Complementary Therapies in Medicine 44 (2019) 44–50

Contents lists available at ScienceDirect

Complementary Therapies in Medicine

journal homepage: www.elsevier.com/locate/ctim

Effect of soy milk consumption on glycemic status, blood pressure, fibrinogen and malondialdehyde in patients with non-alcoholic fatty liver disease: a randomized controlled trial

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ARTICLE INFO

Keywords:
Soy milk
Non-alcoholic fatty liver disease
Insulin resistance
Blood pressure

ABSTRACT

Objective: Diet plays a critical role in the management of non-alcoholic fatty liver disease (NAFLD). Studies on the NAFLD's experimental models have reported that soy had positive effects on the improvement of metabolic parameters. However, there is a lack of clinical trials regarding the efficacy of whole soy foods. Therefore, this study was conducted to determine the effect of soy milk on some of the metabolic characteristics in patients with NAFLD.

Methods: Sixty-sex patients diagnosed with NAFLD were included in this randomized, parallel, controlled trial and were randomly assigned to either the soy milk or control group. Both groups received a 500-deficit calorie diet plan. Also, patients in the soy milk group consumed 240 ml/day soy milk for 8 weeks. Fasting blood sugar (FBS), serum insulin, HOMA-IR, HOMA-β%, and QUICKI as well as serum malondialdehyde (MDA), plasma fibrinogen, and blood pressure (BP) were measured at the beginning and end of the study.

Results: After 8-weeks of intervention, soy milk group had a greater significant reduction in serum insulin (-3.44 ± 5.02 vs. -1.09 ± 3.77 μIU/ml, $P = 0.04$), HOMA-IR (-0.45 ± 0.64 *vs* -0.14 ± 0.47, $P = 0.03$), systolic (-3.81 ± 4.15 vs -1.48 ± 2.93 mmHg, $P = 0.01$) and diastolic (-2.39 ± 2.80 vs. -0.94 ± 2.76 mmHg, $P = 0.04$) BP, and also, a significant increase in QUICKI (0.02 ± 0.032 vs. 0.008 ± 0.018, $P = 0.04$) compared to the control group. While, changes in the FBS, HOMA-β%, fibrinogen, and MDA were not significantly different between the study groups.

Conclusion: A low-calorie diet containing soy milk had beneficial effects on serum insulin, HOMA-IR, QUICKI, and BP in patients with NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease that is characterized by high lipid accumulation in the hepatocytes, usually greater than 5% of the liver weight. [1] If the disease remains untreated, it could be accompanied by progressive degeneration of liver tissue resulting in hepatic fibrosis and cirrhosis. [2] The global prevalence of NAFLD is estimated at 25.24%, with the highest rates reported in the Middle East and South America and the lowest in Africa. [3] NAFLD has been linked to several cardiovascular risk factors including insulin resistance, hypertension, elevated oxidative stress and increased plasma fibrinogen. [4–7] Therefore, the establishment of interventions for the treatment of NAFLD is necessary to prevent further hepatic damages as well as to achieve a favorable metabolic profile that reduces the risk of development of cardiovascular disease. Current evidence has highlighted the significance of dietary

Abbreviations: NAFLD, non-alcoholic fatty liver disease; ChREBP, carbohydrate responsive element binding protein; SREBP-1, sterol-regulatory element binding protein-1; LXR, liver X receptor; RXR, retinoid-X-receptor; BP, blood pressure; MDA, malondialdehyde; BMI, body mass index; FBS, fasting blood sugar; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-β, homeostatic model assessment of β-cell function; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure; DBP, diastolic blood pressure; IPAQ-SF, International Physical Activity Questionnaire-Short Form; SD, standard deviation

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https://doi.org/10.1016/j.ctim.2019.02.020
Received 6 February 2019; Received in revised form 26 February 2019; Accepted 27 February 2019
Available online 28 February 2019
0965-2299/

