Effect of garlic powder supplementation on hepatic steatosis, liver enzymes and lipid profile in patients with non-alcoholic fatty liver disease: a double-blind randomised controlled clinical trial

Abbas Ali Sangouni[1], Mohammad Reza Mohammad Hosseini Azar[2] and Mohammad Alizadeh[3]*

[1]*Student Research Committee, Department of Nutrition, Urmia University of Medical Sciences, PO Box 5756115111, West Azarbaijan Province, Urmia, Iran*

[2]*Gastroenterology and Hepatology Subdivision of Internal Medicine Department, Imam Khomeini Hospital, School of Medicine, Urmia University of Medical Sciences, PO Box 5756115111, West Azarbaijan Province, Urmia, Iran*

[3]*Food and Beverages Safety Research Center, Department of Nutrition, School of Medicine, Urmia University of Medical Sciences, PO Box 5756115111, West Azarbaijan Province, Urmia, Iran*

Abstract

Non-alcoholic fatty liver disease (NAFLD) includes a range of disorders from simple steatosis to non-alcoholic steatohepatitis. There is no proven drug treatment for NAFLD, and diet modification is considered part of the main line of treatment for this disease. The aim of this study was to investigate the efficacy of garlic supplementation in NAFLD patients. The effect of garlic powder supplementation on hepatic steatosis, liver enzymes and lipid profile was investigated in NAFLD patients. Ninety NAFLD patients were randomly assigned to take either a garlic powder supplement or a placebo for 12 weeks. The treatment group received four tablets of garlic daily (each tablet contained 400 mg garlic powder). The control group received four tablets of placebo (each placebo contained 400 mg starch). At the end of the study, hepatic steatosis was significantly reduced in the treatment group compared with the control group ($P = 0.001$). In addition, a significant decrease was seen in the serum concentration of alanine transaminase ($P < 0.001$), aspartate transaminase ($P = 0.002$), γ-glutamyltransferase ($P = 0.003$) as well as total cholesterol ($P = 0.009$), TAG ($P < 0.001$), HDL-cholesterol ($P < 0.001$) and LDL-cholesterol ($P = 0.01$) in the treatment group compared with the control group. No significant difference was seen between the two groups in serum concentration of alkaline phosphatase. Overall, garlic powder supplementation improved hepatic features and lipid profile among NAFLD patients.

Key words: Non-alcoholic fatty liver disease: Garlic: Hepatic steatosis: Liver enzymes: Lipid profile

Non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disease worldwide, includes a range from steatosis to non-alcoholic steatohepatitis[1,2]. Steatosis is a simple fat accumulation without significant inflammation or hepatocellular injury, and fat accumulation along with inflammation and evidence of hepatocellular injury refers to non-alcoholic steatohepatitis[2,3]. The prevalence of NAFLD is estimated to be 25 % in the world[4], and based on a meta-analysis study, its prevalence among Iranian people is 33·95 %[5]. The global rate of the NAFLD mortality was nearly 1·27 million in 2015[6]. Although the complete mechanism of NAFLD pathogenesis still remains unclear, obesity, insulin resistance and oxidative stress are the most important causes associated with pathogenesis of this disease[7,8]. Also, the association of NAFLD with elevated liver enzymes levels and dyslipidaemia has been determined[9]. Dyslipidaemia plays an important role in the pathogenesis of NAFLD by increasing liver NEFA uptake and very-LDL (VLDL) synthesis and also by reducing NEFA oxidation and TAG export[10]. Currently, lifestyle modifications including adherence to a healthy diet, weight loss and regular physical activity are considered as the only proven strategy for NAFLD treatment[11,12].

Garlic has been known as a therapeutic plant in various cultures, throughout history[13]. Bioactive compounds of garlic including allicin, S-allylcysteine, ajoene, diallyl disulphide, S-allylcysteine sulphoxide and S-methylcysteine sulphoxide have caused its therapeutic properties[14,15]. According to the evidence, garlic consumption reduces the risk of CVD and

---

cancer[15,16]. Experimental and clinical investigations have shown the beneficial effects of garlic on insulin resistance and obesity, which are linked to NAFLD pathogenesis, and as a result, garlic may help improve NAFLD[17,18]. Recently, the beneficial effect of garlic on fatty liver and liver enzymes such as alanine transaminase (ALT) and γ-glutamyltransferase (GGT) has been suggested[19,20]. To the best of our knowledge, there is no clinical trial evaluating the effects of garlic consumption on hepatic steatosis and liver enzymes in patients with NAFLD. Also, findings related to the effects of garlic consumption on dyslipidaemia are not integrated[21], and this issue has not been studied in patients with NAFLD. Accordingly, this clinical trial was designed to evaluate the effects of garlic powder supplementation on hepatic steatosis, liver enzymes and lipid profile (as the criterion for dyslipidaemia) among patients with NAFLD.

