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The efficacy of DASH combined with time-restricted feeding (16/8) on metabolic associated fatty liver disease management: a randomized controlled trial

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Recent studies have utilized time-restricted feeding (16/8) (TRF) and dietary approaches to stop hypertension separately to manage metabolic-associated fatty liver disease (MAFLD); however, the effectiveness of combining these two approaches has not been investigated. The objective of this study was to examine the impact of TRF in conjunction with a DASH diet on various factors related to MAFLD. A 12-week randomized controlled trial was conducted to assess the impact of TRF (16/8), along with a DASH diet, compared with a control diet based on standard meal distribution, in patients with MAFLD. An investigation was conducted to examine alterations in anthropometric indices, as well as liver parameters, serum metabolic indices, and an inflammatory marker. The TRF plus DASH diet reduced body mass index ($p = 0.03$), abdominal circumference ($p = 0.005$), controlled attenuation parameter (CAP) ($p < 0.001$), alanine aminotransferase ($p = 0.039$), and aspartate aminotransferase (0.047) compared to the control group. The levels of insulin and homeostasis model assessment of insulin resistance reduced in both groups significantly ($P < 0.05$). In MAFLD patients, TRF (16/8) in combination with a DASH diet is superior to a low-calorie diet in promoting obesity indices, and hepatic steatosis and fibrosis. Further long-term investigations are needed to draw definitive conclusions.

Keywords DASH, TRF, Diet, MAFLD, NAFLD

Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMI	body mass index, CAP, controlled attenuation parameter
DASH	dietary approach to stop hypertension
FBS	fasting blood sugar
GGT	γ-glutamyl transferase
HDL-C	high-density lipoprotein cholesterol
HOMA-IR	homeostasis model assessment of insulin resistance
hs-CRP	high-sensitive C-reactive protein
LDL-C	low-density lipoprotein cholesterol
MAFLD	metabolic associated fatty liver disease, NAFLD, non-alcoholic fatty liver disease

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QUICKI	quantitative insulin sensitivity check index
TC	total cholesterol
TG	triglycerides
TRF	time restricted feeding

Metabolic associated fatty liver disease (MAFLD) is a frequent and worldwide concern with a global prevalence of 6–35%[1]. MAFLD is the predominant etiology of chronic liver disease, characterized by the accumulation of lipid droplets in over 5% of hepatocytes, in the absence of substantial alcohol use[2–4]. Environmental factors, genetic, sex and hormonal status should be considered as crucial factors in the developing of MAFLD[5,6]; nevertheless, the most important cause of death in patients with MAFLD is cardiovascular diseases[7] and particularly, it has been seen that people with MAFLD are more likely to develop type 2 diabetes mellitus[8].The accumulation of triglycerides causes oxidative stress, protein misfolding and mitochondrial damage which ultimately leads to chronic inflammatory response confirmed by the presence of high sensitivity c-reactive protein (hs-CRP) have been recognized as pathogenic factors that contribute to MAFLD[9]. Dietary interventions are known as the most effective approach for management of MAFLD due to the absence of any approved pharmacological therapy[10–12].

Intermittent fasting is one of the dietary patterns of calorie restriction which comprises specific periods of fasting[13]. During fasting, the level of ketone bodies in the blood is raised to produce energy from triglycerides, which has beneficial metabolic effects such as improving glucose regulation, blood pressure and reducing inflammation, independent of its effects on weight loss[14–16]. One type of intermittent fasting that offers time-limited possibilities for food intake throughout the day is called time-restricted feeding (TRF). The 16:8 model is the most widely used kind of TRF. It consists of an 8-hour feeding window followed by a 16-hour fasting window[17,18]. The beneficial effects of Intermittent fasting on different aspects of MAFLD have been reported previously[19,20].

Dietary approaches to stop hypertension (DASH), is a pattern of eating that is low in sodium, high in calories, low in glycemic index, high in phytoestrogens, magnesium, potassium, and dietary fiber[21]. The DASH has less saturated fat, sodium, and sweetened beverages and is high in fruits, vegetables, low-fat or fat-free dairy products, seafood, nuts, and legumes[22]. Moreover, it has been shown to have beneficial effects on weight, body mass index, liver enzymes, insulin metabolism markers, serum triglycerides, very low-density lipoprotein (VLDL), and hepatic steatosis in overweight and obese patients with MAFLD[23–25].

Given the rising prevalence of MAFLD around the world and its strong association with other metabolic disorders, such as diabetes, and cardiovascular disease, and due to the lack of randomized clinical studies examining the impact of DASH with TRF on individuals with MAFLD, we designed this study to evaluate the effects of co-administration of DASH and TRF on hepatic parameters, glucose homeostasis, lipid profile and inflammation in patients with MAFLD. It was hypothesized that combining DASH with TRF diet would be an effective strategy in management of MAFLD.

