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The effect of chitosan supplementation on liver function, hepatic steatosis predictors, and metabolic indicators in adults with non-alcoholic fatty liver disease: a randomized, double-blinded, placebo-controlled, clinical trial

Mohammad Ali Mohsenpoor[1], Karim Parastouei[2], Maryam Taghdir[2,3*], Marzieh Akbarzadeh[4], Sepideh Abbaszadeh[2], Mohammad Ali Abyazi Heris[5], Mohammad Reza Mansouri Rad[6], Mohammad Amin Jafari[7] and Ronak Borzooei[1]

Abstract

Background Non-alcoholic fatty liver disease (NAFLD) is a disease with high complications. An increment in dietary fiber consumption is an approach to NAFLD management, and chitosan as dietary fiber can play a role in the management of NAFLD. Thus, the present study aimed to investigate the effect of chitosan supplementation on liver function, hepatic steatosis predictors, and metabolic indices in adults with NAFLD.

Methods Seventy-two adults with NAFLD were randomly assigned to consume either 1.5 g/day chitosan or placebo along with a low-calorie (– 500 kcal/day) diet for 8 weeks in a parallel, randomized, double-blinded, placebo-controlled, clinical trial. Participants were assessed for dietary intake, physical activity, and anthropometric indices. Blood samples were taken to measure fasting blood sugar (FBS), cholesterol, triglycerides, high- and low-density lipoprotein (HDL and LDL). Liver function indices including alanine aminotransferase (ALT), aspartate transaminase (AST), and gamma-glutamyltransferase (GGT) were evaluated using blood samples as the primary outcomes. Fatty liver index (FLI), hepatic steatosis index (HSI), and triglyceride-glucose index (TyG) were calculated as hepatic steatosis predictors' indices.

Results After 8 weeks of study, 66 participants finished the study. In comparison with placebo, chitosan supplementation reduced weight ($P=0.041$), waist circumference ($P=0.049$), AST ($P=0.040$), ALT ($P=0.001$), and GGT ($P=0.028$). Although the reduction of FBS, triglycerides, cholesterol, LDL, FLI, HSI, and TyG, and increment in HDL was higher in the chitosan group, the results were not significant ($P>0.05$).

*Correspondence:
Maryam Taghdir
mtaghdir@gmail.com; mtaghdir@bmsu.ac.ir
Full list of author information is available at the end of the article

Conclusions Eight-week supplementation with 1.5 g/day chitosan along with a low-calorie diet could possibly reduce weight, waist circumference, AST, ALT, and GGT, and ameliorate NAFLD. Further investigations are recommended.

Trial registration The trial was registered at IRCT.ir as IRCT20140502017522N4 (March 2023).

Keywords Chitosan, Non-alcoholic fatty liver disease, Liver function test, AST, ALT, GGT, FLI, HSI, TyG

Background

Non-alcoholic fatty liver disease (NAFLD), as a prevalent chronic hepatic disease, is caused as a result of fat accumulation in the liver when there is no secondary cause such as excessive alcohol consumption [1]. NAFLD ranges from a benign state of fat accumulation in the liver to non-alcoholic steatohepatitis (NASH). It can also progress to fibrosis and cirrhosis [2]. In addition to the progression of hepatic disease, NAFLD is observed to increase the risk of cardiovascular disease [1]. A recent estimation yielded 30.05% for the global prevalence of NAFLD, with an increment of 50.4% from 1990–2006 to 2016–2019 [3]. The annual mortality rate per 1000 person among NAFLD patients was observed at 12.60, 4.20, 2.83, and 0.92 from all-cause, cardiovascular-related, extrahepatic-cancer, and liver-specific mortality, respectively [3].

NAFLD should be managed to avoid complications and further financial burdens. Lifestyle modifications, dietary amendments, medications, and bariatric surgeries are approaches for NAFLD management. Among proposed approaches, lifestyle modification and dietary strategies have lower risks [4]. Following a Mediterranean diet [5] or low-calorie diet [4, 6], reduction in red meat [7], trans fats [4], and refined grains consumption [8], and increasing fiber consumption [9] are the dietary interventions in NAFLD management [4].

Chitosan is a dietary fiber produced after the deacetylation of chitin (a polymer of N-acetyl-D-glucosamine and D-glucosamine units linked with β-(1–4) glycosidic bonds) which is extracted from the cell wall of fungi and mushroom, the exoskeletons of crustaceans, mollusks, insects [10]. Chitosan has shown weight and body fat reduction properties [11]. In addition, chitosan supplementation has amended insulin resistance indices and lipid profile [12]. Although, chitosan ameliorated NAFLD in animal models [13, 14], no human studies have been conducted.

