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Original paper

Impact of intermittent fasting on laboratory, radiological, and anthropometric parameters in NAFLD patients

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Abstract

Aim of the study: Despite the ample flow of non-alcoholic fatty liver disease (NAFLD) drugs in the pipeline, lifestyle modifications are still the optimal solution of NAFLD. The aim of the study was to assess short term effects of Ramadan fasting (RF) as a sort of intermittent fasting (IF) on biochemical, radiological, and anthropometric parameters of NAFLD patients.

Material and methods: Ninety-eight NAFLD patients were recruited and voluntarily subjected to 16 hours daily fasting for an average of 22-29 days, without special dietary recommendations. Anthropometric, laboratory and radiological parameters were measured before, at 30 days, and one month after fasting (fasting and non-fasting phases).

Results: Patients were mostly rural (76%), hypertensive (34.7%), diabetic (43.9%), and female (76.8%), with overt criteria of metabolic syndrome (67.3%). Liver transaminases (ALT and AST) were ameliorated significantly after fasting ($p \leqslant 0.01$), which continued in the following month ($p \leqslant 0.01$) especially in those with elevated ALT before fasting (46%). Eleven patients (24.4%) experienced ALT normalization after one month of fasting, which was further increased to 15 (33.3%) one month later. Lipid profiles (cholesterol, triglycerides, HDL, LDL, cholesterol/HDL risk ratio) were significantly corrected following IF ($p \leqslant 0.01$) and continuing in the next phase ($p \leqslant 0.010$). Body mass index (BMI) lessened following the fasting ($p \leqslant 0.01$), while no remarkable changes were noted regarding waist, hip, and triceps skin fold thickness ($p \leqslant 0.01$). Glycemic indices (HbA_{1c}, postprandial, HOMA-IR) and fibrosis markers (FIB-4 and APRI) were significantly ameliorated ($p \leqslant 0.01$), while reduction in inflammatory markers was not long lasting ($p \leqslant 0.01$).

Conclusions: Intermittent fasting led to momentous improvements in ultrasonographic, biochemical, and anthropometric parameters of NAFLD especially in early phases and prediabetics.

Key words: nonalcoholic fatty liver disease, intermittent fasting, metabolic syndrome.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) includes a wide spectrum of liver disease, ranging from simple steatosis to steatohepatitis, advanced fibrosis and liver cirrhosis, with histological features of alcohol induced liver disease [1]. Despite the high morbidity and mortality burden of NAFLD, no definite treatment is established yet [2]. Lifestyle modifications are one of the most valued measures for NAFLD amelioration. A lot of dietary regimens have been recommended with proved weight loss, inflammation reduction, along with

improved cardiovascular and metabolic markers [3]. Many regimens have been suggested for NAFLD patients: caloric restriction, low carbohydrate high fat diet, or intermittent fasting diets (IF) [4]. Notably, in IF people are allowed to eat for a restricted time and abstain food and caloric fluids for another time. Remarkably, IF is capable of reversal of insulin resistance, leading to marvelous achievements in most resistant cases. Accordingly, IF is one of the most promising regimens suggested for improving the unhealthy NAFLD parameters [4]. Being an Islamic pillar, Ramadan fasting (RF) might also be considered as a form of dry intermittent fasting ([5] lessan). Despite the reported deleterious impacts of fasting on liver cirrhosis, fruitfully IF had been suggested to improve NAFLD patients [6].

Therefore, this study was designed to assess short term effects (one month) of intermittent fasting on various biochemical, anthropometric, and radiological measurements of NAFLD patients.

Material and methods

This interventional multicenter study was conducted on patients recruited from outpatient clinics of the National Liver Institute, Menoufia University, Talkha Central Hospital and the Society for Care of Liver Disease Patients in Mansoura. Recruitment of cases was initiated at the month of Ramadan in May/June 2017 (Islamic year 1438). 98 cases with NAFLD were included, diagnosed relying on ultrasonographic features of NAFLD. Patients were voluntarily subjected to about 16 hours day of fasting with an average of 22-29 days without any special dietary recommendations. Baseline anthropometric, clinical, laboratory, and radiological data were assembled before patients were subjected to RF, at the end of Ramadan and one month after the end of Ramadan.

Inclusion criteria: adult patients between 18 and 70 years old, planning for completing RF, with ultrasonographic evidence of NAFLD whatever the degree done by a single professional ultrasonographer [7].

