Kocatepe Veterinary Journal

Kocatepe Vet J. (2023) 16(2):131-142

DOI: 10.30607/kvj. 1173180

RESEARCH ARTICLE

Expression Patterns of Some Lipogenic Genes and Fatty Acid Profile of Liver in Food-Restricted Rats

Hüseyin ÖZKAN[1]*, İrem KARAASLAN[2], Ufuk KAYA[3], Sevda DALKIRAN[4], İbrahim ALAKUŞ[5], Baran ÇAMDEVİREN[4], Hasan Hüseyin KEÇELİ[1], Akın YAKAN[1]

[1]*Department of Genetics, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University, Hatay, Türkiye*
[2]*Technology and Research & Development Center (MARGEM),Hatay Mustafa Kemal University, Hatay, Türkiye*
[3]*Department of Biostatistics, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University, Hatay, Türkiye*
[4]*Department of Molecular Biochemistry and Genetics, Institute of Health Sciences, Hatay Mustafa Kemal University, Hatay, Türkiye*
[5]*Department of Surgery, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University, Hatay, Türkiye*

ABSTRACT

This study aimed to determine food restriction effects on the profile of fatty acids and major genes on lipogenesis expressions in liver. 16 *Wistar albino* rats were divided into two groups and different diets were given to groups for 4-weeks. First group was fed ad libitum (Control group), another group was fed the half amount of the daily requirement (Food Restriction group, FR). As well as weekly food consumption and body weight changes, total cholesterol, HDL, LDL, and triglyceride levels were determined at the end of the feeding period. In addition to the fatty acid profile, *FASN* and *SCD-1* genes expression levels were measured in the liver. While the body weight averages decreased after 7 days and remained similar, plasma glucose levels were found lower in the FR. *FASN* was upregulated approximately 6 folds, and *SCD-1* increased insignificantly about 3 folds in the FR. C15:0, C18:1 n9 trans, C18:2 n6 cis, C21:0, C20:2, C20:5 n3, n6 and UFA were lower, while C16:0, C18:2 n6 trans, C20:3 n6, C22:6 n3, C22:1 n9, C22:2 and SFA were higher in FR. In addition to considering the exposure time and rate of food restriction, molecular activity and interactions in other metabolic organs should be investigated.

Keywords: fatty acids, food restriction, liver, *FASN, SCD-1*

***

Gıda Kısıtlaması Yapılan Ratlarda Karaciğerde Bazı Lipojenik Genlerin İfade Düzeyleri Ve Yağ Asidi Profili

ÖZ

Bu çalışma gıda kısıtlamasının karaciğerdeki yağ asidi profili ve majör lipojenik genlerin ekspresyon seviyeleri üzerindeki etkilerini belirlemeyi amaçlamıştır. 16 adet Wistar albino rat iki gruba ayrılarak 4 hafta boyunca farklı diyetlerle beslenmiştir. Birinci grup (Kontrol grubu) ad libitum beslenirken, diğer gruba günlük ihtiyacın yarısı (Yem Kısıtlama grubu, YK) kadar yem verilmiştir. Haftalık besin tüketimi ve vücut ağırlığı değişimlerinin yanı sıra beslenme periyodu sonrasında total kolesterol, HDL, LDL ve trigliserit düzeyleri belirlenmiştir. Yağ asidi profiline ek olarak karaciğerde *FASN* ve *SCD-1* genlerinin ekspresyon seviyeleri ölçülmüştür. FR'nin ortalama vücut ağırlığı 7. günden sonra azalmaya başlarken, YK'de plazma glukoz seviyeleri kontrol grubuna kıyasla daha düşük bulunmuştur. YK grubunda *FASN* geni yaklaşık 6 kat artarken (P<0,05), *SCD-1* önemsiz olacak şekilde yaklaşık 3 kat artmıştır. FR grubunda C15:0, C18:1 n9 trans, C18:2 n6 cis, C21:0, C20:2, C20:5 n3, n6 ve UFA miktarı kontrole göre daha düşükken, C16:0, C18:2 n6 trans, C20:3 n6, C22:6 n3, C22:1 n9, C22:2 ve SFA miktarının daha yüksek olduğu belirlenmiştir. Besin kısıtlamasının, maruz kalma süresi ve oranının dikkate alınmasına ek olarak, diğer metabolik organlardaki moleküler aktivite ve etkileşimler araştırılmalıdır.

Anahtar kelimeler: karaciğer, yağ asitleri, yem kısıtlama *FASN*, *SCD-1*,

To cite this article: Özkan H. Karaaslan İ. Kaya U. Dalkıran S. Alakuş İ. Çamdeviren B. Keçeli H.H. Yakan A. Expression Patterns of Some Lipogenic Genes and Fatty Acid Profile of Liver in Food-Restricted Rats. Kocatepe Vet J. (2023) 16(2):131-142

Submission: 09.09.2022 **Accepted**: 10.04.2023 **Published Online**: 10.05.2023

ORCID ID; *HÖ: 0000-0001-5753-8985, İK: 0000-0002-7485-192X, UK: 0000-0002-4805-0993, SD: 0000-0002-5704-5774, İA: 0000-0002-2031-7035, BÇ: 0000-0003-1508-7869, HHK: 0000-0002-4017-8765, AY: 0000-0002-9248-828X*

*Corresponding author e-mail: hozkan@mku.edu.tr

INTRODUCTION

The incidence of obesity and its complications has increased due to developments in agriculture, advances in technology, and dramatic changes in lifestyle (Ozkan and Yakan 2019). Obesity and its complications have become a major health issue affecting more than 500 million people in the world (Crovesy et al. 2020). Many different methods such as sustained food restriction, intense exercise, and diet regulation are used to control obesity (Laskowski 2012, Hill et al. 2012). Particularly, food restriction (FR) has various effects on energy metabolism (Dumas et al. 2004).