Blood biomarkers and transaminases such as gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), and aspartate transaminase (AST) are the most performed liver function tests for NAFLD diagnosis [15, 16]. However, they have not shown satisfactory results for prediction of NAFLD progression [15]. Hence, some non-invasive indices were introduced to predict NAFLD progression such as fatty liver index (FLI), hepatic steatosis index (HSI), and triglyceride-glucose index (TyG) [16].

Thus, according to the lack of study assessing the effect of chitosan on NAFLD in the human population, and the precision of indices used for diagnosis and prediction of NAFLD progression, the present study aimed to investigate the effect of chitosan supplementation on liver function, hepatic steatosis predictors, and metabolic indicators in adults with NAFLD.

Methods

Study design

An 8-week parallel, randomized, double-blinded, placebo-controlled, clinical trial was designed to investigate the effect of chitosan on liver function, hepatic steatosis predictors, and metabolic indices in adults with NAFLD. The protocol of the study was in accordance with the Declaration of Helsinki for medical research involving human subjects, reviewed and approved by the institutional reviewing board (IRB) and the Research Ethics Committees of Baqiyatallah Hospital (approval ID: IR.BMSU.BAQ.REC.1401.112). The study protocol was also registered in the Iranian Registry of Clinical Trials (irct.ir, registration ID: IRCT20140502017522N4, dated: March 2023). The reporting of the present study was in line with the CONSORT guidelines for reporting clinical trials.

Eligible individuals were verbally informed about the research objectives, possible advantages of the study, and their rights during the study period, prior to participating. In addition, they were assured about the confidentiality of their personal information and that leaving the study would not affect their routine NAFLD care. Then, an informed consent form containing the above-mentioned information was signed before including in the study.

Study population

The sample size was calculated based on the mean differences of liver enzymes as primary outcomes. Among liver enzymes and based on the previous study [17], AST with mean difference of 4.19 between intervention and control group, standard deviations of 4.2 in the intervention and 7.2 in the control group, considering type one error of

0.05 and 80% power, and attrition rate of 10%, yielded the highest sample size of 36.

Adult individuals aged 18–65 years old were eligible to participate if suffered from NAFLD (grades 1–3); had no allergic history to clams or shrimp; had high no alcohol consumption (higher than 10 g for women and 20 g for men); had no history of cancer, viral hepatitis, Hepatocellular carcinoma, diabetes, thyroid, renal, or mental disorders; not being in pregnancy or lactating period; and had not used chitosan or any fiber-containing supplements for 3 months prior to the study. Participants were excluded from the study if reported high alcohol consumption, initiation of pregnancy, consumption of chitosan or any fiber-containing supplements, or allergic reaction to chitosan during the study period.

Eligible participants were allocated to the chitosan or control group using the block randomization method (blocks of two with a 1:1 ratio). An out-of-study person conducted the randomization procedure and prepared a random sequence before the study initiation. The same person concealed the allocated group (coded as A or B) in sealed opaque envelopes before the study for each participant based on the prepared random sequence.

Study procedure

After the diagnosis of NAFLD based on sonographic imaging results by the physicians collaborating in the study, the individual was referred to the research team for checking eligibility. In case of eligibility and willingness to participate, the eligible participant was included in the study after signing the informed consent form. Then, participants' demographic data were recorded. A dietary record and physical activity questionnaire were handed to the participants. Participant recruitment, study, and follow-up were implemented in Moslemin Hospital, Shiraz, Iran.

Participants were asked to present in the hospital laboratory for blood sampling after overnight fasting and fill out the questionnaire during the week before blood sampling. After blood sampling, participants were visited by the research team to collect questionnaires and conduct anthropometric measurements. Then, based on the random sequence, after opening the envelope, participants were allocated to the chitosan or control group and received either intervention or placebo for 8 weeks. Moreover, a low-calorie diet was designed for each participant. In addition, physical activity and dietary record questionnaires were also handed out to participants.

Participants were asked to report any medication changes, not to change their physical activity habits, and avoid consumption of any fiber-containing and chitosan supplements. In addition, participants were asked to report any allergic reactions or possible side effects directly to the research team, immediately.

In the seventh week, the participants received a phone call to fill out the dietary and physical activity form for the next week and attend at medical laboratory of the hospital after an overnight fasting at the end of the eighth week. Participants were again visited by the research team for anthropometric assessments and collecting questionnaires. During the study period, participants received routine phone calls for assurance of compliance with the study protocol.

Intervention

Participants received either 1.5-g chitosan capsules [18] or a placebo (maltodextrin), based on the allocated group, to take half an hour before lunch with 250 ml water. Chitosan and maltodextrin powders (Karen Inc., Tehran, Iran) were capsulated for chitosan and control groups in the Pharmaceutical Laboratory, School of Pharmacy, Shiraz University of Medical Sciences, Iran. In order to blind participants and investigators, Chitosan and placebo Capsules were similar in appearance and size and placed in similar bottles. Bottles were also coded A or B in line with codes allocated to the group by the same person who prepared random sequences.

