



## Original article

# The effect of apple vinegar consumption on glycemic indices, blood pressure, oxidative stress, and homocysteine in patients with type 2 diabetes and dyslipidemia: A randomized controlled clinical trial



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## SUMMARY

**Background:** Some foods and drinks contain special ingredients, causing impressive effects on human health. The aim of the current study was to assess the health effects of apple vinegar in patients with diabetes and dyslipidemia.

**Method:** Seventy participants with type 2 diabetes and hyperlipidemia were randomly assigned into an intervention and control group in order to assess the effect of 20 ml apple vinegar per day using an 8-week parallel study. Fasting blood sugar (FBS), homeostasis model assessment for insulin resistance (HOMA-IR), homeostasis model assessment for b-cell function (HOMA-B), quantitative insulin sensitivity checks index (QUICKI), insulin, malondialdehyde (MDA), 2,20-Diphenyl-1- picrylhydrazyl (DPPH), homocysteine, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured at the beginning and end of the study.

**Results:** The intervention with apple vinegar could significantly improve FBS (mean change:  $-10.16 \pm 19.48$  mg/dl,  $p = 0.006$ ) and DPPH (mean change:  $16.58 \pm 11.56$ ,  $p < 0.001$ ) within intervention group and in comparison with control group ( $p < 0.001$ ). Additionally, the significant increase of MDA in control group ( $p < 0.05$ ) caused a considerable difference between two groups. Glycemic indices containing insulin, HOMA-IR, HOMA-B, and QUICKI decrease significantly in both groups ( $p < 0.05$ ). No considerable effect was observed on blood pressure and homocysteine in intervention group as well as control group.

**Conclusion:** This trial provided some evidences that apple vinegar consumption may cause beneficial effects on glycemic indices and oxidative stress in individuals with diabetes and dyslipidemia.

This randomized clinical trial was registered in the Iranian Registry of Clinical Trials (<https://www.irct.ir/>) as 2013070710826N5.

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## 1. Introduction

Diabetes mellitus is one of the most prevalent metabolic disorders worldwide [1]. The number of individuals with diabetes has

been increasing between 1980 and 2014 from 108 million to 422 million [2], its number will rise to 552 million people by 2030 [3]. Type 2 diabetes mellitus (T2DM), the most common type of diabetes mellitus [4], is associated with deterioration of life quality, causing a considerable reduction in life expectancy [5]. Based on previous studies, diabetes imposes a significant burden on people with diabetes as well as the healthcare system [6]. This disorder leads to several complications such as cardiovascular diseases (CVDs), chronic kidney disorders [7], and some type of cancers [8,9], aggravating the economic burden on communities [10].

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Additionally, hyperglycemia, hyperlipidemia, and hyperinsulinemia are main causes of oxidative stress, which has been proposed to be one of the causative factors of disease severity [11] and complications of diabetes [12] such as insulin resistance and hyperglycemia [13]. Furthermore, diabetes increases the risk of atherosclerosis, as an inflammation response, independently [4,14]. In this case, elevated concentration of homocysteine has a significant role, causing vascular complications of diabetes [15].

The management of blood pressure [16], antioxidant capacity [17], and blood glucose [18] has been suggested as ways to control diabetes complications. Some strategies, especially nutrition, have a striking effect on diabetes management [19,20]. In this case, plants play an impressive role in order to find new therapeutic agents as major sources of antioxidant, hypolipidemic, and hypoglycemic factors [21]. Vinegar especially apple vinegar is consumed more nowadays. Its major components are both polyphenols and acetic acid [22]. Several cross-sectional and prospective epidemiological studies have reported that consumption of foods and beverages rich in polyphenols may cause a better plasma lipids profile, glycemic indices, blood pressure, and biomarkers of inflammation [23,24] and even may decrease the incidence of diabetes [25]. It has been suggested that vinegar components stimulate a protective barrier against oxidative stress in animals [26]. So, it would appear the vinegar specifically apple vinegar treatment lead to the normal oxidative stress [27]. Moreover, several studies showed the beneficial effects of acid acetic in humans. Consuming a beverage containing 750 mg/day acid acetic considerably decrease blood pressure in hypertensive patients [28]. In some experiments, it is proposed that components of vinegar improve serum triglycerides (TG) level and HbA1c in diabetic rats [29]. Additionally, some human studies reported that vinegar intake can improve insulin resistance in healthy participants as well as subjects with diabetes [30,31] and fasting blood sugar (FBS) in participants with diabetes on condition that it is consumed at bedtime [32]. Also, it can suppress postprandial hyperglycemia [30]. Although data are inconsistent regarding the effects of vinegar on the human body. In a study, the insignificant effects of vinegar on FBS, HbA1c, and Homeostasis model assessment for insulin resistance (HOMA-IR) were reported [33], and also it is shown that even 2 months of intervention with vinegar couldn't significantly improve glycemic indices such as HbA1c [34]. Additionally, a systematic review and meta-analysis was revealed an insignificant effect of vinegar on HbA1c, FBS, and fasting insulin [35].