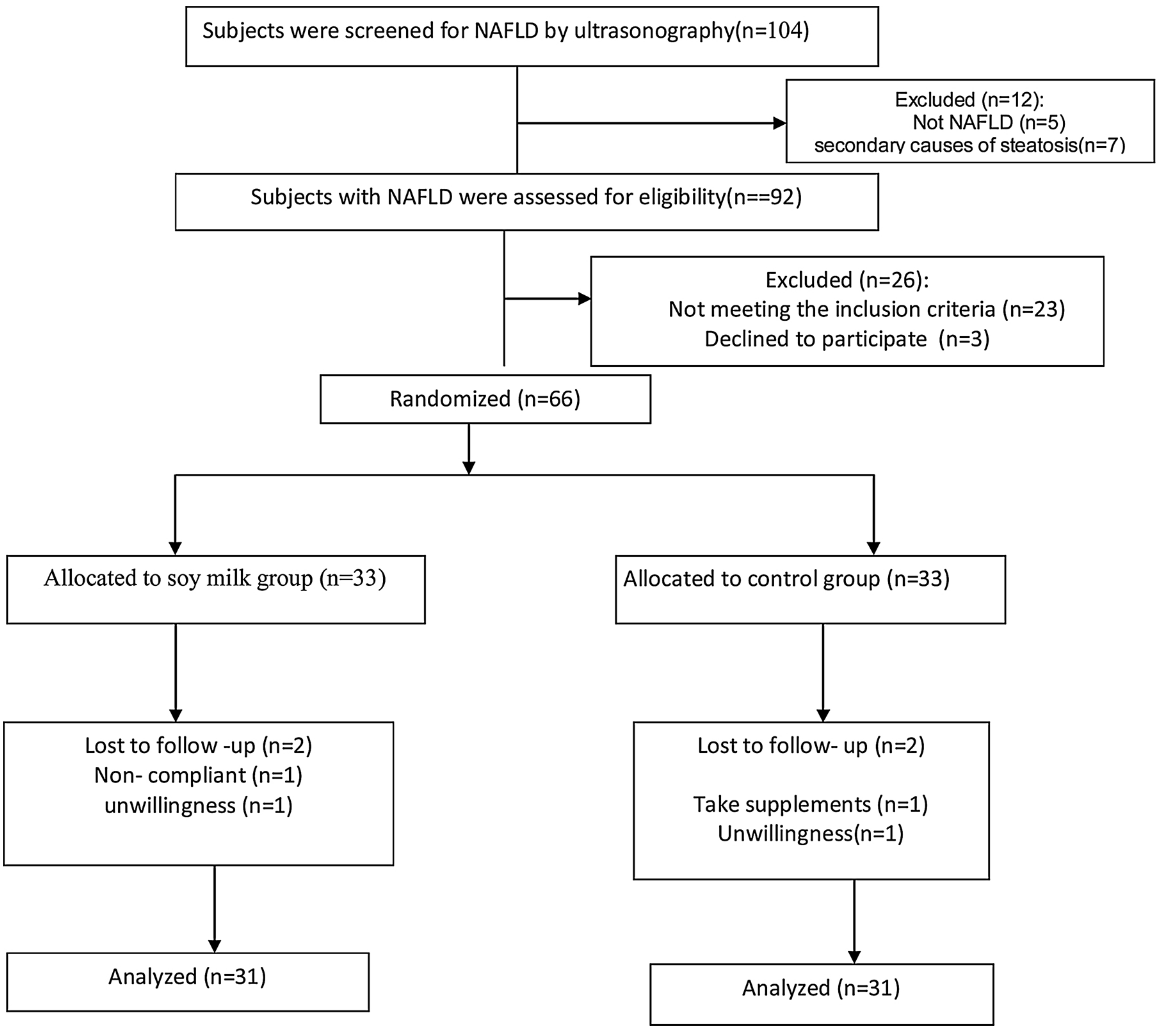

Fig. 1. The patient flow diagram throughout the trial.

Table 1
General characteristics of the study participants.

	Soy milk group (n = 31)	Control group (n = 31)	*P*-value
Age (years)	46.16 ± 10.53[a]	45.16 ± 9.86	0.83 [b]
Sex [n (%)]			
Women	21 (67.7)	22 (71)	0.78 [c]
Men	10 (32.3)	9 (29)	
Weight (kg)	83.03 ± 9.98	84.97 ± 14.02	0.53[b]
Body mass index (kg/ m^2)	30.85 ± 3.53	31.29 ± 3.77	0.64[b]
Physical activity [n (%)]			
Low	12 (38.7)	11 (35.5)	0.87[c]
Moderate	13 (41.9)	15 (48.4)	
High	6 (19.4)	5 (16.1)	
Grade of fatty liver [n (%)]			
Grade 1 (mild)	11(35.5)	16(51.6)	0.32[d]
Grade 2 (moderate)	16(51.6)	10(32.3)	
Grade 3 (severe)	4(12.9)	5(16.1)	

[a] Mean ± SD.
[b] Obtained from Independent sample *t*-test.
[c] Obtained from Chi square test.
[d] Obtained from Fisher Exact Test.

modification particularly a restricted-calorie diet as the cornerstone of the NAFLD management. [4,7] Given the critical role of the diet, many studies have focused on the efficacy of functional foods, as a complementary therapy in the management of NAFLD.

