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The effects of kefir drink on liver aminotransferases and metabolic indicators in patients with nonalcoholic fatty liver disease: a randomized controlled trial

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Abstract

Background and aim Probiotics play an important role in the control and treatment of non-alcoholic fatty liver disease (NAFLD). Kefir drink is a fermented beverage and has indicated some beneficial health effects. The aim of this study was to evaluate the effects of kefir drink on liver aminotransferases, anthropometric indices, glycemic index, lipid profile, blood pressure (BP), high sensitivity C-reactive protein, and malondialdehyde in patients with NAFLD.

Methods In an 8-week randomized clinical trial, 80 patients with NAFLD were randomized into two groups of 40. After a 2-week run-in period, the groups received a dietary plan and dietary plan plus a cup of kefir drink twice a day (500 cc/d), respectively. Also, demographic, anthropometric, laboratory, BP, dietary intake, and physical activity assessments were analyzed before and after the intervention.

Results At last, seventy-two participants completed the study. No significant difference in changes in BP, anthropometric indices, and laboratory data ($P > 0.05$) except HDL-C ($P = 0.02$) and fat-free mass ($P < 0.001$) was observed between the two study groups.

Conclusion Based on the results, Drinking 500 cc/d kefir beverage had no significant effect on liver aminotransferases and metabolic indicators, except for HDL-C and fat-free mass in patients with NAFLD.

Trial registration IRCT20170916036204N6 (2018/08/03).

Keywords Non-alcoholic fatty liver disease (NAFLD), Metabolic dysfunction-associated fatty liver disease (MAFLD), Kefir, Liver aminotransferase, Metabolic indicator

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Introduction

Non-alcoholic fatty liver disease (NAFLD), as the most common chronic liver disease and a serious health problem, increases the risk of morbidity and mortality. NAFLD comprises a wide spectrum of conditions ranging from steatosis (lipid accretion more than 5% liver weight) to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma [1, 2]. The approximate prevalence of NAFLD in the world and Iran is 25% and 33.9%, respectively [3, 4]. In addition, a significant association has been observed between factors such as age, anthropometric indices, lipid profile, glycemic indices, liver aminotransferases, blood pressure, physical inactivity, and food insecurity with nonalcoholic fatty liver disease in the Iranian population [5, 6]. NAFLD is associated with obesity, metabolic syndrome (MetS), insulin resistance, type 2 diabetes mellitus (DM), dyslipidemia, and hypertension (HTN) [7]. The pathogenesis of NAFLD is multifactorial and includes genetic and environmental factors, as well as inflammatory and gut microbiota status [1]. By destroying the gut protective barrier, increasing gut permeability, proinflammatory signaling, aggregating hepatotoxic bacterial products, such as ethanol and ammonia, and increasing de novo lipogenesis, gut microbiota dysbiosis has an important role in the pathogenesis and progression of NAFLD [8, 9]. Lifestyle modification, including weight loss via reducing energy intake and increasing energy expenditure, is recommended for the prevention and treatment of NAFLD [1].

Probiotics, as live microorganisms, have beneficial health effects on the host. Probiotics prevent the progression and development of NAFLD [8, 9]. Probiotics have improving impacts on liver aminotransferases, lipid profile, glycemic index, some anthropometric indices, and oxidative stress and inflammatory markers [10–12]. Also, based on animal studies, consumption of kefir decreased parameters of lipid profile, liver aminotransferases [13], insulin resistance, body weight, and inflammation in rodents [14]. Kefir drink is a fermented beverage [15]. Some beneficial health effects of consuming kefir drink include immunological, antibacterial, hypocholesterolemic, anti-tumoural, anti-carcinogenic effects, and β-galactosidase activity [16].

Given the role of probiotics in the control and treatment of NAFLD and considering that based on our knowledge, no human study has investigated the effect of kefir drink in patients with NAFLD, the purpose of this study was to evaluate the effects of kefir drink on liver aminotransferases, anthropometric indices, glycemic index, lipid profile, blood pressure (BP), high sensitivity C-reactive protein (hs-CRP), and malondialdehyde (MDA) in patients with NAFLD.

Methods and materials

Study design

This study was an 8-week parallel randomized clinical trial that was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (IR.SUMS.REC.1397.107) and conducted at the Motahari Fatty Liver Clinic (Shiraz, Iran) from June 2018 to June 2019. Also, the study was registered in the Iranian Registry of Clinical Trials (IRCT) with a registration number (IRCT20170916036204N6) on 2018/08/03. The study was performed based on the declaration of Helsinki and good clinical practice guidelines and adhered to CONSORT guidelines. A secondary analysis of this study has been previously published [17].