Methods and materials

This randomized controlled trial (RCT) was conducted on patients with MAFLD at the Research Institute for Gastroenterology and Liver Diseases, Tehran, Iran. Inclusion criteria were defined as: age over 18 years, grade 2 MAFLD according to controlled attenuation parameter (CAP) score level of ≥ 260 dB/m, willingness to take part in this study, not having taken medicines such as herbal medicines, anti-inflammatory medications, corticosteroids/any hormone, weight-loss medications, or hepatotoxic medications like phenytoin, amoxicillin, and lithium for >1 weak before enrolling. In addition, patients should not be diagnosed with uncontrolled cardiovascular diseases, stroke, diabetes, acute liver disorders (hepatitis B, C, etc.), kidney diseases, chronic inflammatory disease, depression, cancer, or autoimmune disease, no history of weight loss surgery or weight loss programs in the last 3 months. Participants who cosumed alcohol were not[26] allowed to participate in this study. Only those who met al.l inclusion requirements and were willing to complete the 12 weeks of TRF (16/8) combination with a DASH diet, were included in the study.

Exclusion criteria included current pregnancy or breastfeeding, use of birth control pills during the study, use of drugs that can influence metabolism or liver function (e.g., glucose-lowering agents, blood pressure drugs, and lipid-controlling drugs (, unwillingness to continue the project, and aggravation of the diseases which lead to hospitalization.

Prior to research recruitment, all participants signed a written informed consent form. Registering of the trial was done at www.irct.ir, on 22/07/2023 (IRCT20100524004010N39). The Ethics Committee of the Faculty of Nutrition and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.NNFTRI.REC.1399.019) approved the current trial. The study was conducted according to consort guideline.

Study design and intervention

This study was conducted as a randomized clinical trial. Patients who were diagnosed with MAFLD were stratified into groups by BMI and age. They were then randomly assigned to either the TRF (16/8) combined with the DASH group ($n=27$) or the non-intervention control group ($n=26$) using a computer-generated random-numbers approach. The primary outcomes of the study were liver biomarkers, including enzyme and imaging tests, due to the particular nature of MAFLD. The secondary outcomes included lipid, glycemic, and inflammatory indicators, as well as body composition.

Sample sizes

Various dependent variables were used to determine how many sample sizes would be needed for this study. For the variable that was reliant on the CAP score, the greatest number of samples was obtained. The number of samples that should be chosen to ensure that the average CAP score difference between the intervention

group and the control group is at least 27 dB/m. This difference was mentioned as statistically significant when it reached a power of 80% (β = 20%) and probability of 95% (α = 0.05). It was predicted that the sample size for each group would include 21 patients. Using the following formulas and the deviation from the criterion found in a prior study[27], the sample size was determined.

$$n = \frac{2S^2(Z_{1-\alpha/2} + Z_{1-\beta})^2}{(\mu_1 - \mu_2)^2} = 21$$

Dietary interventions

For a period of 12 weeks, the intervention group was assigned to follow the TRF 16/8 (refraining from eating for 16 h and being allowed to eat for 8 h every day). TRF was chosen over other IF regimens, like alternate-day fasting and Ramadan-style fasting since it might be more practical and convenient for participants. Participants in this group were asked to follow a DASH diet in addition to the TRF program. The DASH diet was low in processed and red meats, and sugar-sweetened beverages, and high in fruits, vegetables, low-fat dairy, and a range of protein sources like fish, chicken, legumes, and nuts[22].

The control group was advised to adhere to weight loss diets, which were assessed by a certified dietitian before the commencement of the trial. Nevertheless, this group was also obligated to comply with the calorie intake, macronutrient composition, and consumption of fruits and vegetables as outlined below.

The caloric intake for patients was determined using the Mifflin-St Jeor equation [28] . The recommended macronutrient distribution was set at 30% of total calories from fat, 18% from protein, and 52% from carbohydrates for both groups in addition to consuming 7–8 servings of fruits and vegetables. Total recommended dietary energy was 500 kcal less than maintenance needed energy per day.

The intervention and control groups were permitted to consume non-caloric fluids such as water, coffee, or tea. The patients were instructed to drink water without any limitations during the study. Therefore, these calorie-free liquids were permitted during both fasting and feeding phases. Each patient was assigned a diet plan for 3 months. During the initial phase of this study, all participants received detailed instructions on how to strictly follow the prescribed diet. Throughout the study, their adherence to the diet was closely monitored by weekly phone calls and monthly interviews, which involved recalling their dietary intake over 24 h on three separate days consisting of two weekdays and one holiday. The participants who failed to comply with the prescribed diet were excluded from the study.

