The Effect of 6-months Fruit-rich Diet on Liver Steatosis, Liver Enzymes, Insulin Resistance, and Lipid Profile in Patients With Non-alcoholic Fatty Liver Disease: a Randomized Clinical Trial

Farkhondeh Alami
Urmia University of Medical Sciences
Mohammad Alizadeh
Urmia University of Medical Sciences
Kamran Shateri (shatterikamran@yahoo.com)
Urmia University of Medical Sciences https://orcid.org/0000-0003-1178-2231

Research

Keywords: non-alcoholic fatty liver disease, fruit, steatosis, triglyceride, insulin resistance, steatohepatitis

Posted Date: October 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-996590/v1

Abstract

Background

Despite various recommended dietary approaches to improve the Non-Alcoholic Fatty Liver Disease (NAFLD), the effect of fruits is not clear. In addition, observational studies reported conflicting results. The present study aimed to evaluate the effect of fruit rich diet (FRD) on liver steatosis, liver enzymes, Insulin resistance, and lipid profile in patients with NAFLD.

Methods

Eighty adults with a diagnosed NAFLD participated in this randomized controlled trial. Subjects were randomly assigned to the FRD group with consumption of at least 4 servings/day or the control group with consumption of less than 2 servings/day. The grade of steatosis, serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), lipid profile, fasting blood sugar (FBS), and insulin resistance were evaluated at the baseline and at the end of the study.

Results

The mean daily intake of fruits during the study was 6.96±0.61 and 1.65±0.17 servings in the FRD and control groups, respectively. After 6 months of intervention, there was a significant increase in BMI ($P<0.001$), the grade of steatosis ($P<0.001$), liver enzymes ($P<0.001$), dyslipidemia ($P<0.001$), FBS ($P<0.001$), and insulin resistance ($P<0.001$) in the FRD group. In contrast, an improvement was observed in BMI ($P<0.001$), steatosis ($P<0.001$), lipid profile ($P<0.05$), and insulin resistance ($P<0.001$) in the control group. Adjusting for the effect of change in energy and other food groups intake, and BMI did not alter the findings of the study.

Conclusion

The present study showed that consumption of fruits more than 4 servings/day exacerbates steatosis, dyslipidemia, and glycemic control in NAFLD patients.

Trial registration

This trial was registered at Iranian randomized clinical trial website with IRCT registration no. IRCT20201010048982N1on October 15, 2020.

Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is characterized by the accumulation of fat in the hepatic parenchymal hepatocytes more than 5% of the liver weight, without a history of high amounts of alcohol consumption. NAFLD can lead to a variety of histological problems, from steatosis to inflammation,

necrosis, fibrosis, cirrhosis, and eventually liver cancer [1]. Epidemiologic studies show that NAFLD is the most prevalent liver disease worldwide with an estimated prevalence of 25% in total population currently, with the highest rate in South America and the Middle East [2]. In recent years, the mortality from chronic liver disease increased and in 2019 it was 10th cause of death worldwide [3]. It has been reported that the NAFLD along with fibrosis increases the mortality rate by 30% [4]. Currently, the tissue biopsy is the gold standard of the NAFLD diagnosis. Since the liver biopsy is an invasive and expensive procedure, it is not suitable for general screening, and ultrasonography, Computed Tomography (CT), and Magnetic Resonance (MRI) may be used to evaluate the amount of liver fat [5]. Ultrasound is a tool for early detection of fatty liver disease, which is less sensitive and specific for grade 1 steatosis than grade 2 and 3 non-alcoholic fatty liver [6]. The sensitivity and specificity of ultrasound to detect hepatic fat content decreases with increasing of body mass index (BMI) and increases with the high degree of fat penetration in the liver and BMI between 18.5 to 30 kg/m^2 and at least 33% of steatosis is optimal for the diagnosis of NAFLD by ultrasonography [7].

The pathology of NAFLD has not been yet well understood and molecular mechanisms are currently being investigated. Macro-vesicular steatosis is the result of increased intake or hepatic synthesis of fatty acids [1]. Impaired regulation of fatty acids and consequent steatosis is mainly caused by elevated levels of insulin, which can make the liver more vulnerable to oxidative damage [8]. Patients with non-alcoholic fatty liver disease often have other conditions such as hypertriglyceridemia, hypertension, and other factors of insulin resistance syndrome [9]. The nutritional risk factors for fatty liver include high intake of saturated fatty acids (SFA), trans fatty acids (TFA), simple carbohydrates (CHO), sweetened beverages, and fructose [10].

Medication for NAFLD is very limited and their long-term effects have not been well understood. Various dietary approaches have been recommended to improve the disease, including the Mediterranean diet [11], replacing SFA with mono-unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) [12], reducing fructose and simple sugars [13], and increasing dietary fiber intake [14]. The effect of fruit, which in most cases is a part of a healthy diet, is not clear in the case of NAFLD. The high content of fiber, antioxidants, flavonoids, carotenoids, vitamins, especially vitamin C, and prebiotic properties of some fruits could have beneficial effects on liver health [15]. On the other hand, the high amount of fructose in fruits has raised concerns about lipogenic properties and its complications, including hepatic steatosis [16]. Observational studies have found conflicting results in relationship between fatty liver and fruit consumption [17, 18]. To the best of our knowledge, no clinical trial has studied the effect of a fruit-rich diet (FRD) on liver function in patients with NAFLD. This study aimed to evaluate the effect of FRD on liver steatosis, liver enzymes, insulin resistance, and lipid profile in patients with NAFLD.

Materials And Methods

Study design and participants (Recruitment and eligibility screening)

A randomized controlled trial was performed to evaluate the effect of the FRD for 6 months on NAFLD outcomes. The sample size was calculated according to the study of Cantero et al. [19] using the following formula:

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 \left(2{\sigma_{diff}}^2\right)}{\delta^2}$$

The α and 1-β were considered equals to 0.05 and 0.90, respectively. The δ were considered as 10 units of change in the ALT levels in intervention group compared to the placebo group. The σ at the baseline and end of the study was considered as 16.5 (the mean of baseline ALT SD of two interventional groups) and 8.0 (the mean of post-intervention ALT SD of two interventional groups), respectively, and σ_{diff} was calculated to be equal to 11.18. This equation estimated 28 cases in each group, and taking into account 30% of the drop-out, the sample size of 40 people for each group was considered. Eighty subjects were recruited between October 2020 to March 2021 from patients with NAFLD referred to the gastrointestinal and liver clinic in Imam Khomeini University Hospital in Urmia., Iran. All participants gave their written informed consent before entering the study. The written informed consent was signed by all study subjects. This study registered in Iranian randomized clinical trial website with IRCT registration no. IRCT20201010048982N1. This study was approved by Ethics committee at the Urmia University of Medical Sciences (Ethic number: IR.UMSU.REC.1398.535, Date: 02/03/2020).Inclusion criteria were defined as age older than 18 years, BMI between 18.5 to 29.9 kg/m^2, and presence of grade 2 or 3 of NAFLD confirmed by gastroenterology and liver specialist. Individuals with viral hepatitis, diabetes mellitus, mental disorders, not-treated hypothyroidism, renal diseases, heart failures, bone diseases, gastrointestinal diseases (such as celiac), α1-antitrypsin deficiency, history of alcohol consumption,; using of nonsteroidal anti-inflammatory drugs (NSAIDs), cholesterol-lowering drugs (such as statins), phenytoin, carbamazepine, and barbiturates (such as phenobarbital); following a certain diet; pregnant, lactating, and menopause women; and smokers (smoking more than 5 cigarettes/week), were not included to the study. The present study was conducted following the deceleration of Helsinki and the Ethics Committee in Urmia University of Medical Sciences approved the protocol of the study.

Randomization and intervention

The flowchart of participants' enrollment was presented in Figure 1. The Stratified Blocked Randomization was performed by an independent statistician by the grade of NAFLD, age, and gender. A blinded person to the aims of the study and patients' baseline status assigned participants to the two groups using sealed envelopes. Patients based on inclusion and exclusion criteria were assigned to the FRD and control groups. Subjects in the FRD group were recommended to consume at least 4 servings of fruits per day and the control group was asked not to consume more than 2 servings of fruit per day. The category of fruits was based on: 1) colored fruits 2) dried fruits 3) and other fruits. To eliminate the effect of pesticides on NAFLD, participants were recommended to unpeel fruits or if they want to eat fruit with the peel, to consume after 20-30 minutes soaking in water. For the same consumption of other food

groups, both groups were advised to follow the recommendations of the Food and Agriculture Organization (FAO) for Iranians [20].