Since the number of human studies to assess the effect of apple vinegar are restricted, we aim to determine the effects of apple vinegar, as a rich source of acetic acid and polyphenols, on human glucose regulation, oxidative stress, and blood pressure by a parallel randomized clinical trial in patients with type 2 diabetes and dyslipidemia. Additionally, in the current trial, the effect of apple vinegar on homocysteine concentration, as an atherogenesis factor, is assessed in patients with type 2 diabetes.

## 2. Materials and methods

### 2.1. Subjects recruitment

This parallel randomized clinical trial was conducted in Yazd, Iran from May 2013 to February 2014. Subjects were recruited from 3 centers (Khatam Al-Anbia, Imam Ali and Diabetes Research Center). Patients with T2DM and dyslipidemia who 1) did not start a new medication or insulin injection, 2) did not have any gastrointestinal, liver, kidney, and heart diseases, 3) were not on a specific diet and 4) females were not pregnant and lactating, were included in the study. Change of dose and type of medications two months prior and during the study, use of either vitamin E, C, D, B, and

antioxidant supplements during the study and use of less than 80 percent of prescribed apple vinegar were considered as the exclusion criteria. The current study was confirmed by the ethics committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran (Ethics code: 52174). In addition, this randomized clinical trial was registered in the Iranian Registry of Clinical Trials (<https://www.irct.ir/>) as 2013070710826N5.

### 2.2. Study procedure

All eligible patients (n = 70) participated in an introductory meeting and informed about the study procedure. All participants signed informed written consent forms. Patients were randomly assigned into apple vinegar and control group (Fig. 1). Randomization was performed by using computer-generated random numbers. Patients with T2DM and dyslipidemia, who aged more than 18 years old were recruited into the study. Individuals with diabetes were screened based on personal medical records (such as polyuria and polydipsia), the use of hypo-glycemic agents and with biochemical tests of FBS  $\geq 126$  mg/dl, HbA1C  $> 6.5\%$ , 2 h post glucose load  $\geq 200$  mg/dl for more than 2 times. Furthermore, dyslipidemia was defined as use of lipid-lowering drugs or having impaired serum lipid levels as total cholesterol  $\geq 200$  mg/dl, TG  $\geq 150$  mg/dl, LDL-C  $\geq 100$  mg/dl and HDL-C  $< 45$  mg/dl in men and  $< 55$  mg/dl in women [36].

Individuals in the treatment group were asked to use a glass of water plus 2 spoons of apple vinegar equal to approximately 10 ml/day, before their lunch and dinner meal. Thus, participants in the treatment group were prescribed to use a total of 20 ml of apple vinegar daily. In this regard, all the participants were provided their apple vinegar for 4 weeks. Apple vinegar was manufactured with the concentration of 5% acetic acid, by Septico Company, Mashad, Iran. In order to provide apple vinegar, Khorasan yellow apple species was used. After controlling for their compliance in their checklist, those who had good compliance were provided with apple vinegar bottles for the next 4 weeks. In order to follow the patients, we called them every 10 days to ensure their consumption. The compliance was defined to use more than 80% of apple vinegar in the intervention period. Patients who were unable to use more than 80% of the prescribed treatment were excluded. Also, in the treatment group compliance was evaluated by the apple vinegar consumption checklist. Individuals were asked to mark the appropriate checkbox after consuming their prescribed apple vinegar each day. We measured participants' physical activity status in a qualitative way by asking them to fill out an international physical activity questionnaire (IPAQ). It categorized into three qualitative subgroups including: The total Met-min/week fewer than 600 was determined as low physical activity, between 600 and 3000 Met-min/week as moderate physical activity, and more than 3000 Met-min/week as severe physical activity [37]. Also, participants were asked not to change their routine physical activity or usual dietary intakes during the study and not to consume any supplements other than the one supplied to them by the researchers.