A gastroenterohepatologist evaluated patients with NAFLD according to study eligibility criteria. (Fig. 1: consort diagram of the study). The informed consent form was completed for all the participants, and dietary recommendations were educated for a 2-week run-in period. After a 2-week run-in period, the groups were assigned to receive a dietary plan and dietary plan plus a cup of kefir drink (Fars Pegah Co., Shiraz, Iran) twice a day (500 cc/d), respectively. This amount of drink was chosen based on an average of doses from previous studies [18–20]. The kefir drink contained 118 kcal per serving (250 cc) and provided 10 g of carbohydrates, 5 g of fat, and 8 g of protein. Additionally, each serving offers 300 mg of calcium. This product also contained.

2×10^9 beneficial bacteria, including Bifidobacterium and Lactobacillus. Also, demographic, anthropometric, laboratory, BP, dietary intake, and physical activity assessments were recorded before and after the intervention. Aminotransferases and weight were established as the primary outcomes, while other biochemical and anthropometric variables, along with BP, were designated as secondary outcomes.

Study population

The sample size was measured according to the power of 80% and a significant level of 0.05 and based on changes in aspartate transaminase (AST) (mean: 16.9 and SD: 23.6) in a previous study [10]. The sample size was calculated as a total of 80 people (40 people in each group). The computer-generated block randomization with a fixed block size of 4 was done, and the analyzer was blinded.

Inclusion criteria included patients aged 18 to 65 years with NAFLD grades 1 to 3, confirmed by a physician through ultrasound results, ALT more than 30 IU/L in men and more than 19 IU/L in women, body mass index (BMI) ≥ 25, controlled DM and lipid profile, no supplements and probiotics consumption during the previous 6 weeks, no alcohol consumption, no pregnancy and lactation, no more than 3 kg weight loss during the previous 3

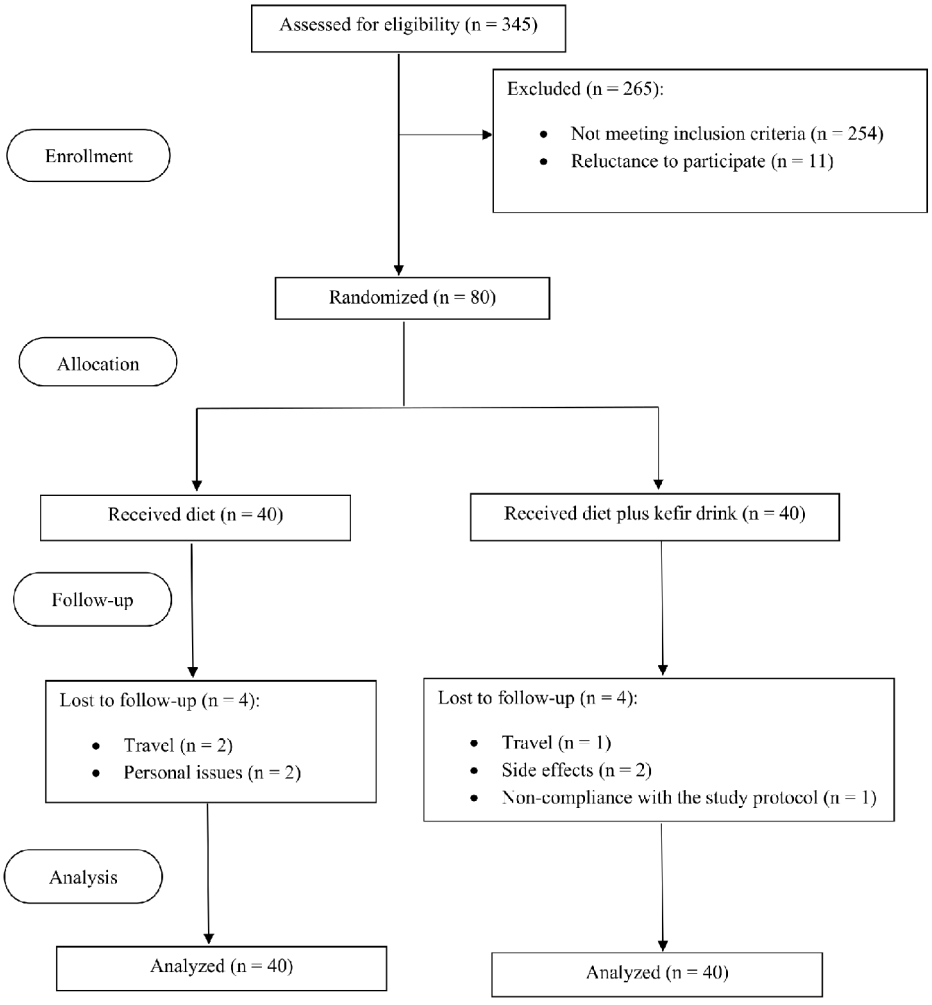

Fig. 1 Consort diagram of the study

months, no other liver disease, hepatitis B and C, cirrhosis, cardiovascular, kidney, autoimmune, hypothyroidism, Wilson, cystic fibrosis, inflammatory bowel diseases, and alpha 1 antitrypsin 1 deficiency. Exclusion criteria included not following the diet and study protocol, taking other supplements and medications during the study, and changing the dose and type of anti-diabetic and anti-lipid medication.