Procedures

At the baseline, the data including gender, age, level of education, family size, duration of NAFLD, physical activity, energy intake, type and dose of medication, herbal medicines and dietary supplements, marital status, place of residence, income, other chronic disease histories, and familial history of the disease was obtained using a general questionnaire. Anthropometric measurements and ultrasonography were performed at the start and end of the study. Moreover, 5 mm of venous blood samples were collected at the baseline and after the intervention to conduct biochemical assessments. After the assignment, participants were asked not to change their physical activity, and medications during the study. To ensure the consumption of fruits within the recommended range, as well as assessing of other food groups consumption, three 24-hours food recalls (two non-consecutive days and one day off) were taken from individuals each month (totally 18 food recalls). In addition, the physical activity was assessed using a metabolic equivalents (MET) questionnaire every month [21]. Patients were called every week and the necessary reminders were made. Those who received less than 4 servings of fruits in the intervention group or more than 2 servings of fruits in the control group were excluded from the study.

Primary outcomes

The primary outcomes of the study were the grade of steatosis, serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL-c), high-density lipoprotein (HDL-c), fasting blood sugar (FBS), insulin, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), and Quantitative Insulin-Sensitivity Check Index (QUICKI).

Secondary outcomes

Secondary outcomes included weight, body mass index (BMI), and waist circumference (WC).

Biochemical assessments

The blood samples were collected at the baseline and end of the study between 7:00 to 9:00 am, after 12 hours of fasting. Blood samples were centrifuged at 4000 rpm for 10 minutes and the isolated serums were stored at -80° C until biochemical analysis. Measurement of serum insulin levels was performed using the enzyme-linked immunosorbent assay (ELISA) kits (Pars Azmoon Co, Tehran, Iran). Serum concentrations of ALT, AST, ALP, GGT, TG, TC, HDL-c, LDL-c, and FBS were assessed using BT1500 autoanalyzer (Biotecnica Instrument SpA, Rome, Italy). The following formulas were used to calculate HOMA-IR and QUICKI:

HOMA-IR= [(Fasting Serum Glucose, mmol/L) × Fasting Serum Insulin, μIU/mL)] / 22.5

QUICKI= [1 / (log (Fasting Serum Insulin) + log (Fasting Serum Glucose))]

Liver steatosis

The liver condition was evaluated under at least 6 hours of fasting by an experienced radiologist. To assess the severity of steatosis the ultrasonography (Siemens ACUSON S2000 Siemens Healthcare, Erlangen, Germany) was performed with previously described methodology [22]. The amount of fat accumulation is associated with an increase in the degree of echogenicity in ultrasound. Accordingly, steatosis was divided into 4 degrees: grade 0 with normal echogenicity, grade 1 or mild in which the echogenicity of the liver increases and the ability to see blood vessels and sound penetration in the liver tissue is normal, grade 2 or moderate in that the vascular wall are seen vaguely and the sound penetration is reduced, and grade 3 or severe, in which the arteries are difficult to see and the sound penetration is very limited. Due to a lower sensitivity and specificity of ultrasonography in diagnosis of grade 1 steatosis, in the present study the subjects with grades 2 and 3 were only recruited. As mentioned earlier, this method is most accurate at BMI between 18.5 and 30, so the participants were recruited in the same range. The size of the liver was also divided into large and normal by the radiologist based on its appearance.

Anthropometric measurements

A digital scale and stadiometer were used to assess the weight and height of the patients with a precision of 100 gr and 0.1 cm, respectively. Measurements were performed with the minimal dress and without shoes. To calculate the BMI, the weight (kg) was divided by the square of height (m^2). WC was measured using a flexible tape at the midpoint of the lowest rib and the iliac crest hip bone. All measurements were repeated 3 times, and the mean of measurements was used to establish the re-test reliability.

Statistical Methods

Quantitative and qualitative variables were presented as mean ± SD and frequency (%), respectively. To calculate the change of dietary intakes, baseline values were subtracted from mean intakes of each food groups throughout the 6 months. The normality of the quantitative variables was evaluated using the Kolmogorov–Smirnov test. The independent sample t-test was used to compare quantitative variables (or their log-transformed) between groups. Also, the paired sample t-test was used to compare the values before and after the study. Moreover, the repeated measure ANOVA was used to compare the change in dietary intake and physical activity in different time frames (baseline, 1st, 2nd, 3rd, 4th, 5th, and 6th months). To adjust the effect of change in BMI and intake of energy and other food groups, the analysis of covariance (ANCOVA) was used. The Chi-square test was used to compare the frequency of qualitative variables between two groups. Statistical analyses were conducted using SPSS software version 25 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY). The P-value < 0.05 was considered statistically significant.

Results

General characteristics

The baseline general characteristics of the participants were presented in Table 1. Totally, 32 males (16 FRD and 16 controls) and 40 females (20 FRD and 20 controls) with a mean age of 46.25 ± 9.80 years participated in the study. Two participants lost to follow-up. In addition, 2 of them discontinued participating in the study. Four participants were excluded from the study due to low compliance. No significant difference was observed between age, education status, family size, duration of disease, gender, and marital status between the intervention and control groups (P>0.05). However, subjects in the control group had significantly higher income compared to the FRD group.

Table 1
General characteristics of the non-alcoholic fatty liver disease participants

Variable		Total (n=72)	FRD[1] (n=36)	Control (n=36)	P[2]
Age (years)		46.25 (9.80)	47.39 (10.29)	45.11 (9.28)	0.33
Education (years)		7.71 (5.14)	7.50 (5.15)	7.92 (5.20)	0.73
Family size (numbers)		4.29 (1.22)	4.39 (1.29)	4.19 (1.16)	0.50
Disease duration		3.53 (1.65)	3.39 (1.55)	3.67 (1.75)	0.48
Monthly income (Million Tomans)		3.42 (0.99)	3.14 (0.79)	3.69 (1.09)	0.02
Gender	Female	40 (55.6)	20 (55.6)	20 (55.6)	1.00
	Male	32 (44.4)	16 (44.4)	16 (44.4)	
Marital status	Married	71 (98.6)	36 (100)	35 (97.2)	1.00
	Single	1 (1.4)	0 (0.0)	1 (2.8)	

Data are presented as mean (SD) for quantitative and frequency (%) for qualitative variables.[1] FRD, fruits rich diet; [2] Calculated using independent sample t-test or chi-square.

Dietary intake and physical activity

Table 2 shows the dietary intake and physical activity of participants during the study. During the study, the mean ± SD intake of fruits in the FRD and control group was 6.96±0.61 and 1.65±0.17 serving/day, respectively. At the end of the study, there was a significant increase in fruits (P<0.001), bread and cereals (P<0.001), meats (P=0.002), vegetables (P=0.01), dairies (P=0.001), fats and oils (P<0.001), and energy intake (P<0.001) and a significant decrease in sugars intake (P=0.001) compared to the baseline in the FRD group. In the control group, a significant decrease in fruit intake (P<0.001) and increase in the intake of bread and cereals (P<0.001), meats (P=0.015), vegetables (P<0.001), dairies (P<0.001), sugars (P<0.001), fats and oils (P<0.001), and energy intake (P<0.001) was observed after 6 months compared to the baseline. Between-group analysis in the change of dietary intake during the study showed that the FRD group compared to control group increased daily servings of fruit intake (+3.59 vs. -0.95, respectively,

P <0.001) and decreased sugar intakes (-1.93 vs. +0.46, respectively, P<0.001). In contrast, a higher intake of vegetables was observed in the control group, compared to the FRD group (+2.29 vs. +0.75, respectively, P<0.001). The mean change of other food groups and energy were not significantly different between two groups. There was no difference in physical activity change between two groups during the study (P=0.792).