Moreover, a low-calorie diet with a reduction of 500 kcal/day based on total energy expenditure was designed for each participant using the Mifflin-St Jeor formula [19]. The diet's macro-nutrient composition consisted of 50–55% carbohydrate, 15–20% protein, and 25–30% fat.

Demographic information

A demographic information checklist was prepared to ask participants' age, gender, marital status, education level, job status, NAFLD grade reported by a physician, level of income as low (under minimum wage), middle (minimum wage to two minimum wages), and high (higher than two minimum wages), smoking history, and alcohol consumption.

Outcomes

In the present study, liver function (AST, ALT, and GGT) were considered as primary out comes. The secondary outcomes were hepatic steatosis predictors (TyG, HSI, and FLI), anthropometric measures, and blood biochemical tests.

Anthropometric assessment

Height was read in a standing position when the participants' heels, buttocks, shoulders, and behind the head touched the wall, using a stadiometer (Seca, Germany) attached to the wall to the nearest 1 cm. Participants were

barefooted with no hat during height assessment. Weight was assessed with the lowest possible clothing when participants were stood on the center of the scale (Seca, Germany) with an accuracy of 100 g. Waist circumference (WC) was measured using an inelastic tape measure with 1 cm accuracy. The measurement was done at the narrowest point between the lowest rib and the iliac crest after a normal exhale in a standing position when the tape was parallel to the ground. Body mass index (BMI) was calculated using the standard formula [weight (kg)/(height (m))^2] [19]. The assessments were done on the day the study began and the last day of the study.

Blood biomarker and hepatic function assessment

On the day of the study initiation and the end of the day, the participants attended at medical laboratory of hospital after 12 h of overnight fasting for blood sampling. Fasting blood sugar (FBS), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were assessed using the colorimetric enzymatic method. AST, ALT, and GGT were measured using the enzyme-linked immunosorbent assay (ELISA) technique.

Hepatic steatosis predictors were calculate using standard formulas. TyG was calculated as [Ln (serum TG (mg/dl) × FBS (mg/dl)/2] [20]. The formula [8 × ALT/AST + BMI + 2 (if suffered from type 2 diabetes mellitus) + 2 (if female)] was used to calculate HSI [21]. FLI result was equal to $[e^{0.953\times \ln(TG) + 0.139\times BMI + 0.718\times \ln(GGT) + 0.053\times WC - 15.745} / (1 + e^{0.953\times \ln(TG) + 0.139\times BMI + 0.718\times \ln(GGT) + 0.053\times WC - 15.745})] \times 100$ [22].

Dietary assessment

Before initiating the study and in the eighth week, participants filled a 3-day food record for 2 weekdays and a weekend. Participants were asked to record all food and beverages consumed with ingredients and preparation methods based on the Iranian household scales. Then, records were converted to grams and analyzed using Nutritionist IV software (First Databank Inc., San Bruno, CA, USA) to record daily energy, carbohydrate, protein, fat, and fiber intake.

Physical activity assessment

The physical activity of participants was evaluated using the short form of the International Physical Activity Questionnaire (IPAQ-SF) for the week before beginning and finishing the study. IPAQ-SF asks about the number of days and duration (minutes) of three categories of physical activity light, moderate, and vigorous intensity. To calculate the physical activity of participants, the duration of each level of physical activity in the past week was multiplied by the coefficient of related intensity (8, 4, and 3.3 for vigorous, moderate, and light activity, respectively). The results were summed up to achieve the physical activity (MET × minutes/week) of participants [23].

Compliance assessment

The participants' compliance with the study protocol was assessed using counting unconsumed capsules. Participants were asked to bring back unconsumed capsules and not to use them on other days. Consuming less than 80% of capsules was known as low compliance and the participant was excluded from the final analysis.

Statistical analysis

Data were reported as frequency and percentage for categorical and mean ± standard deviation (SD) for continuous variables. Categorical variables were analyzed using the chi-square test. Within-group and between-group comparisons were done using paired sample t-tests and independent sample t-tests. The analysis of covariance (ANCOVA) was conducted to adjust the confounder for between-group comparisons of mean differences. The calculation of Hepatic steatosis predictors (FLI, HSI, and TyG) and mean differences (post-intervention–pre-intervention), and data analysis with a per-protocol approach was conducted with SPSS software version 20 (IBM, USA). A *P*-value less than 0.05 was considered significant.