Methods

Recruitment and eligibility screening

One hundred and ten patients with NAFLD were identified and screened consecutively at Imam Khomeini educational and medical centre in Urmia, Iran. Inclusion criteria included patients with grade 1–3 fatty liver and aged ≥18 years. Patients were excluded from the study if one of the following conditions existed: history of alcohol abuse (an average daily alcohol consumption of ≥10 g for women and ≥20 g for men), viral hepatitis, liver cancer, other liver diseases, diabetes mellitus, untreated hypothyroidism, mental diseases, kidney diseases, pregnancy, lactation, low blood pressure, taking blood pressure lowering medications, allergic to garlic and unwillingness to continue the study. Finally, ninety patients were included in the study.

Trial design

This 12-week study was double-blind, randomised controlled clinical trial. Written consent signed by participants was approved by the ethics committee of Urmia University of Medical Sciences. The participants were informed about the risks and benefits of the study and were free to drop out at any time for any reason. Patients, researcher, laboratory staff and statistician were blinded to the study groups until the end of the study. At baseline, patients were randomly divided into two groups. Randomisation lists were computer-generated by a statistician, and then participants were assigned to the study groups. A trained person randomly allocated and assigned the participants to the study groups. The treatment group received 400 mg garlic powder tablets (each coated tablet contained 1·5 mg allicin, approximately 2 g of fresh garlic) four times/d, and the control group received four placebo tablets (each 400 mg coated tablet contained starch) daily along with the usual treatment. The usual treatment for all patients was prescribing milk thistle tablets and recommending weight loss without offering any method. The study protocol was registered at the Iranian clinical trials website (IRCT20170206032417N4).

Intervention

Participants were given garlic and placebo tablets every 3 weeks. Based on the study of Lawson & Hunsaker[22], the effective dose of allicin for clinical trials is 3·6–7·8 mg daily. So, patients took four tablets daily, which provide 6 mg allicin. Participants took two tablets an hour before lunch and two tablets an hour before dinner, due to the reduced garlic bioavailability during protein intake[22]. Garlic and placebo tablets were manufactured at Amin Pharmaceutical Company. Placebo and garlic powder tablets had similar appearance. The consumption of garlic powder and placebo tablets was monitored every 3 weeks. Poor compliance was defined as <80 % of expected tablets taken.

Dietary intake and physical activity assessment

In order to measure average intake of food group servings including dairy products, meats, grains, vegetables, fruits, fats and sugars, a 3-d (one weekend day and two non-consecutive weekdays) 24-h recall questionnaire was used at weeks 0, 6 and 12. In addition, the average consumption of allicin-containing plants including garlic, onion, scallion and leek at weeks 0, 6 and 12 was evaluated. Assessment of physical activity was performed at weeks 0, 6 and 12 by using the metabolic equivalent of task questionnaire.

Laboratory evaluations

Laboratory tests were performed at weeks 0, 6 and 12 to determine serum concentrations of ALT, aspartate transaminase, GGT, alkaline phosphatase (ALP) as well as total cholesterol (TC), TAG, HDL-cholesterol and LDL-cholesterol. Blood was drawn after 12 h fasting. At a speed of 3600 rpm, blood samples were centrifuged for 10 min. Serum samples were evenly poured into the microtubes, and the microtubes were immediately frozen at –80°C. ALT, aspartate transaminase, GGT, ALP as well as TC, TAG, HDL-cholesterol and LDL-cholesterol were measured by using routine enzymatic assays with commercial kits (Pars Azmoon) using an autoanalyzer (AVIDA 1800 chemistry system; Siemens). All laboratory assessments were performed in Department of Nutrition laboratory by the standard laboratory methods.

Liver ultrasonography

At the beginning and end of the study, hepatic steatosis was evaluated by abdominal ultrasound. In order to perform the assessment, participants were in fasting and supine position. The grade of steatosis was determined based on the modified criteria of Kurtz et al.[23] including liver brightness, the contrast ratio of liver:kidney, blurred vessels, narrowing of the lumen of the hepatic veins and attenuation of echogenicity on ultrasonography (Philips Affiniti 50 Ultrasound). Subjects with grade 1–3 identified by ultrasound were included in the study. By using semi-quantitative criteria for steatosis, results were graded including normal: hepatic echogenicity was equal to renal cortex, grade 1: increased diffuse hepatic echogenicity along with visible periportal and diaphragmatic echogenicity, grade 2: increased diffuse hepatic echogenicity along with invisible periportal echogenicity without obscuration of the diaphragm and grade 3: increased diffuse hepatic echogenicity along with invisible periportal echogenicity and obscuration of the

diaphragm. All ultrasound examinations were performed by one professional technician using the same device and criteria.