Table 2
Comparison of dietary intakes and physical activity between FRD and control groups at the baseline and following intervals[1]

Variable[2]		FRD (n=36)	Control (n=36)	P[3]
Total fruits (servings/day)	Baseline	3.37 (1.16)	2.61 (1.17)	**0.007**
	1st month	7.27 (1.28)	1.64 (0.40)	**<0.001**
	2nd month	7.23 (1.43)	1.60 (0.47)	**<0.001**
	3rd month	6.88 (1.35)	1.51 (0.46)	**<0.001**
	4th month	7.09 (1.59)	1.75 (0.40)	**<0.001**
	5th month	6.64 (1.12)	1.61 (0.42)	**<0.001**
	6th month	6.66 (0.76)	1.81 (0.37)	**<0.001**
	Change[4]	3.59 (1.26)	-0.95 (1.19)	**<0.001**
	P[5]	**<0.001**	**<0.001**	
Colored fruits (servings/day)	Baseline	1.61 (0.47)	1.43 (0.50)	**0.126**
	1st month	2.15 (1.53)	0.53 (0.56)	**<0.001**
	2nd month	3.11 (1.85)	0.70 (0.80)	**<0.001**
	3rd month	2.70 (1.44)	0.36 (0.54)	**<0.001**
	4th month	3.20 (2.34)	0.94 (0.78)	**<0.001**
	5th month	4.03 (1.61)	1.26 (0.67)	**<0.001**
	6th month	4.93 (1.50)	1.56 (0.59)	**<0.001**
	Change	1.74 (1.14)	-0.53 (0.56)	**<0.001**
	P[4]	**<0.001**	**<0.001**	
Dried fruits (servings/day)	Baseline	0.65 (0.49)	0.44 (0.47)	0.071
	1st month	2.61 (2.11)	0.20 (0.50)	**<0.001**
	2nd month	2.20 (1.93)	0.31 (0.54)	**<0.001**
	3rd month	2.24 (2.03)	0.21 (0.43)	**<0.001**
	4th month	1.88 (2.05)	0.31 (0.46)	**<0.001**

[1] FRD, fruit-rich diet. [2] data are presented as mean (SD). [3] Calculated using independent sample t-test. [4]The difference between baseline and mean of six values during study [5]Calculated using repeated measure ANOVA to compare intakes during six months.

Variable[2]		FRD (n=36)	Control (n=36)	P[3]
	5th month	1.49 (1.36)	0.11 (0.29)	**<0.001**
	6th month	1.04 (1.08)	0.19 (0.40)	**<0.001**
	Change	1.26 (1.14)	-0.21 (0.55)	**<0.001**
	P[4]	0.056	**<0.001**	
Other fruits (servings/day)	Baseline	1.11 (0.54)	0.73 (0.62)	**0.009**
	1st month	2.45 (2.61)	0.90 (0.74)	**0.001**
	2nd month	1.84 (1.94)	0.64 (0.79)	**0.001**
	3rd month	1.96 (1.98)	0.83 (0.61)	**0.002**
	4th month	1.91 (2.39)	0.47 (0.56)	**0.001**
	5th month	1.12 (1.49)	0.27 (0.48)	**0.002**
	6th month	0.89 (1.48)	0.07 (0.27)	**0.002**
	Change	0.59 (1.29)	-0.20 (0.61)	**0.002**
	P[4]	**0.023**	**<0.001**	
Cereals (servings/day)	Baseline	8.81 (1.26)	8.47 (1.00)	0.201
	1st month	10.54 (1.25)	10.64 (1.51)	0.757
	2nd month	10.31 (1.36)	10.06 (1.36)	0.448
	3rd month	10.80 (1.48)	10.40 (1.19)	0.213
	4th month	10.64 (1.52)	10.22 (1.46)	0.235
	5th month	10.37 (1.51)	10.40 (1.06)	0.924
	6th month	10.44 (1.27)	10.17 (1.15)	0.351
	Change	1.70 (1.44)	1.84 (1.16)	0.637
	P[4]	**<0.001**	**<0.001**	
Meats and poultry (servings/day)	Baseline	4.31 (0.68)	4.41 (0.79)	0.581
	1st month	5.30 (1.34)	5.29 (1.14)	0.973
	2nd month	4.98 (1.31)	4.95 (1.05)	0.918

[1] FRD, fruit-rich diet. [2] data are presented as mean (SD). [3] Calculated using independent sample t-test. [4]The difference between baseline and mean of six values during study [5]Calculated using repeated measure ANOVA to compare intakes during six months.

Variable[2]		FRD (n=36)	Control (n=36)	P[3]
	3rd month	5.34 (1.36)	5.05 (1.48)	0.388
	4th month	5.48 (1.27)	4.76 (1.30)	**0.020**
	5th month	5.37 (1.37)	4.66 (1.26)	0.028
	6th month	4.90 (1.23)	4.81 (1.03)	0.737
	Change	0.91 (1.03)	0.50 (0.93)	0.085
	P[4]	**0.002**	**0.015**	
Vegetables (servings/day)	Baseline	4.48 (1.12)	3.02 (1.69)	<0.001
	1st month	5.03 (1.18)	5.35 (1.21)	0.257
	2nd month	5.43 (1.14)	5.22 (1.25)	0.462
	3rd month	5.11 (1.02)	5.62 (1.14)	0.050
	4th month	5.36 (1.06)	5.38 (1.20)	0.935
	5th month	5.20 (1.16)	5.04 (1.12)	0.574
	6th month	5.28 (1.12)	5.31 (1.45)	0.922
	Change	0.75 (1.06)	2.29 (1.73)	<0.001
	P[4]	**0.010**	**<0.001**	
Dairies (servings/day)	Baseline	1.65 (0.48)	1.42 (0.60)	0.081
	1st month	2.16 (0.86)	2.36 (0.73)	0.288
	2nd month	2.31 (0.86)	2.06 (0.85)	0.231
	3rd month	2.17 (0.69)	2.25 (0.82)	0.630
	4th month	2.35 (0.84)	2.32 (0.84)	0.901
	5th month	2.19 (0.75)	2.53 (0.85)	0.080
	6th month	2.47 (0.78)	2.48 (0.86)	0.978
	Change	0.62 (0.76)	0.91 (0.75)	0.109
	P[4]	**0.001**	**<0.001**	
Sugars (servings/day)	Baseline	5.18 (1.90)	2.88 (1.24)	**<0.001**

[1] FRD, fruit-rich diet. [2] data are presented as mean (SD). [3] Calculated using independent sample t-test. [4]The difference between baseline and mean of six values during study [5]Calculated using repeated measure ANOVA to compare intakes during six months.

Variable[2]		FRD (n=36)	Control (n=36)	P[3]
	1st month	3.12 (0.90)	3.31 (1.13)	0.428
	2nd month	3.40 (1.20)	3.49 (0.87)	0.719
	3rd month	3.34 (1.07)	3.05 (0.77)	0.203
	4th month	3.33 (1.02)	3.21 (0.82)	0.589
	5th month	3.23 (0.99)	3.33 (0.81)	0.636
	6th month	3.05 (1.04)	3.70 (0.82)	**0.004**
	Change	-1.93 (2.26)	0.46 (1.21)	<0.001
	P[4]	**0.001**	**<0.001**	
Fats and oils (servings/day)	Baseline	4.22 (0.88)	4.13 (0.76)	0.645
	1st month	5.72 (1.03)	5.88 (1.07)	0.527
	2nd month	5.70 (1.12)	5.59 (1.16)	0.678
	3rd month	5.77 (1.00)	5.22 (1.21)	0.042
	4th month	5.45 (1.00)	5.58 (1.19)	0.621
	5th month	5.82 (0.94)	5.73 (1.06)	0.707
	6th month	5.38 (1.23)	5.59 (1.18)	0.458
	Change	1.42 (1.01)	1.47 (1.12)	0.845
	P[4]	**<0.001**	**<0.001**	
Energy intake (kcal/day)	Baseline	1900.98 (160.37)	1624.93 (163.97)	**<0.001**
	1st month	2306.89 (213.50)	2018.60 (254.84)	**<0.001**
	2nd month	2321.2 (193.7)	1959.3 (221.7)	**<0.001**
	3rd month	2293.8 (169.7)	1955.8 (205.1)	**<0.001**
	4th month	2299.9 (193.9)	1968.0 (180.9)	**<0.001**
	5th month	2276.8 (185.6)	1981.4 (161.3)	**<0.001**
	6th month	2226.2 (207.5)	2024.5 (164.7)	**<0.001**
	change	487.9 (623.9)	359. (179.3)	0.240

[1] FRD, fruit-rich diet. [2] data are presented as mean (SD). [3] Calculated using independent sample t-test. [4]The difference between baseline and mean of six values during study [5]Calculated using repeated measure ANOVA to compare intakes during six months.

Variable[2]		FRD (n=36)	Control (n=36)	P[3]
	P[4]	**<0.001**	**<0.001**	
Physical activity (METs.hr/day)	Baseline	32.86 (0.93)	32.71 (0.78)	0.471
	1st month	32.91 (1.14)	32.59 (1.17)	0.242
	2nd month	32.44 (1.05)	32.50 (1.06)	0.819
	3rd month	32.52 (1.06)	32.66 (1.16)	0.599
	4th month	33.07 (0.96)	32.66 (1.08)	0.093
	5th month	32.81 (1.04)	32.66 (1.00)	0.559
	6th month	32.86 (0.87)	32.24 (2.20)	0.122
	Change	-0.09 (1.03)	-0.15 (1.14)	0.792
	P[4]	0.131	0.827	

[1] FRD, fruit-rich diet. [2] data are presented as mean (SD). [3] Calculated using independent sample t-test. [4]The difference between baseline and mean of six values during study [5]Calculated using repeated measure ANOVA to compare intakes during six months.