Soybean-derived products such as soy milk are recognized as the functional foods since they are a rich source of isoflavones including genistein, daidzein, and glycitein as well as bioactive peptides, unsaturated fatty acids, and fiber. Several studies in the experimental models of fatty liver have shown an improvement in glycemic measures following supplementation with either soy-isoflavones or -protein. [8–10] It is proposed that soy isoflavones could decrease the activity of intestinal alpha-glucosidase [11] and protein tyrosine kinases. [12,13] Also, they could increase the uptake of glucose mediated by glucose transporter type 4 [14,15] as well as inhibit expression of lipogenesis transcription factors such as carbohydrate responsive element binding protein (ChREBP), sterol-regulatory element binding protein-1 (SREBP-1), liver X receptor (LXR), and retinoid-X-receptor (RXR). [8,10,16] Therefore, they might reduce insulin resistance and improve glucose homeostasis.

In addition to the glycemic status, several studies have reported a positive effect of soy products on blood pressure (BP). [17–19] It is hypothesized that the bioactive peptides found in soy protein could act as the inhibitors of angiotensin-converting enzyme, limit the effect of angiotensin II on vasoconstriction, stimulate the activity of Bradykinin vasodilator and consequently decrease BP. [19] Also, The plant protein in soy has a high content of arginine, which is a precursor of nitric oxide, a

Table 2
Dietary intake of energy and some nutrients in the study groups at the baseline and after 8 weeks.

	Baseline			End of trial		
	Soy milk group (n = 31)	Control group (n = 31)	*P*-value [b]	Soy milk group (n = 31)	Control group (n = 31)	*P*-value [b]
Energy (kcal/d)	2151.35 ± 128.44 [a]	2159.45 ± 78.79	0.76	1778.76 ± 105.85	1783.51 ± 71.62	0.83
Total protein (g/d)	80.66 ± 6.61	77.62 ± 6.16	0.06	63.03 ± 8.36	66.11 ± 8.47	0.15
Total carbohydrates (g/d)	294.50 ± 32.38	290.45 ± 31.98	0.62	248.17 ± 26.86	250.17 ± 30.27	0.78
Total fat (g/d)	80.06 ± 13.28	82.90 ± 20.45	0.51	64.35 ± 11.28	63.20 ± 10.50	0.68
SFA (g/d)	28.72 ± 6.15	27.62 ± 6.19	0.48	21.12 ± 4.94	20.05 ± 3.68	0.33
MUFA (g/d)	24.74 ± 5.01	22.97 ± 4.99	0.16	18.14 ± 4.49	17.13 ± 3.59	0.33
PUFA (g/d)	23.23 ± 4.82	21.67 ± 3.51	0.37 [c]	19.28 ± 3.55	17.90 ± 3.85	0.14
Cholesterol (mg/d)	284.12 ± 93.78	276.12 ± 101.82	0.72 [c]	240.30 ± 105.69	247.02 ± 110.70	0.80
Fiber (g/d)	11.05 ± 2.90	10.98 ± 3.01	0.85 [c]	14.35 ± 2.35	13.28 ± 3.56	0.16
Vitamin E (mg/d)	24.08 ± 4.01	23.87 ± 4.42	0.68 [c]	22.97 ± 4.21	23.01 ± 3.27	0.96
Vitamin C (mg/d)	69 ± 46.25	65.25 ± 43.41	0.84 [c]	77.41 ± 45.33	79.33 ± 42.50	0.86
Vitamin B6 (mg)	1.24 ± 0.31	1.21 ± 0.29	0.67 [c]	1.28 ± 0.39	1.19 ± 0.36	0.37
Calcium (mg)	841.33 ± 100.99	852.56 ± 101.97	0.66	801.89 ± 104.28	809.60 ± 125.12	0.79
zinc (mg/d)	8.35 ± 1.59	8.43 ± 1.51	0.85	7.19 ± 1.76	6.60 ± 1.24	0.13

Abbreviations: SFA, Saturated fatty acid; MUFA, Mono unsaturated fatty acid; PUFA, Poly unsaturated fatty acid.
[a] Mean ± SD.
[b] *P*-values obtained from Independent sample *t*-test unless indicated.
[c] Obtained from Mann-Whitney U test.