Anthropometric assessment

At the beginning of the study, the height of patients was measured by a stadiometer in standing position without shoes with an accuracy of 0·5 cm. Weight and waist circumference (WC) were measured by scale and bioelectrical impedance analyzer (In Body 770) based on standard protocols with light clothes and without shoes, respectively, at weeks 0, 6 and 12. BMI was calculated by the following formula: weight (kg)/height squared (m^2).

Statistical analysis

The appropriate sample size, based on the study of Ashraf *et al.*[24] with $\alpha = 0.05$ and power = 95 %, was estimated to be thirty-seven per study group. Considering a drop-out rate of approximately 20 %, the final sample size needed was estimated to be forty-five per group. In order to finding the confounding factors, independent *t* test (for continuous variables) and χ^2 test (for categorical variables) were used to compare differences in general characteristics and dietary intakes between the two groups at baseline. Differences in NAFLD grade changes at the end of the study (reduction of grade or unchanged) were analysed by χ^2. To determine the effect of garlic powder and placebo on serum concentration of ALT, aspartate transaminase, GGT, ALP as well as TC, TAG, HDL-cholesterol and LDL-cholesterol, we applied a general linear model ANOVA for repeated measurements with Bonferroni *post hoc* analysis. In these analyses, the groups were regarded as between-subject factors and time was considered as a within-subject factor. Data were analysed with the use of SPSS version 24 (SPSS, Inc.). For all analyses, $P < 0.05$ was considered significant and all data are shown as mean values and standard deviations if not indicated otherwise. All of the variables had normal distribution.

Results

Characteristics of the patients

In this randomised controlled clinical trial, recruitment, screening and follow-up consecutively were conducted from August 2018 to March 2019. Ninety participants were randomly divided into two groups. During follow-up, two participants were excluded from trial due to surgery (*n* 1) and lost to follow-up (*n* 1). Eventually, eighty-eight participants completed the trial: treatment group (*n* 45) and control group (*n* 43) (Fig. 1).

Demographic and anthropometric factors

There were no significant differences between the two groups in baseline demographic characteristics (Table 1). No significant difference between the two groups was seen in physical activity ($P > 0.05$) during the study. Based on the 3-d 24-h food recall, no significant differences were observed in the mean intake of energy, consumed food groups and allicin-containing plants ($P > 0.05$) between the two groups.
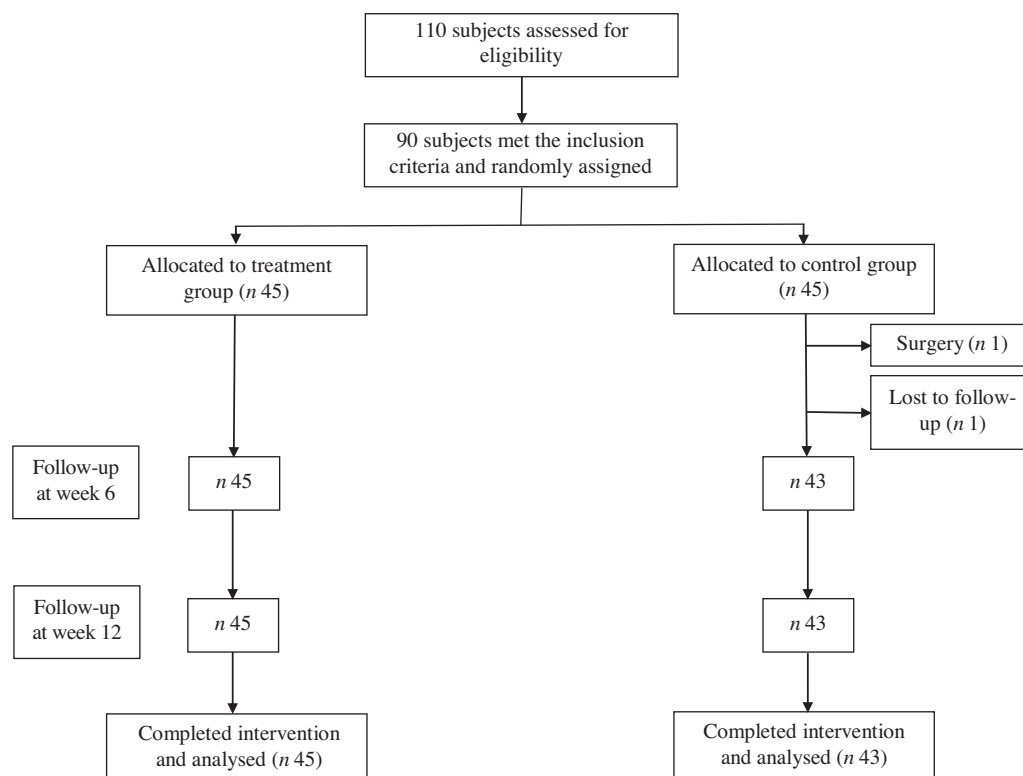