Dietary plan

The energy required by individuals was calculated by a caloric deficit of 500 kcal/day of estimated energy required. Their diet consisted of 55% carbohydrates, 17% protein, and 28% fat. The list was also given to people including allowed and unauthorized foods.

Dietary intake analysis

Dietary intake was recorded by a 3-day food record (2 regular days and a weekend day) and analyzed by Nutritionist 4 software (First Databank Inc., San Bruno, CA, USA). Total energy, macronutrients, fiber, and calcium intakes were reported.

Compliance assessment

Participants attended the clinic every two weeks to receive kefir drinks. A text message was sent to them daily, reminding them to consume kefir. The kefir consumption checklist for individuals was completed. If the patients consumed more than 85% of the recommended dose of kefir drinks during the study, they were considered adherent.

Laboratory assessments

After 8–10 h of fasting, 5 cc of blood was taken from the subjects for laboratory evaluations before and after the intervention. After centrifugation (3000 rpm for 5 min), the sera were stored at -80 °C until further analysis. Total cholesterol (TC), triglyceride (TG), high-density

lipoprotein cholesterol (HDL-C), ALT, AST, and fasting blood sugar (FBS) were measured using standard enzymatic methods through commercial kits (Pars Azmoun, Iran) and calorimetry (auto-analyzer BT-1500, Italy). Low-density lipoprotein cholesterol (LDL-C) was also calculated by Friedewald's Formula [21]. Serum insulin and hs-CRP were measured using ELISA kits (Monobind Inc., USA). MDA was measured by thiobarbituric acid reactive substances (TBARS), using a spectrophotometric assay [22]. Insulin resistance (IR) was defined by the homeostasis model assessment of insulin resistance (HOMA-IR) [23].

Anthropometric assessments

Weight with light clothing and without shoes was measured by scale (Seca, Germany) with an accuracy of 100 g. Height was measured in a standing position by an inelastic tape measure to the nearest 0.5 cm. Then, BMI was calculated using the formula of weight (kg) divided by height squared (m). Waist circumference (WC) was measured using an inelastic tape measure with an accuracy of 0.1 cm, in the middle of the area between the iliac crest and the lowest rib. Body composition (fat and fat-free mass) was measured in a fasting state and constant hydration before performing any intensive physical activity, by a bioelectrical impedance analysis (BIA) device (Inbody S10, South Korea).

Blood pressure measurement

Blood pressure was measured after 10–15 min of rest, with no excitement, and in a sitting position with a mercury sphygmomanometer in the right arm.

Physical activity assessment

Physical activity was assessed according to the International Physical Activity Questionnaire (IPAQ). The questionnaire included 7 questions about the intensity of physical activity (light, moderate, and vigorous) and the duration of physical activity (minutes) during the previous week. The metabolic equivalent (MET) for light, moderate, and vigorous activities was 3.3, 4, and 8, respectively. Then, the amount of physical activity was calculated by multiplying the intensity (MET) by the duration (minutes) of physical activity (MET·min/week).

Statistical analysis

Data were analyzed by Statistical Package for Social Sciences (SPSS) software (version 19.0, SPSS Inc., Chicago, IL, USA) and the statistical significance level was considered $p<0.05$. Data were reported as mean±standard deviation (SD). Within-group and between-group comparisons of variables were carried out by Paired t-test and Independent Sample t-test, respectively. A robust covariance (ANCOVA) analysis was performed to effectively adjust for the impact of covariates, including energy, fat, and carbohydrates. Data analysis was performed on an intention-to-treat (ITT) method.

Results

Eighty participants (35/45, male/female) were randomized into intervention and control groups with a ratio of 1:1. Seventy-two participants completed the study and eight participants were excluded from the study due to side effects such as gastric cramps and bloating ($n=2$), travel ($n=3$), non-compliance with the study protocol ($n=1$), and personal issues ($n=2$). No side effects were observed in phase I/II animal trials [14, 24]. The consort diagram of the study design is displayed in Fig. 1. The mean age of participants was 42.87±10.67. There was no significant difference in terms of baseline demographic variables between the study groups ($P>0.05$) (Table 1). Although there was a significant difference in changes of energy ($P=0.02$), carbohydrate ($P=0.04$), and fat ($P=0.04$) intake between the two groups, no significant difference was observed in changes of protein ($P=0.41$) and fiber ($P=0.90$) intake and physical activity ($P=0.86$) (Table 2). In addition, no significant difference was observed in changes in BP and anthropometric indices, and laboratory parameters ($P>0.05$), except HDL-C ($P=0.02$) and fat-free mass ($P<0.001$) between the two study groups after adjustment for changes of energy, carbohydrate, and fat (Tables 3 and 4).

