Whole Grain Consumption and Its Effects on Hepatic Steatosis and Liver Enzymes in Patients with Nonalcoholic Fatty Liver Disease: A Randomized Controlled Clinical Trial

Running title: Whole grain and NAFLD

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List of abbreviations:

Alanine aminotransferase: ALT

Aspartate aminotransferase: AST

Body mass index: BMI

Diastolic blood pressure: DBP

Fasting blood sugar: FBS

γ –glutamyltransferase: GGT

Homoeostasis model of assessment-estimated insulin resistance: HOMA-IR

Metabolic equivalent of task: MET

Nonalcoholic fatty liver disease: NAFLD

Systolic blood pressure: SBP

Waist to hip circumference ratios: WHR

Abstract

Nonalcoholic fatty liver disease (NAFLD) is a considerable challenge to public health across the globe. Whole grain is highly recommended as an inseparable part of a healthy diet and has been proposed as an effective way to manage NAFLD. The objective in this study was to evaluate the effects of whole grain consumption on hepatic steatosis and liver enzymes as primary outcomes in patients with NAFLD. Over the 12 weeks of this open-label, randomized controlled clinical trial, 112 patients [mean age: 43± 8.7 y; body mass index (BMI) (kg/m^2): 32.2 ± 4.3] were randomly assigned to two groups to receive dietary advice; either to obtain at least half of their cereal servings each day from whole-grain foods or from usual cereals. By the end of the study, the grades of NAFLD showed a significant decrease in the intervention group ($P < 0.001$). In addition, a significant reduction in serum concentration of alanine aminotransferase ($P < 0.001$), aspartate aminotransferase ($P < 0.001$), γ-glutamyltransferase ($P = 0.009$), systolic blood pressure ($P = 0.004$) and diastolic blood pressure ($P = 0.008$) were observed in the intervention group compared to the control group. After adjusting, however, no significant differences were found between the two groups in terms of lipid profile, glycemic status, and anthropometric measurements. Overall, our study demonstrated that consumption of whole grains for 12 weeks had beneficial effects on hepatic steatosis and liver enzymes concentrations in patients with NAFLD.

Keywords: nonalcoholic fatty liver disease, whole grain, liver enzyme, insulin resistance, lipid profile

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in Western and Asian countries [1]. A meta-analysis reported that the prevalence of NAFLD in Iran was 33.95% and that it was increasing at an alarming rate [2]. It has been estimated that NAFLD will be a major cause of liver-related morbidity and mortality by 2030 [3]. NAFLD describes a common clinic-pathological condition characterized by a large number of fatty deposits in hepatocytes, and it is detected by either histology or imaging [4]. NAFLD has been linked to elevation of aminotransferases, dyslipidemia, insulin resistance, hypertension, and obesity [5, 6]. Moreover, NAFLD in the hepatic steatosis stage can be reversed by lifestyle modification such as weight loss, regular physical activity, and adherence to a healthy diet [7].

Whole grains are highly recommended as the inseparable part of a healthy diet, and its significance in chronic disease management is interesting. According to the definition proposed by American Association of Cereal Chemists, whole grains are the intact, ground, cracked, or flaked kernel of grains, whose principal anatomical components are the starchy endosperm, germ, and bran [8]. The 2012 Dietary Guidelines for Americans recommend consuming at least half of the daily grain servings as whole grains with gram recommendations ranging from 48 g/d to 75 g/2000 kcal to reduce the risk of chronic diseases [9].

Previous studies, carried out on animal models suggested that dietary whole grains may have protective effects against chronic diseases [10]. Furthermore, it has been claimed that consumption of whole grain products may facilitate management of metabolic disorders [11]. However, the results of a whole-grain-based case-control study found an inverse association between whole grain consumption and NAFLD [12]. On the other hand, a large cohort study showed a negative significant correlation between whole grain intake and hyperlipidemia and insulin resistance [13].

Similarly, some previous studies have suggested that whole grain foods may improve glucose and lipid biomarkers in healthy obese or hyperglycemic adults [14]. Many randomized controlled trials have also evaluated the effects of whole grain products on blood glucose concentrations and serum lipids, but they have found inconsistent results [15-18].

Despite acknowledgement of the importance of whole grains in the diet by researchers, there is little evidence regarding the association between consumption of whole grains and management of NAFLD-related diseases [19]. Thus, it is hypothesized in this study that dietary whole grains might be effective in the management of NAFLD. To evaluate this hypothesis, this randomized controlled clinical trial was designed to assess whether consumption of whole grains is effective in management of NAFLD in adults or not.

