



Effects of Ramadan Fasting on Hepatic Steatosis and Liver Volume in Individuals without Chronic Liver Conditions

İlyas Dündar*¹, Alpaslan Yavuz²

1. Department of Radiology, Faculty of Medicine, Van Yuzuncu Yil University, Van, Turkey.

2. Department of Radiology, Antalya Training and Research Hospital, Antalya, Turkey.

ARTICLE INFO

Article type:

Research Paper

Article History:

Received: 20 Dec 2020

Accepted: 26 Jan 2021

Published: 25 Aug 2021

Keywords:

Ramadan fasting

Hepatic steatosis,

Chemical shift imaging

Nonalcoholic fatty liver disease

Obesity

ABSTRACT

Introduction: Ramadan fasting (especially in summer) provides an opportunity to evaluate the effects of eating and drinking variances on the metabolism. The present study aimed to assess the effects of Ramadan fasting on hepatic steatosis and liver volume by magnetic resonance imaging (MRI) in overweight and obese individuals and determine the impact of the fasting period on liver function tests and serum lipid profile.

Methods: This study was conducted on 34 individuals (28 males and six females) without chronic liver or systemic diseases who did not use alcohol, had the body mass index (BMI) of ≥ 25 , and were committed to Ramadan fast. Abdominal MRI and blood analysis were performed twice one week before and after Ramadan. In addition, liver fat fractions and volumes were calculated based on the MRI. Data were statistically analyzed and compared.

Results: The mean age of the subjects was 44.5 years (age range: 19-68 years). Before Ramadan, the mean weight and mean BMI were 86.76 kilograms and 30.29 kg/m², respectively. Although the liver fat fraction increased ($2.92 \pm 7.99\%$ vs. $3.44 \pm 8.11\%$; $P > 0.05$) and the liver volume decreased ($1,555.37 \pm 316.92$ vs. $1,546.63 \pm 339.82$; $P > 0.05$) after Ramadan, the differences were not significant. However, significant, positive changes were observed in the serum lipid profile and liver function tests.

Conclusion: Excess food consumption in the evening and at night and a sedentary lifestyle may have affected our findings. Nonetheless, the prolonged avoidance of a predetermined amount of food and drinks could lead to statistical changes in the measured variances. As such, further longitudinal studies on larger sample sizes could be performed to examine individuals with the BMI of less than 25 kg/m² for more accurate results.

► Please cite this paper as:

Dündar İ, Yavuz A. Effects of Ramadan Fasting on Hepatic Steatosis and Liver Volume in Individuals without Chronic Liver Conditions. J Nutr Fast Health. 2021; 9(3): 221-228. DOI: 10.22038/jnfh.2021.54384.1312.

Introduction

Nonalcoholic fatty liver disease (NAFLD) refers to a fatty liver occurring secondary to nonalcoholic causes [1]. NAFLD could potentially progress into cirrhosis due to hepatic damage and increase mortality and morbidity. The incidence of NAFLD has recently been on the rise parallel to the increased obesity. In addition, NAFLD is ranked second in terms of the etiology of chronic liver diseases in Turkey. In the current research, we performed a quantitative analysis to investigate the effects of 29 days of Ramadan fasting with the approximate duration of 16 hours and 50 minutes per day in June 2016 in Van, Turkey. The main objective was to evaluate the effects of fasting on hepatic steatosis and liver volume based on chemical-shift magnetic resonance imaging (MRI) in overweight and obese

individuals with NAFLD and those without underlying diseases. Additionally, the effects on the liver function and serum lipid profile were assessed.

Ultrasonography (US) and computed tomography (CT) could be employed for the diagnostic imaging and follow-up of NAFLD patients. However, the recently defined chemical-shift MRI has come forefront for the diagnosis and quantitative assessment of the disease. The key advantage of MRI over US and CT is that this imaging modality involves no radiation exposure and detected the presence of fats at a cellular level through a chemical shift [2].