Discussion

This 8-week clinical trial study was the first human study to investigate the effect of kefir drink on liver aminotransferases, anthropometric parameters, lipid profiles, glycemic indices, blood pressure, hs-CRP, and MDA in patients with NAFLD. Based on the between-group comparison, drinking 500 cc/d kefir beverage had no significant effect on liver aminotransferases, BP, anthropometric measurements, metabolic indicators, hs-CRP, and MDA, except HDL-C and fat-free mass in patients with NAFLD.

The effects of kefir drink on liver aminotransferases

Liver enzymes such as ALT and AST are useful indicators in assessing the severity of liver damage. In conditions such as excessive accumulation of triglycerides in the liver, the risk of damage due to oxidative stress and inflammation to the hepatocytes increases, so higher levels of liver enzymes are common in these patients. In this study, although within-group comparison indicated a significant decrease in ALT and AST serum levels, no significant decrease was observed in between-group comparison. So far, no study has been performed to evaluate the effect of kefir drink on liver enzymes in people with NAFLD. However, previous studies were performed

Table 1 Demographic characteristics of participants

	Diet	Diet + Kefir	*P*-value
Age yr (mean ± SD)	43.50 ± 11.00	42.25 ± 10.44	0.52[#]
Gender			0.07[†]
Male	13 (32.5)	22 (55.0)	
Female	27 (67.5)	18 (45.0)	
Smoking (yes)*	7 (17.5)	3 (7.5)	0.31[†]
Medication history (anti-hyperglycemic or anti-hyperlipidemia) (Yes)*	18 (45.0)	14 (35.0)	0.49[†]
Disease history (Positive)*			
Diabetes	4 (10.0)	5 (12.5)	1.00[†]
Hypertension	3 (7.5)	3 (7.5)	1.00[†]
Hyperlipidemia	11 (27.5)	10 (25.0)	1.00[†]
Cardiovascular disease	0	0	-
Liver disease	0	1 (2.5)	1.00[†]
Renal disease	2 (5.0)	4 (10.0)	0.67[†]
Hypothyroidism	2 (5.0)	2 (5.0)	1.00[†]
Hyperthyroidism	0	0	-
Mental disorder	3 (7.5)	1 (2.5)	0.61[†]
Rheumatoid disease	0	0	-
Cancer	0	0	-
Allergy	10 (25.0)	4 (10.0)	0.13[†]

* Data are reported for the positive answer as n (%), otherwise, it is stated

[#] Independent Sample t-test

[†] Chi-square test

P-value less than 0.05 considered significant

Table 2 Dietary intake and physical activity of the participants at the beginning and end of the study, as well as their differences

		Pre	Post	*P*-value[#]	Mean difference
Energy (kcal/day)	Diet	2050.52 ± 552.41	1938.29 ± 510.84	0.18	− 112.22 ± 521.57
	Diet + Kefir	2182.80 ± 561.49	1815.42 ± 418.58	< 0.01	− 367.37 ± 461.63
	P-value*	0.29	0.24		0.02
Protein (g/day)	Diet	70.01 ± 19.24	63.02 ± 17.47	0.03	− 6.98 ± 19.62
	Diet + Kefir	71.04 ± 25.36	60.37 ± 21.56	< 0.01	− 10.67 ± 20.75
	P-value*	0.83	0.54		0.41
Carbohydrate (g/day)	Diet	301.97 ± 93.99	286.95 ± 92.09	0.26	− 15.01 ± 84.28
	Diet + Kefir	314.29 ± 88.43	246.33 ± 73.42	< 0.01	− 49.96 ± 71.02
	P-value*	0.54	0.22		0.04
Fat (g/day)	Diet	63.66 ± 20.75	61.60 ± 18.18	0.55	− 2.06 ± 21.69
	Diet + Kefir	70.79 ± 22.19	58.86 ± 10.76	< 0.01	− 11.93 ± 21.29
	P-value*	0.14	0.41		0.04
Fiber (g/day)	Diet	12.04 ± 4.27	13.43 ± 10.18	0.41	1.38 ± 10.56
	Diet + Kefir	14.09 ± 6.60	15.96 ± 20.64	0.59	1.87 ± 21.94
	P-value*	0.10	0.48		0.90
Physical Activity (MET.min/week)	Diet	437.51 ± 86.34	475.32 ± 84.28	0.63	37.81 ± 497.81
	Diet + Kefir	302.23 ± 69.43	322.51 ± 57.25	0.75	20.27 ± 401.96
	P-value*	0.22	0.13		0.86

Data are reported as mean ± SD, except for pre and post for physical activity (mean ± standard error (SE))