Liver enzymes

Table 3 compares the mean ± SD of the liver enzymes between two groups at the baseline and end of the study. According to the paired t-test, at the end of the study, there was a significant increase in the serum levels of ALT, AST, ALP, GGT compared to the baseline in the FRD group (P<0.001). In contrast, there was a significant decrease in all liver enzymes in the control group during the study (P<0.001). After 6 months, the FRD group had higher serum levels of ALT, AST, ALP, and GGT compared to the control group. Adjustments for the effect of change in BMI, energy, bread and cereals, meats, vegetables, dairies, sugars, fats, and oils intake in the ANCOVA models did not change these findings.

Table 3
Comparison of liver enzymes, lipid profile, and glycemic control between FRD and control groups at the baseline and after six months[1]

Variable[2]		FRD (n=36)	Control (n=36)	P[3]	P-adjusted 1[4]	P-adjusted 2[5]	P-adjusted 3[6]
ALT (IU/L)	Baseline*	38.1 (25.3)	50.0 (35.7)	**0.02**	**0.03**	**0.029**	**0.01**
	6th month*	89.1 (92.9)	32.0 (19.2)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Changes*	51.0 (83.3)	-18.0 (26.1)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	P[7]	**<0.001**	**<0.001**				
AST (IU/L)	Baseline*	26.8 (11.0)	36.5 (19.8)	**0.01**	**0.01**	**0.01**	**0.01**
	6th month*	74.5 (107.8)	24.0 (8.5)	**<0.001**	**<0.001**	**0.01**	**<0.001**
	Changes*	47.7 (104.1)	-12.5 (16.8)	**<0.001**	**<0.001**	**0.01**	**<0.001**
	P[7]	**<0.001**	**<0.001**				
ALP (IU/L)	Baseline*	189.4 (73.2)	211.1 (80.7)	0.16	0.26	0.17	0.24
	6th month	273.4 (128.5)	155.0 (43.9)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Changes*	84.0 (95.9)	-56.1 (62.7)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	P[7]	**<0.001**	**<0.001**				
GGT (IU/L)	Baseline*	40.8 (26.4)	55.9 (73.2)	0.43	0.90	0.44	0.84

[1] FRD, food-rich diet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TC, total cholesterol; TG, triglyceride; LDL-c, low-density lipoprotein; HDL-c, high-density lipoprotein; FBS, fasting blood sugar; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, Quantitative Insulin-Sensitivity Check Index.[2] Data are presented as mean (SD). [3] Calculated using independent sample t-test. [4] Calculated using ANCOVA, adjusted for the effect change in energy intake. [5] Calculated using ANCOVA, adjusted for the effect of change in bread and cereals, meats, vegetables, dairies, and oils intake. [6] Calculated using ANCOVA, adjusted for the effect of BMI change. [7]Calculated using paired sample t-test. * Log-transformed were entered into the analysis

Variable[2]		FRD (n=36)	Control (n=36)	P[3]	P-adjusted 1[4]	P-adjusted 2[5]	P-adjusted 3[6]
	6th month*	92.7 (161.2)	21.2 (7.7)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Changes*	51.9 (143.5)	-34.7 (70.8)	**<0.001**	**<0.001**	**0.04**	**<0.001**
	P[7]	**<0.001**	**<0.001**				
TG (mg/dl)	Baseline	183.2 (100.8)	242.5 (109.6)	**0.02**	**0.01**	**0.03**	0.20
	6th month*	248.6 (125.0)	153.5 (84.4)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Changes*	65.4 (123.6)	-88.9 (79.9)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	P[7]	**<0.001**	**0.01**				
TC (mg/dl)	Baseline	174.6 (35.5)	209.4 (38.7)	**<0.001**	**0.01**	**<0.001**	**0.02**
	6th month	206.1 (40.5)	172.7 (42.4)	**0.01**	**0.01**	**0.01**	**0.01**
	Changes	31.6 (28.6)	-36.7 (35.9)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	P[7]	**<0.001**	**<0.001**				
LDL-c (mg/dl)	Baseline	99.9 (29.4)	120.7 (29.3)	**0.01**	**0.01**	**0.01**	0.06
	6th month	126.9 (32.3)	99.8 (29.8)	**<0.001**	**0.01**	**0.17**	**0.01**
	Changes	26.9 (27.5)	-20.9 (27.4)	**<0.001**	**<0.001**	**0.01**	**<0.001**
	P[7]	**<0.001**	**<0.001**				

[1] FRD, food-rich diet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TC, total cholesterol; TG, triglyceride; LDL-c, low-density lipoprotein; HDL-c, high-density lipoprotein; FBS, fasting blood sugar; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, Quantitative Insulin-Sensitivity Check Index.[2] Data are presented as mean (SD). [3] Calculated using independent sample t-test. [4] Calculated using ANCOVA, adjusted for the effect change in energy intake. [5] Calculated using ANCOVA, adjusted for the effect of change in bread and cereals, meats, vegetables, dairies, and oils intake. [6] Calculated using ANCOVA, adjusted for the effect of BMI change. [7]Calculated using paired sample t-test. * Log-transformed were entered into the analysis

Variable[2]		FRD (n=36)	Control (n=36)	P[3]	P-adjusted 1[4]	P-adjusted 2[5]	P-adjusted 3[6]
HDL-c (mg/dl)	Baseline	50.4 (11.1)	42.1 (10.2)	**0.01**	**0.01**	0.01	0.05
	6th month	41.4 (8.9)	53.8 (15.1)	**<0.001**	**0.01**	**0.01**	**<0.001**
	Changes	-9.0 (8.0)	11.7 (11.5)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	P[7]	**<0.001**	**<0.001**				
FBS (mg/dl)	Baseline	96.9 (9.4)	119.1 (49.9)	**0.01**	**0.01**	**0.01**	**0.03**
	6th month	115.5 (30.0)	97.7 (19.0)	**0.01**	**0.01**	**0.06**	**0.01**
	Changes	18.6 (25.7)	-21.4 (39.0)	**<0.001**	**<0.001**	**0.01**	**<0.001**
	P[7]	**<0.001**	**<0.001**				
Insulin (µU/ml)	Baseline*	14.0 (5.7)	18.0 (14.1)	**0.13**	0.19	0.11	0.99
	6th month*	26.6 (15.9)	11.5 (6.4)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Changes*	12.5 (15.3)	-6.5 (12.6)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	P[7]	**<0.001**	**<0.001**				
HOMA-IR	Baseline*	3.32 (1.41)	4.92 (3.45)	**0.01**	**0.01**	0.01	0.33
	6th month*	7.36 (4.37)	2.66 (1.27)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Changes*	4.03 (4.24)	-2.26 (3.13)	**<0.001**	**<0.001**	**<0.001**	**<0.001**

[1] FRD, food-rich diet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TC, total cholesterol; TG, triglyceride; LDL-c, low-density lipoprotein; HDL-c, high-density lipoprotein; FBS, fasting blood sugar; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, Quantitative Insulin-Sensitivity Check Index.[2] Data are presented as mean (SD). [3] Calculated using independent sample t-test. [4] Calculated using ANCOVA, adjusted for the effect change in energy intake. [5] Calculated using ANCOVA, adjusted for the effect of change in bread and cereals, meats, vegetables, dairies, and oils intake. [6] Calculated using ANCOVA, adjusted for the effect of BMI change. [7]Calculated using paired sample t-test. * Log-transformed were entered into the analysis

Variable[2]		FRD (n=36)	Control (n=36)	P[3]	P-adjusted 1[4]	P-adjusted 2[5]	P-adjusted 3[6]
	P[7]	**<0.001**	**<0.001**				
QUICKI	Baseline	0.32 (0.02)	0.31 (0.02)	**0.01**	**0.01**	0.01	0.29
	6th month	0.29 (0.01)	0.33 (0.02)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Changes	-0.03 (0.02)	0.02 (0.01)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	P[7]	**<0.001**	**<0.001**				
Weight (kg)	Baseline	79.4 (9.9)	78.2 (9.7)	0.59	0.71	-	0.82
	6th month	86.4 (9.5)	71.7 (10.2)	**<0.001**	**<0.001**	**-**	**<0.001**
	Changes	7.0 (3.0)	-6.5 (2.8)	**<0.001**	**<0.001**	**-**	**<0.001**
	P[7]	**<0.001**	**<0.001**				
BMI (kg/m^2)	Baseline	28.37 (2.09)	27.78 (2.43)	0.27	0.42	-	0.35
	6th month	31.40 (2.61)	25.68 (2.54)	**<0.001**	**<0.001**	**-**	**<0.001**
	Changes	3.03 (1.36)	-2.09 (1.13)	**<0.001**	**<0.001**	**-**	**<0.001**
	P[7]	**<0.001**	**<0.001**				
WC (cm)	Baseline	109.7 (11.3)	107.1 (8.0)	0.28	0.65	-	0.10
	6th month	113.5 (10.7)	100.5 (7.5)	**<0.001**	**<0.001**	**-**	**<0.001**
	Changes	3.9 (2.5)	-6.6 (5.0)	**<0.001**	**<0.001**	**-**	**<0.001**

