

The Effect of Ramadan Fasting on Non-Alcoholic Fatty Liver Disease (NAFLD) Patients

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ABSTRACT

Purpose: The aim of this study was to compare biochemical tests, body composition and anthropometric parameters in Nonalcoholic Fatty Liver Disease (NAFLD) patients before and after Ramadan fasting.

Methods: fifty NAFLD patients including 33 males and 17 females aged 18-65 year, were recruited. Subjects attended after diagnosis based on ultrasound imaging, with at least 10 hour fasting, before and after Ramadan who have been fasting for at least 10 days. A fasting blood sample was obtained, blood pressure was measured and body mass index (BMI) and fat mass (FM) and fat free mass (FFM) were calculated. Lipid profile, fasting blood sugar (FBS), insulin, ALT and AST enzymes were analyzed on all blood samples.

Result: There was a significant increase in HDL-c, total cholesterol, triglyceride (TG) and fasting blood sugar (FBS) after Ramadan ($P < 0.05$, t test) while there was a significant decrease in systolic blood pressure (SBP), diastolic blood pressure (DBP) and ALT after Ramadan ($P < 0.05$, t test), the change in BMI, LDL-c, FM and FFM after Ramadan were not significant ($P > 0.05$, t test).

Conclusion: This study shows significant effects on NAFLD patient parameters during Ramadan fasting such as decreasing in insulin, ALT enzyme, SBP and DBP and increasing in HDL-c after an average of 27 d fasting. Result from this study suggested that Ramadan fasting may be useful to improve NAFLD, so further studies are needed in this area.

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Introduction

Ramadan is a month for Muslims during which they abstain from eating, drinking and smoking from dawn to dusk. During month of Ramadan there are changes in the quality of food and eating patterns(1). As would be expected, caloric intake is often reduced during this month(2).

The food habits are not similar outside and the proportion of fat, protein and carbohydrate intake can differ during Ramadan. There is a tendency to consume foods and drinks that are richer in carbohydrates than those consumed during other months of the year. The quality of ingested nutrients can also differ during Ramadan compared with the rest of the year(3).

Nonalcoholic fatty liver disease (NAFLD) is gaining increasing recognition as a component of the epidemic of obesity in the United States as well as in other parts of the world. NAFLD is the most common cause of liver dysfunction and affects close to 20 million patients in the US(4). NAFLD is considered to be the liver component of metabolic syndrome (MetS) (5, 6). It is

associated with obesity, dyslipidemia and type 2 diabetes mellitus (DM), (7-9) and also predicts the clustering of risk factors for cardiovascular disease (10). NAFLD is characterized by pathological accumulation of lipids in the liver in the absence of alcohol abuse, but its pathogenesis is still inadequately understood. Generally, fat accumulation arises from an imbalance between hepatic lipid uptake, oxidation, export of lipids from the liver, and de novo lipogenesis (11). NAFLD includes a wide spectrum of liver damage, ranging from simple steatosis to steatohepatitis and advanced fibrosis, with histologic features of alcohol-induced liver disease in individuals who do not consume significant amounts of alcohol(12), the earliest stage is hepatic steatosis, which is characterized by the deposition of TG as lipid droplets in the cytoplasm of hepatocytes. Steatosis is defined as a hepatic TG level exceeding the 95th percentile for lean, healthy individuals (i.e., >55 mg per g of liver) or as the presence of cytoplasmic TG droplets in more than 5% of hepatocytes(11) Fasting in Ramadan