* Independent Sample t-test

[#] Paired t-test

P-value less than 0.05 considered significant

on animal samples. Along with our study, feeding kefir to serum ob/ob mice for 4 weeks led to a significant decrease in ALT and AST [14]. Also, consuming probiotic yogurt in obese NAFLD patients reduced ALT and AST levels [10]. Kefir peptides are probably involved in three ways in reducing liver fat and thus reducing liver enzymes: (1) reducing leptin resistance, which increases signal transducer and activator of transcription 3 (STAT

Table 3 Anthropometrics and blood pressure assessments of the participants at the beginning and the end of the study, as well as their differences

		Pre	Post	*P*-value[#]	Mean difference
Height (cm)	Diet	164.10 ± 10.04			
	Diet + Kefir	168.95 ± 9.65			
	P-value	0.03*			
Weight (Kg)	Diet	80.31 ± 13.52	79.49 ± 13.47	0.01	−0.82 ± 2.06
	Diet + Kefir	85.02 ± 12.99	84.49 ± 13.11	0.02	-0.53 ± 1.39
	P-value	0.11*	0.09*		0.13[†]
BMI (kg/m^2)	Diet	29.71 ± 3.07	29.40 ± 3.08	0.01	−0.30 ± 0.75
	Diet + Kefir	29.72 ± 3.50	29.53 ± 3.55	0.01	−0.19 ± 0.47
	P-value	0.98*	0.86*		0.11[†]
Waist Circumference (cm)	Diet	100.80 ± 8.27	100.15 ± 8.20	0.01	−0.65 ± 1.67
	Diet + Kefir	100.55 ± 7.28	99.47 ± 7.39	< 0.01	−1.07 ± 1.45
	P-value	0.88*	0.70*		0.58[†]
Fat Mass (kg)	Diet	25.04 ± 7.40	24.78 ± 7.55	0.33	−0.26 ± 1.68
	Diet + Kefir	26.79 ± 7.20	25.93 ± 7.29	< 0.01	−0.86 ± 1.66
	P-value	0.28*	0.49*		0.34[†]
Fat-free Mass (Kg)	Diet	53.27 ± 11.09	52.23 ± 11.35	< 0.01	−1.04 ± 1.86
	Diet + Kefir	58.51 ± 12.06	59.29 ± 11.69	0.05	0.77 ± 2.52
	P-value	0.04*	< 0.001*		< 0.001[†]
Systolic BP (mmHg)	Diet	124.17 ± 11.25	122.30 ± 16.79	0.34	−1.87 ± 12.38
	Diet + Kefir	127.12 ± 15.01	125.37 ± 15.37	0.18	−1.75 ± 8.20
	P-value	0.32*	0.39		0.56[†]
Diastolic BP (mmHg)	Diet	81.17 ± 9.53	82.42 ± 11.89	0.49	1.25 ± 11.58
	Diet + Kefir	83.62 ± 14.54	82.75 ± 11.03	0.55	−0.87 ± 9.19
	P-value	0.37*	0.89*		0.17[†]

BMI: Body mass index; BP: Blood pressure

Data are reported as Mean ± SD

* Independent Sample t-test

[#] Paired t-test

[†] ANCOVA test adjusted for mean differences in Energy, carbohydrate, and fat

P-value less than 0.05 considered significant

3), phosphorylated Janus kinase 2 (p-JAK 2) and phosphorylated STAT 3 (p-STAT 3) expression. Following the pathway, the expression of carnitine palmitoyltransferase-1 (CPT-1) increases, which facilitates the transfer of acyl from long-chain fatty acyl to L-carnitine, thereby increasing the oxidation of fatty acids, (2) reducing the expression of sterol regulatory element-binding protein 1 (SREBP-1) and acetyl-CoA carboxylase (ACC) (inhibits ACC lipogenesis), (3) reducing inflammatory response and insulin resistance [13, 14]. By another mechanism, kefir probiotic bacteria reduced pathogenic bacteria that contributed to the development of NAFLD by producing short-chain fatty acids as an antimicrobial factor [10]. In addition, Lactobacillus kefir DH5 increased the expression of CPT1, peroxisome proliferator-activated receptor alpha (PPAR-α) genes, which increased fatty acid oxidation, and fatty acid-binding protein 4 (FABP4) gene, which inhibited lipogenesis and facilitates lipolysis [18]. Consumption of 200 cc/d kefir drink for 21 days in healthy individuals had no significant within-group effect on AST and ALT levels [25]. The difference between the results of this study and the present study can be due to various reasons, such as shorter duration, the lower dose of prescribed kefir, the same low-calorie diet used in both groups, and the selection of healthy individuals as the study population in this study.