known vasodilator, which could reduce BP. [20,21] Besides, some types of soy isoflavones such as genistein are shown to have a diuretic activity and in this way might reduce BP. [22]

Soy milk is a popular soybean-derived food, which obtained by wetting soybeans in water and crushing and pressing the soaked soy. [23] It is a good source of isoflavones, mono- and poly-unsaturated fatty acids. [24,25] To date, several clinical trials have evaluated the effect of soy milk on insulin resistance and BP in overweight/obese individuals or patients with diabetes. However, to the best of our knowledge, there is a lack of evidence regarding the efficacy of soy milk as a complementary therapy in the management of cardio-metabolic health in patients with NAFLD. With this regard, the present clinical trial aimed to determine the effect of soy milk consumption on glycemic status, as the primary outcome of this study, as well as BP, fibrinogen, and malondialdehyde (MDA), as the secondary outcomes, in patients with NAFLD.

2. Material and methods

2.1. Participants

The present study was conducted as a parallel randomized clinical trial for 8 weeks in patients with non-alcoholic fatty liver disease referring to the Gastroenterology clinic of Hazrat-e Rasool General Hospital (Tehran, Iran). The criteria for entering the study were as follows: 1) the age range of 18–60 years, 2) diagnosis of NAFLD with heaptic ultrasonography, and the absence of any secondary cause for steatosis based on the American Gastrointestinal and Liver Association criteria [26] including the history of alcohol use, hemochromatosis or Wilson disease, history of using the hepatotoxic drugs (methotrexate, aminodarone, tamoxifen, corticosteroids, valproate and antiviral drugs), history of hepatitis C, and known autoimmune diseases, 3) not being affected by other diseases including chronic and acute liver disorders, celiac disease, diabetes, cancer, thyroid disorders, cardiovascular, renal and pulmonary diseases as well as inflammatory and autoimmune diseases, 4) body mass index (BMI) in the range of 25–40 $K\,g/m^2$, 5) lack of allergy or intolerance to soy milk, 6) not using any nutritional supplements over the past two months, 7) not using any medication including glucose-lowering or BP-lowering drugs as well as the anti-inflammatory drugs; 8) not being pregnant or breastfed, and 9) willingness to participate in the study and signing a written informed consent.

The exclusion criteria were: 1) unwillingness to continue cooperation, 2) allergy or intolerance to soy milk during the study, 3) lack of compliance with the daily intake of soy milk, 4) presence of any illness during the study, which requires the special treatments.

The sample size was calculated based on the data on serum insulin, as the key variable, obtained from a similar previous study. [9] Considering a power of 80% and type I error of 0.05, 30 subjects per group were required. Anticipating 10% drop-out rate during the intervention, this number increased to 33 per group.

The Ethical Committee of Iran University of Medical Sciences (Tehran, Iran) approved the study protocol (IR.IUMS.REC 1395.9411468009). All participants were informed of the study objectives and procedures and signed a written informed consent. Also, this study was registered in the Iranian Registry of Clinical Trials (IRCT no. IRCT201701162709N40(

2.2. Study design

Before the beginning of the study, a 2-week run-in period was considered for each patient. During this period, it was recommended that patients not consume any soy products such as soy milk, soy protein, soy flour, soy oil, soy yogurt, soy cheese, and soy nut. After this period, patients were randomly assigned to the soy milk and control groups in a 1:1 ratio. Both groups received a 500-kcal deficit diet plan comprised 55% of calories from carbohydrate, 30% from fat, and 15% from protein. [27] Also, patients in the soy milk group consumed 240 ml/ day soy milk in an exchange with one serving of either grains/starches and fats/oils food groups based on the food exchange list. [28] While patients in the control group just followed the low-calorie diet for 8 weeks and were not allowed to consume soy milk or other soy products. Also, patients in the soy milk group were asked to not consume any types of soy foods other than the soy milk provided to them during the intervention. All patients were advised to get involved in the moderate-intensity exercises such as brisk walking for at least 30 min/ day, 5 days a week. All participants were regularly monitored in terms of their adherence to the treatment protocol as well as any adverse symptoms following the soy milk consumption using telephone interviews and monthly visits in the clinic. The soy milk was provided for this study by Saina Ghaza Part Co. (Alborz, Iran). Each serving of this product (equivalent to 240 ml) was comprised 99.6 kcal, 6.75 g protein, 4 g total fat, 9.15 g total carbohydrate, 21 mg total isoflavones, 100 mg calcium, and 98 mg sodium.