Ramadan fasting is compulsory for healthy adult Muslims, and the duration of fasting is 29-30 days in the 9th month of the lunar calendar. According to the Islamic calendar (Hijri

* Corresponding author: İlyas Dündar MD, Assistant Professor, Van Yuzuncu Yil University, Dursun Odabasi Medical Center, Department of Radiology, 65080, Campus, Tusba, Van, Turkey. Tel: +904322150470; Email: dundarilyas262@hotmail.com.

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calendar), Muslims must refrain from eating and drinking for 13-14 hours per day (from dawn to sunset). However, ill individuals, pregnant/lactating and menstruating women, the elderly, and travelers are exempt from Ramadan fasting. In this month, eating and drinking is allowed during the night from sunset to approximately 1.5-2 hours before sunrise. In many countries, one meal is traditionally taken at *Sahur* (before sunrise) as breakfast, and the other meal is taken at *Iftar* (after sunset) as dinner. In some countries, more than two meals could be taken from *Iftar* to *Sahur*.

Previous studies have shown that nutrients ingested at unusual times may exert different metabolic effects [3-5]. Ramadan fasting (especially in summer) provides an opportunity to evaluate the effects of eating and drinking variances on the metabolism. NAFLD has been reported to be a complication and sequela of overweight and obesity, with a higher prevalence among obese individuals [6, 7]. In the present study, we hypothesized that the prolonged daytime fasting in Ramadan in June may have positive metabolic effects on the lipid profile (especially on the liver metabolism) in overweight and obese individuals with NAFLD. Lipid profile [3, 8-12] and liver function [5, 13, 14] have been previously analyzed during Ramadan using blood tests.

The present study aimed to investigate the effects of Ramadan fasting on hepatic steatosis and liver volume through blood tests and MRI. To the best of our knowledge no prior studies have measured hepatic steatosis and volume by MRI in Ramadan.

Materials and Methods

Patient Selection

This study was conducted on 34 out of 85 patients aged more than 18 years who referred to the clinics of the medical centers of our institute, presenting with hepatic steatosis, obesity, and impaired serum lipid profile. The body mass index (BMI) of the patients was calculated, and those with the BMI of ≥ 25 kg/m² were enrolled in the study. On the other hand, 22 patients who were not able to fast during Ramadan were excluded for different reasons, including pregnancy (n=5), menstruation (n=8), severe diseases (n=4), and chronic drug use (n=5). In addition, 16 patients with the BMI of < 25 kg/m² and 13 patients with chronic liver

disease, systemic disease, history of malignancies, and chronic medication use affecting the liver were excluded.

The required permit for the study was obtained from the Clinical Research Ethics Committee of our institute (05.05.2016; No. 03), and the study was conducted on 34 patients, including 28 males (age: 18-70 years) and six menopausal females (age: 45-70 years), who committed to Ramadan fasting during June 2016. None of the participants had a known chronic liver disease or alcohol use.

Imaging Technique

MRI was carried out using a 1.5 Tesla MRI device (Syngo MR B17, Siemens MAGNETOM Symphony, Germany, 2009) with an abdominal coil. The upper abdominal MRI of the patients was obtained twice, with the first process performed in the week before Ramadan fasting (BR) and the second in the week after Ramadan (AR). The interval between the BR and AR tests was planned to be at least 30 days, with the maximum of 34 days (including the 29-day fasting period). The breath-hold T1-weighted gradient echo dixon sequences were used for the imaging. In this sequence, the voxel dimensions were determined as 1.9×1.2×3.0 (SNR:1.00), with the section slice thickness of three millimeters (TR:7.71; TE:2.38), flip angle of 10, and bandwidth of 340 HZ/Px. In each examination, 64 three-dimensional (3D) section images were also obtained in four different sequences, including in-phase (IP), out-phase (OP), water (W), and fat (F).

Laboratory Serum Values

Simultaneously with the imaging studies and after at least eight hours of fasting, biochemical tests were carried out to measure triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TCL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and direct bilirubin (DB) twice at the BR and AR.