The effects of kefir drink on anthropometric parameters

Due to the close association of obesity with the development of insulin resistance, steatosis, and the progression of fatty liver to nonalcoholic steatohepatitis, weight loss and reduction of fat stores are one of the most important NAFLD treatment strategies. The results of this study indicated a significant decrease in body weight, WC, and BMI in both study groups compared to the initial value. The decrease in the mean body fat mass and increase in fat-free mass were significant in the intervention group. In the within-group of the control group, fat-free mass decreased, and body fat mass increased significantly. Studies on the effect of kefir drink on weight and body composition are limited. Most studies on the effect of dairy or probiotic supplements on weight have been done

Table 4 Laboratory assessment of the participants at the beginning and end of the study, as well as their differences

		Pre	Post	*P*-value[#]	Mean difference
ALT (IU/L)	Diet	44.97 ± 28.52	39.02 ± 23.77	< 0.01	− 5.95 ± 13.11
	Diet + Kefir	42.92 ± 25.74	33.50 ± 14.64	< 0.01	− 9.42 ± 14.76
	P-value	0.73*	0.21		0.29[†]
AST (IU/L)	Diet	33.32 ± 15.66	28.95 ± 12.36	< 0.01	− 4.37 ± 9.05
	Diet + Kefir	33.10 ± 20.92	25.75 ± 10.27	< 0.01	− 7.35 ± 13.17
	P-value	0.95*	0.21		0.31[†]
FBS (mg/dl)	Diet	95.00 ± 22.08	93.87 ± 22.90	0.55	− 1.12 ± 11.98
	Diet + Kefir	91.10 ± 17.84	84.30 ± 10.86	< 0.01	− 6.80 ± 15.73
	P-value	0.38*	0.02		0.12[†]
Insulin (μIU/ml)	Diet	21.79 ± 16.92	24.21 ± 17.84	0.37	2.42 ± 17.14
	Diet + Kefir	16.64 ± 12.58	20.22 ± 17.42	0.09	3.57 ± 13.10
	P-value	0.12*	0.31		0.42[†]
HOMA-IR	Diet	5.05 ± 3.87	5.69 ± 4.35	0.33	0.64 ± 4.13
	Diet + Kefir	3.90 ± 3.35	4.41 ± 4.27	0.35	0.50 ± 3.42
	P-value	0.15*	0.18		0.72[†]
TC (mg/dl)	Diet	175.30 ± 29.33	169.55 ± 30.98	0.16	− 5.75 ± 25.79
	Diet + Kefir	163.35 ± 37.38	160.37 ± 38.68	0.46	− 2.97 ± 25.75
	P-value	0.11*	0.24		0.37[†]
LDL-C (mg/dl)	Diet	95.70 ± 20.08	92.37 ± 22.20	0.28	− 3.32 ± 19.40
	Diet + Kefir	94.40 ± 36.24	93.62 ± 34.27	0.76	− 0.77 ± 16.33
	P-value	0.84*	0.84		0.38[†]
HDL-C (mg/dl)	Diet	41.27 ± 10.19	40.42 ± 9.47	0.38	− 0.85 ± 6.07
	Diet + Kefir	39.47 ± 9.57	41.02 ± 8.37	0.03	1.55 ± 4.38
	P-value	0.41*	0.76		0.02[†]
TG (mg/dl)	Diet	177.25 ± 87.75	165.82 ± 77.20	0.29	− 11.42 ± 68.55
	Diet + Kefir	151.12 ± 93.29	129.25 ± 53.45	0.02	− 21.87 ± 59.87
	P-value	0.20*	0.01		0.49[†]
hs-CRP (pg/ml)	Diet	2184.37 ± 636.74	2088.37 ± 665.44	0.10	− 96.00 ± 365.50
	Diet + Kefir	2159.32 ± 680.10	2002.57 ± 617.16	0.31	− 156.75 ± 976.92
	P-value	0.86*	0.55		0.73[†]
MDA (μmol)	Diet	2.59 ± 0.59	2.47 ± 0.50	0.29	− 0.12 ± 0.71
	Diet + Kefir	2.66 ± 0.51	2.52 ± 0.52	0.12	− 0.14 ± 0.57
	P-value	0.58*	0.70		0.59[†]

ALT: Alanine transaminase; AST: Aspartate transaminase; FBS: Fasting blood sugar; HOMA-IR: HOMA-IR (Homeostatic Model Assessment for Insulin Resistance); TC: Total cholesterol; LDL-C: low-density lipoprotein; HDL-C: high-density lipoprotein; TG: Triglyceride; hs-CRP: high-sensitivity C-reactive protein; MDA: Malondialdehyde; IU: international unit