Table 3
Comparison of biochemical measures and blood pressure at baseline, end of trial, and changes from baseline between the treatment groups.

		Soy milk group (n = 31)	Control group (n = 31)	*P*-value[b]
FBS (mg/dL)	Baseline	92.80 ± 11.86 [a]	93.22 ± 10.56	0.88
	Week 8	89.48 ± 12.27	91.70 ± 10.34	0.44
	Changes	−3.32 ± 10.16	−1.51 ± 11.48	0.51
	P-value [c]	0.07	0.46	
Insulin (μIU/ml)	Baseline	12.70 ± 4.40	12.86 ± 2.56	0.86
	Week 8	9.26 ± 4.42	11.76 ± 4.05	**0.02**
	Changes	−3.44 ± 5.02	−1.9 ± 3.77	**0.04**
	P-value	**0.001** [d]	0.11 [d]	
HOMA-IR	Baseline	1.63 ± 0.55	1.66 ± 0.32	0.68 [c]
	Week 8	1.18 ± 0.54	1.51 ± 0.52	**0.01**
	Changes	−0.45 ± 0.64	−0.14 ± 0.47	**0.03**
	P-value	**0.001** [d]	0.13 [d]	
HOMA-β	Baseline	129.89 ± 45.98	131.04 ± 36.60	0.91
	Week 8	112.89 ± 51.89	125 ± 41.20	0.11 [e]
	Changes	−17 ± 41.01	−6.04 ± 8.31	0.35 [e]
	P-value	**0.02** [d]	0.33 [d]	
QUICKI	Baseline	0.329 ± 0.021	0.0326 ± 0.010	0.50
	Week 8	0.350 ± 0.027	0.334 ± 0.020	**0.01** [e]
	Changes	+0.022 ± 0.032	+0.008 ± 0.018	**0.04**
	P-value	**0.001** [d]	**0.03** [d]	
SBP (mmHg)	Baseline	127.41 ± 7.15	125.51 ± 7.16	0.3
	Week 8	123.61 ± 7.07	124.03 ± 7.21	0.81
	Changes	−3.81 ± 4.15	−1.48 ± 2.93	**0.01**
	P-value	< **0.001**	0.08	
DBP (mmHg)	Baseline	83.48 ± 4.78	82.06 ± 4.98	0.25
	Week 8	81.09 ± 4.08	81.12 ± 4.52	0.95 [e]
	Changes	−2.39 ± 2.80	−0.94 ± 2.76	**0.04** [e]
	P-value	< **0.001** [d]	**0.04** [d]	
Fibrinogen (mg/dL)	Baseline	262.28 ± 71.54	259.12 ± 63.77	0.85
	Week 8	247.58 ± 62.79	250.51 ± 45.66	0.83
	Changes	−14.70 ± 50.27	−8.61 ± 44.53	0.61
	P-value	0.11	0.29	
MDA (μmol/L)	Baseline	4.99 ± 1.54	5.3 ± 1.7	0.41 [e]
	Week 8	4.07 ± 1.09	4.6 ± 1.27	0.08 [e]
	Changes	−0.92 ± 1.26	−0.63 ± 0.97	0.31
	P-value	**0.001** [d]	**0.001** [d]	

Abbreviations: FBS, Fasting plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-B, homeostasis model assessment of Beta-cell function; QUICKI, Quantitative insulin sensitivity check index; SBP, Systolic blood pressure; DBP, diastolic blood pressure; MDA, malondialdehyde.

[a] Mean ± SD.
[b] *P*-values obtained from Independent sample *t*-test unless indicated.
[c] *P*-values obtained from Paired *t*-test unless indicated.
[d] Obtained from Wilcoxon test.
[e] Obtained from Mann-Whitney U test. Bold *P*-values are statistically significant (< 0.05).

2.3. Biochemical analysis

After 10–12 hours fasting, 7 ml of venous blood samples were taken from each patient at baseline and at the end of trial. Then their serum and plasma were isolated and stored at -70 °C before the analysis. Fasting blood sugar (FBS) was measured by enzymatic calorimetric method using Pars-Azmoon kit (Pars-Azmoon Co.,Tehran,Iran). Serum levels of insulin were determined by enzyme-linked immunosorbent assay using commercial reagents (Monobind, Tehran, Iran).