[1] FRD, food-rich diet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TC, total cholesterol; TG, triglyceride; LDL-c, low-density lipoprotein; HDL-c, high-density lipoprotein; FBS, fasting blood sugar; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, Quantitative Insulin-Sensitivity Check Index.[2] Data are presented as mean (SD). [3] Calculated using independent sample t-test. [4] Calculated using ANCOVA, adjusted for the effect change in energy intake. [5] Calculated using ANCOVA, adjusted for the effect of change in bread and cereals, meats, vegetables, dairies, and oils intake. [6] Calculated using ANCOVA, adjusted for the effect of BMI change. [7]Calculated using paired sample t-test. * Log-transformed were entered into the analysis

Variable[2]		FRD (n=36)	Control (n=36)	P[3]	P-adjusted 1[4]	P-adjusted 2[5]	P-adjusted 3[6]
	P[7]	**<0.001**	**<0.001**				

[1] FRD, food-rich diet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TC, total cholesterol; TG, triglyceride; LDL-c, low-density lipoprotein; HDL-c, high-density lipoprotein; FBS, fasting blood sugar; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, Quantitative Insulin-Sensitivity Check Index.[2] Data are presented as mean (SD). [3] Calculated using independent sample t-test. [4] Calculated using ANCOVA, adjusted for the effect change in energy intake. [5] Calculated using ANCOVA, adjusted for the effect of change in bread and cereals, meats, vegetables, dairies, and oils intake. [6] Calculated using ANCOVA, adjusted for the effect of BMI change. [7]Calculated using paired sample t-test. * Log-transformed were entered into the analysis

Lipid profile

After 6 months of intervention, subjects in the FRD group had higher levels of TG (P<0.001), total cholesterol (P<0.001), and LDL-c (P<0.001), and a lower level of HDL-c (P<0.001) compared to the baseline. In the control group, a decrease in TG (P=0.003), TC (P<0.001), and LDL-c (P<0.001) and an increase in HDL-c (P<0.001) was observed. Between-groups analysis showed that the FRD group had a higher level of TG (P<0.001), total cholesterol (P=0.001), and LDL-c (P<0.001) and lower levels of HDL-c (P<0.001) at the end of the study compared to the control group. However, the difference between the two groups in the LDL-c was not significant after adjusting for the effect of BMI change (P=0.17). Adjustment for changes in energy and dietary intakes and the BMI in the ANCOVA model did not change these results for other variables.

Glycemic control

The before-after comparison showed a significant increase in the serum FBS (P<0.001), insulin (P<0.001), and HOMA-IR (P<0.001) and a significant decrease in QUICKI (P<0.001) in the FRD group. The control group had a significant reduction in the FBS (P<0.001), serum insulin (P<0.001), and HOMA-IR (P<0.001), and a significant increase in the QUICKI (P<0.001) at the end of the study compared to the baseline. Following 6 months of intervention, the FRD group had a higher FBS, serum insulin, and HOMA-IR and a lower QUCKI compared to the control group. Nevertheless, the between-groups difference in the FBS was not statistically significant after adjusting for the effect of BMI change (P=0.06). Other findings were not changed after adjustment of the effect of changes in energy and dietary intakes and BMI.

Anthropometric measures

The results showed a significant increase in weight, BMI, and WC in the FRD group after 6 months of intervention (P<0.001). The analysis in the control group showed a significant decrease in all of these

variables (P<0.001). At the baseline, there was no difference between the two groups in weight (P=0.82), BMI (P=0.35), and WC (P=0.10). However, at the end of the study the FRD group had a higher weight (P<0.001), BMI (P<0.001), and WC (P<0.001).

Liver sonography

Figure 2 shows the frequency of subjects with a mild, moderate, or severe grade of steatosis in two groups. Before study (2A) there was no difference between groups in grade of steatosis (P=1.00). After 6 months (2B) the frequency of severe and moderate steatosis was significantly higher in the FRD group (P<0.001).

As shown in figure 3A, there was no significant difference in the size of the liver before the study. At the end of the study (3B), most of the participants in the FRD group had a large liver, but the size of the liver in the control group was normal (P<0.001).

Discussion

The present study investigated the effect of a FRD compared to the low-fruit diet on liver steatosis, lipid profile, and glycemic control in NAFLD. Surprisingly, after 6 months of intervention, exacerbation of steatosis, dyslipidemia, and glycemic control were observed in the FRD group. In contrast, patients in the low fruit diet gruop had an improvement in their conditions.

There are limited studies on the relationship between fatty liver and fruit consumption. Randomized clinical trials (RCT) are even more scarce. Cantero, I. et al. [23] showed that calorie restriction along with fruit fiber intake (≥8.8 g/day) improved fatty liver index, hepatic steatosis index, and serum levels of GGT, ALT, and AST in obese subjects with NAFLD. In the mentioned study, in addition to intervention with fruit fiber intake, the energy intake (-30% of subject's requirement) and the distribution of macronutrients of total caloric value (40% carbohydrate, 30% protein, and 30% lipids in intervention group vs. 55% carbohydrate, 15% protein, and 30% lipids in control group) were altered, each of which could have an independent effect on fatty liver. In addition, the dietary habits were changed, with at least 7 meals/day in the intervention group, compared to the 5 meals/day in the control group. Therefore, the observed changes cannot be attributed only to the intake of fruit fiber.

There are other reports of an improvement in hepatic function or lipids metabolism due to intake of specific fruits or compounds that naturally occur in fruits. Previous studies have shown the hepatoprotective effect of antioxidants including polyphenols, carotenoids, glucosinolates, and fibers [24, 25]. Among them, resveratrol, which is found in the family of plums and grapes, can increase the oxidation of fatty acids [26]. Quercetin is a flavonoid found in a variety of plants, including berries, whose antioxidant activity has been well established [27]. Moreover, anthocyanins found in many fruits have shown some anti-liver damage activity in experimental studies [28]. Carotenoids are other substances that generally accumulate in the liver where they attach to lipoproteins. Dietary carotenoids can purify physiologically active oxygen species, which can prevent liver damage. Also, due to the role of

carotenoids in regulating the polarization activity of macrophages, they can prevent the formation and progression of nonalcoholic steatohepatitis (NASH) [29]. Despite this evidence, contradictory results have also been obtained in some studies. Fakhoury-Sayegh et al. [18] Showed in a case-control study that a fruit-rich dietary pattern (more than 2-3 serving/day of fruits and >20 gr/day of fructose) was directly related to NAFLD. Earlier, Kobayashi et al. [30] reported that people with fatty liver were even more likely to eat fruits and sweets than people with diabetes. In addition, Xia et al. [31] found in a relatively large study (with a sample size of more than 27,000 people) that consuming oranges seven times a week was associated with an increased chance of fatty liver.

In our study, no calorie restriction was considered, and also an increase in energy intake was observed in both groups. Since the weight loss is one of the first approaches in controlling fatty liver [2], it is probably recommended to the patients in the treatment process. So, it is important to consider the weight reduction of participants in addition to the other procedures or treatments to control NAFLD. In the present study, there was an increase in the BMI of the FRD group and a decrease in the control group. However, further analysis in the present study showed that the findings are independent from changes in the BMI, energy or other food groups intake. A cross-sectional study showed that controlling for the effect of BMI eliminates the association between fruit intake and NAFLD [32]. Therefore, more clinical trials should investigate the interaction between fruit consumption and weight changes on the consequences of NAFLD during other treatments. Although, Some observational studies found a lower intake of fruits in patients with NAFLD [33], moreover, dietary habits and eating behaviors are other important factors in NAFLD patients [34]. It is also important to consider the intake of other food groups. In the present study, an increase of more than 2 servings/day of vegetable and about 0.5 serving of sugars and a decrease of about 1 serving/day of fruits were observed in the control group. In contrast, in the FRD group the intake of sugars decreased about 2 servings/day and an increase was observed in the consumption of fruits and vegetables 3.6 and 0.75 servings/day, respectively. Although some beneficial effects of reduced fruit diet could attributed to increased intake of vegetables, [35] however, fruit consumption may play a more important role in the accumulation of fats in the liver in FRD group. The reason for these observations could be traced to the lipogenic potential of fructose compared to the glucose. There is an evidence that fructose leads to a greater increase in liver fat content than glucose [36]. It has been suggested that lipogenic effect of fructose is due to downregulation of fatty acids oxidation rather than its production [36]. Lactate and glucose are two metabolites of fructose that in the skeletal muscles spares fatty acids from oxidation. Decreased fatty acid oxidation in skeletal muscle induces the free fatty acids flux to the liver, thereby increasing the hepatic fat deposition [37]. On the other hand, fructose may increase hepatic fat content through de novo lipogenesis from acetate [38]. After absorption, glucose is mainly metabolized by peripheral tissues, while fructose is transported directly to the liver. Due to the lack of feedback control, fructose is metabolized faster and enters the path of lipogenesis compared to the glucose [39]. Also, fructose induces lipogenesis more efficiently than glucose through upregulation of carbohydrate-responsive element-binding protein (ChREBP) and sterol regulatory element-binding protein 1c (SREBP1c) signaling pathways in the hepatocytes [40]. In addition, fructose could intensify bacterial growth in the small intestine, which increases endotoxin levels in the portal vein and can lead to