Measurements

Body weight, height, abdominal circumference (AC), and body composition were measured for all participants at the start and the end of the trial. Additionally, fasting blood samples were collected in the morning following a 10 to 12-hour period of abstaining from food. In addition, the degree of steatosis and hepatic fibrosis was assessed using the FibroScan® 502 Touch equipment (Echosens, Paris, France) at the beginning and end of the 12-week trial. In addition, the researchers evaluated physical activity levels by utilizing the metabolic equivalent of the task (MET) questionnaire[29] and 24-hour recalls (one weekend day and two workdays)[30,31] at baseline and end of the study. Furthermore, patients also completed demographic questionnaires. The dietary energy and macronutrient composition were analyzed using Nutritionist IV software, developed by (First Databank Inc., Hearst Corp., San Bruno, CA, USA),

Anthropometric measurements and body composition

Body weight was assessed with precision to the nearest 0.1 kg using a digital scale (Seca 808, Germany) that has an accuracy of ± 0.1 kg. Participants were instructed to wear lightweight clothing throughout the measurement. The participants' standing height was measured with a wall stadiometer (Seca), using standard protocols[32], without shoes, and rounded to the nearest 0.5 cm. The BMI was computed by dividing the weight (in kilograms) by the square of the height (in meters). To mitigate measurement errors, all measurements were conducted by a single individual.

Abdominal circumferences (AC) and waist-to-hip ratio (WHR) were measured using bioelectrical impedance analysis (BIA; Tanita-BC 418, Arlington Heights, MA, USA) at the baseline and end of the study[33] .The measurements were conducted following 12 h of abstaining from food. Patients were instructed to abstain from ingesting alcoholic beverages for 24 h before the test and to refrain from engaging in physical exercise for 12 h before the test. Participants were instructed to void their bladders 30 min before the test and to promptly remove any metal objects before the test[34] .

Blood sample measurements

The participants' blood samples, measuring 10 mL, were collected between the hours of 7 and 10 A.M. Subsequently, the samples were subjected to centrifugation at a force of 2000 g at room temperature for 20 min[35]. At the commencement and end of the study, serum biomarkers were examined.

Liver enzymes, including (alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transferase (GGT), as well as the lipid profile, which includes (total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG)), were measured using the recommended procedures outlined by Delta-dp diagnostic kits (Roche, Germany).

The concentrations of fasting blood glucose (FBG) were determined using an auto analyzer equipped with the glucose oxidase method (Cobas c311, Roche Diagnostics, Risch-Rotkreuz, Switzerland). The ELISA kit (Monobind Inc. Lake Forest, CA, USA) was utilized to assess serum insulin levels.

To evaluate insulin resistance (IR), we computed the homeostasis model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) using the following formulas:

[HOMA-IR = glucose (mg/dL) × insulin (mU/L) /405].

QUICKI = 1/ [log fasting serum insulin (μU/ml) + log fasting plasma glucose (mg/dl)]

The high-sensitivity C-reactive protein (hs-CRP) was quantified using the enzyme-linked immunosorbent assay (ELISA) technique provided by (ZellBio GmbH, located in Ulm, Germany).

Statistical analysis

Statistical analysis was conducted with SPSS version 22. The data is shown as mean ± SD. The Kolmogorov-Smirnov tests were utilized to assess how well the data fit the normal distribution. For non-normally distributed data, log transformation was used. The data set comprised only those who successfully finished the RCT, as determined by per-protocol analysis. Quantitative factors were compared between groups using independent sample t-tests at baseline and before and after the intervention for baseline and anthropometric factors. For qualitative variables, chi-squared tests were employed. Quantitative variables within each of the two groups were also compared before and after the intervention using the student's paired samples t-tests. To compare the means of other variables between groups, analysis of covariance (ANCOVA) was used to adjust the confounding variables (metabolic equivalents, BMI, dietary energy intake, and baseline values of the outcomes). $P < 0.05$ for statistical significance was applied.

The data were analyzed using both per-protocol, and intention-to-treat (ITT) principle. For ITT analysis, patients missing laboratory measurements of the secondary outcome measures were imputed. A multiple imputation procedure was used based on Multivariate Imputation by Chained Equations. In the multiple imputation procedure, 5 imputed data sets were generated. The results of the 5 imputed data sets were pooled to obtain data estimates. Since the results of both analysis were similar, we reported the per-protocol analysis.

Results

Among the 102 patients who were randomly assigned, 9 patients were not interested in participating in the study and 40 patients did not meet inclusion criteria. As a result, there were 27 subjects in the intervention group (TRF with DASH diet) and 26 subjects in the control group. After four weeks of the trial, six more subjects withdrew from the treatment group and five subjects withdrew from the control group due to difficulties in their schedules and personal reasons. Thus, the study was completed by 21 patients in the intervention group and 21 patients in the control group (Fig. 1). The anthropometric factors and baseline characteristics of the participants are shown in (Table 1). There was no significant difference between the groups in age ($p = 0.833$), gender ($p = 0.533$), smoking ($p = 0.500$) and height ($p = 0.195$).