Homeostatic model assessment of insulin resistance (HOMA-IR), homeostatic model assessment of β-cell function (HOMA-β%), and the quantitative insulin sensitivity check index (QUICKI) were calculated as follows [29] :

HOMA-IR = [FBS (mg/dL) × Fasting insulin (μIU/mL)] / 405

% HOMA-β = [Fasting insulin (μIU/mL) × 360] / [FBS (mg/ dL) - 63] %

QUICKI = 1 / [log Fasting insulin (μIU/mL) + log FBS (mg/ dL)].

Plasma concentration of fibrinogen was measured by the clauss method using Stago kit (MAHSA-YARAN, Iran). Serum MDA was quantified by colorimetric method using ZellBio kit (ZellBio, Germany)

2.4. Measurement of blood pressure

Systolic (SBP)- and diastolic (DBP)- BP were measured from the left arm twice, in sitting position, and after 5 min of rest, using mercuric sphygmomanometer and the average of two measurements was reported for the analysis.

2.5. Assessment of other variables

Patients' liver steatosis was assessed by ultrasonography using the Siemens SONOLINE device (Hamburg, Germany) and it was graded as mild (grade 1), moderate (grade 2), and severe (grade 3), based on the liver echogenicity. [30] Weight was measured using Seca scale (Seca co., Hamburg, Germany) as the patients were minimally clothes, barefoot, and in standing position. Similarly, height was measured by a Seca stadiometer (Seca co., Hamburg, Germany). BMI was determined as weight/ height^2 (kg/m^2). Dietary intakes of study participants were collected using the 24-hour recall questionnaires for three non - consecutive days. The intakes of energy, macro- and micro-nutrients were analyzed using Nutritionist IV software (First Data Bank; Hearst Corp, San Bruno, CA, USA). Physical activity level was estimated by International Physical Activity Questionnaire-Short Form (IPAQ-SF), and it was reported as low, moderate and high. [31]

2.6. Statistical analysis

Data analysis was done using SPSS software version 22. Kolmogorov-Smirnov test was used to check the normality of the data. Independent sample *t*-test or, if necessary, its nonparametric equivalent (Mann-Whitney U-test) was applied to compare the quantitative variables between the study groups, while the qualitative data were compared using the Chi-square test. To evaluate within-group differences, paired *t*-test group or, if necessary, its non-parametric equivalent (Wilcoxon test) was used. Quantitative data were reported by mean and standard deviation (SD), and the qualitative data were presented with frequency and percentage. In this study, $P < 0.05$ was considered as statistically significant.

3. Results

Fig.1 shows the flowchart of the selection and follow-up of the study participants. Of 104 subjects that were underwent hepatic ultrasonography, 92 had been diagnosed with NAFLD. Sixty-six out of 92 patients had met the inclusion criteria and were included in the trial. During the trial, 4 patients were excluded due to the following reasons: 1) not-compliant with the daily consumption of soy milk (n = 1), 2) unwillingness to continue the study (n = 2) and 3) using nutritional supplements (n = 1). Finally, a total of 62 patients were completed the trial, and their data were used for statistical analysis. The patients reported no adverse complication following soy milk consumption.

Table 1 demonstrates the general characteristics of the study participants. The mean (SD) age of the study participants was 45.89 ± 10.03 years-old (women: 47.95 ± 9.19; and men: 41.21 ± 10.52 years-old). Also, women comprised 69.35% (n = 43) of the study subjects. There was no significant difference between the study groups in terms of age, sex, weight, BMI, physical activity level, and grade of fatty liver. Also, the dietary intakes of energy and nutrients were not significantly different between the two groups at baseline and at the end of the intervention (Table 2).

Table 3 compares the glycemic status, SBP, DBP, MDA, and fibrinogen within and between the study groups. The baseline values of the metabolic parameters were not significantly different between the treatment groups. After 8-weeks of intervention, there was a significant decrease in serum insulin ($P = 0.001$), HOMA-IR ($P = 0.001$), HOMA-β ($P = 0.02$), SBP ($P < 0.001$), DBP ($P < 0.001$), and MDA ($P < 0.001$) in the soy milk group, while QUICKI was significantly increased ($P = 0.001$). In the control group, there was a significant decrease in serum MDA ($P = 0.001$), DBP ($P < 0.04$), and also a significant increase in QUICKI ($P = 0.03$).

After the intervention, serum insulin and HOMA-IR were significantly lower in the soy milk compared to the control group, while QUICKI was significantly higher (9.26 ± 4.42 vs. 11.76 ± 4.05 μIU/ml for insulin, respectively, $P = 0.02$; 1.18 ± 0.54 vs. 1.51 ± 0.52 for HOMA-IR, respectively, $P = 0.01$; 0.350 ± 0.027 vs. 0.334 ± 0.020 for QUICKI, respectively, $P = 0.01$).