Energy metabolism is one of the leading factors for the continuation of healthy life due to metabolic regulation and related biological systems (Zhang et al. 2017). The energy needed by the organism is provided by the stored carbohydrates in the case of FR. Energy homeostasis is maintained by hydrolysis of triglycerides stored in satiety with the continuation of FR. As a result of FR, the lipolysis pathway is activated, and body weight decreases by the rapidly diminishing of adipose tissue (Barzilai and Gabriely 2001, Smith et al. 2010). Moreover, FR increases oxidation of fatty acids and decreases synthesis of fatty acids for a certain period of time. In addition, it has been reported restriction of food or calorie cause downregulation of genes as Fatty Acid Synthase (*FASN*) and Stearoyl-CoA Desaturase (*SCD-1*) (Margolis et al. 2016). However, in the case of long-term FR, the balance in the lipolysis and lipogenesis pathways is rearranged and homeostasis is maintained.

The regulation of molecular pathways associated with FR and energy metabolism causes significant changes in the liver fatty acid profile. As well as other functions in the organism, fatty acids have important functions in the liver. Moreover, fatty acids are particularly essential molecules to provide glucose production for metabolic energy regulation in the FR state (Rui 2014).

Although many studies have reported different effects of FR on the organism depending on the varying exposure time, limited information is known about the activity of major genes including the lipogenesis pathway and fatty acid profile of the liver (Bruss et al. 2010, Mulligan et al. 2008). In this study, as well as changes of body weights and some biochemical parameters, it has been aimed to determine the effects of 4-week FR on the profile of fatty acids and expression levels of *FASN* and *SCD-1* in the liver of rats. Moreover, the relationships between fatty acids and studied genes have been investigated. In this context, it has been aimed to better understand the effects of 4-week FR on fatty acid metabolism at the molecular levels in the liver.

MATERIAL AND METHOD

Animals and experimental design

This study was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Decision No: 2021/04-02). *Wistar albino* male rats aged approximately 8 weeks were used in the study. Rats were fed without any restrictions for a week for acclimatization. Rats' food consumption was recorded during the acclimatization period for one week. Therefore, the daily food consumption was calculated (30.44±2.2 g/rat/day). In addition, the sample size of the study was calculated with the G*Power software version 3.1.9.2. The statistical sample size calculation showed that at least 16 rats were needed, considering an effect size of 1.6, an alpha value of 0.05, and a power of 0.80. Sixteen rats were divided randomly into 2 groups (n=8). While the first group was fed with commercial food (Bil-Yem Ankara) *ad libitum* (Control group, Cont), the other group was fed with half amount of the daily

requirement (50% Food Restriction group, FR) (Gardner et al. 2010, Smyers et al. 2015). The protein and energy requirements were appropriate to the suggestion of the NRC (NRC, 1995). The ingredients of standard food were presented in Table 1.

During the feeding period, the animals were housed in transparent cages, with one animal in each cage. Environmental conditions in the place where the animals were housed; 12 hours of light and 12 hours of darkness (07.00-19.00 light / 19.00-07.00 dark) and the humidity was regulated as 55%. The ambient temperature was adjusted to be 21±2°C. The rats used in the study were checked twice daily (morning and evening), and in addition to weekly body weight changes, the consumption of food and water were recorded. Water was given *ad libitum* to all rats.

Table 1. Standard chow ingredient

	Ingredient (%)
Corn maize	29.00
Full-fat soybean	28.00
Sunflower seed meal	7.00
Wheat middling	13.00
Alfalfa meal	14.00
Meat bone meal	4.00
Molasses	1.75
Limestone	2.00
Dicalcium phosphate	0.50
NaCl	0.50
Vitamin-mineral premixes	0.25
Nutrient composition (calculated)	
Metabolic Energy (kcal/kg)	2600.00
Dry Matter (%)	88.40
Crude Protein (%)	22.50
Crude Cellulose (%)	8.10
Fat (%)	3.15
Carbohydrate (%)	54.72
Insoluble ash in HCl (%)	1.05
Ca (%)	1.30
P (%)	0.80

Euthanasia and sample collection

Following the four-week feeding period, the rats fasted for 12 hours while allowed to access water *ad libitum*. After 12 hours of fasting, the animals were euthanized by collected blood from the heart under Ketamine and Xylazine anesthesia. While the blood samples were kept at +4°C, liver tissues were collected from the animals and sectioned into two parts. One of the pieces was quickly frozen in liquid nitrogen for molecular analysis, while another piece was transported to -80°C in the cold chain for fatty acid analysis.

Biochemical analysis

Centrifugation was performed on the blood samples at 3000 xg at +4°C for 10 min. Obtained plasma was collected in new sterile and nuclease-free tubes. The plasma samples were stored at -80°C until analysis. Total cholesterol, glucose, triglyceride, HDL, and LDL parameter were determined with an auto-analyzer (Siemens Advia 1800, Japan).

Isolation of RNA and synthesis of cDNA

RNA isolation from liver samples was performed by the applying the modified Trizol method (Rio et al 2010). Approximately 50 mg of liver tissue exposed to 1 mL TRIzol Reagent (Thermo Fisher Scientific, USA). Following isolation of RNA, the RNA pellets were left to dry at room temperature for approximately 10 min. Then, samples were diluted with nuclease-free water (NFW). The quality of RNA samples was controlled by a nucleic acid meter (Merinton-SMA 1000). Also, RNA integrity was assessed on 1% agarose gel electrophoresis (100 V, 25 min).