Data are reported as Mean ± SD

* Independent Sample t-test

[#] Paired t-test

[†] ANCOVA test adjusted for mean differences in Energy, carbohydrate, and fat

P-value less than 0.05 is considered significant

separately. Previous studies with various interventions such as kefir, probiotics, or dairy intake revealed decreasing effects on anthropometric indices [11, 26]. Based on recent studies, the consumption of kefir had no significant effect on fat mass and fat-free mass [27, 28]. Consuming kefir drink changes the intestinal microbial population to increase the ratio of Lactobacillus/ Lactococcus and Candida. This change in the population of intestinal microbes at the molecular level leads to increased expression of the PPAR-α gene in adipose tissue. By activating PPAR-α by down-regulating the genes involved in mitochondrial transmission and beta-oxidation and peroxisome fatty acids, it increases fatty acid uptake, catabolism, and utilization, thus reducing fat accumulation and obesity [29]. Another mechanism of the kefir effect on obesity is related to low-grade systemic inflammation. Blood interleukin 6 (IL-6) concentration is associated with obesity and abdominal obesity [30]. Monocyte chemoattractant protein 1 (MCP-1) is also an inflammatory chemokine that leads to adipogenesis and an increase in the number of adipocytes [31]. Kefir drink can be effective in reducing obesity by reducing IL-6 and reducing the expression of MCP − 1 [26]. In the present study, decreasing the energy intake of individuals due to a 500 kcal/day deficit

of estimated energy needs may be the cause of weight loss in both groups.

The effects of kefir drink on lipid profile

Dyslipidemia is one of the most common conditions in patients with NAFLD and is one of the main underlying factors in cardiovascular disease in NAFLD patients. In the present study, kefir drink significantly increased the serum HDL-C level. Although levels of LDL-C, TC, and TG decreased in both groups, neither was statistically significant. In the same line with the present study, the level of HDL-C was significantly increased by receiving probiotic yogurt in previous studies [20, 32]. Also, daily administration of kefir to diabetic rats led to a significant increase in HDL-C [24]. HDL-C increasing effect of kefir can be due to its sphingolipids and its fat distribution [32]. It should be noted that, in addition to the sphingolipids in milk fat used in the production of kefir, the cell membrane of kefir bacteria also contains sphingolipids. Some studies have shown the effect of sphingolipids in increasing the HDL-C [33]. Other studies have also shown that the increase in HDL-C is due to the combination of saturated fatty acids (12 and 14 carbons) in the milk fat [34, 35]. Although in some studies, kefir did not have a significant effect on reducing TC, LDL-C, and TG [36, 37], in some other studies, kefir caused a significant reduction in these parameters [20, 38]. Different species of lactic acid bacteria may have different effects on serum cholesterol levels [39]. The variety of microbial composition of kefir depends on the source of kefir and production and cultivation methods [40]. Therefore, the lack of effect of kefir on LDL-C, TC, and TG can be due to the diversity of microbial composition of kefir in different studies. Furthermore, in the present study, most participants had normal serum levels of TG, TC, and LDL-C. Therefore, kefir may be effective only at increased levels of lipid profile.

The effects of kefir drink on the glycemic index

High serum glucose levels are very common in patients with NAFLD. Insulin resistance, even in normal-weight individuals, can contribute to the development of NAFLD. Studies have shown that insulin sensitivity in patients with fatty liver is reduced at the level of the skeletal muscle, liver, and adipose tissue. In the present study, although FBS levels decreased in the kefir group, there was no significant difference in the changes in FBS, insulin levels, and insulin resistance between the two groups at the end of the study. Based on previous studies, the FBS level decreased by consuming kefir [24, 37, 41]. Many mechanisms have been suggested for the hypoglycemic effect of kefir. One of these mechanisms is the production of insulinotropic polypeptides and glucagon-like peptide (GLP) by kefir probiotics that increase glucose uptake by the muscle [37]. Moreover, bioactive exopolysaccarides of kefir activate gastric inhibitory peptide (GIP), GLP 1, and the enzyme adenylate cyclase, which activates protein kinase A via cyclic adenosine monophosphate (cAMP), thereby increasing insulin secretion from the pancreatic beta cells [41, 42]. In the present study, kefir intake had no significant effect on insulin levels and HOMA-IR. In contrast, in previous studies, kefir reduced insulin levels [41] and HOMA-IR [43]. Also, based on the results of a meta-analysis, probiotic intake reduced insulin levels in diabetic patients [44]. The lack of effect of kefir on insulin resistance may be due to the short duration of the intervention in this study.

The effects of kefir drink on BP

In the present study, despite a decrease in systolic and diastolic BP, no significant change was found. In this regard, the results of the studies with probiotic yogurt intervention were consistent with those of our study [45, 46]. Contrary to the present study, kefir intervention in rats reduced BP in some studies [47, 48]. Kefir peptides which have angiotensin-converting enzyme (ACE) inhibitory properties could be the cause of lowering BP [49, 50]. The difference in the probiotics used seems to be a reason for not observing a significant change. In addition, most participants in the present study were in the normal BP (29%) or the pre-hypertensive stage (46%), and kefir might have been effective in cases of hypertension.