Exclusion criteria: 1. Patients who did not complete RF, 2. Patients with medical conditions in which fasting is contraindicated (e.g.: renal impairment, recurrent hypoglycemia or stroke), 3. Active alcohol consumption, 4. Patients with recent history of hepatotoxic drug intake or medications known to alter the measured parameters apart from oral hypoglycemic for diabetics (e.g. statins), 5. Patients with a history of chronic liver disease other than NAFLD.

Informed written consent and approval from the ethical committee in the National Liver Institute, Menoufia University were prerequisites before the study commencement.

Accordingly, 98 NAFLD patients aged 18-70 years had undergone voluntary Islamic intermittent fasting with about 16 hours of daily fasting for 22-29 days. Anthropometric, laboratory, and radiological parameters were measured before and after 30 days of intermittent fasting (fasting phase). The same parameters were re-assessed before and after 30 days of non-fasting (non-fasting phase). Subjects were screened 2 days before, at the end of Ramadan, and one month later. At each visit all patients were subjected to the following:

1. Laboratory measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), serum albumin, complete blood count (CBC), C-reactive protein (CRP), serum uric acid, thyroid stimulating hormone (TSH). High density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG), cholesterol, fasting blood sugar (FBS), postprandial blood sugar (PPBS) and HbA_{1c} and fasting insulin level.
2. Abdominal ultrasound, diagnosis of NAFLD patients was based on abdominal ultrasound which was performed before and after 30 days of fasting and after 30 days by the same radiologist who was unaware of the subjects' medical histories and laboratory findings.
3. Anthropometric parameters such as weight, height, waist circumference, hip circumference and triceps skin fold. Body mass index (BMI). Waist/hip ratio (WHR) calculated as 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 in a horizontal plane using a non-stretching tape with an insertion buckle at one end. Hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor, triceps skin fold (TSF) calculated at the level of the mid-point between the acromial and the radial process, on the mid-line of the posterior surface of the relaxed arm with the palm of the hand supinated. A vertical pinch, parallel to the long axis of the arm, is made at the landmark.
4. Bioelectrical impedance analysis (BIA): It is a commonly used method for estimating body composition, in particular body fat, muscle mass and total body water (TBW). It was available to be performed for a nested group of 61 patients of the 98 patients.
5. HOMA-IR calculation was done by the equation: fasting insulin × fasting glucose/405). Multiple fibrosis scores were calculated.

Scores evaluating fibrosis in NAFLD cases:

NAFLD fibrosis score – it is a non-invasive scoring system based on several laboratory tests that help to estimate the amount of scarring in the liver in cases of NAFLD [8].

NFS = –1.675 + 0.037 × age (years) + 0.094 BMI (kg/m^2) + 1.13 × impaired glucose tolerance/diabetes (yes = 1, no = 0) + 0.99 AST/ALT ratio – 0.013 × platelets (× 10^9/l) – 0.66 × albumin (g/dl)

AST to platelet ratio index (APRI) [9]:

$$\text{APRI} = \frac{\text{upper normal value (IU/l)}}{\text{platelet count } (\times 10^9/\text{l})} \times 100$$

Fibrosis-4 (FIB-4) [10]:

$$\text{FIB-4 score} = \frac{\text{age} \times \text{AST (IU/l)}}{\text{platelet count } (\times 10^9/\text{l})} \times \sqrt{\text{ALT (IU/l)}}$$

Statistical analysis

Data were collected, tabulated, and statistically analyzed using an IBM personal computer with SPSS version 20 where the following statistics were applied: Descriptive statistics, in which quantitative data were presented in the form of mean (*X*), standard deviation (SD), range, and qualitative data were presented in the form of numbers and percentages. Analytical statistics includes paired samples *t*-test for normally distributed parameter variables before and after Ramadan and the Wilcoxon signed ranks test for non-normally distributed parameters. The relationship between quantitative variables was assessed by the paired sample *t* test and Pearson's correlation for quantitative data. Statistical significance was considered at $p < 0.05$ for all tests.