Fig. 1. Flow diagram of the study participants.

Table 1. Demographic characteristics of patients with non-alcoholic fatty liver disease (NAFLD)*
(Mean values and standard deviations; numbers and percentages)

	Treatment group (n 45)		Control group (n 43)		
	Mean	SD	Mean	SD	P
Age (years)	45·2	12·4	44·2	11·1	0·68
Sex					0·08
Male					
n	33		24		
%	73·3		55·8		
Female					
n	12		19		
%	26·7		44·2		
NAFLD duration (months)	7·67	4·0	8·07	4·0	0·31
Serum liver enzymes (IU/l)					
ALT	30·9	15·7	24·9	15·4	0·07
AST	22·8	11·1	21·2	7·3	0·41
GGT	32·0	21·0	28·7	12·0	0·36
ALP	203·8	56·9	190·9	63·1	0·31
Serum lipids (mmol/l)					
TC	5·3	0·9	5·2	1·0	0·57
TAG	2·1	0·9	2·1	0·9	0·94
HDL-cholesterol	1·1	0·2	1·2	0·3	0·08
LDL-cholesterol	3·2	0·7	2·9	0·7	0·11
Metabolic characteristics					
Height (cm)	172·2	9·8	168·8	11·0	0·13
Weight (kg)	89·8	11·9	90·0	13·6	0·95
BMI (kg/m^2)	30·2	3·1	31·6	3·8	0·08
WC (cm)	105·6	9·8	108·4	10·4	0·19
MET-h/d	28·8	3·8	27·8	3·6	0·23
Energy intake (kcal/d)†	2041	123	2034	136	0·80

ALT, alanine transaminase; AST, aspartate transaminase; GGT, γ-glutamyltransferase; ALP, alkaline phosphatase; TC, total cholesterol; WC, waist circumference; MET-h, metabolic equivalent task hours.
*P values were computed by using independent t test for continuous variables and by χ^2 for categorical variables.
†To convert energy values from kcal to kJ multiply values by 4·184.

Table 2. Effects of garlic powder on anthropometric indices of patients with non-alcoholic fatty liver disease*
(Mean values and standard deviations)

Anthropometric indices	Treatment group (n 45)		Control group (n 43)		P_{Time}	P_{Group}	$P_{Time \times Group}$
	Mean	SD	Mean	SD			
Weight (kg)					0·02[a,b]	0·42	0·86
Week 0	89·8	11·9	90·0	13·6			
Week 6	89·5	11·8	90·0	13·6			
Week 12	89·2	11·8	89·8	13·4			
Mean difference	−0·6	1·2	−0·1	1·5			
BMI (kg/m^2)					0·02	0·06	0·12
Week 0	30·2	3·1	31·6	3·8			
Week 6	30·1	3·1	31·6	3·8			
Week 12	30·0	3·1	31·5	3·8			
Mean difference	−0·19	0·4	−0·03	0·5			
WC (cm)					0·001[a,b]	0·008	0·001
Week 0	105·6	9·8	108·4	10·4			
Week 6	104·9	9·7	108·9	10·9			
Week 12	104·0	9·7	108·4	10·8			
Mean difference	−1·58	2·1	−0·03	1·9			

WC, waist circumference.
Post hoc test: [a]significant difference between baseline and week 12; [b]significant difference between week 6 and week 12.
*P values were computed by general linear model ANOVA for repeated measurements.

The anthropometric indices of patients with NAFLD are shown in Table 2. At baseline, no significant difference was observed in anthropometric measurements including weight, BMI and WC. At the end of the study, only WC in the treatment group was significantly decreased compared with the control group ($P = 0.001$) (Table 2). According to the significant improvement of WC at the end of the study, mean change of WC was entered as a covariate in the analysis.