MRI-based Hepatic Steatosis Measurement

The MRI images were examined by radiologists with 15 and seven years of clinical experience. Considering the craniocaudal size of the liver, the measurements were performed at the upper, middle, and lower levels by division into three equal segments in order to ensure homogeneity. In addition, the region of interest

(ROI) was drawn polygonal to the liver in the IP and OP images using the picture archiving communication system, including the entire extent of the liver parenchyma. The mean ROI values were measured in the IP and OP images separately at the same level in each region (Figure 1). The IP and OP values at the upper, middle, and lower levels of the BR and AR were

also recorded separately. The values were applied to the fat signal fraction (FF) formulas ($FF = [IP - OP] / [2 \times IP]$ or $FF = F / [W + F]$) for the BR and AR regions separately, and the values were obtained as percentages. The mean FF of each region was calculated, and the values were recorded.

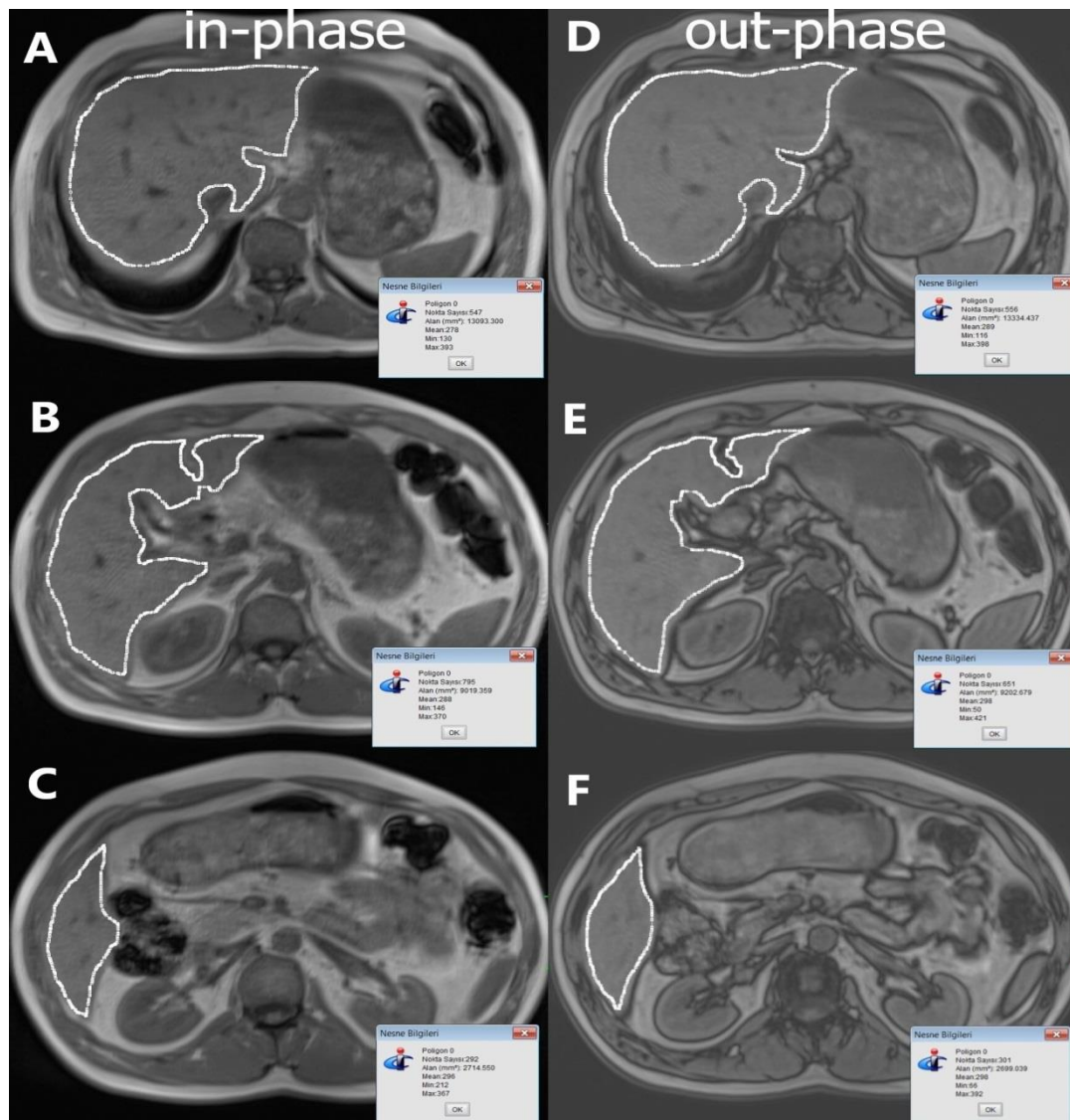


Figure 1. Polygon-shaped region of interest (ROI) drawing of the liver contours in the in-phase (IP) (A, B, C) and out-phase (OP) (D, E, F) images using the Picture Archiving Communication Systems (PACS) in three separate areas of the liver, upper (A, D), middle (B, E) and lower (C, F), and calculating the average ROI values.

MRI-based Liver Volume Measurement

The obtained 3D images were uploaded into a Linux operating system-based XIO treatment planning system program (version 4.34; CMS