Table 1. Effect of Ramadan fasting (RF) on anthropometric measures

Anthropometric measures	Before Mean ±SD	After Mean ±SD	*P* value	After 1 month Mean ±SD	*P* value
Weight	97.44 ±16.41	96.69 ±16.22	⩽ 0.01	96.41 ±16.22	0.545
BMI	37.03 ±6.56	36.74 ±6.46	⩽ 0.01	36.65 ±6.45	0.778
Waist	114.53 ±12.08	114.34 ±11.96	0.655	114.27 ±11.94	0.889
Hip	116.95 ±11.5	116.67 ±11.47	0.755	116.54 ±11.41	0.852
Waist/hip ratio	0.98 ±0.06	0.97 ±0.07	0.682	0.97 ±0.06	0.885
Triceps skin fold	2.45 ±0.77	2.42 ±0.77	0.623	2.42 ±0.76	0.945

BMI – body mass index

Table 2. Effect of Ramadan fasting (RF) on metabolic and biochemical parameters

Improved Parameters	Before Mean ±SD	After Mean ±SD	*P* value	After 1 month Mean ±SD	*P* value
FBG	130.19 ±64.13	121.47 ±51.26	⩽ 0.01	120.45 ±50.25	0.078
Insulin (fasting)	7.5 ±5.2	7.3 ±4.9	⩽ 0.01	7.3 ±4.9	0.341
PPBG	183.79 ±96.27	168.14 ±70.73	⩽ 0.01	166.61 ±69.77	⩽ 0.01
HbA_{1c}	6.1 ±1.1	5.9 ±1	⩽ 0.01	5.8 ±1	⩽ 0.01
HOMA-IR	2.13 ±1.45	1.96 ±1.19	⩽ 0.01	1.95 ±1.2	0.222
ALT	36.5 ±20.2	32.7 ±16.6	⩽ 0.01	30.7 ±14.6	⩽ 0.01
AST	38.5 ±19.6	32.8 ±13.6	⩽ 0.01	31 ±12.1	⩽ 0.01
ALP	167.5 ±64.4	160.9 ±52.5	⩽ 0.01	161.1 ±49.6	⩽ 0.01
GGT	41.1 ±36.3	37.1 ±22	0.047	36.1 ±20.3	⩽ 0.01
Cholesterol	241.1 ±60.99	218.48 ±52.2	⩽ 0.01	210.63 ±44.3	⩽ 0.01
Triglyceride	171.01 ±90.23	157.49 ±79.08	⩽ 0.01	152.6 ±74.87	⩽ 0.01
HDL	45.3 ±7.4	48.5 ±7.5	⩽ 0.01	49.6 ±7.2	⩽ 0.01
LDL	162 ±60	150.9 ±49.4	⩽ 0.01	147 ±45.9	⩽ 0.01
CRP	7.84 ±6.86	6.31 ±6.41	⩽ 0.01	8.63 ±8.53	0.323

FBG – fasting blood glucose, PPBG – postprandial blood glucose, HOMA-IR – Homeostatic Model Assessment of Insulin Resistance, ALT – alanine transaminase, AST – aspartate transaminase, ALP – alkaline phosphatase GGT – γ-glutamyl transferase, HDL – high-density lipoprotein, LDL – low-density lipoprotein, VLDL – very low-density lipoprotein

Table 3. Effect of Ramadan fasting (RF) on non-alcoholic fatty liver disease (NAFLD) steatosis grades

Grades of steatosis	Before fasting		After fasting		*P* value	After 1 month		*P* value
	n	%	*n*	%		*n*	%	
Grade 1	59	60.2	63	64.3	⩽ 0.01	63	64.3	*p*1 = 1.0
Grade 2	39	39.8	35	35.7		35	35.7	
Grade 3	0	0	0	0		0	0	

Table 4. Effect of Ramadan fasting (RF) on fibrosis associating non-alcoholic fatty liver disease (NAFLD)

NAFLD fibrosis stage	Before (*n*)	After (*n*)	*P* value	After 1 month (*n*)	*P* value
F0-F2	5	6	0.317	6	1.0
F3-F4	93	92		92	
FIB-4	1.48 ±0.69	1.33 ±0.57	⩽ 0.01	1.28 ±0.54	⩽ 0.01
APRI	0.61 ±0.33	0.52 ±0.23	⩽ 0.01	0.49 ±0.22	⩽ 0.01

Table 5. Comparison of the data of the 4 improved patients to those who did not improve