Characteristics	Time-restricted feeding (16/8) combined with DASH diet ($n=21$)	Control diet ($n=21$)	P-value[b]
Age (years)	45.38 ± 11.52	46.14 ± 11.65	0.833
Gender n (%)			
Male	13(61.9)	11(52.4)	0.533
Female	8(38.1)	10(47.6)	
Smoking n (%)			
Yes	1(4.8)	2(9.5)	0.500
No	20(95.2)	19(90.5)	
Anthropometric indices			
Height (cm)	168.24 ± 12.15	163.24 ± 12.40	0.195
Body weight (kg)			
Before	87.23 ± 20.36	85.15 ± 13.90	0.701
After	79.13 ± 17.90	81.61 ± 13.10	0.611
P-value[a]	< 0.001	< 0.001	
BMI (kg/m^2)			
Before	30.74 ± 5.56	31.92 ± 3.74	0.423
After	27.82 ± 4.37	30.64 ± 3.73	0.030
P-value[a]	< 0.001	< 0.001	
Abdominal circumference (cm)			
Before	97.61 ± 11.37	102.00 ± 6.67	0.135
After	91.22 ± 10.35	99.33 ± 7.1	0.005
P-value[a]	< 0.001	0.011	
Waist to hip ratio			
Before	0.93 ± 0.049	0.93 ± 0.06	0.979
After	0.90 ± 0.046	0.92 ± 0.05	0.126
P-value[a]	< 0.001	0.300	

Table 1. Characteristics, and anthropometric indices of the participants. BMI, body mass index. Values are expressed as mean ± SD. [a]Within-group comparison (a paired samples t-test was used for testing two values within group). [b]Between-group comparison (an independent sample t-test was used for quantitative variables).