Results

The present study was carried out between October 2023 and February 2024. Among 160 individuals assessed for eligibility, 88 were not eligible based on the predefined eligibility criteria or refused to participate in the study. Finally, 72 eligible adults with NAFLD were randomly assigned to the chitosan or control group (36 per group). During the study period, 6 did not successfully finish the investigation (three from chitosan and three from the control group) and were excluded from the final analysis. Thus, 66 participants aged 39.64 ± 7.91 years finished the study. Figure 1 depicts the CONSORT flow diagram of the study, as well as the reason for the exclusion of participants. Moreover, no side effects were observed after consumption of the chitosan.

Table 1 summarizes the baseline and demographic characteristics of participants. Of 33 participants who finished the study in the chitosan group, 54.5% were female, while 60.6% of participants who finished the study in the control group were female ($P > 0.05$). Analysis did not show a significant difference in marital status, education level, job status, income, and NAFLD grade ($P > 0.05$). Participants reported no alcohol consumption. Among participants, one, in the control group, reported smoking (data are not shown).

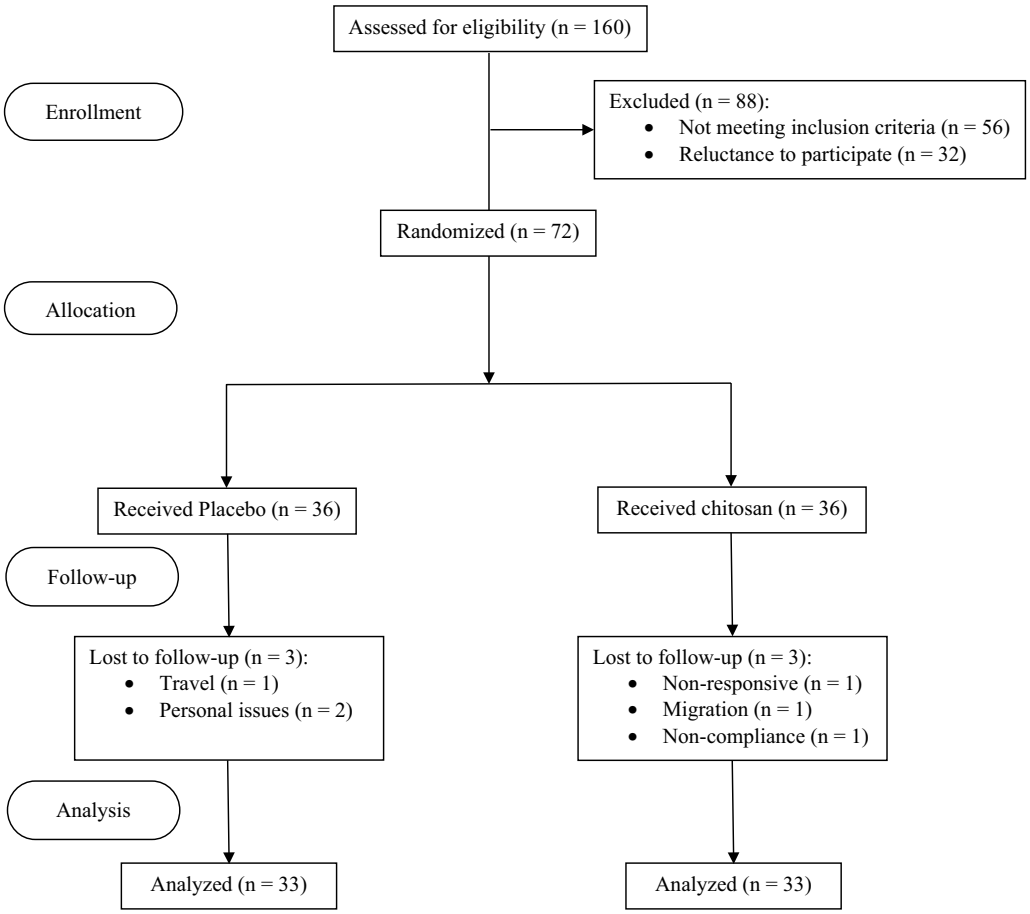

Fig. 1 CONSORT diagram of the study

Between-group analysis for age, height, and BMI did not show statistical differences ($P > 0.05$), this is while weight was significantly higher in the chitosan group ($P = 0.026$).

Dietary intake and physical activity

Table 2 shows the dietary intake and physical activity of participants before the study, at the end of the study, and changes during the study period. Within-group analysis indicates a significant reduction of energy, protein, carbohydrate, and fat during the study period in both groups ($P < 0.05$). Fiber consumption was significantly reduced in the chitosan group (-1.99 ± 4.32 g/day, $P = 0.012$), while the reduction of fiber intake was not significant in the control group (-0.38 ± 1.64 g/day, $P = 0.191$). However, between-group comparisons did not show any statistically significant difference in dietary intake before and after the study, as well as changes during the study period ($P > 0.05$). The analysis did not state significant differences in physical activity.