DNA digestion was applied with a commercial kit (DNase I, RNase free, Thermo Fisher Scientific, USA) to the samples for elimination of gDNA. After this process, the synthesis of cDNA was applied to the RNA samples with RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). The reaction was in three steps: Following the 10 min at 25°C, the temperature was arranged as 37°C for 120 min, and 85°C for 5 min. End of the reaction, the volume of samples was completed to 150 µL with NFW. Until RT-qPCR application, samples were kept at -20°C.

RT-qPCR application

FASN and *SCD-1* genes amplifications were performed in duplicate from each sample. qPCR (Rotor Gene Q MDx 5plex HRM, Qiagen, ABD) application was performed with a commercial kit (Power SYBR Green PCR Master Mix, Thermo Fisher Scientific, USA). The reaction protocol in qPCR was as follows: 10 minutes at 95°C, followed by 15 seconds at 95°C, 60 seconds at 60°C, and 40 cycles. *PPIA* gene was used as the normalized gene for internal control (Table 2).

Table 2. Forward and reverse sequences of primers

Genes	Forward and Reverse Sequences	P.L.	Reference
SCD-1	F:5'-CCTTAACCCTGAGATCCCGTAGA-3' R:5'-AGCCCATAAAAGATTTCTGCAAA-3'	95	(Yasari et al. 2010)
FASN	F: 5'-GCTGCTACAAACAGGACCATC-3' R: 5'- TCCACTGACTCTTCACAGACCA-3'	98	(Mock et al. 2017)
PPIA	F: 5'-CAGACAAAGTTCCAAAGACAGCA-3' R: 5'-CACCCTGGCACATGAATCCT-3'	117	(Dos Santos et al. 2016)

P.L: Product Length

Fatty acid analysis of liver

Fatty acids were analyzed by using lipid extracts. Lipids were extracted with ether. Liver tissues were homogenized in ether. Then the tubes were shaked for 3 hours at room temperature. After 3 hours, they were placed in a heater to evaporate ether and lipids were collected. Fatty acids of samples were transmethylated to their fatty acid methyl esters (FAME) using methanolic NaOH and methanolic Boron Trifluoride (BF_3). The FAME composition was determined by a gas chromatograph Schimadzu GC-2025 equipped with a Rt-2560 Restek column having 0.25 mm ID, 100 m length, 0.20 μm film thickness. The column temperature program was initiated at 100°C held for 2 min, raised by 4°C/min up to 250°C held for 15 min. The carrier gas was

hydrogen with a flow rate of 1.2 mL min-1 and the split ratio was 50: 1. One μL was injected by using an autosampler. The temperatures of the injector and detector were 250°C. As a reference standard, Restek FAME mix was used to recognize individual fatty acids. Fatty acid indexes were also calculated.

Statistical analysis

Before performing the significance tests, all variables were examined for assumptions of normality and homogeneity of variances. The differences in liver fatty acids and biochemical parameters were determined by Student's t test. Evaluation of rat weights in terms of group and time was examined with two-way mixed ANOVA. In addition, any significant terms were compared with Bonferroni adjustment. Expression analyzes of genes were performed according to the $2^{-\Delta\Delta Ct}$ method and calculated as fold change (Livak and Schmittgen 2001). The relationships between genes expression levels (*FASN* and *SCD-1*), liver fatty acids, and biochemical parameters were determined by Pearson correlation coefficient and shown as a Heatmap. Significance level was $P<0.05$. For the analysis of data, IBM SPSS 23.0 was used.

RESULTS

The average daily food consumption of rats in Cont group was as follows: 30.15 g (1st week), 35.01 g (2nd week), 36.14 g (3rd week), and 34.26 (4th week). The weekly body weight gain of the rats in the Cont group was continuous and the body weight averages in all weeks were significantly different from each other during the feeding period ($P<0.001$). Body weight averages, which were similar at the beginning, were significantly different between the groups from the 1st week ($P<0.001$). The FR group body weight averages had a significant decrease after 7 days of the feeding period ($P<0.001$). On the other hand, body weight averages in the FR group, which were lower than at the beginning of the study, remained at similar levels throughout the study (Table 3).

Table 3. Changes in weekly body weight averages in groups (Mean ± SE)

	Parameter	**Body Weights Averages (g)**	
	Groups	**Cont**	**FR**
Feeding Period	**Initial BW**	277.11 ± 8.60^{e}	276.89 ± 9.19^{a}
	1. week	$317.94 \pm 6.83^{d, A}$	$241.29 \pm 7.31^{c, B}$
	2. week	$350.29 \pm 7.14^{c, A}$	$254.56 \pm 7.63^{b, B}$
	3. week	$368.39 \pm 8.20^{b, A}$	$260.67 \pm 8.77^{b,B}$
	4. week	$380.29 \pm 8.87^{a, A}$	$259.21 \pm 9.48^{b, B}$
P	**Group**	<0.001	
	Time	<0.001	
	Group*Time	<0.001	

BW: Body Weights; **Cont**: Control; **FR**: Food Restriction

^{A,B}: Groups with different letters on the same line are different from each other.

^{a,b,c,d,e}: Groups with different letters in the same column are different from each other.

While plasma total cholesterol, triglyceride, HDL, and LDL levels plasma were similar in both the Cont and the FR groups, it was determined that the amount of plasma glucose was significantly lower in the FR group ($P<0.05$) (Figure 1).