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has been shown to have some effects on the circulating levels of several biochemical markers known to be associated with vascular and metabolic disorders including lipid profile (1, 13, 14). Farshidfar et al reported a significant increase in high density lipoprotein - cholesterol (HDL-C) and a decrease in low density lipoprotein - cholesterol (LDL-C) at day 28 of Ramadan (15). Also, studies among patients with type II diabetes mellitus reported decreased total cholesterol (TC), triglyceride (TG), and LDL-C as well as increased HDL-C levels after fasting in Ramadan (16). It has been established that a given nutrient ingested at an unusual time can induce different metabolic effects (3). Lipid profile is affected by dietary habit, percentage of fat in the daily diet and its saturation, percentage of simple sugar, and exercise (17, 18) and the metabolic response to starvation has been well described and involves homeostatic mechanisms that result in energy expenditure being derived from the oxidation of lipids and proteins (19). Yet, studies on the effects of Ramadan fasting on blood lipids, blood pressure, anthropometric parameters and other cardiovascular risk factors are scarce, and have given inconclusive results (20). There is no study till now to survey the effect of Ramadan fasting on NAFLD patients so in current research, the effect of Ramadan fasting on NAFLD patients and its parameters has been investigated.

Materials and methods

This was a prospective observation that performed during the month of Ramadan in June/ July 2014 (Islamic year 1435) in city of Mashhad, Iran. The subjects were recruited from Ghaem teaching hospital clinic via poster advertisement or direct invitation. Inclusion criteria were male and female between 18 and 65 years old with NAFLD that diagnosed fatty liver by ultrasoundography and at least 10 hour fasting. Exclusion criteria were pregnant and lactating women, subjects with the age of below 18 and 65 y old and those who fasted for less than 10 d, subjects with any type of acute inflammation (hs-CRP >5), take medication that had influence on ALT and AST and exogenous insulin, Body Mass Index (BMI) <25 kg/m², any definite or suspected alcohol abuse, alcohol over-consumption (more than 30 gr/ day in men

and > 20 gr/day in women) or signs of alcohol as a cause of liver disease led to exclusion from study. In the diagnostic work-up, other causes of chronic liver disease (hepatitis B and C, and Wilson's disease) were ruled out. Additionally, patients with severe heart, lung, brain, or kidney diseases were excluded.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Research Ethics Committee of Mashhad University of Medical Sciences (approval number 930229). Written informed consent was obtained from all subjects.

Participants were interviewed private face to face in the clinic using a questionnaire that gathered information on demographic characteristics, medical history, medication use and health-related habits including questions on smoking, alcohol intake, and all subjects completed a 72 hour food recall questioner before and after Ramadan month.

Subjects attended the metabolic unit after 10–12 h fasting, in two stages; between 7 d before and 2 first days of starting Ramadan and from the 27th of Ramadan till 6 d after end of this month. An 8 mL fasting blood sample was collected from the median cubital vein. The blood samples were analyzed in an University affiliated laboratory for measuring ALT, AST, High density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Triglyceride (TG), cholesterol, Fasting Blood Sugar (FBS) and insulin.

Anthropometric parameters such as height, body weight and waist circumference were measured in both phases. Body mass index (BMI) was calculated as body weight (kg) divided by squared height in meters (m²). Height and weight were measured, using standardized procedures. Height was measured using a portable stadiometer (OTM, Tehran, Iran). Height measurement was taken to the nearest 0.1 cm, without shoes, with the subject stretching to the maximum height and the head positioned in the Frankfort plane. Weight was measured using Rassa weight scale (Rassa, Tehran, Iran) to the nearest 100 g with subjects removing shoes and in light clothing. Waist circumference was measured to the nearest 0.1 cm at the mid-point between the lower rib and

the upper margin of the iliac crest (21) in a horizontal plane by using a no stretching tape with an insertion buckle at one end. Fat free mass (FFM) and fat mass (FM) also measured with Bioelectrical Impedance Analysis(BIA).

Data analysis

The Kolmogorov-Smirnov test was performed to assess the normal distribution. Differences between results were analyzed, using paired samples t- test for normally distributed parameters and Wilcoxon Signed Ranks test for not normally distributed parameters. Relationship between dichotomous and quantitative variables was assessed by independent sample t test. Two-way repeated measures ANOVA were used to assess interactions among variables seem to be effective on parameter differences (P<0.15 in Pearson's correlation or independent sample t test) on variables before and after Ramadan. Quantitative data were expressed as the mean \pm SD for all parameters. Statistical significance was considered at P<0.05 for all tests. These statistical analyses were conducted by using SPSS statistical software (version 11.5, SPSS Inc.Chicago, IL).