Parameter		Mean	SD	*T*	*P* value	CI Lower	CI Upper
BMI	No response	37.19	6.649				
	Improved steatosis	33.27	1.12	4.41	⩽ 0.01	2.04	5.78
Waist	No response	114.87	12.19				
	Improved steatosis	106.5	4.43	3.28	⩽ 0.01	1.9	14.84
Hip	No response	117.13	11.7				
	Improved steatosis	112.5	1.0	3.55	⩽ 0.01	2.03	7.24
BSF	No response	0.45	0.12				
	Improved steatosis	0.32	0.05	4.62	⩽ 0.01	0.05	0.2
ALT	No response	36.92	20.51				
	Improved steatosis	27.52	3.69	3.34	⩽ 0.01	3.41	15.38
AST	No response	38.8	19.97				
	Improved steatosis	30.75	3.45	2.99	⩽ 0.01	2.37	13.73
APRI	No response	0.61	0.336				
	Improved steatosis	0.5	0.068	2.4	⩽ 0.01	0.01	0.22

BMI – body mass index, BSF – biceps skin fold, ALT – alanine transaminase, AST – aspartate transaminase

Results

Demographically, this cohort comprised mainly women aged over forty (78.6%), with about 87% of them suffering from obesity, and 77.6% living in rural areas. Only weight and BMI showed a statistically significant decrease after fasting, while none of the other anthropometric measures showed a statistically significant difference after fasting compared to before fasting, or even after one month (Table 1).

There was a significant improvement in insulin resistance (HOMA-IR) after RF and this improvement was sustained for another one month. All glycemic parameters were significantly decreased after fasting. Postprandial blood glucose (PPBG) and HbA_{1c} further decreased one month later. In contrast, for fasting blood glucose (FBG), insulin and HOMA-IR the reductions after cessation of fasting were insignificant (Table 2). 10/27 (37%) glycemic prediabetic participants turned into normoglycemic participants after fasting and the number was still nearly the same after 1 month (Table 2). Before fasting we had 39 cases of grade 2 steatosis, and by the end of Ramadan we had only 35 patients as 4 cases turned into grade 1, and this achievement was maintained following the non-fasting month (Table 3).

Regarding fibrosis grade on NAFLD fibrosis score, only 5 cases were between F0 and F2 and 93 cases were between F3 and F4. After RF one case turned from F3/F4

to F1/F2 with persistence for another non-fasting month (Table 4). The fibrosis scores (FIB-4 and APRI) showed significant decline on RF and another consecutive non-fasting month (Table 4).

In the studied group, four cases had a significant improvement in hepatic steatosis by ultrasonography from grade 2 to grade 1 with statistically significant improvement in BMI, waist, hip, biceps skin fold (BSF), AST, ALT and APRI (Table 5).

Discussion

Ramadan fasting, in which Muslims fast from dawn to sunset, is one of the five Pillars of Islam. Physicians and religious scholars were getting closer in their approach to advise patients based on objective risk assessment [11].

Marvelous improvements have been reported in metabolic markers following RF [11].

It is established that changes in lifestyle and losing at least 5% of body weight lead to a significant improvement in ALT enzyme in NAFLD patients [12]. Regarding liver transaminases in the current study, both were significantly lessened following RF with continued amelioration one month later. This significant decrease after RF was in accordance with Unalacak *et al*., who confirmed the positive impact of RF on liver enzymes in NAFLD patients [13]. Dissimilar were the results of Furuncuoglu *et al*. and El-Mitwalli *et al*., who denied any significant changes in liver transaminases following RF [14, 15]. These discrepant results might be due to different cohort sizes, or timing of analysis.

Improvement of insulin sensitivity as reflected by change of HOMA index and the amelioration of lipid profile or by mild loss of weight secondary to the refined dietary adjustment done by our cohort might be the factors responsible for the fruitful outcomes in the pattern of liver enzymes as well as dampening of inflammation as mirrored by decline of CRP [16].

C-reactive protein in our study was significantly decreased after RF ($p \leq 0.015$). Unfruitfully, after fasting, these levels were raised again. Similar were the results of Adawi *et al*., who found that after a month of intermittent fasting, CRP levels were significantly decreased [17]. These results might enhance the concept of RF immunological modulations of RF modulating the anti-inflammatory responses. Suggestions might add a role of RF in amelioration of immunologically based inflammatory disorders. However, more targeted studies are still required.

In relation to lipid profile in the current study, it was demonstrated that there was a marvelous amelioration in all parameters of lipid profile before, after fasting, and one month later. These results were in line with the previous studies; Adlouni *et al*. observed a marked HDL cholesterol increase during Ramadan [18]. The increase of HDL cholesterol was due to loss of body weight and change of meal frequency. Moreover, it is also reported that eating one large quantity daily meal leads to a significant increase in HDL cholesterol [19]. According to Adlouni *et al*., their study showed that the total cholesterol and LDL cholesterol are significantly decreased during Ramadan and these findings showed that LDL is an atherogenic lipoprotein [18]. The low concentration of both indicators contributed to low frequency of ischemic heart disease. It also showed that a significant reduction in LDL occurred regardless of the tendency of fried food consumption to increase during Ramadan compared to before Ramadan [18]. On the other hand, Alsubheen *et al*. reported that the HDL and LDL levels were significantly increased, with no significant changes in the levels of total cholesterol and TGs during fasting in the detraining period group [20].