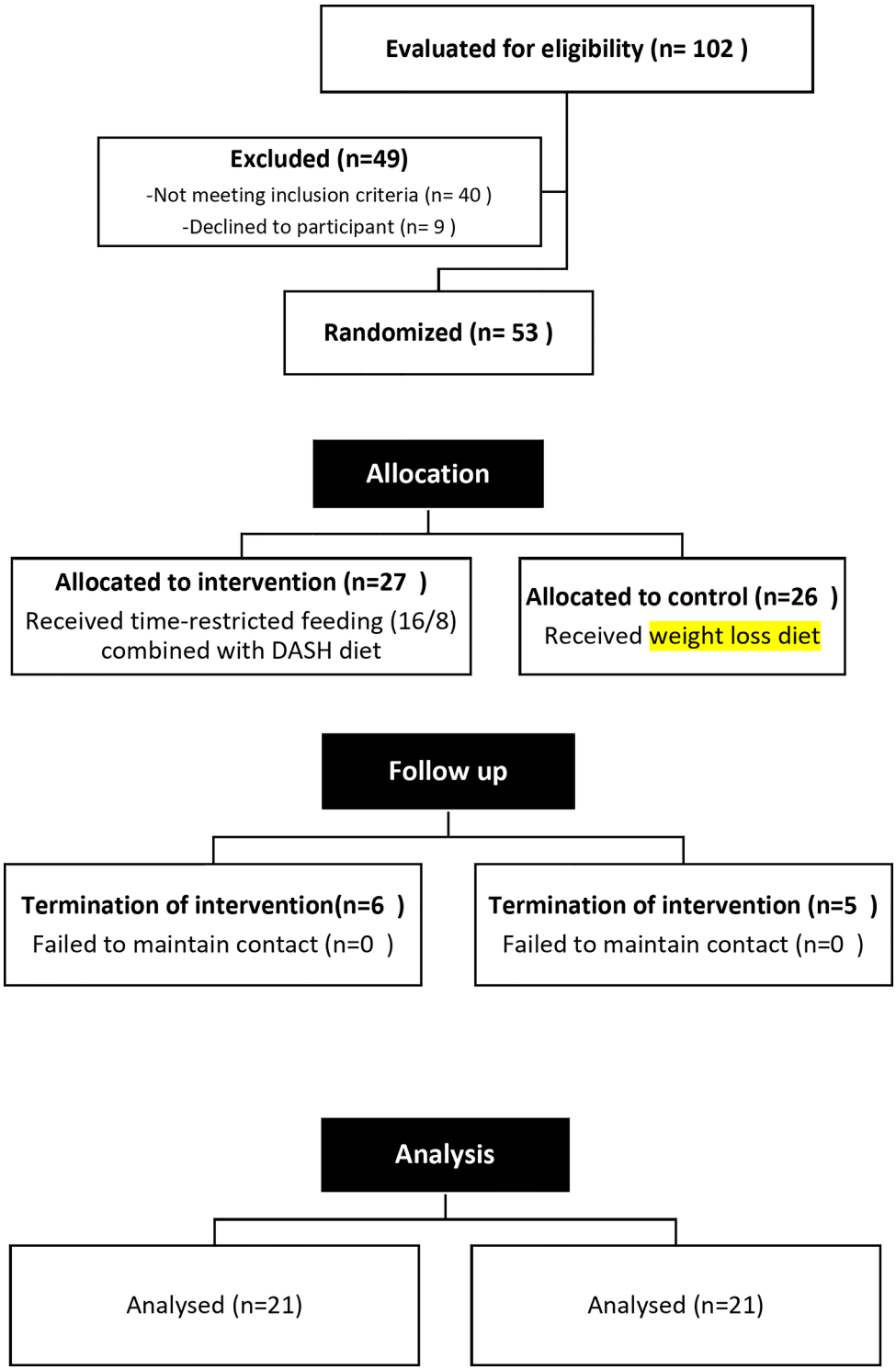

Figure 1. Consort diagram of the study

The data on the anthropometric indices and body composition of the patients is presented in Table 1. At the beginning of the study, there were no significant differences between the groups in terms of body weight, BMI, AC and WHR ($P > 0.05$). After 12 weeks of intervention, significant differences were found between the groups in BMI ($P = 0.030$), and AC ($P = 0.005$). Within-group comparisons showed that both groups experienced significant changes in body weight, BMI, and AC. However, a significant difference in WHR was seen only in the intervention group (0.93 ± 0.049–0.90 ± 0.046, $P < 0.001$) compared to the control group (0.93 ± 0.06–0.92 ± 0.05, $P = 0.30$).

Variables	Intervention group (n = 21)	Control group (n = 21)	$P^{\dagger}$	$P^{\dagger\dagger}$
Physical activity (MET hr/day)				
Baseline	27.22 ± 3.42	31.83 + 5.05	0.001	0.371
After intervention	28.12 ± 4.60	32.32 ± 4.70	0.006	
P	0.185	0.590		
Mean change of Physical activity	0.89 ± 2.99	0.48 ± 4.08	0.714	
Total energy (kcal/day)				
Baseline	1588.09 ± 655.72	2257.03 ± 559.74	0.001	0.022
After intervention	1261.70 ± 440.27	1856.88 ± 482.87	< 0.001	
P	0.014	0.002		
Mean change of Total Energy	-326.38 ± 558.63	-400.15 ± 523.37	0.661	
Total carbohydrate (g/day)				
Baseline	233.51 ± 103.03	309.62 ± 95.62	0.017	0.011
After intervention	167.71 ± 72.27	246.31 ± 64.39	0.001	
P	0.002	0.004		
Mean change of Total carbohydrate	-65.80 ± 85.76	-63.30 ± 88.27	0.926	
Total protein (g/day)				
Baseline	50.48 ± 25.93	85.99 ± 28.15	< 0.001	0.622
After intervention	57.65 ± 27.56	80.60 ± 23.06	0.006	
P	0.282	0.230		
Mean change of Total Protein	7.17 ± 29.73	-5.38 ± 19.95	0.116	
Total fat (g/day)				
Baseline	52.60 ± 29.40	82.78 ± 22.06	0.001	0.116
After intervention	42.71 ± 21.04	66.77 ± 24.37	0.001	
P	0.196	0.003		
Mean change of Total Fat	-9.88 ± 33.83	-16.01 ± 21.54	0.489	
Saturated fatty acids (SFA) (g/d)				
Baseline	13.05 ± 8.51	23.58 ± 9.38	< 0.001	0.058
After intervention	10.64 ± 5.43	18.81 ± 8.00	< 0.001	
P	0.298	0.013		
Mean change of SFA	-2.40 ± 10.32	-4.77 ± 7.99	0.411	
MUFA (g/d)				
Baseline	16.70 ± 9.13	27.22 ± 7.13	< 0.001	0.974
After intervention	15.73 ± 7.81	23.68 ± 10.37	0.008	
P	0.611	0.064		
Mean change of MUFA	-0.97 ± 8.60	-3.54 ± 8.28	0.330	
PUFA- w-6 (g/d)				
Baseline	10.95 ± 3.89	6.65 ± 1.84	< 0.001	0.608
After intervention	8.16 ± 2.60	5.23 ± 1.94	< 0.001	
P	< 0.001	< 0.001		
Mean change of PUFA- w-6	-2.78 ± 2.00	-1.41 ± 1.32	0.013	
PUFA- w-3 (g/d)				
Baseline	0.60 ± 0.81	1.24 ± 1.03	0.033	0.844
After intervention	0.83 ± 0.69	1.20 ± 0.99	0.167	
P	0.128	0.813		
Mean change of PUFA- w-3	0.22 ± 0.65	-0.04 ± 0.76	0.233	
Continued				

