the F60 was lower than those of the F30, but were similar to those of the other groups. The results show that 30% fructose solution causes a significant increase in BW. It was reported that 20% fructose solution did not cause change in both BW and biochemical parameters (2). In this study, as well as BW, liver weights were similar in F15 and F60. However, the heaviest liver was in F30.

Glucose, insulin, HDL, and total cholesterol were found similar in all groups. These results were similar to those of some studies (17, 31). Although a high concentration of fructose solution does not cause a significant change in BW and other parameters, it does increase LDL and metabolic disorders are triggered (17, 31). Hypertriglyceridemia was observed in all fructose consuming groups. Fructose increased plasma triglyceride levels even though the consumption was low (7). The findings indicate that the percentage of fructose in the solution may be an important factor for hypertriglyceridemic effect.

Increased *TNFα* expression in liver triggers triglyceride production and leads to steatosis (9, 18). It has been shown that fructose at a concentration of 30% and above can upregulate *TNFα* expression (18). In a study, it has been found that 4-week consumption of 60% fructose solution increased *TNFα* expression in liver (36). We found that *TNFα* was upregulated in F30 and F60. The activity of this gene was found to be consistent with biochemical parameters as triglyceride and LDL and histopathological findings. *IL-1β* and *IL-6* genes also upregulated in F30 and F60. It has been reported that the expression level of *IL-1β* increased 3-fold in fructose fed rats (4). In our study, *IL-1β* was upregulated in F60 with the highest levels (3-fold). *IL-6*, usually co-regulated with *TNFα* and *IL-1β*, has also been reported to be involved in the regulation of triglyceride secretion (3). Plasma triglyceride levels and expression levels of this cytokine in fructose-fed groups have been confirmed this information. *TNFα, IL-1β* and *IL-6* expressions confirmed the histopathological findings of inflammation in the liver of F30 and F60.

SREBP-1c increased approximately 2-fold in F15 and F30 but the most upregulation was in F60. However, *ChREBP* upregulated in all experimental groups. Although SREBP-1c was reported to be affected by the activation of *ChREBP* and *LXRα*, it was thought that this increase might have been influenced by additional factors with fructose consumption (5). *LXRα* has been reported to be involved in lipid biosynthesis in the liver and regulate the amount of triglyceride (24). *LXRα* activity varies depending on the amount of insulin and increased approximately 2-fold in F15. However, expression levels of *LXRα* in other experimental groups were similar. It was thought that standard chow consumption and fructose concentrations might be caused these results.

Jegatheesan et al. (17) were reported that the body weight was lower in rats fed with 60% fructose for 8 weeks compared to the normally fed animals (17). However, triglyceride and LDL levels were significantly higher, similar to the F60 in this study. In the same study, it was reported that *SREBP-1c* and *ChREBP* expression levels increased, although BW was lower than those of the control (17).

FAS, ACACA and *SCD-1* were upregulated in all experimental groups. Similar to our study, it was reported that without changes in BW, triglyceride levels and lipogenic genes expression levels were increased in rats fed with 60% fructose for 4 weeks (16). The lipogenic effect of fructose is mostly seen by the effect of *SREBP-1c* and *ChREBP*. But also, *FAS, ACACA* and *SCD-1* activities are conspicuous. Fatty acid production during the lipogenesis process is caused by *ACACA*-mediated *FAS* catalysis (6, 30).

Studies conducted with animal models showed that high fructose consumption significantly affects *ACACA* in the liver and lipogenesis is significantly influenced by activity in this gene (15, 20, 34). Fructose induces more *ACACA* than other energy sources in the lipogenesis pathway (33). According to our study, although the low concentration of fructose solution affects the activity of this gene, increased lipogenic activity was found in F30 and F60.

SCD-1 has a central role in energy metabolism and is required for lipogenesis in combination with dietary fructose in SREBP-1c-dependent and independent pathways (25). *SCD-1* has been reported to increase energy consumption and insulin sensitivity, and the activity of this gene is closely associated with liver steatosis (25, 28). It has been understood from the results of this study that *SREBP-1c* has a close relationship with *SCD-1*. This gene upregulated more than 10-fold in F60. This result confirmed the histopathological findings of liver steatosis.

According to the histopathological examination, although the most severe changes were in F60, livers in F30 were also damaged. The storage of excessive fat increases liver sensitivity and makes it more susceptible to damaging agents and causes fibrosis and hepatosteatosis (1). In F30 and F60, lipogenic genes and histopathological results showed that high fructose caused liver damage due to fattening. Inflammatory genes results confirmed the damage in F30 and F60. However, it was found that animals fed with 15% fructose solutions were similar to the Cont in terms of liver histopathology. Although it was determined that feeding with 15% fructose solution for 8 weeks increased plasma triglyceride levels, it did not lead to significant pathological changes. Histopathological and biochemical results in F30 were similar to those of other studies (30, 31). Inflammation, fatty degeneration and passive hyperemia parameters were found to have the highest values in F60. Also, degenerative and necrotic changes were at the highest levels in F60.

Recently, with the consumption of fructose the activity of lipogenic genes in other tissues has been investigated as well. In a study, *SREBP-1c* and *ACACA* gene expression increased in the hypothalamus with 20% fructose consumption, while *FAS* was downregulated (2). In addition, it was reported that food intake was suppressed through *FAS* inhibitors in the hypothalamus, preventing body weight gain (2). With this study, lipogenic genes activity has been determined that strongly changed with the fructose consumption in liver. However, further study is needed on the activity of lipogenic genes in other tissues.

In conclusion, the results of this study show that NAFLD can occur without increasing overall body

weight. In addition, high fructose solution consumption causes significant histopathological changes with lipogenic and inflammatory effects at the molecular levels in the liver. The concentration of the fructose solution was found to be a very important factor. This study is thought to provide important information about production animals modeling metabolic syndrome with fructose.

Financial Support

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Ethical Statement

The study was ethically approved by the Animal Experiments Local Ethics Committee of Hatay Mustafa Kemal University (Decision number: 2018/2-8).

Conflict of Interest

The authors declared that there is no conflict of interest.

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