Results

Duration of daily fasting during Ramadan 2014 was between 14 h and 42 min in first day and 13 h and 35 min in last day in Mashhad,

Iran. The average days subjects fasted in this study were 27.3 ± 5 (range 25–30 d). Fifty volunteers including 33 male and 17 female, age 18–65 y, average 40.52 ± 10.90 y, with non-alcoholic fatty liver were contributed in this study.

The anthropometric, body composition, blood pressure and macronutrients intake data of groups according to gender during Ramadan fasting were listed in table 1. As indicated there is a significant decrease in SBP and DBP in male patients during Ramadan (130.00(113.0-139.0) mmHg - 120.00(107.0-130.0) mmHg .P< 0.001 and 70.50(70.0-80.0) mmHg - 70.00(60.0-70.0) mmHg, P= 0.003 respectively). There is no significant change in body composition in two genders during Ramadan. In nutrients, total calorie intake was significantly decreased in two gender (123 ± 68.71 Kcal/d, 89 ± 83.02 Kcal/d, P<0.001 respectively) and total carbohydrate intake decreased in male patients alone (29.77 ± 6.28 gr/d, P<0.001).

Table 2 indicated the effect of Ramadan fasting on lipids profile and clinical parameters before and after Ramadan according to gender category. As shown in the table HDL increases 7.32 ± 6.38 mg/dl in female patient during Ramadan (P=0.04) while cholesterol and FBS were significantly increased in two gender (P<0.001). But TG had a significantly increased in male patients (138.00 (114.0- 196.5)-

Table 1. The effect of fasting on anthropometrics, body composition and dietary parameters before and after Ramadan according to gender category

Variable	Male N=33			N=17 Female		
	Before Intervention Mean \pm SD δ	After Intervention Mean \pm SD	p-value	Before Intervention Mean \pm SD	After Intervention Mean \pm SD	p-value
*BMI(kg/m ²)	29.50 (27.0-31.8)	29.00 (26.9-31.1)	0.07	34.15 (28.9-37.7)	33.40 (29.8-37.5)	0.09
WC	101.11 \pm 8.44	100.10 \pm 8.51	0.11	98.04 \pm 10.34	99.04 \pm 10.88	0.32
*SBP(mmHg)	130.00 (113.0-139.0)	120.00 (107.0-130.0)	<0.001	120.00 (101.1-122.5)	120.00 (111.0-130.0)	0.54
*DBP(mmHg)	70.50 (70.0-80.0)	70.00 (60.0-70.0)	0.003	78.00 (60.0-81.5)	80.00 (60.0-80.0)	0.75
Fat mass (%)	24.2 (21.9-28.2)	24.2 (21.5-29.5)	0.63	41.7 (36.0-45.3)	41.05 (35.6-44.5)	0.23
FFM (%)	65.20 \pm 9.60	64.54 \pm 9.95	0.10	48.06 \pm 6.65	49.39 \pm 6.33	0.12
Total calorie (kcal)	2279 \pm 389.33	2156 \pm 458.04	0.05	2076 \pm 320.07	1987 \pm 403.09	0.04
Carbohydrate(gr)	317.27 \pm 52.57	287.5 \pm 46.29	<0.001	292.82 \pm 64.60	284.29 \pm 65.85	0.53
Protein(gr)	62.75 \pm 15.22	59.43 \pm 15.76	0.23	54.58 \pm 9.93	54.20 \pm 18.66	0.92
*Fat(gr)	76.0 (65.5-92.5)	79.0 (66.0-89.0)	0.62	67.0 (58.5-78.0)	62.0 (53.5-83.0)	0.39

δ SD: Standard deviation

For data with normal distribution Paired Samples t- Test and Mean \pm SD were used

* For data with non-normal distribution, Wilcoxon signed ranks test and Median (IQR) were used

BMI: Body Mass Index, WC: Waist circumference, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, FFM: Fat Free Mass