In terms of changes in variables from baseline, consumption of soy milk was accompanied by a significantly greater reduction in serum insulin, HOMA-IR, SBP, and DBP compared with the control group (-3.44 ± 5.02 vs. -1.09 ± 3.77 μIU /ml for insulin, respectively, $P = 0.04$; -0.45 ± 0.64 vs -0.14 ± 0.47 for HOMA-IR, $P = 0.03$; -3.81 ± 4.15 vs -1.48 ± 2.93 mmHg for SBP, respectively, $P = 0.01$; and -2.39 ± 2.80 vs. -0.94 ± 2.76 mmHg for DBP, respectively, $P = 0.04$). Also, changes in QUICKI were significantly higher in the soy milk than the control group (0.022 ± 0.032 vs. 0.008 ± 0.018 for QUICKI, respectively, $P = 0.04$). While, changes in FBS, HOMA-β, MDA, and fibrinogen were not significantly different between the treatment groups.

4. Discussion

The findings of this clinical trial indicated that daily consumption of soy milk in the context of a restricted-calorie diet was accompanied by a significant improvement in insulin resistance and BP in overweight/obese patients with NAFLD. However, we could not find such a significant effect on fibrinogen and MDA.

Our findings showed a significant decrease in the serum insulin and HOMA-IR and also a significant increase in QUICKI following consumption of soy milk, while, it had no significant effect on FBS. To date, only one clinical trial conducted by Hashemi-Kani et al [18] had investigated the effect of a whole soy food on glycemic status in patient with NAFLD. They reported that consumption of 30 g/day soy nuts in combination with a low-calorie, low-carbohydrate diet led to a significant decrease in serum insulin. Although, unlike our findings, it reduced significantly FBS compared to the control diet. It should be noted that in Hashemi-Kani et al. study, [18] the low-carbohydrate diet consisted of 45% of calories from carbohydrates and 35% from fats while these figures for the low-calorie diet plan in our study were 55% and 30%, respectively. Evidence from some clinical trials has shown that low-carbohydrate diet had caused a greater reduction in the glycemic measures including FBS, insulin, and HbA1c compared to the low-fat, low-calorie or usual diets. [32–34] Therefore, it seems that the differences in the macronutrient composition of the prescribed diet in our study with the one as mentioned above might be a possible reason for the non-significant effect on FBS.

Clinical trials investigating the effect of soy milk consumption on glycemic measures in patients with other chronic diseases have yielded conflicting results. In a cross-over trial among the overweight/obese, but otherwise healthy women, it was observed that consumption of 240 ml/day soy milk instead of 240 ml/day cow's milk in a low-calorie diet (200 to 500-deficit calorie diet) for 4 weeks, had no favorable effect of FBS and serum insulin. [35] Similarly, Miraghajani et al. [19] reported that consumption of 240 ml/day soy milk in the context of a standard diet without calorie restriction for 4 weeks did not lead to a significant reduction in FBS and insulin among type 2 diabetic patients with nephropathy. The non-significant outcomes reported by these studies might be due to the short duration of follow-up, the differences in the prescribed diets or baseline health status of the study participants.

In the present study, consumption of soy milk for 8 weeks had a favorable effect on SBP and DBP. Such a beneficial effect was already reported among patients with NAFLD following a low-calorie, low-carbohydrate diet containing 30 g soy nuts for 8 weeks. [18] Moreover, in a cross-over trial by Azadbakht et al. [17] overweight/obese women consumed 240 ml/d soy milk instead of 240 ml/d cow's milk in conjunction with a restricted-calorie diet (200 to 500-deficit calorie diet) for 6 weeks. At the end of follow-up, it was found that soy milk led to a greater significant reduction in SBP compared to cow's milk (-4.0% vs. -1.7%, respectively), and also, it significantly decreased DBP (-0.4% vs. 0.4%, respectively). In addition, a similar cross-over trial among diabetic patients with nephropathy reported that consumption of soy milk for 4 weeks was accompanied by a significant decrease in SBP compared to the cow's milk (−4.50% vs. 5.89%, respectively). [19] However, our findings were not in agreement with several previous clinical trials. keshavarz et al. [35] found a non-significant reduction in SBP and DBP following consumption of 240 ml/d soy milk for 4 weeks in the overweight/obese women. They proposed that the baseline health status of study subjects might be the possible reason for not achieving a significant effect on BP since they were normotensive and non-menopausal women.