Materials and Methods

Recruitment and eligibility screening

Two-hundred and two adult patients diagnosed with NAFLD, who had visited Ghods Clinic in Urmia, Iran, between March and May 2017, were enrolled in the study, consecutively. NAFLD diagnosis was based on the presence of ultrasonographic evidence of fatty liver and no other type of liver injury or steatosis. All patients with NAFLD were asked to participate and were recruited provided they met the inclusion criteria and accepted to participate in the study. The reason behind this was to eliminate any bias in patient selection. Men and women at the age of fertility, that is 18 years or older, with fatty liver grades 1, 2 or 3 and BMI ≤ 40 were included in the study. Excluding factors consisted of a history of alcohol abuse; evidence of any acute and chronic gastrointestinal diseases other than fatty liver; autoimmune disease; type 1 or 2 diabetes; uncontrolled thyroid conditions; psychiatric disorders; impaired renal function; use of drugs to treat NAFLD; use of drugs having the potential to affect glucose and lipid metabolism during the

study and over the last six months; history of using medications that increase the risk of NAFLD, such as perhexiline, methotrexate, amiodarone, diltiazem, tamoxifen, nifedipine, estrogens, chloroquine, and other possible hepatotoxic agents; pregnancy and breast-feeding for women. Participants who took at least half of their daily grain servings as whole grains before the start of the study were also excluded from the study.

Study design

This parallel, open-label randomized controlled clinical trial was conducted from June to August 2017. Written informed consents were obtained from all participants after they became informed about the aims and procedures of the study. The study protocol was approved by the Ethics Committee at Urmia University of Medical Sciences and was registered at the Iranian Registry of Clinical Trials website (IRCT20170206032417N3). Randomization lists were computer-generated by a statistician, and the laboratory staff, radiologist, and statistician were blinded to the intervention assignment until the end of the trial.

Intervention

At baseline, patients were randomly allocated to two groups to receive dietary advice either to obtain at least half of their cereal servings each day from whole-grain foods [intervention group (n=56)] or from usual cereals [control group (n=56)] for 12 weeks. This time period was designated based on those previous studies showing beneficial effects of whole grains on metabolic markers in six and eight weeks [16, 17]. A dietitian met with participants at baseline to explain the 2012 Dietary Guidelines for Americans [9] used in the study and also to provide educational information to facilitate their understanding and adherence. Participants in the intervention group were given a list and description of whole-grain foods and were advised to consume at least half of the daily grain servings as whole grains based on recommendations in

the 2012 Dietary Guidelines for Americans [9]. In addition, participants in both groups were asked to eat, 2-3 servings of low-fat dairy products, five servings of fruits and vegetables, and two servings of lean meat, poultry, or fish on a daily basis, as recommended in the 2012 Dietary Guidelines for Americans [9]. At baseline, patients were requested to maintain their levels of physical activity throughout the trial period. Compliance with the guidelines was determined every week by a telephone call and dietary assessment on four occasions (at weeks 0, 4, 8 and 12) during the study.

Liver ultrasonography

All participants underwent a liver ultrasonographic examination performed by one operator and one expert radiologist (intra-inter-observer variation around 20% and 25%, respectively) at baseline and at the end of the study. NAFLD was defined as the presence of an ultrasonographic pattern according to the following criteria: liver-kidney echo discrepancy, attenuated echo penetration, visibility of diaphragm, and narrowing of the lumen of the hepatic veins on ultrasonography (Philips Affiniti 50 Ultrasound). Fatty liver was graded as normal, grade 1, 2, and 3, according to the modified criteria of Kurtz et al. [20].

Biochemical measurements

Biochemical testing was performed at weeks 0 and 12 to determine serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyltransferase (GGT), total cholesterol, triacylglycerol, HDL cholesterol, LDL cholesterol, fasting blood sugar (FBS), and insulin concentrations using routine automated assay methods at the Urmia University of Medical Sciences reference laboratory after an overnight fast. Serum concentrations of ALT, AST, ALP, total cholesterol, triacylglycerol, HDL cholesterol, LDL cholesterol, and FBS were measured using routine enzymatic assays with commercial kits (Pars Azmoon, Tehran, Iran) and

an automatic biochemical analyzer (BT 4500, Biotechnica, Italy). Furthermore, serum concentrations of insulin were measured by the electrochemiluminescence method on the Cobas E411 analyser (Roche Diagnostics GmbH, Mannheim, Germany). Homeostatic model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were also calculated based on suggested formulas [21].