Anthropometric and blood biochemical

During the study period, participants in the chitosan group showed a significant weight reduction ($P = 0.013$), BMI ($P = 0.016$), and WC ($P < 0.001$), while the reduction was just observed for WC in the control group ($P = 0.048$). Between-groups comparison for mean differences indicated that the decrement in weight (chitosan: -1.70 ± 3.73 kg, control: -0.08 ± 2.45 kg; $P = 0.041$) and WC (chitosan: -1.81 ± 2.37 cm, control: -0.72 ± 2.03 cm; $P = 0.049$) were statistically higher in the chitosan group. Analysis for blood biomarkers showed insignificant higher changes in the chitosan group in comparison with the control group for FBS, TG, cholesterol, HDL, and LDL. Table 3 demonstrates anthropometric and blood biomarker assessments of participants.

Liver function and hepatic steatosis predictors

Table 4 summarizes the observation of liver function and hepatic steatosis predictors during the study. Supplementation with chitosan significantly reduced

Table 1 Demographic and baseline characteristics of participants

	Chitosan (n = 33)	Control (n = 33)	*P*-value
Age (years) mean ± SD	40.27 ± 7.20	39.00 ± 8.62	0.518*
Height (cm) mean ± SD	169.00 ± 10.19	167.63 ± 11.29	0.608*
Weight (kg) mean ± SD	87.13 ± 15.40	78.89 ± 13.87	0.026*
BMI (Kg/m^2) mean ± SD	30.37 ± 3.97	28.40 ± 5.69	0.108*
Gender			
Male	18 (54.5)	13 (39.4)	0.218#
Female	15 (45.5)	20 (60.6)	
Marital Status			
Single	5 (15.2)	7 (21.2)	0.523#
Married	28 (84.8)	26 (78.8)	
Education level			
Diploma or lower	17 (51.5)	12 (36.4)	0.118#
Bachelor	11 (33.3)	19 (57.6)	
Master or higher	5 (15.2)	2 (6.1)	
Job status			
Unemployed	5 (15.2)	4 (12.1)	0.895#
Employed	24 (72.7)	24 (72.7)	
Self-employed	4 (12.1)	5 (15.2)	
Income			
Low	4 (12.1)	1 (3.0)	0.355#
Middle	27 (81.8)	29 (87.9)	
High	2 (6.1)	3 (9.1)	
NAFLD grade			
1	12 (36.4)	15 (45.5)	0.453#
2	21 (63.6)	18 (54.5)	

BMI Body Mass Index, *NAFLD* Non-alcoholic Fatty Liver Disease

Data are presented 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 participants during the study based on the groups

	Chitosan (n = 33)	Control (n = 33)	*P*-value#
Energy (kcal/day)			
Before	2171.82 ± 653.75	2003.12 ± 443.52	0.224
After	1671.14 ± 484.19	1559.25 ± 472.04	0.345
P-value*	< 0.001	< 0.001	
Mean difference	− 500.68 ± 445.59	− 443.86 ± 209.94	0.511
Protein (gr/day)			
Before	78.24 ± 22.08	71.50 ± 21.75	0.216
After	60.99 ± 16.22	53.54 ± 17.91	0.081
P-value*	< 0.001	< 0.001	
Mean difference	− 17.24 ± 19.44	− 17.95 ± 15.71	0.870
Carbohydrate (gr/day)			
Before	297.89 ± 90.53	283.31 ± 66.77	0.459
After	223.41 ± 63.00	221.02 ± 72.60	0.887
P-value*	< 0.001	< 0.001	
Mean difference	− 74.48 ± 77.56	− 62.29 ± 51.32	0.455
Fat (gr/day)			
Before	76.55 ± 38.21	67.54 ± 26.16	0.269
After	61.49 ± 29.49	53.36 ± 22.57	0.213
P-value*	0.001	< 0.001	
Mean difference	− 15.05 ± 24.70	− 14.18 ± 14.95	0.862
Fiber (gr/day)			
Before	6.39 ± 4.23	4.88 ± 1.52	0.058
After	4.39 ± 1.37	4.49 ± 1.75	0.804
P-value*	0.012	0.191	
Mean difference	− 1.99 ± 4.32	− 0.38 ± 1.64	0.052
Physical activity			
Before	3980.15 ± 2939.87	4716.84 ± 3109.82	0.326
After	4059.03 ± 2740.25	4906.48 ± 2788.26	0.218
P-value*	0.672	0.373	
Mean difference	78.87 ± 922.96	189.63 ± 1205.28	0.677

Mean differences are calculated as: After–Before

Results are shown as mean ± SD

*Paired sample t-test

Independent sample t-test

P-value less than 0.05 considered significant

AST (− 2.27 ± 3.13 U/l, $P < 0.001$), ALT (− 3.90 ± 4.83 U/l, $P < 0.001$), and GGT (− 1.69 ± 4.51 U/l, $P = 0.039$), this is while consumption of placebo led to insignificant increment in AST (0.30 ± 6.37 U/l, $P = 0.787$), ALT (0.36 ± 4.64 U/l, $P = 0.656$), and GGT (0.30 ± 4.63 U/l, $P = 0.710$). After adjustment for weight change, between-group comparisons resulted in significant differences in ALT ($P = 0.040$), AST ($P = 0.001$), and GGT ($P = 0.028$).