Software, St. Louis, MO). The visible outer contours in each section of the liver were drawn manually using the program, and the areas within the drawn contours were reconstructed by the program to calculate the liver volume

(Figure 2). Moreover, the BR and AR liver volume values were recorded separately.

Statistical Analysis

Data analysis was performed in SPSS version 20.0 (IBM Corporation, Armonk, NY) using descriptive statistics for the continuous variables, which were expressed as mean,

standard deviation, minimum, and maximum. The categorical variables were expressed as numbers and percentages. The within-group comparisons of the continuous variables were also performed using dependent t-test, and the level of statistical significance was considered to be 5%.

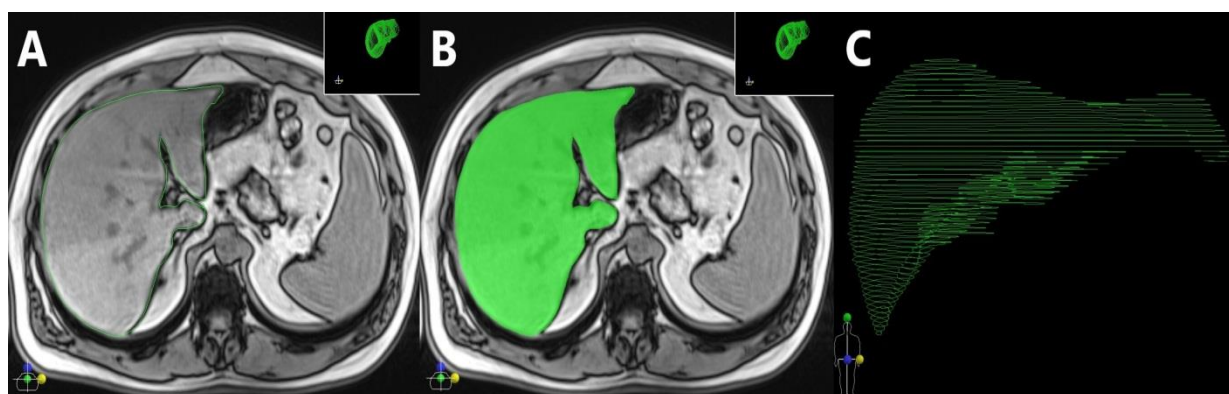


Figure 2. Considering the craniocaudal size of the liver, manual drawing of all external contours seen in all sections of the liver through the XIO (v4.34, CMS Software, St. Louis, MO) Treatment Planning System program (A, B). Obtaining liver volumes by reconstructing the areas within the contours that are drawn through the program (C).

Results

Baseline Characteristics

In total, 34 patients were enrolled in the study, including 28 males (82.4%) and six females (17.6%) with the mean age of 44.5 ± 10.81 years (age range: 19-68 years). The mean weight of the patients was 86.76 ± 12.82 kilograms (range: 63-111 kilograms), and the mean BMI was 30.29 ± 3.40 kg/m² (range: 25.46-38.06 kg/m²).