Outcomes

We observed hepatic steatosis significantly reduced in the treatment group compared with the control group ($P = 0.001$) (Table 3). Also, ALT ($P < 0.001$), aspartate transaminase ($P = 0.002$) and GGT ($P = 0.003$) in the treatment group were significantly decreased compared with the control group (Table 4).

In addition, a significant decrease was observed in TC ($P = 0.009$), TAG ($P < 0.001$), HDL-cholesterol ($P < 0.001$) and LDL-cholesterol ($P = 0.01$) in the treatment group compared with the control group (Table 4).

At the end of the study, there was no significant difference between the two groups in ALP. After controlling the effect of WC as a confounder, the results remained unchanged.

Discussion

To our knowledge, this study was the first clinical trial to evaluate the effect of garlic powder supplementation on hepatic steatosis, liver enzymes and lipid profile in patients with NAFLD. The study found that 12-week garlic powder supplementation improved hepatic steatosis, liver enzymes (except for ALP) and lipid profile as the criterion for dyslipidaemia. Possible mechanisms of the effect of garlic on improving hepatic steatosis and consequently liver enzymes could be the regulation of lipogenesis by decreasing the activity of enzymes involved in liver fat production, insulin resistance, NF-κB pathway activity and gene expression of oxidative stress markers[19,25]. Consistent with our study, Maeda et al.[19] demonstrated that aged garlic extract ameliorates fatty liver in a mouse model of insulin resistance. Also, in the study of Xiao et al.[26] after garlic-derived S-allylmercaptocysteine intake, liver steatosis, fibrosis and NAFLD activity score significantly improved in rats with NAFLD. Usually, elevated liver enzymes are a consequence of NAFLD, though not always. In line with our results, Kim et al.[20] showed a significant reduction of ALT and GGT in patients with elevated levels of serum GGT. Also, Xiao et al.[26] showed garlic-derived S-allylmercaptocysteine improved ALT in NAFLD rats.

As mentioned above, dyslipidaemia is an important risk factor in NAFLD pathogenesis[10]. The beneficial effects of garlic on dyslipidaemia could be due to mechanisms such as decreasing activity of enzymes involved in liver fat production and regulation of lipogenesis, decreasing intestinal absorption of TAG and increasing adiponectin levels[27,28]. In the study of Ashraf et al.[24], consumption of raw garlic resulted in a significant decrease in LDL-cholesterol and TC levels as well as a significant increase in HDL-cholesterol among type 2 diabetes patients with dyslipidaemia; however, there was no significant decrease in serum TAG levels. Also, Heidarian et al.[29] showed improvement in LDL-cholesterol, VLDL and TAG after garlic intake in hyperlipidaemic rats. Contrary to the results of our study, in the randomised controlled clinical trial of Jung et al.[30], no significant improvement was observed in TAG, TC and LDL-cholesterol levels after black garlic extract supplementation and there was only a significant increase in HDL-cholesterol levels. Also, Turner et al.[31] showed no significant improvement in TAG, TC, LDL-cholesterol and HDL-cholesterol levels in healthy subjects after garlic powder supplementation. The contradictory

Table 3. Comparison of pre- and post-intervention grades of liver steatosis assessed by ultrasonography in patients with non-alcoholic fatty liver disease (NAFLD)*
(Numbers and percentages)

| | Week 0 | | | | | | Week 12 | | | | | | | | |
| | Grade 1 | | Grade 2 | | Grade 3 | | 1 grade reduction | | 2 grade reduction | | Without change | | 1 grade increase | | Number of grade reduction units | | |
Group	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	P
Treatment group	11	24·4	29	64·4	5	11·2	28	62·2	2	4·4	15	33·4	0	0·0	32	71·1	0·001
Control group	12	27·9	29	67·4	2	4·7	11	25·6	0	0·0	30	69·7	2	4·7	9	20·9	

* On the basis of χ^2 test, there were no significant differences between groups with regard to baseline grades of liver steatosis ($P = 0.52$). P value was computed by χ^2 test to compare the difference in number of NAFLD grade reduction units between two groups at week 12.