No significant differences were observed for hepatic steatosis predictors in within- and between-group comparisons, even after adjustment for weight changes in the comparisons of changes for TyG, HSI, and FLI. However, TyG (− 0.04 ± 0.16) and FLI (− 1.47 ± 5.51) were reduced after chitosan supplementation but increased after consumption of placebo (TyG: 0.04 ± 0.25, FLI: 0.65 ± 5.38). Both groups showed insignificant reduction for HSI, this is while the reduction for the chitosan group was higher than the control group (chitosan: − 0.67 ± 2.03, control: − 0.22 ± 1.60).

Discussion

The present study was the first to investigate the effect of chitosan supplementation on liver function, hepatic steatosis predictors, and metabolic indices in adults with NAFLD. After 8 weeks of supplementations, chitosan consumption along with a low-calorie diet could possibly improve liver function, and reduce weight and WC. However, no promising effect was observed for blood biomarkers and hepatic steatosis predictors.

Table 3 Anthropometric and blood biochemical assessments of participants based on the groups

	Chitosan (n = 33)	Placebo (n = 33)	*P*-value[#]
Weight (kg)			
Before	87.13 ± 15.40	78.89 ± 13.87	0.026
After	85.43 ± 13.87	78.81 ± 12.88	0.049
P-value*	0.013	0.852	
Mean differences	− 1.70 ± 3.73	− 0.08 ± 2.45	0.041
BMI (Kg/m^2)			
Before	30.37 ± 3.97	28.40 ± 5.69	0.108
After	29.83 ± 3.65	28.34 ± 5.73	0.215
P-value*	0.016	0.740	
Mean differences	− 0.54 ± 1.22	− 0.05 ± 0.99	0.080
WC (cm)			
Before	107.87 ± 14.73	99.96 ± 16.08	0.041
After	106.06 ± 13.91	99.24 ± 15.33	0.063
P-value*	< 0.001	0.048	
Mean differences	− 1.81 ± 2.37	− 0.72 ± 2.03	0.049
FBS (mg/dl)			
Before	91.72 ± 16.74	96.09 ± 20.56	0.348
After	88.39 ± 8.15	95.18 ± 18.13	0.054
P-value*	0.099	0.455	
Mean differences	− 3.33 ± 11.27	− 0.90 ± 6.90	0.296
TG (mg/dl)			
Before	147.57 ± 62.77	151.12 ± 76.04	0.837
After	141.78 ± 52.35	150.69 ± 45.05	0.461
P-value*	0.188	0.956	
Mean differences	− 5.78 ± 24.70	− 0.42 ± 43.60	0.541
Cholesterol (mg/dl)			
Before	198.06 ± 95.39	177.03 ± 31.67	0.234
After	169.48 ± 41.82	181.75 ± 23.51	0.147
P-value*	0.160	0.115	
Mean differences	− 28.57 ± 114.20	4.72 ± 16.74	0.102
HDL (mg/dl)			
Before	41.12 ± 11.31	40.51 ± 6.56	0.791
After	43.87 ± 8.51	41.24 ± 5.88	0.148
P-value*	0.007	0.347	
Mean differences	2.75 ± 5.46	0.72 ± 4.37	0.100
LDL (mg/dl)			
Before	121.18 ± 73.86	107.54 ± 28.23	0.326
After	104.69 ± 27.26	108.00 ± 19.32	0.572
P-value*	0.116	0.849	
Mean differences	− 16.48 ± 58.53	0.45 ± 13.58	0.110

BMI Body Mass Index, *WC* Waist Circumference, *FBS* Fasting Blood Sugar, *TG* Triglycerides, *HDL* High-density lipoproteins, *LDL* Low-density lipoproteins

Mean differences are calculated as: After–Before

Results are shown as mean ± SD

*Paired sample t-test

[#] Independent sample t-test

P-value less than 0.05 considered significant

Table 4 Liver function and hepatic steatosis indices assessments of participants based on the groups