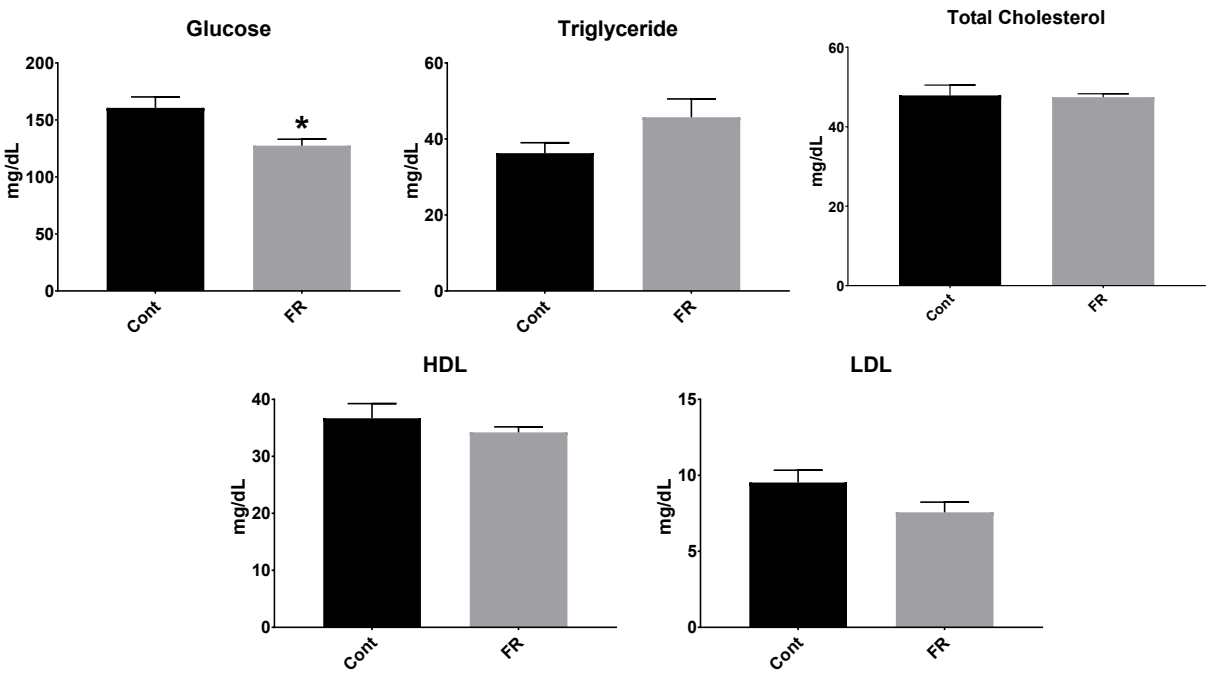

Figure 1: Plasma biochemical parameters in groups (Mean±SE).
Cont: Control group; FR: Food Restriction group; *: P<0.05

According to the gene expression results, expression levels of *FASN* gene were approximately 6 folds more in the FR group compared to the Cont group (P<0.001). Furthermore, approximately 3 folds insignificant upregulation was detected on the expression levels of *SCD-1* gene in the FR group (Figure 2).

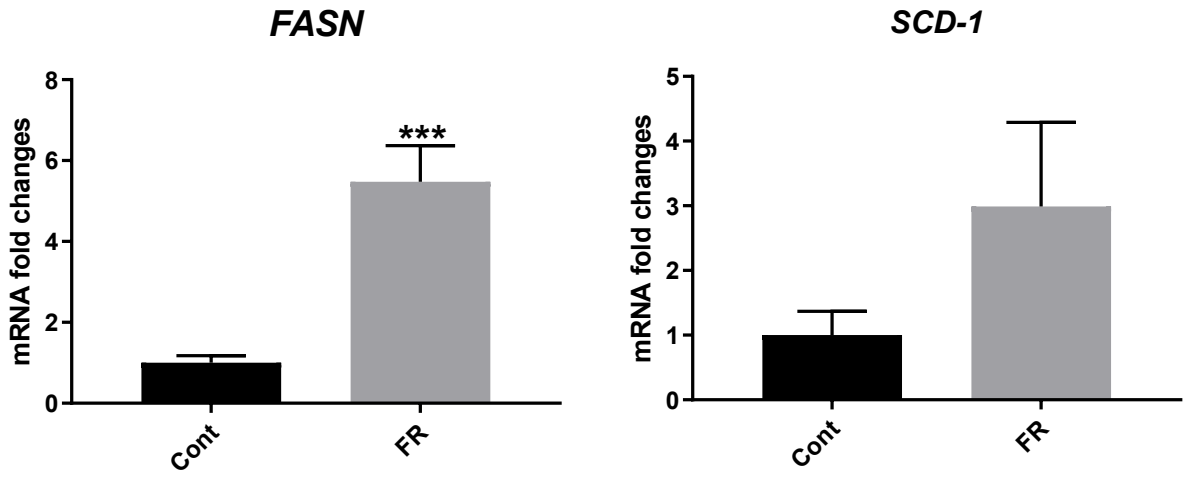

Figure 2: *FASN* and *SCD-1* genes expression levels in liver (Mean±SE).
Cont: Control group; FR: Food Restriction group; ***: P<0.001

Compared to Cont group, the amounts of C15:0, C18:1 n9 trans, C18:2 n6 cis, C21:0, C20:2, C20:5 n3, n6 and UFA were found to be significantly lower in the FR group (P<0.05). On the other hand, C16:0, C18:2 n6 trans, C20:3 n6, C22:2, C22:6 n3, C22:1 n9, fatty acids amounts and Saturated Fatty Acids (SFA) contents were higher in the FR group, significantly (P<0.05) (Table 4).