Blood pressure and anthropometric measurements

At weeks 0 and 12, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken by the same person, during the same period of the day, and after five min of rest in the sitting position employing a validated mercury sphygmomanometer device (MicrolifeBP AG1-10). Furthermore, anthropometric measures were taken in the standing position, with participants wearing light clothing and no shoes, at baseline and week 12. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2); height and weight were measured using a digital scale and stadiometer (BSM370; Inbody Co.). Moreover, waist and hip circumferences were measured following recommendations by the World Health Organization [22].The waist to hip circumference ratios (WHR) were calculated as well.

Dietary intake and physical activity assessment

At baseline and every four weeks, 3-d (one weekend day and two nonconsecutive week days) 24-h diet recall interview was performed by a trained dietitian. The dietary recalls were analyzed using the Nutritionist 4 (N4) software (First Databank, Inc.; Hearst Corporation) modified for Iranian foods. The obtained data were then used to estimate the mean of consumed dairy, vegetables, fruits, cereals, whole grains, meat, and fat group servings. If a whole-grain food did not exist in the N4 software, it would be replaced with a proper whole-grain with similar micro- and macronutrient and dietary fiber contents. According to the 2012 dietary guidelines for

Americans, grain servings were defined as 1/2 cup (120 mL) of cooked rice, pasta, or cereal; one slice of bread (30 g); and one ounce (28 g) of ready-to-eat cereals such as puffed products, biscuits, shred products, and other conventional cereal piece shapes. Physical activities were investigated using the metabolic equivalent of task (MET) questionnaire [23] at weeks 0, 6 and 12.

Primary and secondary outcomes

The primary outcome measures were changes in liver steatosis grades and serum concentrations of liver enzymes. Secondary outcome measures were changes in serum concentrations of lipid profiles, glycemic variables, blood pressure, and anthropometric variables.

Statistical analysis

The obtained data were analyzed using SPSS software version 21 (SPSS, Inc.). For all analyses a *P* value <0.05 was considered to be significant. Continuous and categorical data were presented as means ± SD and frequency, respectively. To calculate the sample size, the standard formula suggested for parallel clinical trials was applied, considering type one error (α) of 0.05 and type two error (β) of 0.10 (power=90 %) (based on a study conducted by Katcher et al. [24] and a mean change in the total cholesterol). Based on this, the sample size was estimated to be 47 per study group. Assuming 19% dropouts from each group, the final sample size was estimated to be 56 per group. Differences in general characteristics between groups at baseline were compared by applying independent-samples Student's *t* test (for continuous variables) and chi-square (for categorical variables). Marginal Model and Generalized Estimating Equations (GEE) with a cumulative logit link function were used to compare changes in the fatty liver grade among groups during the intervention. To assess confounders, the changes in fatty liver grade for baseline value of the outcome, mean change in food groups, MET value, energy intake, weight, BMI, and WHR were adjusted by using the GEE models. To identify within-group differences,

paired-samples *t* tests were used. Repeated measures ANOVA, using the general linear model with group (intervention and control) as the between-subjects factor, was conducted to identify changes in dietary intakes and serum concentrations of liver enzymes, lipid profile, glycemic variables, blood pressure over time and also differences between the groups. Moreover, to assess confounders, all analyses for baseline value of the outcome, mean change in food groups, MET value, energy intake, weight, BMI, and WHR were adjusted by applying the ANCOVA test.

Results

A total of 112 participants were randomly assigned to two groups. After randomization, 18 participants were excluded, due to failure to follow-up. In total, 94 participants completed the trial: the intervention group (n = 47) and control group (n = 47) (**Figure 1**). No significant differences were found between the two groups regarding baseline demographic characteristics (**Table 1**). On the basis of 3-d 24-h dietary recalls obtained at study baseline and throughout the intervention, significant changes between the groups, in terms of energy intake and consumed food groups, with the exception of dairy and fruit intakes were observed (**Table 2**).

Primary outcomes

Ultrasound grades decreased significantly in the intervention group compared with the control group ($P < 0.001$). In the intervention group, 66% of patients showed a reduction in grade of fatty liver (**Table 3**).

Changes in serum concentrations of ALT ($P < 0.001$), AST ($P < 0.001$), and GGT ($P = 0.009$) were significantly different between the two groups. The intervention group showed a greater reduction in serum concentrations of these enzymes than did the control group (**Table 4**). These significant differences between the groups were observed even after adjustments were made to

the baseline value of the outcome, mean changes in food groups, and MET value, and after more adjustments were made to the mean changes in energy intake, weight, BMI, and WHR (**Table 5**).