	Chitosan (n = 33)	Placebo (n = 33)	*P*-value
AST (U/l)			
Before	27.36 ± 8.91	26.06 ± 10.58	0.591[#]
After	25.09 ± 7.32	26.36 ± 9.11	0.534[#]
P-value*	< 0.001	0.787	
Mean differences	− 2.27 ± 3.13	0.30 ± 6.37	0.040[†]
ALT (U/l)			
Before	37.72 ± 20.35	29.45 ± 19.31	0.095[#]
After	33.81 ± 17.61	29.81 ± 19.05	0.379[#]
P-value*	< 0.001	0.656	
Mean differences	− 3.90 ± 4.83	0.36 ± 4.64	0.001[†]
GGT (U/l)			
Before	38.78 ± 31.17	34.72 ± 23.73	0.554[#]
After	37.09 ± 29.71	35.03 ± 22.05	0.750[#]
P-value*	0.039	0.710	
Mean differences	− 1.69 ± 4.51	0.30 ± 4.63	0.028[†]
TYG			
Before	8.72 ± 0.48	8.77 ± 0.53	0.712[#]
After	8.67 ± 0.40	8.82 ± 0.35	0.128[#]
P-value*	0.104	0.290	
Mean differences	− 0.04 ± 0.16	0.04 ± 0.25	0.159[†]
HSI			
Before	41.86 ± 5.32	38.33 ± 7.79	0.036[#]
After	41.19 ± 4.66	38.11 ± 7.54	0.051[#]
P-value*	0.067	0.427	
Mean differences	− 0.67 ± 2.03	- 0.22 ± 1.60	0.705[†]
FLI			
Before	71.16 ± 27.87	58.97 ± 31.40	0.100[#]
After	69.69 ± 26.26	59.62 ± 29.58	0.149[#]
P-value*	0.134	0.493	
Mean differences	− 1.47 ± 5.51	0.65 ± 5.38	0.571[†]

AST Aspartate Transferase, *ALT* Alanine Transaminase, *GGT* Gamma-glutamyl Transferase, *TYG* Triglyceride Glucose index, *HSI* Hepatic Steatosis Index, *FLI* Fatty Liver Index

Mean differences are calculated as: After–Before

Results are shown as mean ± SD

*Paired sample t-test

[#] Independent sample t-test

[†] ANCOVA test, adjusted for changes in weight

P-value less than 0.05 considered significant

The findings of the present study indicated that chitosan supplementation could reduce weight and WC. This result supports the finding of a previous study conducted by Mhurchu et al. [24]. Mhurchu [24] observed that 24 weeks of chitosan supplementation was able to reduce weight and BMI in obese and overweight adults, but the results were not significant for WC. The findings of a recent meta-analysis conducted by Huang [11] were also in line with our findings. This meta-analysis

indicated that chitosan consumption reduced weight, BMI, and WC. It should be noted that the subgroup analysis showed supplementation duration lower than 12 weeks has higher effects on body weight and BMI reduction [11]. The dose of chitosan used in the studies included in Huang's meta-analysis was between 300 mg per day and high doses of 4.5 g per day. Subgroup analysis indicated that doses lower than 2.4 g/day were not able to reduce BMI [11]. To support this finding, Fatahi et al. [12] observed that 3 g/day of chitosan supplement for 12 weeks can reduce weight, BMI, and WC. Thus, it justifies our non-significant findings for BMI.

Our findings did not show any significant results for FBS and lipid profile. Fatahi et al. [12] also did not observe promising effects of chitosan on FBS and lipid profiles. It should be noted that the intervention done by Fatahi had a higher dose (3 gr/day) and duration (12 weeks) [12]. Guo et al. [18] conducted a meta-analysis on the effect of chitosan on glycemic indices in individuals with metabolic syndrome. Their result showed FBS lowering effect of chitosan. Moreover, the sub-group analysis revealed that this finding was independent of the dosage of interventions. However, supplementation with durations longer than 13 weeks in comparison with shorter duration had a significant effect [18]. After conducting a meta-analysis on randomized controlled trials, Moraru et al. [25] concluded that chitosan could ameliorate total cholesterol, TG, and LDL, but not HDL. Considering the wide range of participants' health status, dose, and length of study in the investigations included in Moraru's meta-analysis, the results for lipid profile have been facing high heterogeneity [25].

Although our study did not show significant results for FBS and lipid profile, the reduction of FBS, TG, cholesterol, and LDL were higher in the chitosan group in comparison with the control group. Similar results were observed for the increment in HDL during the study period. It should be noted that between-group changes for HDL were not significant, thus, the HDL increments could be due to the calorie restriction during the study period [26]. Thus, the low sample size could justify the inconsistency of results with previous meta-analyses [18, 25]. In addition, the insignificant effect of chitosan supplementation in Fatahi's [12] and our study on FBS could be justified by the sub-group analysis of intervention duration in Guo's meta-analysis [18]. Hence, it can be concluded that chitosan supplementation should be done for a longer duration than 13 weeks to achieve promising effects on FBS, regardless of the dosage. Moreover, for the general population, 3 g of chitosan consumption has been recommended for promoting lipid profile and blood glucose [27]. This is while, according to the nature of the present study and its participants a lower dosage was used which this lower dosage could justify non-significant results for blood sugar and lipid profile. In addition, our participants did not suffer from hyperlipidemia or increased FBS at baseline which could justify the inconsistency between our findings and previous studies for blood glucose and lipid profile-promoting effects.