Table 4. Fatty acid profile of liver in groups (Mean ± SE).

Fatty acids (%)	Cont.	FR	P
C10:0	0.10 ± 0.02	0.10 ± 0.03	-
C12:0	0.18 ± 0.04	0.24 ± 0.03	-
C14:0	0.38 ± 0.03	0.44 ± 0.05	-
C14:1	0.06 ± 0.003	0.05 ± 0.01	-
C15:0	0.33 ± 0.02	0.25 ± 0.01	*
C15:1	0.10 ± 0.02	0.11 ± 0.03	-
C16:0	20.35 ± 0.60	23.65 ± 1.20	*
C16:1	0.91 ± 0.15	0.73 ± 0.05	-
C17:0	0.75 ± 0.04	0.67 ± 0.08	-
C17:1	0.18 ± 0.02	0.16 ± 0.01	-
C18:0	14.74 ± 0.90	16.37 ± 0.43	-
C18:1 n9 trans	2.03 ± 0.33	0.72 ± 0.17	**
C18:1 n9 cis	12.07 ± 0.77	11.58 ± 0.57	-
C18:2 n6 trans	0.19 ± 0.01	0.26 ± 0.03	*
C18:2 n6 cis	18.25 ± 0.75	12.96 ± 0.56	***
C20:0	0.50 ± 0.06	0.33 ± 0.08	-
C18:3 n6	0.34 ± 0.06	0.55 ± 0.13	-
C20:1	0.17 ± 0.02	0.17 ± 0.01	-
C21:0	0.75 ± 0.04	0.59 ± 0.03	**
C18:3 n3	0.31 ± 0.02	0.26 ± 0.03	-
C20:2	3.65 ± 0.58	2.04 ± 0.31	*
C22:0	0.76 ± 0.08	0.52 ± 0.08	-
C20:3 n6	0.55 ± 0.10	0.93 ± 0.13	*
C22:1 n9	0.96 ± 0.11	1.56 ± 0.13	**
C20:3 n3	0.74 ± 0.09	0.95 ± 0.08	-
C20:4 n6	18.18 ± 1.12	18.29 ± 1.29	-
C22:2	0.58 ± 0.10	2.84 ± 0.65	*
C20:5 n3	0.48 ± 0.05	0.26 ± 0.04	**
C22:6 n3	0.36 ± 0.12	0.85 ± 0.12	*
SFA	39.37 ± 0.99	44.00 ± 1.20	**
MUFA	16.99 ± 1.05	15.81 ± 0.68	-
PUFA	43.64 ± 0.55	40.19 ± 1.67	-
UFA	60.63 ± 0.99	56.00 ± 1.20	**
n6	37.51 ± 0.98	33.00 ± 1.50	*
n3	1.90 ± 0.12	2.32 ± 0.23	-

Cont: Control; **FR**: Food Restriction; **SFA**: Saturated Fatty Acids; **MUFA**: Monounsaturated Fatty Acids; **PUFA**: Polyunsaturated Fatty Acids; **UFA**: Unsaturated Fatty Acids; **n6**: Omega 6; **n3**: Omega 3-: P>0.05; *: P<0.05; **: P<0.01; ***: P<0.001

A strong and positive correlation was detected between *FASN* and *SCD-1* genes expression levels in the liver (P<0.01). In addition, it was determined that the *FASN* gene had a positive correlation with C16:0, C18:3 n6, C20:3 n6, C22:1 n9, C22:2 fatty acids, and SFA parameters at varying levels of significance. However, the *FASN* gene has a significant negative correlation with C18:1 n9 trans, C18:2 n6 cis, C20:0, C21:0, C20:5 n3, PUFA, UFA, n6 and LDL (P<0.05). The results were presented in Figure 3. In terms of the relationship of the *SCD-1* gene with fatty acids and biochemical parameters, it showed a positive correlation with C14:0, C16:0, C18:3 n6, and SFA content, and a significant negative correlation with PUFA, UFA, n6, and LDL (P<0.05). Glucose was negatively correlated with SFA, while it was positively correlated with C18:1 n9 trans, C18:2 n6 cis, C20:1, C21:0, C20:2 and UFA parameters. On the other hand, triglyceride was negatively correlated with C14:1, C18:1 n9 trans, and C20:2, and positively correlated with C15:1 and C22:1 n9. As expected, positive correlation was found between HDL and total cholesterol, while LDL was negatively correlated with C20:3 n6 and positively correlated with C18:2 n6 cis, as well as target genes (Figure 3).