Dietary intakes of individuals throughout the study were assessed by means of 3-day dietary records. The dietary records were based on estimated values in household measurements. To measure nutrient intakes of participants based on these 3-day food diaries, the Nutritionist IV software (First Databank, San Bruno, CA, USA) which is modified for Iranian foods was used.

### 2.3. Anthropometric and blood pressure assessments

A well-trained person at the centers performed the anthropometric measurements at the beginning and the end of the study.

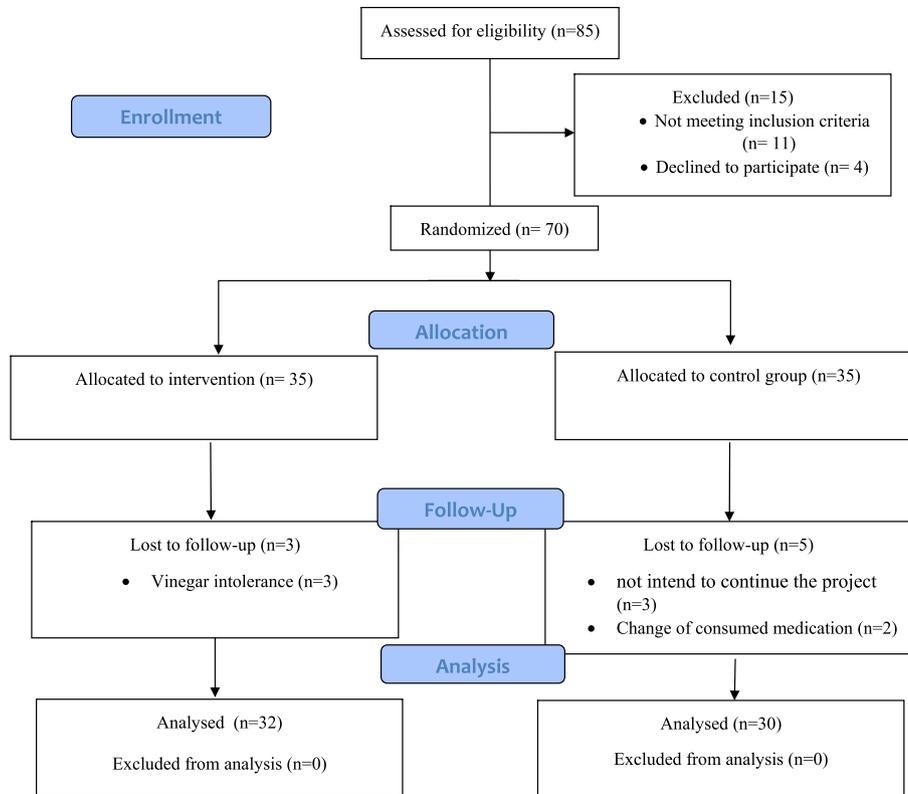


Fig. 1. Flow diagram for study selection process. Summary of participants' flow.

Body weight was measured to the nearest 0.1 kg, while participants were without shoes and in minimal clothing, using digital scales (Seca, Hamburg, Germany). Height was measured by a non-stretched tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared. Blood pressure was measured at the beginning and the end of the trial, in the sitting mode after 5 min rest, using a mercury sphygmomanometer (Riester) [38].

#### 2.4. Biochemical measurements

10 ml blood samples were collected at the beginning and 8 weeks later, in the early morning after an overnight fast. Blood samples were immediately centrifuged (Hettich D-78532; Hettich GmbH) at 3000 rpm for 10 min to separate serum. Afterward, Samples were stored at  $-70^{\circ}\text{C}$  before analysis at the central laboratory of Shahid Sadoughi University of Medical Sciences. Fasting blood glucose (FBS), the concentration was measured by using commercial kits (Pars Azmoon). The intra- and inter-assay CVs for FBS were 2.0% and 3.5%, respectively. All HOMA-IR, b-cell function (HOMA-B) and quantitative insulin sensitivity check index (QUICKI) were measured based on suggested formulas [39]. Plasma Malondialdehyde (MDA) was determined using the thiobarbituric acid reactive substance (TBARS) spectrophotometric test. The CVs for MDA was 3.5%. Serum insulin was measured by using an ELISA kit (Monobind) with the inter- and intra-assay CVs for serum insulin were 2.9% and 5.9%, respectively. In order to assess total antioxidant capacity (TAC) 2,20-Diphenyl-1-picrylhydrazyl (DPPH), based on Janaszewska method, was evaluated [40]. Plasma total homocysteine concentrations were measured by use of frozen samples by HPLC (High-performance Liquid Chromatography) method (KNAUER, Germany), The intra- and inter-assay coefficient of variation for homocysteine samples were 3.9% and 5.9% respectively.