The effects of kefir drink on MDA

Accumulation of fat in the liver cells and increased levels of fatty acids cause damage to the mitochondrial electron transport chain of the hepatocytes. Damage to this chain increases the oxidation of fats in the microsomal pathway. This increases the production of reactive oxygen species, which, in turn, depletes the antioxidant capacity. At the end of this pathway, increased oxidative stress, production of inflammatory cytokines, DNA damage, and cell death take place [51]. MDA, as a marker of fat oxidation, can be a good indicator of oxidative stress. In the present study, although MDA decreased, this decrease was not statistically significant. In this regard, some former studies had similar results to those of our study [52, 53]. Nonetheless, MDA decreased significantly in the other studies related to kefir or other probiotics [54, 55]. The mechanism of action of kefir in reducing MDA is decreasing oxidative stress due to its antioxidant effect. The antioxidant activity of kefir can be due to lactic acid bacteria [56]. Likewise, bioactive peptides produced by the fermentation of casein and its protein by proteolytic bacteria are involved in its antioxidant activity [55]. The lack of significant MDA change in the present study may be due to differences in the type of lactic acid bacteria applied in various studies.

The effects of kefir drink on hs-CRP

Since inflammatory conditions are one of the main stimuli of damage to the hepatocytes and predispose them to the progression of the disease to fibrosis [57], control and mitigation of inflammation are essential in the treatment process of NAFLD patients. In the present study, hs-CRP was measured as an inflammatory marker. Although the intervention with kefir reduced the level of hs-CRP, this change was not statistically significant. Consistent with the present study, probiotic supplementation alone did not affect hs-CRP, but hs-CRP was significantly reduced only in the group that was supplemented with probiotics and prebiotics [12]. On the other hand, some prior studies with the intervention of probiotics in different patients resulted in a reduction in hs-CRP level [58, 59]. The non-significant decrease in hs-CRP in our study may be due to different combinations of probiotics, the short study period, or the low-grade inflammatory status of participants.

In conclusion, the reason for the lack of effect of kefir on some parameters and the reason for the difference in results between studies can be attributed to the differences in individual microbiota, study duration, and dose of interventions. Therefore, it is suggested that future studies be conducted by measuring the amount of individual microbiota with different doses of probiotic strains.

Limitations

Due to the limitations of this study, such as the short duration of the study, small sample size, baseline nutritional differences, lack of blindness, and lack of measurement of the fatty liver using non-invasive methods, it is suggested that studies should be conducted in the future in accordance with these limitations.

Conclusion

Drinking 500 cc/d kefir beverage in addition to a balanced, restricted-energy diet may increase the HDL-C level and fat-free mass in NAFLD patients, so more studies are recommended on this issue.

Abbreviations

NAFLD	Non-alcoholic fatty liver disease
BP	Blood pressure
hs-CRP	High sensitivity C-reactive protein
MDA	Malondialdehyde
HDL-C	High-density lipoprotein cholesterol
NASH	Non-alcoholic steatohepatitis
MetS	Metabolic syndrome
DM	Diabetes mellitus
HTN	Hypertension
ALT	Alanine transaminase
AST	Aspartate transaminase
BMI	Body mass index
TC	Total cholesterol
TG	Triglyceride
FBS	Fasting blood sugar
LDL-C	Low-density lipoprotein cholesterol
TBARS	Thiobarbituric acid reactive substances
IR	Insulin resistance
HOMA-IR	Homeostasis model assessment of insulin resistance
WC	Waist circumference
BIA	Bioelectrical impedance analysis
IPAQ	International physical activity questionnaire
MET	Metabolic equivalent
STAT 3	Signal transducer and activator of transcription 3
p-JAK 2	Phosphorylated Janus kinase 2
p-STAT 3	Phosphorylated STAT 3
CPT-1	Carnitine palmitoyltransferase-1
SREBP-1	Sterol regulatory element-binding protein 1
ACC	Acetyl-CoA carboxylase
PPAR-α	Peroxisome proliferator-activated receptor alpha
FABP4	Fatty acid-binding protein 4
IL-6	Interleukin 6
MCP-1	Monocyte chemoattractant protein 1
GLP	Glucagon-like peptide
GIP	Gastric inhibitory peptide
ACE	Angiotensin-converting enzyme

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Author contributions

FM: formal analysis, investigation, methodology, writing – original draft, writing – review & editing. NR: conceptualization, data curation, investigation, methodology, project administration, writing – review & editing. MAM: formal analysis, investigation, methodology, writing – review & editing. MAN: investigation, methodology, project administration, writing – review & editing. MHE: investigation, methodology, project administration, writing – review & editing. NH: conceptualization, methodology, project administration, supervision, writing – review & editing.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (IR.SUMS.REC.1397.107). The study was also conducted following the Helsinki Declarations of Ethics. The informed consent form was completed for all the patients.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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