There was a statistically significant decline in anthropometric measurements in the current study: both BMI and body weight.

Pallayova *et al*. reported that the changes in anthropometric measurements were not significant, but physical activities increased significantly after Ramadan [21]. In relation to glycemia-related parameters, postprandial blood glucose and HbA_{1c} showed a significant decrease after fasting, with a further decrease one month later. On the other hand, FBG, insulin (fasting) and HOMA-IR had considerably decreased after cessation of fasting. Fasting had substantially impacted prediabetics ($p = 0.015$), 10/27 (37%), turning them to normoglycemics, continuing for the next month. Also, a significant improvement in insulin resistance (HOMA-IR) after RF ($p = 0.03$) was reported.

Ramadan fasting has various biochemical and physiological effects on body weight, glucose, and lipid profile. In the study of Alsubheen no changes were observed in the lipid, anthropometric, and body composition profiles on fasting. However, increased FBG levels after Ramadan were observed in the women. The authors inferred that to improve the favorable biochemical parameters after Ramadan, lifestyle modifications such as increased intake of healthy diets and increased physical activity should be adopted [20]. Furthermore, Kiyani *et al*. reported minimal weight reduction following fasting [22]. Nevertheless, Khan *et al*. observed significant reductions in glucose level, total cholesterol, low-density lipoprotein cholesterol (LDL), and triglycerides (TGs), but also some reduction in high-density lipoprotein cholesterol (HDL).

Also, BMI, body adiposity index, and visceral adiposity index were reduced following RF [23].

Notably, energy intake distribution and macronutrients were excessively studied with no significant effects of RF [24, 25].

In our study, classifying patients according to grade of fibrosis on NAFLD fibrosis score, only 5 patients were between F0 and F2, while 93 were between F3 and F4. Following RF one case turned from F3/F4 to F1/F2, which persisted in the next month. On the other hand, both fibrosis scores (FIB-4 and APRI) were significantly decreased after RF, with sustainable reductions in the next month (both p values are $p \leq 0.010$).

In the current study, there were significant changes in steatosis ultrasonographic grades in the fasting phase ($p = 0.04$), as 4 cases of steatosis grade 2 turned to grade 1. Analysis of the data relevant to those four cases revealed a consistent improvement in BMI, waist, hip, BSF, ALT, AST and APRI score. Similar were the results of Ebrahimi *et al.*, who noted remarkable improvement in grades of steatosis following RF [26].

Systemic blood pressure (SBP) monitoring before, during, and after RF proved to be not affected, compatible with the results in our study; neither systolic nor diastolic pressures were affected by RF. Also, no significant differences were seen in the trends of systolic and diastolic BP between the hypertensive and non-hypertensive groups during and after RF [25]. In the study of Ebrahimi *et al.*, significant improvements in SBP during fasting were reported, with increasing levels on Iftar [26]. These results might be explained by the undefined quality and quantities of food taken after long fating. Most Muslims tend to have high carbohydrate, fatty, and salty diets. Modified diet regimens might be capable of amending these defects.

Evaluation of kidney function tests in our study revealed significant rises of serum creatinine and uric acid in the fasting phase; however, these increases are still within normal ranges. Similarly, Kara *et al*. reported an increase in serum creatinine levels by 0.3 mg/dl (26.5 μmol/l) from baseline during or within 3 months after Ramadan. The increased metabolic rate with more energy production might be responsible for these increases in metabolites affecting the kidneys [27].

In conclusion, RF with its extraordinary ameliorations in most vital body functions surpassed its role as an Islamic devotion to be honorably placed as a disease modulator in patients with metabolic disorders such as NAFLD. Adopting the regimen of RF for longer periods over the year as a lifestyle modification might hasten the betterment process. Also, adding diet modifications along with exercises would guarantee remarkable nonpharmacologic accomplishments in NAFLD control.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the National Liver Institute, Menoufia University no 20-0122.

Consent to participate and publish

Written informed consent was obtained from the parents.

Disclosure

The authors declare no conflict of interest.

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