#### 2.5. Sample size calculation

Sample size was determined by  $N = 2 [(Z_{1-\alpha/2} + Z_{1-\beta})^2 [f_x] S^2] / d^2$  [41], while type 1 ( $\alpha$ ) and type 2 errors ( $\beta$ ) were considered as 0.05 and 0.2 (power = 80%) respectively. By using formula, the sample size of 20 participants in each group was calculated.

#### 2.6. Statistical analysis

Kolmogorov–Smirnov was applied to examine the normal distribution of variables. Therefore, Log transformation was used for non-normally distributed variables. Independent sample t-test was used to detect the differences in means of energy intake, mean of biomarkers at both beginning and end of the study and changes in biomarkers between apple vinegar group and control group. Baseline and endpoints values were compared within each group by paired t-tests. Both crude and a multivariate-adjusted model (adjusted for changes in body weight and age) were performed to compare mean differences between the intervention and control groups. Independent student t-test and analysis of covariance were applied in crude and in multivariate-adjusted models, respectively. Continuous data are represented as mean and standard deviations. All the analysis was performed by using SPSS version 20 statistical software.

### 3. Results

Among 70 individuals with diabetes and dyslipidemia, who were randomized to intervention and control groups, 62 participants completed the study and finally were included in the analysis. As it is shown in Fig. 1, since vinegar intolerance, not intend to continue the project, and consumed medication changes, 8 participants withdrew from the study.

Baseline characteristics of the participants are represented in Table 1. Mean age of participants in each group were  $49.47 \pm 8.02$  and  $52.1 \pm 7.87$ , respectively. Additionally, no significant differences were found in weight ( $P = 0.28$ ) and BMI ( $P = 0.87$ ) between mentioned groups. Table 2 subscribes no considerable difference in dietary intake in both pre-intervention and post-intervention ( $P > 0.05$ ) in each group, furthermore, no differences were observed in dietary intakes between intervention and control groups ( $P > 0.05$ ).

Table 3 represents the effect of apple vinegar on glycemic indices in the intervention group and its comparison with the control group. Within-group analysis indicated a significant decrease in insulin ( $P < 0.001$ ), HOMA-IR ( $P < 0.001$ ), HOMA-B ( $P < 0.001$ ), and QUICKI ( $P < 0.001$ ) in both intervention and control groups, and a significant decrease in FBS level in participants consuming apple vinegar ( $P = 0.006$ ). Between-groups analysis demonstrated a considerable difference in FBS level among participants. It showed that apple vinegar can decrease FBS concentration from  $138.62 \pm 31.82$  mg/dl at the beginning to  $128.46 \pm 29.74$  mg/dl at the end of the study (Mean change:  $-10.16 \pm 19.48$  mg/dl) which is considerably different compared with control group ( $P < 0.001$ ).

The effects of the intervention on oxidative stress markers, homocysteine level, and blood pressure are shown in Table 4. Apple vinegar can decrease MDA, homocysteine, and SBP. However, its effects were not significant ( $P > 0.05$ ). Also, DPPH, blood pressure, and homocysteine did not substantially change in the control group ( $P > 0.05$ ). As it is shown in Table 4, DPPH was increased significantly in apple vinegar group (pre:  $35.15 \pm 12.31$ , post:  $51.73 \pm 7.85$ , mean change:  $16.58 \pm 11.56$ ,  $P < 0.001$ ) and the change of MDA and DPPH were considerably different between two groups ( $P < 0.001$ ).

#### 4. Discussion

The current randomized controlled clinical trial indicates that the consumption of apple vinegar, a rich source of acetic acid and phytochemicals, can improve glycemic indices (insulin, HOMA-IR,

HOMA-B, and QUICKI) and in comparison with participants consuming just lipid-lowering drugs apple vinegar can significantly decrease FBS by 10.16 mg/dl. Additionally, it was demonstrated that DPPH increases more in patients who consumed the mentioned juice than the control group, and the increase of MDA level was much more in the control group in comparison with apple vinegar group, which led to a significant difference between apple vinegar and control groups.