Outcomes of Serum Lipid Profile

The mean values of TG, LDL, TCL, and HDL BR were 232 ± 167.76 , 102.38 ± 35.37 , 190.06 ± 39.89 , and 42.44 ± 8.66 mg/dl, respectively, while they were estimated at 156.85 ± 134.45 , 129.65 ± 41.82 , 200.65 ± 36.42 , and 39.09 ± 7.13 mg/dl AR, respectively. The comparison of the measured values BR and AR indicated a very significant reduction in the TG and HDL ($P < 0.001$), as well as a very significant increase in the LDL ($P < 0.001$), and a significant increase in the TCL ($P = 0.01$) (Table 1).

Outcomes of Liver Function Tests

The mean values of DB, AST, and ALT BR were 0.22 ± 0.10 mg/dl, 22.97 ± 10.27 IU/l, and 26.15 ± 13.48 IU/l, respectively, while they were estimated at 0.28 ± 0.10 mg/dl, 21.74 ± 6.92 IU/l, and 21.85 ± 10.80 IU/l AR, respectively. The comparison of the measured values BR and AR

indicated a significant decrease in ALT ($P = 0.01$), as well as a significant increase in DB ($P < 0.001$), and an insignificant reduction in AST ($P = 0.20$) (Table 1).

Outcomes of Hepatic Steatosis Measurement

The mean liver fat fractions measured BR and AR at the upper, middle, and lower levels of the liver were calculated to be $2.47 \pm 7.67\%$, $2.77 \pm 8.04\%$, $3.51 \pm 8.42\%$, and $3.08 \pm 7.9\%$, $3.31 \pm 8.25\%$, and $3.93 \pm 8.33\%$, respectively. In addition, the mean values of fat fraction measured at these three levels of the liver BR and AR were estimated at $2.92 \pm 7.99\%$ and $3.44 \pm 8.11\%$, respectively. The comparison of the fat fraction values measured BR and AR indicated that despite the increase in the rate of fat fractions at all the levels, the obtained values had no statistically significant difference in this regard ($P > 0.05$) (Table 1).

Outcomes of Liver Volume Measurement

The mean values of liver volume BR and AR were $1,555.37 \pm 316.92$ and $1,546.63 \pm 339.82$ cc, respectively. The comparison of the values measured BR and AR indicated that despite the decreased liver volume, the obtained values had no statistically significant difference in this regard ($P > 0.05$) (Table 1).

Table 1. Average and comparison of the values before and after Ramadan

Variables	Before Ramadan (mean ± std. deviation)	After Ramadan (mean ± std. deviation)	Paired Samples Test				*p values
			Paired Differences		95% Confidence Interval of the Difference		
			Mean Difference	Std. Deviation	Lower	Upper	
Fat Fraction 1 (%)	2.47 ± 7.67	3.08 ± 7.9	-0,61	2,73	-1,56	0,35	,204
Fat Fraction 2 (%)	2.77 ± 8.04	3.31 ± 8.25	-0,54	2,68	-1,47	0,40	,252
Fat Fraction 3 (%)	3.51 ± 8.42	3.93 ± 8.33	-0,42	3,21	-1,54	0,70	,447
Mean Fat Fraction (%)	2.92 ± 7.99	3.44 ± 8.11	-0,52	2,72	-1,47	0,43	,272
Liver Volume (cc)	1555.37 ± 316.92	1546.63 ± 339.82	8,75	151,02	-43,95	61,44	,738
Triglyceride (mg/dl)	232 ± 167.76	156.85 ± 134.45	75,15	104,05	38,84	111,45	,000
Low Density Lipoprotein (mg/dl)	102.38 ± 35.37	129.65 ± 41.82	-27,26	28,24	-37,12	-17,41	,000
Total Cholesterol (mg/dl)	190.06 ± 39.89	200.65 ± 36.42	-10,59	23,53	-18,80	-2,38	,013
High Density Lipoprotein (mg/dl)	42.44 ± 8.66	39.09 ± 7.13	3,35	4,44	1,80	4,90	,000
Direct Bilirubin (mg/dl)	0.22 ± 0.10	0.28 ± 0.10	-0,06	0,07	-0,08	-0,04	,000
Aspartat Aminotransferaz (IU/L)	22.97 ± 10.27	21.74 ± 6.92	1,24	5,55	-0,70	3,17	,204
Alanin Aminotransferaz (IU/L)	26.15 ± 13.48	21.85 ± 10.80	4,29	9,63	0,93	7,66	,014

*p <0.05 statistically significant.