Table 4. Effects of garlic powder on serum levels of liver enzymes and lipid profile*
(Mean values and standard deviations)

| | Treatment group | | | | | | Control group | | | | | | | | |
| | Total (n 45) | | Men (n 33) | | Women (n 12) | | Total (n 43) | | Men (n 24) | | Women (n 19) | | P_{Time} | P_{Group} | $P_{Time \times Group}$ |
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Serum liver enzymes															
ALT (IU/l)													0.01[a,c]	0.77	<0.001
Week 0	30.9	15.7	35.1	16.1	19.4	5.6	24.9	15.4	30.0	17.8	18.5	8.6	0.004†	0.054†	<0.001†
Week 6	29.6	13.6	32.5	14.0	19.4	5.9	29.7	18.3	36.0	21.8	21.8	7.5			
Week 12	26.0	13.2	29.5	13.8	16.5	4.0	28.5	17.6	33.9	20.2	21.7	10.7			
Mean difference	−4.90	6.5	−5.63	7.1	−2.88	3.7	+3.56	8.4	+3.93	7.6	+3.10	9.4			
AST (IU/l)													0.07[c]	0.55	0.002
Week 0	22.8	11.1	24.7	12.4	17.7	3.1	21.2	7.3	22.5	7.8	19.5	6.5	0.07†	0.76†	0.01†
Week 6	21.9	8.3	23.6	9.1	17.3	2.1	24.3	9.8	26.6	11.8	21.4	5.5			
Week 12	20.6	8.6	22.3	9.5	15.9	2.0	23.1	9.4	24.6	10.8	21.1	7.2			
Mean difference	−2.29	4.2	−2.46	4.6	−1.80	2.6	+1.90	7.4	+2.10	7.4	+1.64	7.47			
GGT (IU/l)													0.10	0.67	0.003
Week 0	32.0	21.0	36.0	23.1	21.0	6.0	28.7	12.0	29.9	13.9	27.1	9.3	0.32†	0.76†	0.02†
Week 6	30.9	20.0	34.9	21.8	19.9	5.9	29.4	11.4	30.8	13.2	27.6	8.7			
Week 12	29.0	16.4	32.3	17.9	20.0	5.3	29.5	12.3	30.5	13.8	28.2	10.4			
Mean difference	−3.01	6.0	−3.73	6.7	−1.05	2.5	+0.82	5.8	+0.58	3.2	+1.12	8.2			
ALP (IU/l)													0.051	0.32	0.63
Week 0	203.8	56.9	207.9	62.3	192.6	38.2	190.9	63.1	197.3	78.2	182.8	36.6	0.08†	0.38†	0.65†
Week 6	206.7	53.4	209.9	57.6	197.9	40.6	195.1	56.5	195.7	69.8	194.3	34.8			
Week 12	200.3	49.0	202.5	53.1	194.2	36.6	190.0	56.7	188.1	68.7	192.3	38.3			
Mean difference	−3.51	21.1	−5.36	22.7	+1.58	15.8	−0.95	29.2	−9.20	33.3	+9.47	19.1			
Serum lipids															
TC (mmol/l)													0.002[b,c]	0.77	0.009
Week 0	5.3	1.0	5.2	1.0	5.6	0.6	5.2	1.0	5.2	1.1	5.1	1.0	0.007†	0.81†	0.02†
Week 6	5.2	1.0	5.1	1.0	5.6	0.7	5.3	1.0	5.2	1.0	5.4	1.0			
Week 12	5.0	0.9	4.9	0.9	5.2	0.8	5.2	0.9	5.1	0.9	5.3	0.9			
Mean difference	−0.3	0.5	−0.3	0.5	−0.4	0.6	0	0.6	−0.2	0	+0.2	0.7			
TAG (mmol/l)													0.01[b]	0.27	<0.001
Week 0	2.1	0.9	2.1	1.0	2.2	0.6	2.1	0.9	2.2	1.0	2.0	0.8	0.01†	0.58†	<0.001†
Week 6	2.0	0.9	1.9	1.0	2.1	0.5	2.1	0.9	2.3	1.0	2.0	0.8			
Week 12	1.7	0.7	1.7	0.8	1.8	0.3	2.2	0.9	2.3	1.0	2.1	0.9			
Mean difference	−0.4	0.6	−0.4	0.6	−0.4	0.5	+0.1	0.5	0	0.3	+0.1	0.7			
HDL-cholesterol (mmol/l)													<0.001[a,b,c]	0.98	<0.001
Week 0	1.1	0.2	1.1	0.2	1.2	0.1	1.2	0.3	1.2	0.2	1.3	0.3	<0.001†	0.83†	<0.001†
Week 6	1.3	0.2	1.2	0.2	1.4	0.2	1.2	0.2	1.2	0.2	1.3	0.2			
Week 12	1.2	0.2	1.2	0.2	1.3	1.2	1.2	0.2	1.1	0.2	1.3	0.2			
Mean difference	+0.1	0.1	+0.1	0.1	+0.1	0.1	0	0.1	0	0.1	0	0.1			
LDL-cholesterol (mmol/l)													<0.001[b,c]	0.32	0.01
Week 0	3.2	0.7	3.1	0.7	3.3	0.6	2.9	0.7	2.9	0.7	3.0	0.6	<0.001†	0.22†	0.01†
Week 6	3.1	0.8	3.0	0.8	3.3	0.4	2.9	0.6	2.9	0.5	3.0	0.6			
Week 12	2.9	0.6	2.8	0.7	3.0	0.6	2.9	0.6	2.8	0.6	3.0	0.5			
Mean difference	−0.3	0.4	−0.3	0.4	−0.3	0.3	−0.1	0.4	−0.2	0.4	0	0.4			