Secondary outcomes

Changes in serum concentrations of total cholesterol ($P = 0.004$), LDL cholesterol ($P = 0.014$), SBP ($P = 0.004$), and DBP ($P = 0.008$) were significantly different between the two groups. The intervention group showed a greater reduction in these parameters than did the control group. In addition, no significant differences were found between the two groups in terms of serum concentrations of triacylglycerol, HDL cholesterol, insulin, FBS, and HOMA-IR, weight, BMI, or WHR.

After adjusting for baseline value of the outcome, mean changes in food groups, and MET value and after adjusting more for mean changes in energy intake, weight, BMI, and WHR, the differences in changes of serum, total cholesterol and LDL cholesterol concentrations between the two groups became non-significant. Moreover, other findings were not significantly changed after the adjustments were made (Table 4).

Discussion

NAFLD poses substantial challenges to the public health across the globe. Therefore, to improve NAFLD risk factors, researchers have become interested in the effectiveness of different dietary patterns. Consequently, in this study, it is hypothesized that patients with NAFLD who included whole-grain foods in their diet would show a more considerable improvement in laboratory liver tests.

In the present study, we showed that the consumption of whole grains for 12 weeks led to statistically and clinically significant improvements in grade of steatosis and liver enzymes. Significant differences in hepatic steatosis and liver enzymes were observed between the groups,

even after adjustment with covariates. This indicates that whole grain by itself and independent of weight loss, improved the above outcomes. Data on the effects of whole grains on hepatic steatosis and liver enzymes among NAFLD patients, however, are scarce. Results from a case–control study with 34 nonalcoholic steatohepatitis patients suggested that whole grains were potentially useful for improving hepatic steatosis and liver enzymes [25]. Also, in a study by Abdelmalek et al [26], it was found that betaine, as a beneficial factor in whole grain, improved hepatic steatosis. Tovar et al. [15] observed a significant decrease in GGT concentrations after consumption of whole grain barley product supplementation, compared with a control diet, in overweight women. The results of study conducted by Angelino et al. [14], however, showed that whole grain consumption for 12 weeks had no effect on liver enzymes. The discrepancy was probably due to an approximate doubling in total daily fiber intake in the study by Tovar et al. [15]. This efficacy can at least be partially attributed to the effects of the whole grains on modulation of the microbiota composition in humans, with corresponding changes to gut microflora metabolites including short-chain fatty acids [27].

At an anatomical location, liver is directly supplied with blood flow from the intestinal. As a result, changes in intestinal permeability caused by dysbiosis gut microbiota allow the translocation of bacterial products into the portal vein and development of obesity-related metabolic diseases such as NAFLD. The portion of gut microbiota in the progression of NAFLD is mainly based on increased hepatic reactive oxygen species due to the increased pro-inflammatory markers in the intestinal lumen. Some studies have shown associations between whole grain consumption and inflammatory markers [28-31]. It is assumed in our study that the reduction in oxidative stress and inflammatory markers due to the consumption of whole grains has been responsible for the decrease in liver enzymes and steatosis; nonetheless, further

investigations are needed. On the other hand, whole-grain foods are a good source of micronutrients such as copper, which is identified as a key player in various metabolic disorders [32]. Reduced serum and hepatic copper concentrations characterize NAFLD patients and seem to have an important role in metabolic diseases progression.

Moreover, findings from the current study showed that the consumption of whole grains did not affect serum FBS and insulin concentrations. In a report from the Framingham Offspring Cohort Study, the number of daily servings of whole grain foods was inversely related with insulin resistance and fasting insulin concentrations [13]. In the Insulin Resistance Atherosclerosis Study, a greater intake of whole grains was associated with higher insulin sensitivity [33]. Similar results were observed in a cross-sectional study of 285 adolescents [34]. This discrepancy might be due to the number of daily servings of whole grain foods, nature of the disease,
and duration of intervention.

Whole-grain foods are thought to have a beneficial effect on glycemic variables due to their greater fiber content, which leads to a slower or reduced digestion of macronutrients, reduced blood glucose burden and lower insulin concentrations when supplemented in clinical trials [35]. Also, bioactive compounds in whole grain foods, such as phenolic acids and alkylresorcinols, seem to contribute to the antioxidant and anti-inflammatory effects [36].

The lipid profile results of the present analyses are in line with the Framingham Offspring Cohort Study that showed whole-grain foods were related to lower LDL-cholesterol and total cholesterol concentrations and not to HDL cholesterol or triacylglycerol concentrations [14]. In Tehran, Lipid and Glucose Study with 827 men and women, revealed that intake of traditional Iranian whole-grain foods was associated with lower triacylglycerol concentrations but not with any other lipid variables [11]. Similarly, an improvement in the lipid profiles has also been found

in overweight or obese subjects, after consumption of oat-based products for 12 weeks [37] and in subjects with intake of β-glucan-rich products for eight weeks [15]. The beneficial effects of whole-grain foods has been, at least partly, ascribed to their lipid lowering properties, particularly for whole grains containing relatively high amounts of viscous soluble fiber [38].