inflammation in the NASH [41]. Studies suggest that fructose restriction decreases steatosis and serum levels of hepatic enzymes [42]. However, the hypothesis of an increased odds of NAFLD as a result of high fructose intake was rejected in a cross-sectional study, and an inverse association between NAFLD and fructose intake was reported [17].

To the best of our knowledge, limited studies investigated the effect of fruit intake on NAFLD outcomes. Clinical trial design, stratified randomization, including only grade 2 and 3 of NAFLD and limiting participants to a range of BMI between 18.5 to 29.9 kg/m^2 (Which eliminates the diagnostic bias of ultrasound), and controlling for the effect of change in BMI, energy, and dietary intake with 18 food recalls are the strengths of the present study. However, some limitations should be noted. Given the lack of differences between the two groups in terms of changes in energy intake and physical activity, it should be determined what factor led to weight loss in the control group. Also, perhaps setting a specific range and limiting the maximum amount of fruit intake could help patients improve their condition. In addition, it may be better to determine participants' fruit daily servings based on the individual energy requirement in future studies.

Conclusion

In the present study, 6 months of intervention with FRD exacerbated steatosis, dyslipidemia, and glycemic control of NAFLD patients. It seems that excessive fruit consumption (about 7 servings per day) makes worse the condition of patients with fatty liver. According to the findings of the study, fruits intake increases the fat content of the hepatocyte probably through lipogenic effect of fructose. To clarify the issue, more studies specifying a range for fruit intake (with minimum and maximum values) and considering the energy requirements are warranted.

Abbreviations

NAFLD: Non-Alcoholic Fatty Liver Disease; FRD: fruit rich diet; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyltransferase; FBS: fasting blood sugar; TC: total cholesterol; TG: Triglyceride; LDL-c: low-density lipoprotein; HDL-c,: high-density lipoprotein; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; QUICKI: Quantitative Insulin-Sensitivity Check Index; MUFA: mono-unsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; TFA: trans fatty acids; BMI: body mass index; ChREBP: carbohydrate-responsive element-binding protein; SREBP1c: sterol regulatory element-binding protein 1c.

Declarations

Acknowledgements:

The authors would like to express their gratitude towards the Urmia University of Medical Sciences, for the facilities and financial support. The authors would like to thank the patients who participated in the

present study.

Informed consent:

Informed consent was obtained from all individual participants included in the study.

Author's contributions:

The authors' responsibilities were as follows MA and FA: conceived and designed the study and collected of blood sample and analyzed the data; KS: provided material and technical support, FA: wrote the manuscript; MA: critically revised the manuscript for important intellectual content; all authors: read and approved the final manuscript.

Funding:

This study was supported by Urmia University of Medical Sciences.

Availability of data and material:

Data are available upon reasonable request.

Ethics approval and consent to participate:

This study was approved by Ethics committee at the Urmia University of Medical Sciences (Ethic number: IR.UMSU.REC.1398.535, Date: 02/03/2020). This study registered in Iranian randomized clinical trial website with IRCT registration no. IRCT20201010048982N1. The written informed consent was signed by all study subjects.

Consent for publication:

All authors support the submission to this journal.

Competing interests:

The author reports no conflict of interest in this work.

References

1. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ: **Mechanisms of NAFLD development and therapeutic strategies.** *Nature medicine* 2018, **24:**908-922.
2. Huang DQ, El-Serag HB, Loomba R: **Global epidemiology of NAFLD-related HCC: Trends, predictions, risk factors and prevention.** *Nature Reviews Gastroenterology & Hepatology* 2021, **18:**223-238.
3. Asrani SK, Devarbhavi H, Eaton J, Kamath PS: **Burden of liver diseases in the world.** *Journal of hepatology* 2019, **70:**151-171.

4. Le MH, Devaki P, Ha NB, Jun DW, Te HS, Cheung RC, Nguyen MH: **Prevalence of non-alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States.** *PLoS One* 2017, **12:**e0173499.
5. Bedossa P: **Current histological classification of NAFLD: strength and limitations.** *Hepatol Int* 2013, **7 Suppl 2:**765-770.
6. Esterson YB, Grimaldi GM: **Radiologic Imaging in Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis.** *Clin Liver Dis* 2018, **22:**93-108.
7. Khov N, Sharma A, Riley TR: **Bedside ultrasound in the diagnosis of nonalcoholic fatty liver disease.** *World journal of gastroenterology: WJG* 2014, **20:**6821.
8. de Castro GS, Calder PC: **Non-alcoholic fatty liver disease and its treatment with n-3 polyunsaturated fatty acids.** *Clinical nutrition* 2018, **37:**37-55.
9. Alsahhar JS, Elwir S: **Epidemiology and Natural History of Chronic Liver Disease.** In *The Critically Ill Cirrhotic Patient.* Springer; 2020: 1-9
10. de Wit NJ, Afman LA, Mensink M, Müller M: **Phenotyping the effect of diet on non-alcoholic fatty liver disease.** *Journal of hepatology* 2012, **57:**1370-1373.
11. Gelli C, Tarocchi M, Abenavoli L, Di Renzo L, Galli A, De Lorenzo A: **Effect of a counseling-supported treatment with the Mediterranean diet and physical activity on the severity of the non-alcoholic fatty liver disease.** *World journal of gastroenterology* 2017, **23:**3150.
12. Georgoulis M, Kontogianni M, Margariti A, Tiniakos D, Fragopoulou E, Zafiropoulou R, Papatheodoridis G: **Associations between dietary intake and the presence of the metabolic syndrome in patients with non-alcoholic fatty liver disease.** *Journal of Human Nutrition and Dietetics* 2015, **28:**409-415.
13. Chiu S, Sievenpiper J, De Souza R, Cozma A, Mirrahimi A, Carleton A, Ha V, Di Buono M, Jenkins A, Leiter L: **Effect of fructose on markers of non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of controlled feeding trials.** *European journal of clinical nutrition* 2014, **68:**416-423.
14. Lei S, Liu ZW, Yun L, Cai G, Zhang H, Song LJ, Huang CY, Ming L: **The prevalence of nonalcoholic fatty liver disease and its association with lifestyle/dietary habits among university faculty and staff in Chengdu.** *Biomedical and environmental sciences* 2012, **25:**383-391.
15. Karasawa MMG, Mohan C: **Fruits as prospective reserves of bioactive compounds: a review.** *Natural products and bioprospecting* 2018, **8:**335-346.
16. Ter Horst KW, Serlie MJ: **Fructose consumption, lipogenesis, and non-alcoholic fatty liver disease.** *Nutrients* 2017, **9:**981.
17. Kanerva N, Sandboge S, Kaartinen NE, Männistö S, Eriksson JG: **Higher fructose intake is inversely associated with risk of nonalcoholic fatty liver disease in older Finnish adults.** *The American journal of clinical nutrition* 2014, **100:**1133-1138.
18. Fakhoury-Sayegh N, Younes H, Heraoui GN, Sayegh R: **Nutritional profile and dietary patterns of lebanese non-alcoholic fatty liver disease patients: a case-control study.** *Nutrients* 2017, **9:**1245.