ALT, alanine transaminase; AST, aspartate transaminase; GGT, γ-glutamyltransferase; ALP, alkaline phosphatase; TC, total cholesterol; WC, waist circumference.
Post hoc test: [a]significant difference between baseline and week 6; [b]significant difference between baseline and week 12; [c]significant difference between week 6 and week 12.
*P values were computed by general linear model ANOVA for repeated measurements.
†Adjusted based on mean changes of WC and baseline values of parameters.

findings regarding the effect of garlic consumption on lipid profile can be attributed to the use of different types of garlic as well as different design of studies. In this regard, it seems that consumption of raw garlic (but not black garlic) by patients with dyslipidaemia has the best results in normalising lipid profile and no significant change was observed in lipid profile among healthy subjects. During the study, no serious side effects were reported by participants.

The most important strengths of the present study include high rate of compliance (97·7 %) and evaluation of the consumption of allicin-containing plants.

One of the limitations of the study is the use of liver ultrasonography due to its low cost, non-invasiveness and availability, despite biopsy is a 'gold standard' of liver steatosis assessment[32].

This clinical trial demonstrated that garlic powder supplementation for 12 weeks improves hepatic steatosis, liver enzymes and lipid profile as an indicator for dyslipidaemia. As a result, it seems that using garlic is logical in modified diet of patients with NAFLD, which is a main part of NAFLD treatment strategy. Further clinical studies with longer duration are needed to determine whether these effects of garlic will be sustained.