In the current study, we found a 3 mm Hg reduction in SBP and a 1 mm Hg reduction in DBP. These results are also consistent with results of previous studies that reported the beneficial effects of whole grains on blood pressure [39, 40]. Contrary to our study, Angelino et al. [14] found no significant variations in systolic and diastolic blood pressure, probably because of low consumption of daily whole grains. The beneficial effects of whole grains on blood pressure could be explained by potential bioactivity of the micronutrients such as calcium, magnesium, zinc, potassium, and vitamin D [32].

Based on an assessment of dietary intake at baseline and every four weeks, participants in the intervention group had a greater reduction in energy intake than did participants in the control group. This might be explained by the fact that whole grains could reduce appetite; however, further investigations are required. Participants in the intervention group significantly improved their diet quality by increasing their intake of dietary fibers to almost 14 g/1000 kcal, which is the amount recommended in the 2012 dietary guidelines for Americans [9]. Participants in the whole-grain group also increased their intake of beneficial micronutrients which potentially contribute to management of NAFLD [32]. Thus, the consumption of whole-grain foods would be expected to benefit metabolic diseases, such as obesity, type 2 diabetes, and NAFLD.

Strength of the present study rests on its high completion and compliance rate. Furthermore, consumption of whole-grain food is a low cost management strategy for NAFLD patients. Limitation of this study is its short duration of interventions.

In conclusion, this open-label randomized controlled clinical trial provided some evidence that consumption of whole-grain foods improved hepatic features in patients with NAFLD. Nevertheless, future clinical studies examining larger sample sizes for longer periods are required to determine the long-term health benefits of whole grains.

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Transparency Declaration

The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported. The reporting of this work is compliant with CONSORT guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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TABLE 1

Demographic characteristic of patients with NAFLD[1]

	Intervention group (n = 47)	Control group (n = 47)	P value[2]
Age (y)	43.1 ± 8.9	42.4 ± 8.6	0.73
Sex [n (%)]			0.67
Male	21 (44.7)	18 (38.3)	
Female	26 (55.3)	29 (61.7)	
Height (cm)	162.5 ± 9.6	161.6 ± 9.1	0.71
Weight (kg)			
Baseline	85.7 ± 12.0	82.8 ± 10.3	0.21
Week 12[*]	84.2 ± 11.8[††]	82.0 10.6[†]	0.34
WHR (cm)			
Baseline	1.0 ± 0.06	1.0 ± 0.04	0.09
Week 12[*]	1.0 ± 0.06	1.0 ± 0.05[†]	0.10
BMI (kg/m^2)			
Baseline	32.5 ± 4.1	31.8 ± 4.4	0.43
Week 12[*]	32.0 ± 4.2[††]	31.6 ± 4.6[†]	0.65

MET (h/d)[**]			
Baseline	28.4 ± 7.7	32.6 ± 11.7	0.044
Week 6	28.7 ± 7.2	33.4 ± 11.3	0.019
Week 12	27.7 ± 8.4	32.5 ± 11.7	0.025

[1]Values are means ± SD unless otherwise indicated. BMI, body mass index; MET, metabolic equivalent of task; NAFLD, nonalcoholic fatty liver disease; and WHR, waist to hip circumference ratios.

[2]*P* values were computed by the independent-samples *t* test for continuous variables and by chi-square for categorical variables.

[*]*P* values were computed by paired-samples *t* test: [†]$P < 0.05$ and [††]$P < 0.001$

[**]*P* values were computed by analysis of general linear model ANOVA for repeated measurements: $P_{Time} = 0.10$, $P_{Group} = 0.024$ and $P_{Time \times Group} = 0.21$

TABLE 2

Dietary intakes of study participants at weeks 0, 4, 8 and 12 of the study[1]