Several mechanisms could be suggested for the weight reduction, and lipid and FBS lowering of chitosan. First, chitosan can lower lipid absorption through the gastrointestinal tract by its fat-binding properties [28, 29]. Second, in animal models, it was observed that chitosan regulates adipokine secretions and consequently inhibits adipogenesis [30]. Third, in vivo investigations revealed chitosan consumption can increase the concentration of leptin and lower C-reactive protein which finally triggers weight loss [31, 32].

Chitosan consumption amended liver function in the present study. A previous study of chitosan on rats with hepatotoxicity indicated that supplementation with chitosan recovered ALT and AST levels [33]. In line with our study, oral administration of chitosan to rats reduced ALT, AST, and GGT [34]. Akbarzadeh et al. [35] showed intervention with fiber supplements along with a weight reduction diet in NAFLD patients could ameliorate NAFLD by reduction in weight, and lowering liver enzymes, which supports our findings. Moreover, chitosan was observed to improve NAFLD in mice [13]. On the other hand, in a randomized controlled trial, celery powder with high fiber content did not affect ALT or AST [36].

On the other hand, the result of the present study did not show a beneficial effect of chitosan on hepatic steatosis predictors. On the contrary, previous studies indicated that high-fiber supplements could improve hepatic steatosis [37, 38]. Although our results were not significant for hepatic steatosis predictors, TyG, HSI, and FLI were reduced in the chitosan group, while increased in the control group. This observation could be due to the insufficiency of sample size for interpreting these predictors. Weight reduction is a cornerstone in the management of NAFLD which was observed to improve liver function and hepatic steatosis [4]. Thus, considering our findings, it can be concluded that chitosan supplementation could possibly improve liver function and hepatic steatosis through weight reduction.

The present study was the first to investigate the effect of chitosan on liver function and hepatic steatosis predictors. Although, including participants after confirming the NAFLD with sonographic imaging results, we did not use this test to observe the effect of intervention. Moreover, the relatively lower dosage and duration of the study in comparison with other studies limited our findings. In addition, assessment of serum insulin and insulin

resistance indices could help to achieve a better understanding of the chitosan effect on the glycemic status of participants.

Conclusions

This study showed that an 8-week intervention with 1.5 g/day chitosan along with a low-calorie diet can possibly reduce weight and waist circumference, and improve liver function, and consequently ameliorate NAFLD. Further investigations with different dosages, longer duration, and higher sample sizes are suggested to achieve more accurate results and to observe the effect of these interventions on hepatic steatosis predictors, fasting blood sugar, and lipid profile.

Abbreviations

ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate transaminase
BMI	Body mass index
ELISA	The enzyme-linked immunosorbent assay
FBS	Fasting blood sugar
FLI	Fatty liver index
GGT	Gamma-glutamyltransferase
HDL	High-density lipoprotein
HIS	Hepatic steatosis index
IPAQ-SF	International Physical Activity Questionnaire-Short form
IRB	Institutional reviewing board
LDL	Low-density lipoprotein
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
SD	Standard deviation
TC	Total cholesterol
TG	Triglycerides
TyG	Triglyceride-glucose index
WC	Waist circumference

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Author contributions

MAM, MT, and MA have designed the study. MAM, KP, SA, MAA, MRM, MAJ, and RB conducted the study. MAM, MT, and MA have performed statistical analysis. MAM, KP, SA, MAA, MRM, MAJ, and RB have prepared the first draft of the manuscript. MT and MA have revised the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

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

Declarations

Ethics approval and consent to participate

The study protocol was adhered to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Research Ethics Committees of Baqiyatallah Hospital (approval ID: IR.BMSU.BAQ.REC.1401.112). The study protocol was also registered in the Iranian Registry of Clinical Trials (irct.ir, registration ID: IRCT20140502017522N4, dated: March 2023). In addition, all participants signed informed consent form prior to the participation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Student Research Committee, Baqiyatallah University of Medical Sciences, Tehran, Iran. [2]Health Research Centre, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran. [3]Department of Nutrition and Food Hygiene, Faculty of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran. [4]Nutrition Research Center, Department of Community Nutrition, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran. [5]Baqiyatallah Research Center for Gastroenterology and Liver Diseases (BRCGL), Baqiyatallah University of Medical Sciences, Tehran, Iran. [6]Marine Medicine Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran. [7]Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran.

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