19. Cantero I, Abete I, Monreal JI, Martinez JA, Zulet MA: **Fruit Fiber Consumption Specifically Improves Liver Health Status in Obese Subjects under Energy Restriction.** *Nutrients* 2017, **9**.
20. **http://www.fao.org/home/en/**
21. Forde C: **Scoring the international physical activity questionnaire (IPAQ).** *University of Dublin* 2018.
22. Lee SS, Park SH: **Radiologic evaluation of nonalcoholic fatty liver disease.** *World J Gastroenterol* 2014, **20:**7392-7402.
23. Cantero I, Abete I, Monreal JI, Martinez JA, Zulet MA: **Fruit Fiber Consumption Specifically Improves Liver Health Status in Obese Subjects under Energy Restriction.** *Nutrients* 2017, **9:**667.
24. Ferramosca A, Di Giacomo M, Zara V: **Antioxidant dietary approach in treatment of fatty liver: New insights and updates.** *World journal of gastroenterology* 2017, **23:**4146.
25. Van De Wier B, Koek GH, Bast A, Haenen GR: **The potential of flavonoids in the treatment of non-alcoholic fatty liver disease.** *Critical reviews in food science and nutrition* 2017, **57:**834-855.
26. Mercader J, Palou A, Bonet ML: **Resveratrol enhances fatty acid oxidation capacity and reduces resistin and Retinol-Binding Protein 4 expression in white adipocytes.** *The Journal of nutritional biochemistry* 2011, **22:**828-834.
27. Ozgen S, Kilinc OK, Selamoğlu Z: **Antioxidant activity of quercetin: a mechanistic review.** *Turkish Journal of Agriculture-Food Science and Technology* 2016, **4:**1134-1138.
28. Zhang P-W, Chen F-X, Li D, Ling W-H, Guo H-H: **A CONSORT-compliant, randomized, double-blind, placebo-controlled pilot trial of purified anthocyanin in patients with nonalcoholic fatty liver disease.** *Medicine* 2015, **94:**e758-e758.
29. Ni Y, Zhuge F, Nagashimada M, Ota T: **Novel action of carotenoids on non-alcoholic fatty liver disease: macrophage polarization and liver homeostasis.** *Nutrients* 2016, **8:**391.
30. Kobayashi Y, Tatsumi H, Hattori M, Sugiyama H, Wada S, Kuwahata M, Tanaka S, Kanemasa K, Sumida Y, Naito Y: **Comparisons of dietary intake in Japanese with non-alcoholic fatty liver disease and type 2 diabetes mellitus.** *Journal of clinical biochemistry and nutrition* 2016**:**16-17.
31. Xia Y, Lu Z, Lu M, Liu M, Liu L, Meng G, Yu B, Wu H, Bao X, Gu Y: **Raw orange intake is associated with higher prevalence of non-alcoholic fatty liver disease in an adult population.** *Nutrition* 2019, **60:**252-260.
32. Tajima R, Kimura T, Enomoto A, Saito A, Kobayashi S, Masuda K, Iida K: **No association between fruits or vegetables and non-alcoholic fatty liver disease in middle-aged men and women.** *Nutrition* 2019, **61:**119-124.
33. Hattar LN, Wilson TA, Tabotabo LA, Smith EOB, Abrams SH: **Physical activity and nutrition attitudes in obese Hispanic children with non-alcoholic steatohepatitis.** *World Journal of Gastroenterology: WJG* 2011, **17:**4396.
34. Yasutake K, Kohjima M, Kotoh K, Nakashima M, Nakamuta M, Enjoji M: **Dietary habits and behaviors associated with nonalcoholic fatty liver disease.** *World Journal of Gastroenterology: WJG* 2014, **20:**1756.

35. Li H, Wang X, Ye M, Zhang S, Zhang Q, Meng G, Liu L, Wu H, Gu Y, Wang Y, et al: **Does a high intake of green leafy vegetables protect from NAFLD? Evidence from a large population study.** *Nutrition, Metabolism and Cardiovascular Diseases* 2021, **31:**1691-1701.
36. Dusilová T, Kovář J, Drobný M, Šedivý P, Dezortová M, Poledne R, Zemánková K, Hájek M: **Different acute effects of fructose and glucose administration on hepatic fat content.** *The American journal of clinical nutrition* 2019, **109:**1519-1526.
37. Geidl-Flueck B, Hochuli M, Németh Á, Eberl A, Derron N, Köfeler HC, Tappy L, Berneis K, Spinas GA, Gerber PA: **Fructose- and sucrose- but not glucose-sweetened beverages promote hepatic de novo lipogenesis: A randomized controlled trial.** *Journal of Hepatology* 2021, **75:**46-54.
38. Parks EJ, Skokan LE, Timlin MT, Dingfelder CS: **Dietary sugars stimulate fatty acid synthesis in adults.** *The Journal of nutrition* 2008, **138:**1039-1046.
39. Hannou SA, Haslam DE, McKeown NM, Herman MA: **Fructose metabolism and metabolic disease.** *The Journal of clinical investigation* 2018, **128:**545-555.
40. Softic S, Gupta MK, Wang G-X, Fujisaka S, O'Neill BT, Rao TN, Willoughby J, Harbison C, Fitzgerald K, Ilkayeva O: **Divergent effects of glucose and fructose on hepatic lipogenesis and insulin signaling.** *The Journal of clinical investigation* 2017, **127:**4059-4074.
41. Basaranoglu M, Basaranoglu G, Sabuncu T, Sentürk H: **Fructose as a key player in the development of fatty liver disease.** *World journal of gastroenterology: WJG* 2013, **19:**1166.
42. Schwimmer JB, Ugalde-Nicalo P, Welsh JA, Cordero M, Harlow KE, Alazraki A, Durelle J, Knight-Scott J, Newton KP, Cleeton R: **Effect of a low free sugar diet vs usual diet on nonalcoholic fatty liver disease in adolescent boys: a randomized clinical trial.** *Jama* 2019, **321:**256-265.

Figures

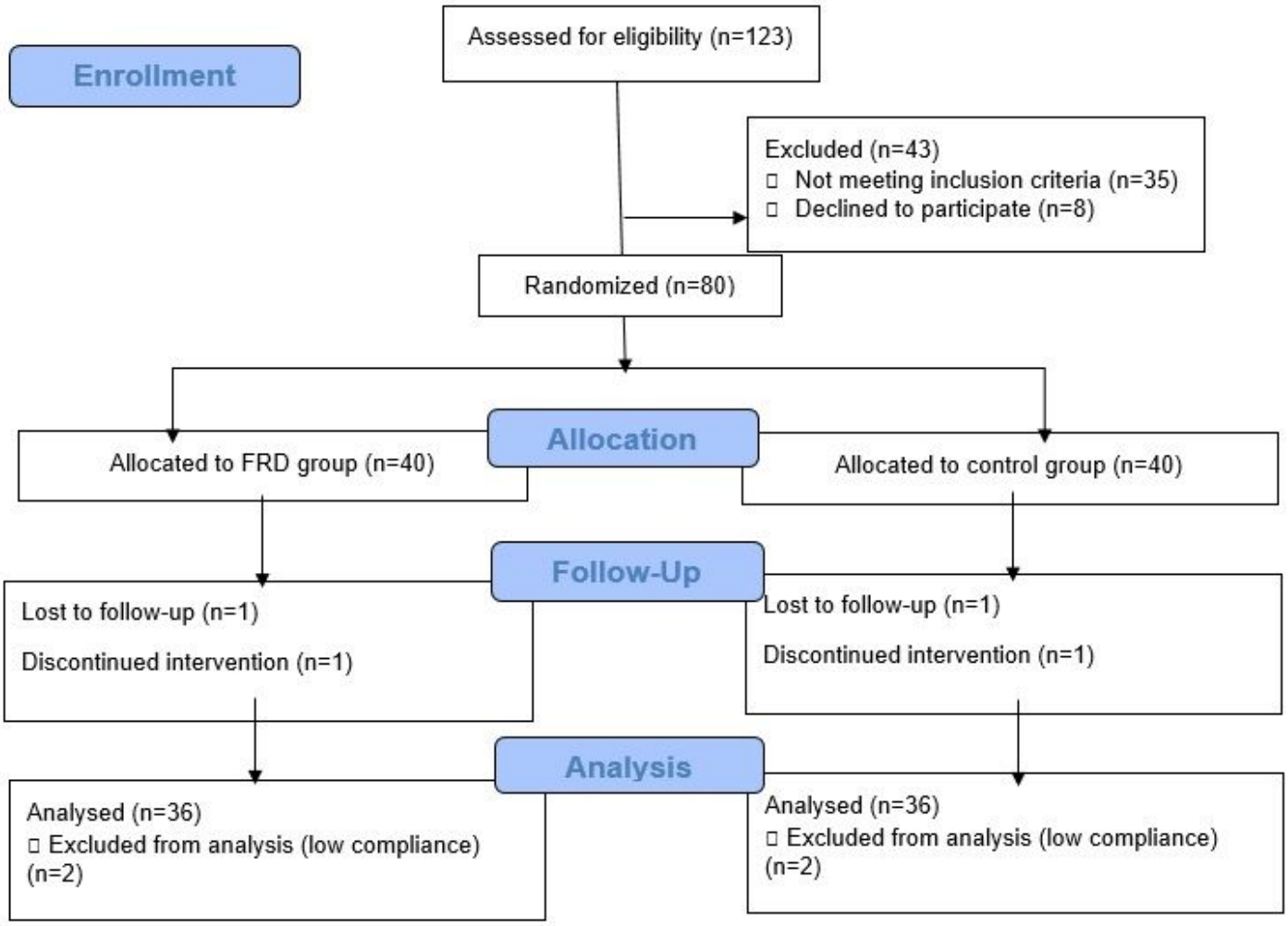

Figure 1

The CONSORT flow diagram of the study participants.