	Intervention group (n = 47)				Control group (n = 47)				P value[2]		
	Baseline	Week 4	Week 8	Week 12	Baseline	Week 4	Week 8	Week 12	Time	Group	Time × group
Dairy	0.2 ± 0.1	0.3 ± 0.3	0.3 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.10	0.31	0.56
Vegetable	1.5 ± 1.1	1.4 ± 0.9	1.5 ± 0.8	1.5 ± 0.8	2.0 ± 1.2	2.0 ± 1.2	2.0 ± 1.0	2.0 ± 1.1	<0.001	0.030	0.52
Fruit	1.8 ± 1.6	1.8 ± 1.2	1.6 ± 1.1	1.8 ± 1.0	1.2 ± 0.7	1.6 ± 0.7	1.7 ± 1.0	1.9 ± 0.9	0.004	0.33	0.36
Grain	12.6 ± 2.9	11.0 ± 2.6	11.2 ± 2.5	11.0 ± 2.1	14.1 ± 3.1	14.1 ± 2.8	13.6 ± 3.0	13.4 ± 2.6	0.034	<0.001	0.34
Whole grain	0	7.8 ± 1.8	7.7 ± 2.3	8.1 ± 2.3	0	0	0	0	0.32	<0.001	0.32
Meat	3.5 ± 1.1	3.7 ± 1.1	3.5 ± 1.3	3.7 ± 1.1	4.4 ± 1.2	4.6 ± 1.0	4.7 ± 1.2	4.3 ± 1.2	0.20	0.011	0.018
Fat	3.9 ± 1.3	5.4 ± 1.4	5.8 ± 1.3	5.6 ± 1.1	4.9 ± 1.2	5.0 ± 1.4	4.7 ± 1.4	4.6 ± 1.2	<0.001	<0.001	0.37

[1] Values are means ± SD. NAFLD, nonalcoholic fatty liver disease.

[2] P values represent the effect of time, group, and time × group interaction (computed by analysis of general linear model ANOVA for repeated measurements).

TABLE 3

Changes in fatty liver grade during the 12-week diet period in patients with NAFLD in the intervention and control groups[1]

Group	Grade of fatty liver	Baseline, *n* (%)	Week 12, *n* (%)	Changes, *n* (%)	*P* value[2]		
					P_1	P_2	P_3
Intervention group	Normal	0	17 (36.2)	2 degree reduction in grade: 6 (12.5)			
	Grade1	24 (51.1)	23 (48.9)	1 degree reduction in grade: 25 (53.2)			
	Grade 2	20 (42.6)	7 (14.9)	Without change: 15 (31.9)			
	Grade3	3 (6.4)	0 (0)	1 degree of deterioration: 1 (2.1)			
					< 0.001	0.003	0.004
Control group	Normal	0 (0)	5 (10.6)	2 degree reduction in grade: 0			
	Grade1	26 (55.3)	23 (48.9)	1 degree reduction in grade: 12 (25.5)			
	Grade 2	20 (42.6)	19 (40.4)	Without change: 31 (66.0)			
	Grade3	1 (2.1)	0 (0)	1 degree of deterioration: 14 (8.5)			

[1]Intervention group: baseline, $n = 56$ and week 12, $n = 47$. Control group: baseline, $n = 56$ and week 12, $n = 47$.

[2] Based on marginal model and generalized estimating equations with a cumulative logit link function there were significant differences between groups regarding grades of liver steatosis ($P < 0.001$). On the basis of generalized estimating equations there were significant differences between groups in grades of liver steatosis after adjusting for baseline value of the outcome, mean change in food groups, and MET value ($P = 0.003$) and after more adjusting for mean change in energy intake, weight, BMI, and WHR ($P = 0.004$).

TABLE 4

Changes in liver enzymes, lipids profile, glycemic variables, and blood pressure during the 12-week diet period in patients with NAFLD in the intervention and control groups[1]

	Intervention group (n = 47)			Control group (n = 47)			P value[2]		
	Baseline[3]	Week 12	Change[4]	Baseline[3]	Week 12	Change[4]	Time	Group	Time × group
Serum liver enzymes, IU/L									
ALT	34.6 ± 12.5	24.1 ± 12.2	-10.5 ± 13.9††	32.1 ± 16.9	32.5 ± 18.2	0.5 ± 13.3	0.001	0.39	<0.001
AST	27.7 ± 13.6	21.9 ± 6.8	-5.8 ± 9.6††	25.5 ± 9.5	26.5 ± 11.0	1.0 ± 8.7	0.012	0.56	<0.001
GGT	26.3 ± 11.4	21.8 ± 7.0	-4.4 ± 9.0†	26.8 ± 11.1	28.5 ± 19.2	1.7 ± 13.2	0.25	0.13	0.009
Serum lipids, mg/dL									
Total cholesterol	192.4 ± 40.9	174.1 ± 37.3	-18.3 ± 32.3††	183.1 ± 38.2	183.4 ± 36.5	0.3 ± 29.0	0.006	0.99	0.004
triacylglycerol	167.9 ± 13.6	156.7 ± 11.6	-11.2 ± 7.5	180.5 ± 15.6	190.8 ± 15.7	10.3 ± 10.9	0.94	0.22	0.11
HDL cholesterol	41.2 ± 7.0	43.0 ± 5.9	1.8 ± 4.9†	44.8 ± 7.5	43.8 ± 9.5	-1.1 ± 8.2	0.047	0.11	0.54
LDL cholesterol	114.4 ± 4.5	101.5 ± 4.2	-12.8 ± 4.4†	98.9 ± 4.8	99.7 ± 4.7	0.9 ± 3.3	0.032	0.14	0.014
Glycemic variables									
FBS, mg/dL	88.1 ± 10.2	86.9 ± 8.2	-1.2 ± 9.5	87.5 ± 10.0	88.9 ± 10.9	1.4 ± 10.6	0.91	0.70	0.20
Serum insulin, mU/L	17.0 ± 9.3	14.9 ± 7.9	-2.1 ± 6.4†	14.7 ± 7.1	14.5 ± 7.9	-0.3 ± 5.7	0.63	0.39	0.15