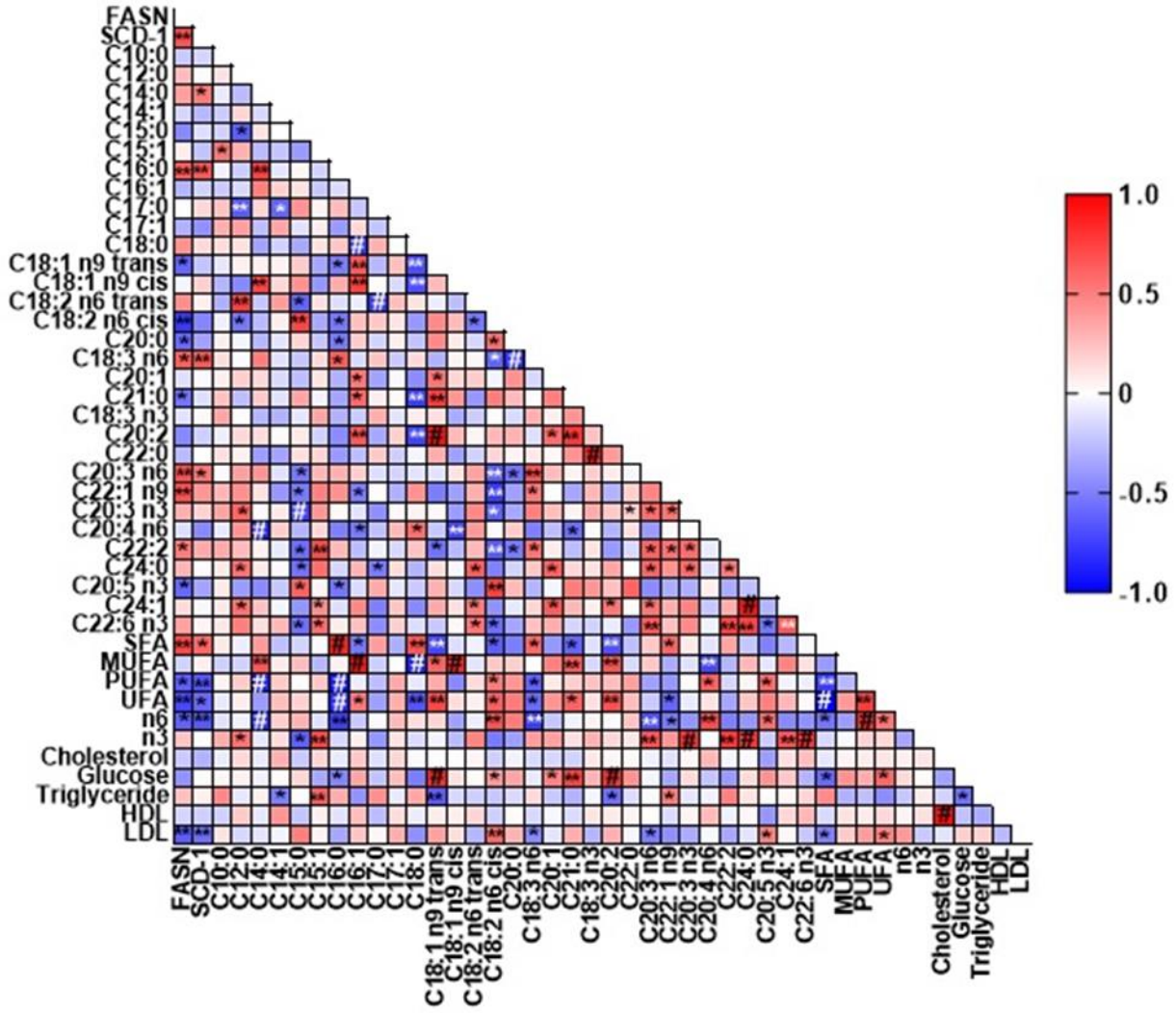

Figure 3: Correlations between studied parameters in heat map form

*: *P< 0.05*; **: *P< 0.01*; #: P<0.001

DISCUSSION

FR, which is applied at a changeable rate in all species including studies on metabolism, has important positive effects such as weight loss, prevention of chronic diseases, and prolongation of a lifetime (Mulligan et al. 2008). The effectiveness of FR is closely related to metabolic resistance (Levin and Dunn-Meynell 2000). Similar to our study findings, it has been reported in some studies that the body weights of rats treated with FR decreased (Bruss et al. 2010, Moraes et al. 2016, Margolis et al. 2016). However, Bruss et al. (2010) reported in their study that there was a loss of body weight at the end of 1 week in rats treated with FR compared to rats fed *ad libitum*, and there was no significant change in body weight averages for the next 4 weeks with FR. In a study, it was reported that FR, which was applied approximately 60%, caused a loss in body weight in the first week and a tendency to gain weight in the following weeks (21-28 days) (Moraes et al. 2016). In this study, it was determined that body weight averages gradually increased in rats fed *ad libitum* during the 4-week feeding period. Similar to the findings of Bruss et al. (2010) body weight loss occurred rapidly in the first week in the FR group, and body weight averages were maintained from the second week to the end of the feeding period in relation to metabolic resistance (Levin and Dunn-Meynell 2000).

Changes in body weight averages are closely related to metabolic regulation. Anabolic and catabolic events are re-regulated in the organism according to the caloric changes in the diet and exercise status. The protected body weight averages in FR group has continued the following weeks from the second week in this study. It has been deduced that the rate and exposure time to food and/or calorie restriction are one of the most important factors for the formation of this situation (Fu and Klaassen 2014).

It was determined that FR application during the 4-week feeding period did not cause a significant change in total cholesterol, HDL, LDL and triglyceride levels compared to the *ad libitum* fed group. Unlike humans, it was reported that mice and rats could tolerate dietary changes without alterations of plasma lipoproteins such as LDL (Flowers and Ntambi 2008). However, it was thought that the significant decrease in plasma glucose levels might be related to metabolic activity and body weight averages. Although Moraes et al. (2016) reported that 60% FR restriction applied for 3 weeks resulted in a significant decrease in total cholesterol and triglyceride levels along with glucose, it was thought that the main difference causing this situation might be related to the restriction rate and feeding period (Moraes et al. 2016, Zhang et al. 2021).