Variables	Intervention group (n = 21)	Control group (n = 21)	$P^{\dagger}$	$P^{\dagger\dagger}$
Vitamin E (mg/d)				
Baseline	14.70 ± 9.99	16.13 ± 3.69	0.545	0.738
After intervention	12.23 ± 7.43	13.25 ± 3.48	0.575	
P	0.41	0.007		
Mean change of Vitamin E	-2.47 ± 5.18	-2.88 ± 4.39	0.783	

Table 2. Dietary intakes and physical activity of the study participants. MET, metabolic equivalents. Values are presented as mean ± standard deviation (SD). P: resulted from comparisons within groups by paired t-test P †: resulted from comparisons between two groups by independent t-test. P ††: resulted from comparisons mean changes between two groups by Univariate analysis of covariance after adjusting for body mass index(BMI) and abdominal circumference(AC).

The data on dietary intake and physical activity (MET.hr/day) is shown in Table 2. A significant difference in physical activity was observed between the two groups at both the beginning and the end of the study ($P < 0.05$). However, within-group comparisons did not show any significant differences. In terms of other variables, significant differences between the groups were found in all variables, except for Selenium ($P = 0.673$) and Vitamin E ($P = 0.545$) at the beginning of the trial. The intervention group showed a significant change in PUFA- w-6 (-2.78 ± 2.00 vs. -1.41 ± 1.32; $P = 0.013$) compared to the control group. Within-group comparisons represented significant reductions in total fat (82.78 ± 22.06–66.77 ± 24.37, $P = 0.003$), SFA (23.58 ± 9.38–18.81 ± 8.00, $P = 0.013$), and Vitamin E (16.13 ± 3.69–13.25 ± 3.48, $P = 0.007$) consumptions in the control group compared to the intervention group.

The liver parameters assessment findings are presented in Table 3. At baseline, the values for ALT ($P = 0.363$) and AST ($P = 0.395$) did not differ significantly between the two groups. The level of GGT significantly reduced in the intervention group compared to the control group (-5.71 ± 12.46 vs. -1.03 ± 9.53). After the intervention, significant reductions were observed in the intervention group compared to the control group in ALT (-15.23 ± 18.30 vs. -4.73 ± 13.12, $P = 0.039$), AST (-7.52 ± 8.31 vs. -3.47 ± 3.11, $P = 0.047$) and steatosis score (-68.57 ± 30.94 vs. -30.19 ± 29.49, $P = < 0.001$). According to group comparisons, the results showed that the fibrosis score decreased significantly in the intervention group (5.97 ± 1.83 to 5.03 ± 1.25 kPa, $P = 0.001$) compared to the control group (7.43 ± 2.48 to 6.99 ± 2.22 kPa, $P = 0.083$). Both groups showed a significant reduction in Steatosis score/CAP, but the reduction in the intervention group was significantly greater than that in the control group (-68.57 dB/m .vs -30.19 dB/m). The only meaningful differences were observed after adjusting for BMI and AC in AST ($P = 0.003$) and fibrosis score ($P = 0.004$).

The intervention group showed a significant decrease in TG concentrations (171.00 ± 89.06 to 138.47 ± 92.06 mg/dL, $P = 0.049$), in comparison to the control group (Table 3). However, there were no significant changes in FBS, HDL-C, TC, hs-CRP and LDL-C in either group ($P > 0.05$). Significantly differences in the results of insulin and HOMA-IR were seen in both the intervention and control groups.

Discussion

This is the first study that evaluated the effects of the TRF + DASH diet on obesity index, liver parameters, glycemic indices, lipid profile, and inflammation among patients with MAFLD in comparison with the control diet. The superiority of the TRF + DASH diet over the control diet was observed in the reduction of BMI, abdominal circumference, ALT, AST, hepatic fibrosis, and steatosis; however, this superiority effect was diminished in the case of glycemic, and lipid indices, and inflammation.

The beneficial effects of TRF on patients with MAFLD have been reported in previous studies[19,20]. In such a way TRF significantly has improved liver stiffness, liver steatosis, waist circumference, visceral fat volume and insulin resistance in these patients. Enhanced fatty acid mobilization and ketone body production, the stimulation of browning in white adipose tissue, increased insulin sensitivity, lowering of leptin and increased human growth hormone, ghrelin and adiponectin circulating levels, improved autophagy by sirtuin-1 activity stimulation, modification of apoptosis and a shift in the gut microbiota composition are possible metabolic pathways explaining the beneficial effects of TRF on patients with MAFLD[36,37]. However, Cai et al.'s study reported that adherence to the TRF regime with ad libitum intake on feeding day did not improve liver stiffness in 176 patients with MAFLD[38]. Moreover, Wei et al.'s study was conducted to compare the effectiveness of two diets with equal calorie restriction, one with a time limit on food intake (eating with a time limit) and the other with the usual timing of meals in patients with MAFLD. The results of this study showed that compared to receiving meals at the usual time, applying a time restriction on food intake (eating period from 8:00 am to 4:00 pm) is not effective in reducing intrahepatic triglyceride content in patients with MAFLD. In this study, both diets were equally effective in changes in plasma glucose level, HOMA-IR, liver enzyme levels and lipid levels for 12 months[39]. Wei et al.'s study results are comparable with our results. Our results showed that TRF combined with the DASH diet, can be effective on more risk factors of MAFLD including general and abdominal obesity, ALT, AST, Fibrosis score and Steatosis score.