Discussion

Several studies have investigated the epidemiology, pathogenesis, natural course, and treatment of NAFLD, and prevalence studies have been carried out in the community and on various risk groups. However, the evaluation of these studies may be technically challenging. The lack of a standardized definition for NAFLD, growing number of the asymptomatic cases, unavailability of specific diagnostic tests, and impracticality of liver biopsy as a screening method are among the main limitations in regards to the disease.

The prevalence of NAFLD has been reported to be 20% with US is used as the screening tool, while the rate increase 2-3 fold with liver biopsy [15]. This discrepancy is mainly due to the fact that invasive liver biopsy is only used under special circumstances when it is unavoidable.

The prevalence of NAFLD has also been reported to be higher in specific risk groups, such as patients with type II diabetes mellitus, obesity, and hyperlipidemia, compared to the general population [7, 16-18]. As such, we focused on overweight and obese cases in the present study.

Currently, liver biopsy is considered to be the 'gold standard' for NAFLD diagnosis and determining the level of steatosis. The procedure could be affected even by inconsequential factors, such as the angle of the liver biopsy needle upon insertion, length of the sample, and differences in number, while there is also the possibility of numerous minor and major complications due to its invasive nature [19, 20]. Therefore, the diagnosis and follow-up of the patients are primarily performed using US, CT, and MRI [21]. Liver stiffness could be measured by transabdominal US with

elastography, which is a novel and accurate method for evaluating the severity of NAFLD [22, 23]. Notably, the available MRI methods are chemical-shift imaging modalities and MR spectroscopy (MRS) [24].

The main advantage of MRI is its direct chemical specificity for TG, the accumulation of which is a histopathological sign of steatosis. Furthermore, MRI has been reported to have high sensitivity and specificity in the detection of mild steatosis [21, 24, 25]. In a meta-analysis, MRI and MRS were proven superior in the differentiation of various mild types of the disease [26].

In the current research, chemical-shift MRI was employed for determining the level of hepatic steatosis as a noninvasive approach involving no radiation, with an easier application and examination compared to MRS. MRS has some other limitations as well; for instance, it is only available in academic centers, the process is time-consuming, the evaluation requires specialty training, and only the area of examination could be evaluated rather than the entire liver.

Variable results have been reported regarding the effects of Ramadan fasting on the lipid profile. In a study of 30 premenopausal women, a significant decrease was observed in TG and LDL, and a significant increase was reported in the HDL values, with no significant difference in the TCL values [8]. In another study conducted on 81 healthy subjects, the reported values demonstrated a significant increase in the TG of the subjects with the BMI of less than 25 kg/m², while a significant decrease was denoted in TG, a significant increase was reported in LDL, and a significant decrease was observed in HDL. In the mentioned study, the TCL levels remained

unchanged in the subjects with the BMI of ≥ 25 kg/m² [3].

According to the findings of Shehab A. et al., TG significantly increased in women and decreased in men, and they also reported the significant reduction of LDL, the significant increase of HDL, and increased TCL in the women and decreased TCL in men, while no significance was denoted in 65 healthy volunteers with the mean BMI of 27.5 kg/m² [12]. In another study in this regard, a significant decrease was observed TG and LDL, a significant increase was reported in HDL, and TCL was also observed to increase in 33 healthy volunteers [10]. Another research conducted on 28 healthy women also demonstrated increased TG, as well as the significant reduction of LDL and TCL and the significant increase of HDL [9].