References

1. Rinella ME (2015) Nonalcoholic fatty liver disease a systematic review. *JAMA* **313**, 2263–2273.
2. Chalasani N, Younossi Z, Lavine JE, *et al.* (2012) The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* **55**, 2005–2023.
3. Polyzos SA & Mantzoros CS (2016) Nonalcoholic fatty future disease. *Metabolism* **65**, 1007–1016.
4. Araújo AR, Rosso N, Bedogni G, *et al.* (2018) Global epidemiology of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: what we need in the future. *Liver Int* **38**, Suppl. 1, 47–51.
5. Moghaddasifar I, Lankarani KB, Moosazadeh M, *et al.* (2016) Prevalence of non-alcoholic fatty liver disease and its related factors in Iran. *Int J Organ Transplant Med* **7**, 149–160.
6. Estes C, Razavi H, Loomba R, *et al.* (2018) Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology* **67**, 123–133.
7. Day CP & James OFW (1998) Steatohepatitis: a tale of two "hits"? *Gastroenterology* **114**, 842–845.
8. Polyzos SA, Kountouras J & Mantzoros CS (2017) Adipose tissue, obesity and nonalcoholic fatty liver disease. *Minerva Endocrinol* **42**, 92–108.
9. Gao X & Fan JG (2013) Diagnosis and management of non-alcoholic fatty liver disease and related metabolic disorders: consensus statement from the Study Group of Liver and Metabolism, Chinese Society of Endocrinology. *J Diabetes* **5**, 406–415.
10. Sangouni AA, Ghavamzadeh S & Jamalzehi A (2019) A narrative review on effects of vitamin D on main risk factors and severity of non-alcoholic fatty liver disease. *Diabetes Metab Syndr* **13**, 2260–2265.
11. Zelber-Sagi S, Godos J & Salomone F (2016) Lifestyle changes for the treatment of nonalcoholic fatty liver disease: a review of observational studies and intervention trials. *Therap Adv Gastroenterol* **9**, 392–407.
12. Clark JM (2006) Weight loss as a treatment for nonalcoholic fatty liver disease. *J Clin Gastroenterol* **40**, Suppl. 1, S39–S43.
13. Bayan L, Koulivand PH & Gorji A (2014) Garlic: a review of potential therapeutic effects. *Avicenna J Phytomed* **4**, 1–14.
14. Iciek M, Kwiecień I & Włodek L (2009) Biological properties of garlic and garlic-derived organosulfur compounds. *Environ Mol Mutagen* **50**, 247–265.
15. El-Bayoumy K, Sinha R, Pinto JT, *et al.* (2006) Cancer chemoprevention by garlic and garlic-containing sulfur and selenium compounds. *J Nutr* **136**, Suppl. 1, 864S–869S.
16. Chan JYY, Yuen ACY, Chan RYK, *et al.* (2013) A review of the cardiovascular benefits and antioxidant properties of allicin. *Phytother Res* **27**, 637–646.
17. Padiya R, Khatua TN, Bagul PK, *et al.* (2011) Garlic improves insulin sensitivity and associated metabolic syndromes in fructose fed rats. *Nutr Metab* **8**, 53.
18. Soleimani D, Paknahad Z, Askari G, *et al.* (2016) Effect of garlic powder consumption on body composition in patients with nonalcoholic fatty liver disease: a randomized, doubleblind, placebo-controlled trial. *Adv Biomed Res* **5**, 2.
19. Maeda T, Miki S, Morihara N, *et al.* (2019) Aged garlic extract ameliorates fatty liver and insulin resistance and improves the gut microbiota profile in a mouse model of insulin resistance. *Exp Ther Med* **18**, 857–866.
20. Kim HN, Kang SG, Roh YK, *et al.* (2017) Efficacy and safety of fermented garlic extract on hepatic function in adults with elevated serum gamma-glutamyl transpeptidase levels: a double-blind, randomized, placebo-controlled trial. *Eur J Nutr* **56**, 1993–2002.
21. Zeng T, Zhang CL, Zhao XL, *et al.* (2013) The roles of garlic on the lipid parameters: a systematic review of the literature. *Crit Rev Food Sci Nutr* **53**, 215–230.
22. Lawson LD & Hunsaker SM (2018) Allicin bioavailability and bioequivalence from garlic supplements and garlic foods. *Nutrients* **10**, 812.
23. Kurtz AB, Dubbins PA, Rubin CS, *et al.* (1981) Echogenicity: analysis, significance, and masking. *Am J Roentgenol* **137**, 471–476.
24. Ashraf R, Aamir K, Shaikh AR, *et al.* (2005) Effects of garlic on dyslipidemia in patients with type 2 diabetes mellitus. *J Ayub Med Coll Abbottabad* **17**, 60–64.
25. Trio PZ, You S, He X, *et al.* (2014) Chemopreventive functions and molecular mechanisms of garlic organosulfur compounds. *Food Funct* **5**, 833–844.
26. Xiao J, Ching YP, Liong EC, *et al.* (2013) Garlic-derived S-allyl-mercaptocysteine is a hepato-protective agent in non-alcoholic fatty liver disease *in vivo* animal model. *Eur J Nutr* **52**, 179–191.
27. Ha AW, Ying T & Kim WK (2015) The effects of black garlic (*Allium satvium*) extracts on lipid metabolism in rats fed a high fat diet. *Nutr Res Pract* **9**, 30–36.
28. Lin MC, Wang E-J, Lee C, *et al.* (2002) Garlic inhibits microsomal triglyceride transfer protein gene expression in human liver and intestinal cell lines and in rat intestine. *J Nutr* **132**, 1165–1168.
29. Heidarian E, Jafari-Dehkordi E & Seidkhani-Nahal A (2011) Effect of garlic on liver phosphatidate phosphohydrolase and plasma lipid levels in hyperlipidemic rats. *Food Chem Toxicol* **49**, 1110–1114.
30. Jung ES, Park SH, Choi EK, *et al.* (2014) Reduction of blood lipid parameters by a 12-wk supplementation of aged black garlic: a randomized controlled trial. *Nutrition* **30**, 1034–1039.
31. Turner B, Mølgaard C & Marckmann P (2004) Effect of garlic (*Allium satvium*) powder tablets on serum lipids, blood pressure and arterial stiffness in normo-lipidaemic volunteers: a randomised, double-blind, placebo-controlled trial. *Br J Nutr* **92**, 701–706.
32. Saleh HA & Abu-Rashed AH (2007) Liver biopsy remains the gold standard for evaluation of chronic hepatitis and fibrosis. *J Gastrointestin Liver Dis* **16**, 425–426.