In our study, the TRF + DASH diet was able to reduce obesity indices and liver parameters much more than the control diet. As we mentioned before, adherence to TRF increases fat burning, and this fat burning is independent of calorie intake[40]. In addition, adding the DASH diet has been effective in observing these results

Variables	Intervention group (n = 21)	Control group (n = 21)	$P^{\dagger}$	$P^{\dagger\dagger}$
ALT (U/L)				
Baseline	39.85 ± 23.6	34.59 ± 10.9	0.363	0.052
After intervention	24.61 ± 9.2	29.86 ± 9.0	0.070	
P	0.001	0.114		
Mean change of ALT	-15.23 ± 18.30	-4.73 ± 13.12	0.039	
AST (U/L)				
Baseline	25.80 ± 11.0	28.64 ± 10.3	0.395	0.003
After intervention	18.2 ± 5.2	25.16 ± 11.0	0.015	
P	0.001	< 0.001		
Mean change of AST	-7.52 ± 8.31	-3.47 ± 3.11	0.047	
GGT (U/L)				
Baseline	28.38 ± 9.8	36.87 ± 14.3	0.031	0.392
After intervention	22.66 ± 15.3	35.84 ± 20.4	0.023	
P	0.049	0.624		
Mean change of GGT	-5.71 ± 12.46	-1.03 ± 9.53	0.180	
Fibrosis score (kPa)				
Baseline	5.97 ± 1.8	7.43 ± 2.4	0.037	0.004
After intervention	5.03 ± 1.25	6.99 ± 2.2	0.001	
P	0.001	0.083		
Mean change of Fibrosis score	-0.94 ± 1.16	-0.44 ± 1.11	0.164	
Steatosis score/CAP (dB/m)				
Baseline	340.57 ± 39.0	300.10 ± 29.7	0.001	0.302
After intervention	272.00 ± 38.7	269.90 ± 35.7	0.856	
P	< 0.001	< 0.001		
Mean change of Steatosis score	-68.57 ± 30.94	-30.19 ± 29.49	< 0.001	
TG (mg/dL)				
Baseline	171.00 ± 89.0	215.27 ± 110.2	0.160	0.080
After intervention	138.47 ± 92.0	206.08 ± 101.2	0.029	
P	0.049	0.334		
Mean change of TG	-32.52 ± 71.15	-9.18 ± 42.53	0.206	
TC (mg/dL)				
Baseline	182.14 ± 42.6	177.99 ± 42.2	0.753	0.401
After intervention	180.42 ± 45.2	168.65 ± 43.4	0.395	
P	0.806	0.087		
Mean change of TC	-1.71 ± 31.62	-9.33 ± 23.76	0.383	
HDL-C (mg/dL)				
Baseline	39.78 ± 5.8	38.16 ± 8.7	0.485	0.306
After intervention	41.76 ± 8.1	39.18 ± 7.0	0.278	
P	0.142	0.323		
Mean change of HDL-C	1.97 ± 5.93	1.01 ± 4.60	0.562	
LDL-C (mg/dL)				
Baseline	99.56 ± 36.0	101.95 ± 46.8	0.854	0.179
After intervention	102.61 ± 33.6	92.17 ± 47.1	0.414	
P	0.619	0.102		
Mean change of LDL-C	3.05 ± 27.71	-9.77 ± 26.11	0.130	
FBS (mg/dL)				
Baseline	98.52 ± 18.7	106.01 ± 24.1	0.268	0.835
After intervention	98.52 ± 13.4	103.57 ± 22.2	0.380	
P	1.000	0.148		
Mean change of FBS	00.00 ± 11.92	-2.44 ± 7.45	0.430	
Insulin (mU/L)				
Baseline	13.02 ± 8.1	12.49 ± 5.9	0.814	0.935
After intervention	9.89 ± 7.5	9.66 ± 3.6	0.900	
P	0.034	0.005		
Mean change of Insulin	-3.12 ± 6.29	**-2.83** ± 4.14	0.860	
Continued				