In the study by Vardarli MC et al., a significant increase was observed in LDL, and a significant decrease was reported in HDL [11]. In the present study, TG significantly decreased, which is consistent with the previous studies in this regard. On the other hand, we observed a significant increase in LDL and TCL and a significant reduction in HDL, which is inconsistent with the previous findings. Table 2 shows the complex variables observed in the serum lipid profile and a comparison with the literature in this regard. Previous findings on the effects of Ramadan fasting on serum lipid profile are inconsistent and often contradictory, which could be due to variables such as the genetic structure, seasonal differences, duration of Ramadan fasting, type of consumed foods, socioeconomic status of fasting individuals, and frequency of physical activity.

Table 2. Comparison of our study with the variable data observed in the blood-lipid profile in the literature

	Our Study (n=34)	Culha C et al. (n=30)	Ziaee V et al. (n=81)	Shehab A et al. (n=65)	Ismail WI et al. (n=33)	Akhtaruzzaman M et al. (n=30)	Vardarli MC et al. (n=24)
Triglyceride (mg/dl)	↓ *	↓ *	↑* (N BMI) ↓* (≥ 25 BMI)	↑* (Female) ↓ (Male)	↓ *	↑	
LDL-Cholesterol (mg/dl)	↑ *	↓	↑ *	↓ *	↓ *	↓ *	↑ *
HDL-Cholesterol (mg/dl)	↓ *	↑ *	↓ *	↑ *	↑ *	↑ *	↓ *
Total Cholesterol (mg/dl)	↑ *			↑ (Female) ↓ (Male)	↑	↓ *	

↑: Increase, ↓: Decrease, *: p < 0.05 statistically significant

Few studies have been focused on the effects of Ramadan fasting on the liver function. In a study

of 34 patients with NAFLD, ALT levels were reported to increase significantly [5]. In

addition, Ibrahim NSI et al. observed a significant decrease in the AST and ALT levels in 18 healthy young males [14]. Another study performed on 57 healthy individuals indicated a significant increase in the AST and total bilirubin levels, as well as a significant decrease in the ALT levels [14]. In the present study, ALT decreased significantly, which is consistent with the previous studies conducted on healthy participants, while inconsistent with some other findings. Furthermore, AST decreased in the current research although the reduction was not considered significant. On the other hand, DB significantly increased, which is in line with the previous studies in this regard. Although our patients had NAFLD, they had no underlying chronic liver or systemic diseases, which differs with the previous studies [5] in which the ALT levels have been reported to increase. As such, our findings may be in congruence with the studies performed on healthy individuals.

For the first time, we evaluated the effects of short-term fasting on hepatic steatosis and liver volume based on the steatosis level using MRI as the most sensitive and reliable quantitative measurement method. According to the obtained results, the fat signal fraction increased at the upper, middle, and lower levels of the liver, while the liver volume decreased; notably, these changes were not considered significant. Although only a few studies have been conducted in this regard so far, we suggest that the duration of daily fasting short as 29 days is probably not sufficient for a significant morphological change to occur in the liver MRI. We also believe that significant results may be obtained with longer periods of fasting. Although it was not visually detected in the liver MRI of our patients, the quantitative measurements indicated the hepatic steatosis to be larger in the lower levels of the liver in both the BR and AR regions.

The main limitations of the present study were the lack of participants with the BMI of <25 kg/m², no sonographic grading of the hepatic steatosis, and the small sample size.

Conclusion

Initially, we hypothesized that prolonged daily fasting in healthy individuals with NAFLD and no underlying diseases may have positive metabolic effects on hepatic steatosis and planned to demonstrate these effects using

imaging modalities. However, no significant increase was observed in the liver fat signal fraction, and no significant decrease was denoted in the liver volume as measured by MRI during the one-month period of the study. In contrast, positive, significant changes were observed in several biochemical blood parameters in this period. The excess consumption of food in the evening and at night and a sedentary lifestyle may have affected our findings, while the prolonged avoidance of a predetermined amount of food and drinks regularly may also lead to statistical changes. Therefore, it is recommended that larger longitudinal studies in this regard be conducted on subjects with the BMI of <25 kg/m².

Acknowledgements

This research did not receive any specific grants from any funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

None declared.

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