FASN and *SCD-1* genes expression levels tended to be upregulated in the FR group. *FASN* has a central role of lipogenesis in liver (Dorn et al. 2010). Mulligan et al. (2008) reported that as well as the decrease in body weight averages, *FASN* gene expression levels decreased in subcutaneous adipose tissue and liver in the first week, but tended to increase in the following periods in mice with long-term FR. In another study, it was reported that *SCD-1* gene expression levels increased in adipose tissues due to the prolongation of food restriction (Turyn et al. 2012). *FASN*, which is the protein encoded by the *FASN* gene, is involved in the synthesis of long-chain fatty acids using Acetyl-CoA, Malonyl-CoA and NADPH, in other words, it shows more lipogenic activity (Dorn et al. 2010). *SCD-1*, which is also involved in lipogenesis, is responsible for the regulation of lipogenesis in relation to genes involved in the lipogenesis pathway such as *FASN* and *SREBP-1c (*Ozkan and Yakan 2019). It has been thought that the upregulation in the related genes may

have been shaped by the metabolic resistance that developed to response to food restriction.

It was reported that food restriction increases fatty acid synthesis in the body (Bruss et al. 2010). In this study, although a significant calorie restriction was applied for 4 weeks in the FR group, C16:0 fatty acid and SFA levels with some of the long-chained fatty acids were found to be higher compared to the control group, basically consistent with *FASN* and *SCD-1* genes expression levels. It is known that *FASN* catalyzes the final stage of fatty acid biosynthesis and it is a crucial role to generate fatty acid de novo lipogenesis (Dorn et al. 2010). The regulation of fatty acid synthesis is a complex and intricate process in the liver. However, it has been understood that while the amount of SFA increased in the liver with the application of food restriction, the amount of UFA and n6 decreased. It has been thought that this situation might be caused by the signaling of the synthesis of essential fatty acids by the upregulation of genes responsible for more saturated fatty acid production such as *FASN* and *SCD-1* in the liver after metabolic regulation was rearranged. Activation of the *FASN* gene in the liver has a leading role especially in C16:0 biosynthesis and catalyzes the biosynthesis of saturated fatty acids in de novo lipogenesis (Dorn et al. 2010, Jensen-Urstad and Semenkovich 2012). Similar to our results, it has been reported there is a positive correlation between *SCD-1* and C16:0 and C18:0 fatty acids (Miyazaki and Ntambi 2003). On the other hand, Kunešová et al. (2006) reported in their study that after restricted dietary regulation in obese people, the amount of C16:0 increased, while the amount of C18:2 n6 and C20:3 n6 decreased. Although energy regulation in rodents and humans is mostly similar, it is conceivable that minor differences may be due to species differences.

In addition to differences in measured parameters between groups, variable interactions were determined between genes and other parameters. In addition to LDL, *FASN* and *SCD-1* genes expressions were found co-correlated with many fatty acid parameters. LDL was detected negatively correlated with *FASN* and *SCD-1*. It was reported that *SCD-1* deficiency led to a decrease the synthesis of LDL in the liver (Flowers and Ntambi 2008). As expected, strong and negative correlations were found between these genes and PUFA, UFA and n6 parameters. *FASN* and *SCD* were positively correlated with C16:0, which has a key role in de novo lipogenesis (Dorn et al. 2010, Jensen-Urstad and Semenkovich 2012). Interestingly, these genes were positively correlated with the various significances with long and unsaturated fatty acids such as C20:3 n6. This showed that the related genes may have important roles related to regulation of unsaturated and long-chained fatty acids in addition to their main functions in the liver.

Undoubtedly, metabolic regulation is closely related to molecular activity at the transcriptional level. The obtained findings of this study suggest that increased SFA contents with food restriction in the liver are regulated by upregulated lipogenic genes mainly *FASN* and *SCD-1*.

CONCLUSION

In conclusion, remarkable changes occur with the exposure time and ratio of restriction in FR. Considering that metabolism is also related to muscle and adipose tissues, it is thought that more studies are needed to understand the interactions with other organs at the molecular levels in food restriction.

Ethical Approval: This study was ethically approved by HMKU HAYDEK with the number 2021/04-02 and the date 16.06.2021.

Conflict of interest: The authors declared that there are no actual, potential, or perceived conflicts of interest for this article.

Authors contribution rate: HO: %25, IK: %15, UK: %15, SD: %10, İA: %5, BÇ: %5, HHK: %5, AK: %20

REFERENCES

Barzilai N, Gabriely I. The role of fat depletion in the biological benefits of caloric restriction. J Nutr. 2001; 131(3): 903S-906S.

Bruss MD, Khambatta CF, Ruby MA, Aggarwal I, Hellerstein MK. Calorie restriction increases fatty acid synthesis and whole body fat oxidation rates. Am J Physiol Endocrinol Metab. 2010; 298(1): E108-E116.

Crovesy L, Masterson D, Rosado EL. Profile of the gut microbiota of adults with obesity: a systematic review. Eur J Clin Nutr. 2020; 74(9): 1251-1262.

Dorn C, Riener MO, Kirovski G, Saugspier M, Steib K, Weiss TS, Gäbele E, Kristiansen G, Hartmann A, Hallerbrand C. Expression of fatty acid synthase in nonalcoholic fatty liver disease. Int J Clin Exp Pathol. 2010; 3(5): 505.

Dos Santos BP, da Costa Diesel LF, da Silva Meirelles L, Nardi NB, Camassola M. Identification of suitable reference genes for quantitative gene expression analysis in rat adipose stromal cells induced to trilineage differentiation. Gene. 2016; 594(2): 211-219.