HOMA-IR	3.5 ± 0.3	3.2 ± 0.3	-0.5 ± 0.2†	3.2 ± 0.2	3.1 ± 0.2	-0.03 ± 0.2	0.10	0.35	0.16
QUICKI	0.3 ± 0.02	0.3 ± 0.02	0.007 ± 0.02	0.3 ± 0.02	0.3 ± 0.02	0.007 ± 0.02	0.70	0.41	0.25
Blood pressure, mm Hg									
SBP	125.5 ± 7.4	122.3 ± 5.5	-3.2 ± 5.5	125.1 ± 8.3	125.5 ± 7.1	0.4 ± 6.2	0.026	0.31	0.004
DBP	79.7 ± 4.7	78.0 ± 5.0	-1.7 ± 4.3	79.9 ± 5.6	80.7 ± 4.4	0.8 ± 4.8	0.37	0.10	0.008

[1]Values are means ± SD. ALT, alanine aminotransferase; AST, aspartate aminotransferase; DBP, diastolic blood pressure; FBS, fasting blood sugar; GGT, γ - glutamyltransferase; HOMA-IR, homoeostasis model of assessment-estimated insulin resistance; NAFLD, nonalcoholic fatty liver disease; QUICKI, Quantitative insulin sensitivity check index; and SBP, systolic blood pressure.

[2]*P* values represent the effect of time, group, and time × group interaction (computed by analysis of general linear model ANOVA for repeated measurements).

[3]On the basis of independent-samples *t* test there were no significant differences between groups with regard to baseline values ($P > 0.05$).

[4]*P* values were computed by paired-samples *t* test: $^{\dagger}P < 0.05$ and $^{\dagger\dagger}P < 0.001$

TABLE 5

Adjusted changes in Liver enzymes, lipids profile, glycemic variables, and blood pressure during the 12-week diet period in patients with NAFLD in the intervention and control groups[1]

	Intervention group (n = 47)	Control group (n = 47)	P value[2]
ALT, IU/L			
Model 1*	-8.5 ± 2.0	-1.5 ± 2.1	0.022
Model 2**	-9.0 ± 1.9	-1.0 ± 1.9	0.012
AST, IU/L			
Model 1	-4.8 ± 1.4	0.01 ± 1.4	0.019
Model 2	-5.2 ± 1.4	0.3 ± 1.4	0.010
GGT, IU/L			
Model 1	-5.9 ± 2.0	3.1 ± 2.0	0.006
Model 2	-5.6 ± 2.1	2.9 ± 2.1	0.012
Total cholesterol, mg/dL			
Model 1	-15.2 ± 4.5	-2.7 ± 4.5	0.11
Model 2	-15.9 ± 5.1	-2.1 ± 5.0	0.09
triacylglycerol, mg/dL			

Model 1	-5.6 ± 1.3	4.7 ± 1.2	0.53
Model 2	-6.5 ± 1.1	5.6 ± 1.1	0.47
HDL cholesterol, mg/dL			
Model 1	-2.3 ± 1.4	-0.5 ± 1.4	0.32
Model 2	-2.5 ± 1.4	-0.3 ± 1.4	0.24
LDL cholesterol, mg/dL			
Model 1	-9.8 ± 4.3	-2.2 ± 4.3	0.25
Model 2	-10.0 ± 4.3	-1.9 ± 4.3	0.25
FBS, mg/dL			
Model 1	-0.9 ± 1.6	1.2 ± 1.5	0.35
Model 2	-0.1 ± 1.6	1.2 ± 1.6	0.36
Serum insulin, mU/L			
Model 1	-1.4 ± 0.9	-0.9 ± 0.9	0.79
Model 2	-1.4 ± 1.0	-1.0 ± 1.0	0.79
HOMA-IR			
Model 1	-0.3 ± 0.3	-0.2 ± 0.3	0.76
Model 2	-0.3 ± 0.3	-0.2 ± 0.3	0.75
QUICKI			
Model 1	-0.02 ± 0.01	-0.02 ± 0.01	0.86
Model 2	-0.02 ± 0.01	-0.02 ± 0.01	0.86