Table 2. The effect of fasting on lipids profile and clinical parameters before and after Ramadan according to gender category

Variable	N=33 Male		p- value	N=17 Female		p- value
	Before Intervention Mean±SD δ	After Intervention Mean±SD		Before Intervention Mean±SD	After Intervention Mean±SD	
HDL(mg/dl)	42.72±8.11	45.27± 9.98	0.22	46.52±10.16	53.91±16.54	0.04
LDL(mg/dl)	119.06±33.80	121.61± 36.48	0.71	124.06±23.56	127.71±38.19	0.61
*TG(mg/dl)	138.00 (114.0-196.5)	190.00 (157.0-276.0)	<0.001	197.0 (119.5-244.5)	233.00 (152.0-301.0)	0.23
cholesterol(mg/dl)	190.60±47.43	220.72±59.96	0.001	199.95±45.51	229.1±47.87	0.001
*FBS(mg/dl)	85.5 (80.0-92.5)	133.56 (93.0-108.2)	<0.001	100.0 (87.5-102.5)	120.23 (102.2-121.0)	<0.001
*ALT(units/L)	18.00 (11.0-23.5)	13.00 (9.0-18.0)	<0.001	14.0 (10.0-32.0)	11.00 (9.0-17.5)	0.001
*AST(IU/L)	27.00 (20.0-31.5)	27.00 (18.5-34.5)	0.72	24.0 (16.0-33.5)	19.00 (16.5-32.5)	0.69
Insulin(mg/dl)	13.77±6.49	13.40±7.53	0.67	15.91±7.06	12.67±4.61	0.01

HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, TG: Triglyceride, FBS: Fasting Blood Sugar, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase
 δ SD: Standard deviation

For data with normal distribution Paired Samples t- Test and Mean \pm SD were used

* For data with non-normal distribution, Wilcoxon signed ranks test and Median (IQR) were used

190.00(157.0-276.0), $P<0.001$) and this rising was not significant in female ($P=0.23$). ALT significantly decreased during Ramadan in two groups while fasting insulin decreased 3.24 ± 2.45 mg/dl in female patients during Ramadan ($P<0.001$, $P=0.01$ respectively). There were no significant relation between LDL-c and AST during Ramadan month.

Discussion

During Ramadan, Muslims refrain from eating, drinking, smoking and sexual relations from sunrise (Sahur) until sunset (Iftar). They are allowed to eat during the remaining hours. It has been established that a given nutrient ingested at an unusual time can induce different metabolic effects (3). This study shows the effect of Ramadan fasting on NAFLD parameters, to our knowledge, there was no similar study among NAFLD patients in this regard. Most of the studies evaluated changes of some clinical, biological and anthropometric factors among metabolic syndrome, diabetic, hyperlipidemia patients, cardiovascular patients and or healthy adults (20, 22-24). In comparing findings with other studies that conducted on different target population. In our study there was a significant increasing in total cholesterol, HDL and TG and decreasing in SBP and DBP after Ramadan Fasting in NAFLD patients, in some studies also an increase in plasma cholesterol ,TG and decreasing in SBP was shown but in healthy population(20, 25, 26), Some studies reported that at the initial stages of weight loss there was

an increase in blood TG level, which may have been due to mobilization of body fat(27, 28).Elevated blood TG levels observed may be due to the consumption of high-carbohydrate diets accompanied by less exercise during this month and there is a tendency for higher sugar consumption during Ramadan(29, 30). Bugianesi et al stated that steatosis can result from an increased delivery of circulating lipids to the liver due to a reduced antilipolytic effect of insulin in adipose tissue. Within the liver, part of this non-esterified fatty acids excess is oxidized and the remainder is re-esterified to be exported into the circulation as triglyceride-rich lipoproteins (12), furthermore, the increased lipid flux that occurs during the fasting state results not only from insulin resistance, but also from "accelerated starvation", with the early recruitment of alternative fuels for energy needs and for gluconeogenesis (as the glycogen stores of the cirrhotic liver are significantly reduced)(31).