Dumas JF, Roussel D, Simard G, Douay O, Foussard F, Malthiery Y, Ritz P. Food restriction affects energy metabolism in rat liver mitochondria. Biochim Biophys Acta Gen Subj. 2004; 1670(2): 126-131.

Flowers MT, Ntambi JM. Role of stearoyl-coenzyme A desaturase in regulating lipid metabolism. Curr Opin Lipidol. 2008; 19(3): 248.

Fu ZD, Klaassen, CD. Short-term calorie restriction feminizes the mRNA profiles of drug metabolizing enzymes and transporters in livers of mice. Toxicol Appl Pharmacol. 2014; 274(1): 137-146.

Gardner CD, Kim S, Bersamin A, Dopler-Nelson M, Otten J, Oelrich B, Cherin, R. Micronutrient quality of weight-loss diets that focus on macronutrients: results from the A TO Z study. Am J Clin Nutr. 2010; 92(2): 304-312.

Hill JO, Wyatt HR, Peters JC. Energy balance and obesity. Circulation. 2012; 126(1): 126-132.

Jensen-Urstad AP, Semenkovich CF. Fatty acid synthase and liver triglyceride metabolism: housekeeper or messenger? Biochim Biophys Acta Mol Cell Biol Lipids. 2012; 1821(5): 747-753.

Kunešová M, Braunerova R, Hlavatý P, Tvrzická E, Stanková B, Skrha J, Hilgertová J, Hill M, Kopecký J, Wagenknecht M, Hainer V, Matoulek M, Parízková J, Zák A, Svacina S. The influence of n-3 polyunsaturated fatty acids and very low calorie diet during a short-term weight reducing regimen on weight loss and serum fatty acid composition in severely obese women. Physiol Res. 2006; 55(1): 63-72.

Laskowski ER. The role of exercise in the treatment of obesity. PM&R. 2012; 4(11): 840-844.

Levin BE, Dunn-Meynell AA. Defense of body weight against chronic caloric restriction in obesity-prone and-resistant rats. Am J Physiol Regul Integr Comp Physiol. 2000; 278(1): R231-R237.

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 2001; 25(4): 402-408.

Margolis LM, Rivas DA, Ezzyat Y, Gaffney-Stomberg E, Young AJ, McClung JP, Fielding RA, Pasiakos SM. Calorie restricted high protein diets downregulate lipogenesis and lower intrahepatic triglyceride concentrations in male rats. Nutrients. 2016; 8(9): 571.

Miyazaki M, Ntambi JM. Role of stearoyl-coenzyme A desaturase in lipid metabolism. Prostaglandins Leukot Essent Fatty Acids. 2003; 68(2): 113-121.

Mock K, Lateef S, Benedito VA, Tou JC. High-fructose corn syrup-55 consumption alters hepatic lipid metabolism and promotes triglyceride accumulation. J Nutr Biochem. 2017; 39: 32-39.

Moraes CD, Oliveira CAD, Amaral, MECD, Landini GA, Catisti R. Liver metabolic changes induced by conjugated linoleic acid in calorie-restricted rats. Arch Endocrinol Metab. 2016; 61: 45-53.

Mulligan JD, Stewart AM, Saupe KW. Downregulation of plasma insulin levels and hepatic PPARγ expression during the first week of caloric restriction in mice. Exp Gerontol. 2008; 43(3): 146-153.

NRC. Nutrient Requirements of Laboratory Animals. Fourth Revised Edition. Washington (DC): National Academies Press (US) 1995; 11-58.

Ozkan H, Yakan A. Dietary high calories from sunflower oil, sucrose and fructose sources alters lipogenic genes expression levels in liver and skeletal muscle in rats. Ann Hepatol. 2019; 18(5): 715-724.

Rio DC, Ares M, Hannon GJ, Nilsen TW. Purification of RNA using TRIzol (TRI reagent). Cold Spring Harb Protoc. 2010; 2010(6): pdb-prot5439.

Rui L. Energy metabolism in the liver. Compr Physiol. 2014; 4(1): 177.

Smith DLJ, Nagy TR, Allison DB. Calorie restriction: what recent results suggest for the future of ageing research. EJCI. 2010; 40(5): 440-450.

Smyers ME, Bachir KZ, Britton SL, Koch LG, Novak CM. Physically active rats lose more weight during calorie restriction. Physiol Behav. 2015; 139: 303-313.

Turyn J, Mika A, Stepnowski P, Swierczynski J. Unusual increase of Scd1 and Elovl6 expression in rat inguinal adipose tissue. Open Life Sci. 2012; 7(2): 192-200.

Yasari S, Prud'homme D, Wang D, Jankowski M, Levy É, Gutkowska J, Lavoie JM. Exercise training decreases hepatic SCD-1 gene expression and protein content in rats. Mol Cell Biochem. 2010; 335(1): 291-299.

Zhang L, Huang YJ, Sun JP, Zhang TY, Liu TL, Ke B, Shi XF, Li H, Zhang GP, Ye ZY, Hu J, Qin J. Protective effects of calorie restriction on insulin resistance and islet function in STZ-induced type 2 diabetes rats. Nutr Metab. 2021; 18(1): 1-10.

Zhang X, Heckmann BL, Campbell LE, Liu J. G0S2: A small giant controller of lipolysis and adipose-liver fatty acid flux. Biochim Biophys Acta Mol Cell Biol Lipids. 2017; 1862(10): 1146-1154.