BP, mm Hg			
Model 1	-3.4 ± 0.9	0.7 ± 0.9	0.004
Model 2	-4.0 ± 0.9	1.2 ± 0.9	<0.001
DBP, mm Hg			
Model 1	-1.6 ± 0.8	1.1 ± 0.8	0.007
Model 2	-1.9 ± 0.8	1.1 ± 0.8	0.012

[1]Values are means ± SD. ALT, alanine aminotransferase; AST, aspartate aminotransferase; DBP, diastolic blood pressure; FBS, fasting blood sugar; GGT, γ -glutamyltransferase; HOMA-IR, homoeostasis model of assessment-estimated insulin resistance; NAFLD, nonalcoholic fatty liver disease; QUICKI, Quantitative insulin sensitivity check index; and SBP, systolic blood pressure.

[2]*P* values were computed by univariate ANCOVA.

[*]Adjusted for baseline value of the outcome, mean change in food groups, and MET value.

[**]Adjusted for baseline value of the outcome, mean change in food groups, energy intake, weight, BMI, WHR, and MET value.

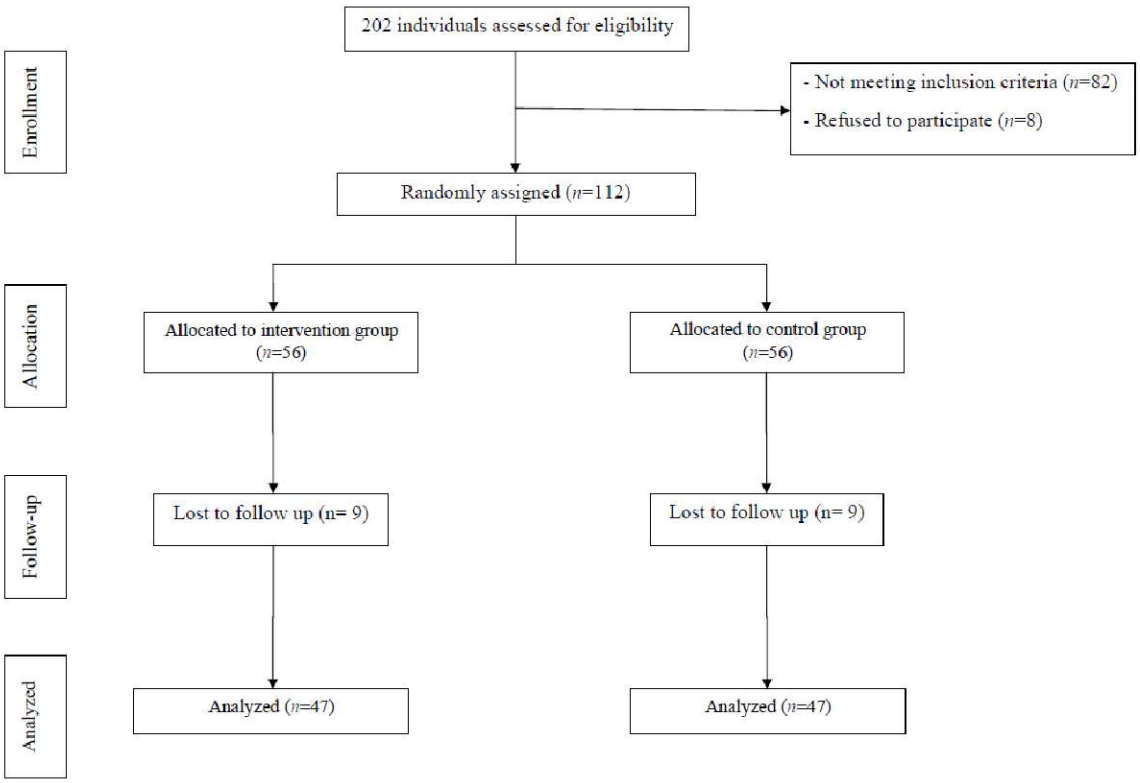

Figure 1 Summary of patient flow diagram.