In one study on suncus indicated that plasma levels of free fatty acids increase as a result of fasting and the ability to secrete VLDL is also impaired, causing increased amounts of triglyceride in the liver. In suncus, however, a very short period of starvation, e.g., 8 h, induced a striking accumulation of triglyceride in the liver, suggesting that there is an unusual mechanism unique to these animals and also plasma lipid levels were very low and decreased significantly after 1-day fasting, suggesting that the transporting mechanism of

the lipids from liver may be impaired(32) but in our study that for the first time investigating the effect of fasting on the NAFLD lipid profile, shows that the serum TG and cholesterol were increased but in present study the liver TG content was not measured and because the fat dietary intake in these patients in pre and post Ramadan fasting didn't had a significant difference so can concluded the mechanism for transporting lipids from the liver may have been improved.

The evidence that a marked increase in plasma HDL-C occurs after Ramadan fasting is promising (26, 33, 34). There was a striking non pharmacologic improvement in plasma HDL-C in our study, which were most probably induced by eating one large evening meal a day. In the present study HDL-c increased in females, body weight did not change and no alcohol was consumed. Furthermore, we find an increase in plasma HDL than that published by others, even when hypolipidemic drug therapy was a factor (35) while parameters such as exercise, body weight, smoking, moderate alcohol intake, and hypolipidemic drugs, do not apply to our study.

One of the other main finding in present study was the decreasing fasting insulin and increasing FBS in NAFLD patients during Ramadan month, this finding is in contrast with the M'guil et al results that investigating the effect of Ramadan fasting on type 2 diabetes patients(36). A significant decrease in insulin and insulin resistance among men patients with type II diabetes after Ramadan fasting was reported by Yarahmadi et al(37). In a study by Nematy et al shown that there is no difference in insulin and FBS after Ramadan fasting(20). The diurnal insulin hypo secretion observed during fasting would also favor a predominant lipolytic state. Such an assertion is sustained by data concerning both the respiratory quotient and nutrient oxidation rates throughout the circadian cycle.(38)

In the present investigation, carbohydrate consumption decreased during Ramadan, which may have resulted in decreased carbohydrate oxidation and increased fat oxidation. However, due to the fact that we did not measure the respiratory exchange ratio, we cannot know this with certainty. Despite a decrease in carbohydrate intake during Ramadan, blood glucose increased, which may be due to an

increase in gluconeogenesis but other investigations have reported a similar lack of effect of Ramadan fasting on blood glucose(2, 39).

The ALT enzyme decreases significantly after Ramadan in the present study that was along with Unalacak et al findings(40) and in other studies there is no significant change in ALT or AST enzymes(41, 42), change in life style and losing at least 5% of body weight have a significant improvement on ALT enzyme in NAFLAD patients (43) in our study decreasing in energy and carbohydrate intake and insulin can lead to a change in liver function of NAFLD patients.

Limitation

There was no control group in this study. Pre Ramadan sampling in Ramadan was performed in the morning after an overnight fasting, while post Ramadan blood sampling was performed in the afternoon, after at least 10 h fasting during day time. This difference in the time of blood sampling was inevitable to have at least 10 h fasting. As the circadian rhythms of nutrition-related biological variables shows some degree of changes during Ramadan (44), there might be concerns about amount of daily activities or circadian rhythm of hormones like cortisol which might affect FBS levels. In the present study, we had only two measurements during the study, possibly evaluation in the middle of the month and after a time after Ramadan would reveal more details and shows us the trend of changes. Level of physical activity of the study population was not measured in this study.

For the better understanding of change and improvement in liver function, Ultrasound images and liver fat content was needed.

Conclusion

This study for the first time shows significant effects on NAFLD patient parameters during Ramadan fasting such as decreasing in insulin, ALT enzyme, SBP and DBP and increase in HDL-c after an average of 27 d fasting, Result from this study suggested that Ramadan fasting may be useful to improve NAFLD, but further studies are needed in this area.

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