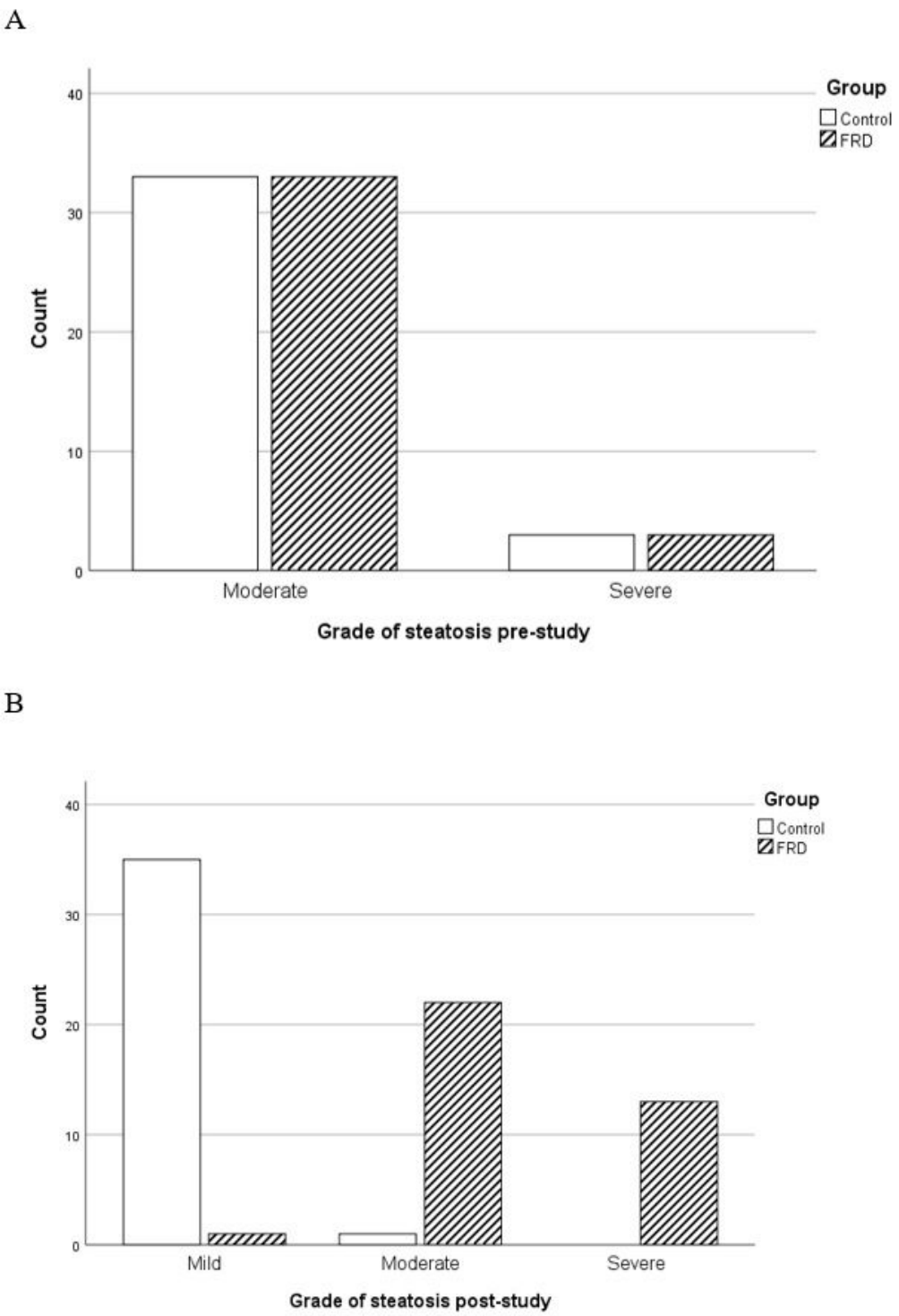

Figure 2

The grade of steatosis according to sonography in two groups before (A) and after (B) study. The P-value of difference between groups were 1.000 and <0.001 at the baseline and after study, respectively.

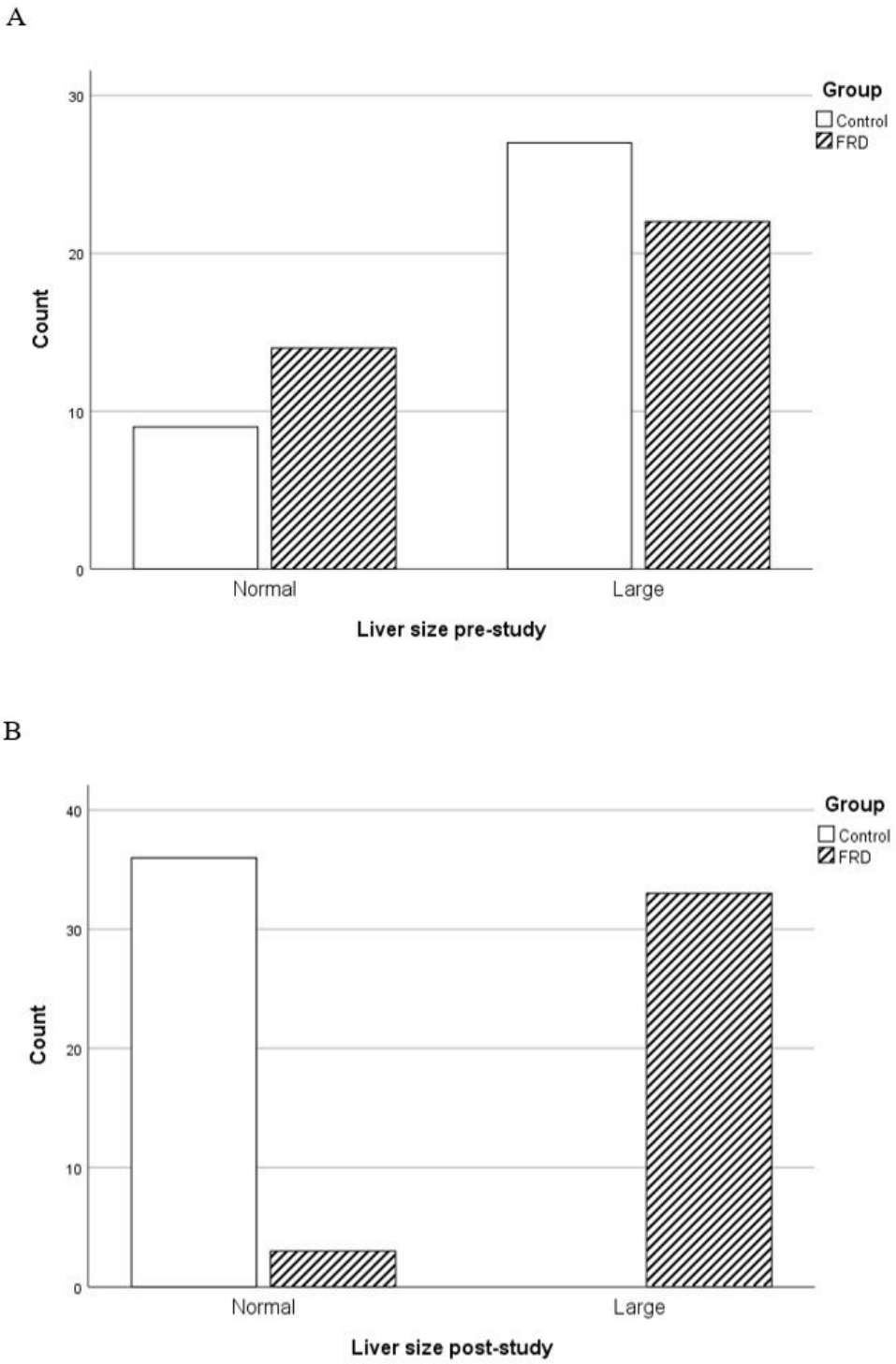

Figure 3

The liver size according to sonography in two groups before (A) and after (B) study. The P-value of difference between groups were 0.312 and <0.001 at the baseline and after study, respectively.