In addition, a study by Amanat et al [36] among patients with NAFLD, reported that daily supplementation of 250 mg genistein for 8 weeks had no significant effect on SBP and DBP. Although, mean values of BP were within the normal ranges, which might have limited the efficacy of isoflavones to reduce the BP since a recent meta-analysis of clinical trials indicated that soy isoflavones had a BP-lowering effect only in hypertensive subjects, but not in the normotensive ones. [37] However, in our study as well as several earlier studies [17–19] that reported a beneficial effect, the mean values of SBP and DBP were within the normal ranges. Therefore, it supports the hypothesis that whole soy foods might have a greater BP-lowering effect rather than soy isoflavones alone. This effect could be mediated by their nutrient composition, which characterized by high amounts of bioactive peptides, unsaturated fatty acids, and isoflavones. [38]

In this study, consumption of soy milk could not lead to a significant decrease in plasma fibrinogen and serum MDA. Also, other clinical trials could not find any significant changes in fibrinogen [35,39] or MDA [40] following consumption of 240 ml/d soy milk for 4 weeks. In contrary to these findings, intake of 30 g/d soy nuts containing a total of 102 mg, for 8 weeks significantly decreased serum MDA and plasma fibrinogen in patients with NAFLD. [9] Moreover, genistein supplementation at the dosage of 250 mg/d for 8 weeks was accompanied by a significant reduction in serum MDA in NAFLD patients. [36] It seems that the small amount of isoflavones that exist in one portion of soy milk (equivalent to 240 ml) might be limited our ability to observe the significant effects on MDA and fibrinogen. However, this effect could not be explained merely by the amount of isoflavones in the soy product, since it is suggested that several factors might influence the pharmacokinetics of isoflavones including food matrix (i.e., liquid vs. solid type of soy foods), background diet, gut microbiome, gastrointestinal transit time, ethnic differences, the process and storage conditions of soy foods. [41]

To the best of our knowledge, the present study was the first clinical trial that had investigated the effect of a low-calorie diet containing soy milk on glycemic status, BP, MDA, and fibrinogen in patients with NAFLD. Also, no adverse complication was reported by the participants in the soy milk group, and thus, it was well tolerated. However, this study had several limitations. First, the participants were informed of the type of interventions during the trial. Although, the blinding was difficult since this study was a dietary intervention. Second, due to the financial limitations, we could not measure serum or urine isoflavones to evaluate the compliance to the intervention more accurately. Although, we had evaluated compliance with the soy milk consumption regularly using face-to-face and telephone interviews as well as the dietary recalls. Third, To the best of our knowledge, the present study was the first clinical trial that had investigated the effect of a low-calorie diet containing soy milk on glycemic status, BP, MDA, and fibrinogen in patients with NAFLD. Also, no adverse complication was reported by the participants in the soy milk group, and thus, it was well tolerated. However, this study had several limitations. First, the participants were informed of the type of interventions during the trial. Although, the blinding was difficult since this study was a dietary intervention. Second, due to the financial limitations, we could not measure serum or urine isoflavones to evaluate the compliance to the intervention more accurately. Although, we had evaluated compliance with the soy milk consumption regularly using face-to-face and telephone interviews as well as the dietary recalls. Third [39], and therefore, might not reflect the changes in oxidative stress accurately. Nevertheless, there is accumulating evidence that MDA is a valuable biomarker in various metabolic disorders such as metabolic syndrome and hepatic steatosis. [42] Forth, we did not perform an ultrasound evaluation at the end of intervention to assess the changes in steatosis, which could provide more precise information about the efficacy of soy milk on hepatic steatosis.

5. Conclusion

In conclusion, this clinical trial found that consumption of soy milk in conjunction with a low-calorie diet for a short-term period had beneficial effects on serum insulin, HOMA-IR, SBP, and DBP in patients with NAFLD. Although, it had no significant effect on FBS, MDA, and fibrinogen. Further clinical trials with higher dosages of soy milk and a longer duration of follow-up are needed to confirm these findings.

Declarations of interest

None

Funding

This work was financially supported by Vice Chancellor for Research, Iran University of Medical Sciences, Tehran, Iran.

Acknowledgments

The authors thank Saina Ghaza Part Company for their support in providing soy milk. Also, thank all the study participants as well as the staff of Velayat hospital and Hakim medical laboratory for their cooperation.

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