Variables	Intervention group (n = 21)	Control group (n = 21)	$P^{\dagger}$	$P^{\dagger\dagger}$
HOMA-IR				
Baseline	3.13 ± 1.9	3.40 ± 1.9	0.661	0.921
After intervention	2.39 ± 1.7	2.51 ± 1.1	0.799	
P	0.033	0.011		
Mean change of HOMA-IR	-0.74 ± 1.48	-0.88 ± 1.44	0.748	
QUICKI				
Baseline	0.33 ± 0.03	0.32 ± 0.0	0.357	0.228
After intervention	0.39 ± 0.1	0.33 ± 0.02	0.107	
P	0.055	0.006		
Mean change of QUICKI	0.54 ± 0.12	**0.01** ± 0.01	0.131	
hs-CRP (mg/L)				
Baseline	2.75 ± 2.7	3.94 ± 2.8	0.179	0.372
After intervention	1.92 ± 2.2	2.95 ± 2.2	0.148	
P	0.100	0.085		
Mean change of hs-CRP (mg/L)	-0.82 ± 2.20	-0.99 ± 2.50	0.824	

Table 3. Lipid profile, glycemic indices, liver enzymes, inflammatory biomarker, and indices of hepatic steatosis and fibrosis. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; FBS, fasting blood sugar; GGT, γ-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitive C-reactive protein; LDL-C, low-density lipoprotein cholesterol; QUICKI, quantitative insulin sensitivity check index; TC, total cholesterol; TG, triglycerides. Values are presented as mean ± standard deviation (SD). P: resulted from comparisons within groups by paired t-test P †: resulted from comparisons between two groups by independent t-test. P ††: resulted from comparisons mean changes between two groups by Univariate analysis of covariance after adjusting for BMI and AC.

according to previous studies[24,25]. Because in previous studies, TRF regimes alone were not effective in reducing liver parameters for long duration[39]. Considering the low energy density and high dietary fiber content of the DASH diet[41], following the TRF + DASH diet is more effective in increasing satiety, and reducing energy and carbohydrate intake in comparison with the control diet (as we see in Table 2). Therefore, it is not surprising that it has been able to improve obesity indices in MAFLD patients more than in the control group. The high dietary fiber content of the DASH diet[41], can lead to delayed carbohydrate absorption and decrease accumulation of fat in the liver[42]. DASH diet contains high calcium and magnesium that can stimulate microsomal triglyceride transfer protein (MTP) in the liver[43] and increasing the expression of this protein has been considered as a treatment for non-alcoholic steatohepatitis[44]. The standard DASH diet limits salt intake and decreased sodium negatively influenced markers of liver steatosis and fibrosis[45]. Dorosti et al. reported that consumption of whole grains (healthy DASH food), beneficially affected liver enzyme concentrations, and fatty liver in patients with MAFLD[46]. Adding DASH to the TRF regimen is effective in solving two important challenges of the TRF regimen. Many people find a lot of appetite in the hours after fasting, especially for high-calorie foods, so individuals fully compensate during fed periods for the negative energy balance incurred during extended periods of fasting between eating bouts[47]. Furthermore, the DASH diet can be effective in reducing this desire to eat by increasing the feeling of fullness. Long fasting in a TRF diet can reduce the quality of a person's diet and nutrient intake[48]. Also, the high nutrient density of the DASH diet[49] can help in solving this problem and therefore, the combination of these two regimens becomes more clinically important.

Limitations of our study should be addressed. Certainly, a longer intervention period not only provides the possibility of examining the chronic effects of this type of combined diet but also provides the possibility of evaluating the level of continuous adherence to the TRF + DASH diet in the long term. Adding another intervention group to the study that was only affected by the TRF regimen was very helpful in comparing and interpreting the results. The present study also had several strengths; the RCT design of this study is a practical method that leads to the development of evidence-based clinical recommendations. Combining TRF with the DASH diet is a new nutritional strategy that has been investigate in this study for the first. The TRF program can be considered a convenient and sustainable diet[50]. The DASH diet is also a flexible diet that is easy to follow because it does not restrict entire food groups. The DASH eating plan is easily adaptable to other styles of eating and dietary preferences[51]. Therefore, the combination of these two diets can be easily used in clinical practice.

Conclusions

In MAFLD patients, TRF (16/8) in combination with a DASH diet is superior to a low-calorie diet in promoting obesity indices, and hepatic steatosis and fibrosis. Further long-term investigations are needed to draw definitive conclusions.

Data availability

The data are available upon request to corresponding author.

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Declarations

Competing interests
The authors declare no competing interests.

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Declaration of competing interest
The authors declare no conflict of interest.

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Ethical approval
The study protocol was approved by the ethics committee of the National Nutrition and Food Technology Research Institute (Ethics committee reference number IR.SBMU.NNFTRI.1402.001) and all participants signed a written informed consent form. This study complies with the Declaration of Helsinki and was performed according to ethics committee approval.

Additional information

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