Vinegar is an important source of acid acetic and can provide a considerable amount of phytochemicals in the human diet [22]. In order to improve the condition of patients with diabetes, improvement of FBS is a vital pharmacologic treatment aim [42]. Apple vinegar may lead to this improvement. The current project shows a statistically significant decrease in FBS in individuals who consumed apple vinegar in comparison with patients that just consumed related medications. In contrast, Siddiqui and et al. in a systematic review and meta-analysis based on 103 participants, which aimed to assess the effect of vinegar consumption as a diabetic control treatment, showed that vinegar cannot have a significant effect on FBS ( $P = 0.15$ ) [35]. Moreover, another study reported an insignificant effect of apple vinegar in 19 patients with diabetes on FBS [43]. On the other hand, a one-month parallel intervention on 30 participants with diabetes indicated that consuming 15 ml/day apple vinegar can decrease FBS level considerably by 18 mg/dl in the participants [44]. In addition, Yoon and et al. reported the significant reduction of FBS due to consuming other types of vinegar by  $-1.19$  mmol/l ( $P = 0.021$ ) in individuals with diabetes [45]. Also, our analysis indicates the improvement effect of apple vinegar on fasting insulin and some other glycemic indices as well as FBS in apple vinegar group, which was inconsistent with other studies [35,45,46]. Several mechanisms can clarify the effect of vinegar on glycemic indices. A study which considers the effect of vinegar on postprandial blood glucose found that acetic acid can decrease the rate of gastric emptying which may explain the causes of glycemic improvement effect of vinegar [47]. Additionally, this acid can reduce starch digestion [48]. While it was proposed that acetic acid can change the activity

**Table 1**  
baseline characteristics of patients with diabetes and dyslipidemia in both intervention and control group.

Variables	Apple vinegar group (n = 32) <sup>a</sup>	Control group (n = 30) <sup>a</sup>	p-value <sup>b</sup>
Age (year)	$49.47 \pm 8.02$	$52.1 \pm 7.87$	0.19
Male/Female	10/22	10/20	0.788
Height (Cm)	$159.34 \pm 9.74$	$159.71 \pm 8.5$	0.87
Weight (Kg)	$73.41 \pm 11.82$	$76.77 \pm 12.73$	0.28
BMI (Kg/m <sup>2</sup> )	$28.96 \pm 4.31$	$28.85 \pm 4.24$	0.87

<sup>a</sup> All values are presented as Mean  $\pm$  SD, except for gender which is reported as number.

<sup>b</sup> Calculated by student t-test.

**Table 2**  
Nutrients and calorie intake in patients with diabetes and dyslipidemia in both intervention and control group.

Variables <sup>a</sup>	Apple vinegar group (n = 32)		Control group (n = 30)		p-value <sup>b</sup>	p-value <sup>c</sup>	p-value <sup>d</sup>	p-value <sup>e</sup>
	Before	After	Before	After				
Calorie (Kcal)	$1326.62 \pm 308.8$	$1246.07 \pm 283.13$	$1358.55 \pm 169.05$	$1283.25 \pm 235.17$	0.98	0.65	0.32	0.26
Carbohydrate (gr)	$145.04 \pm 51.58$	$143 \pm 42.41$	$149 \pm 49.55$	$142.85 \pm 49.82$	0.8	0.99	0.83	0.43
Protein (gr)	$38.22 \pm 11.92$	$34.74 \pm 9.52$	$38.2 \pm 8.68$	$36.43 \pm 10.96$	0.99	0.6	0.1	0.35
Fat (gr)	$46.33 \pm 9.96$	$46.42 \pm 9.61$	$45.93 \pm 12.42$	$46 \pm 10.6$	0.9	0.89	0.96	0.95
Cholesterol (mg)	$50.51 \pm 43.7$	$59.74 \pm 57.66$	$61.84 \pm 62.08$	$58.63 \pm 62.33$	0.5	0.95	0.48	0.53
Fiber (gr)	$7.46 \pm 3.52$	$6.91 \pm 2.93$	$7.96 \pm 3.58$	$7.86 \pm 4.6$	0.65	0.43	0.59	0.90

<sup>a</sup> All values are presented as Mean  $\pm$  SD.

<sup>b</sup> p-value between groups before treatment. Calculated by student t-test.

<sup>c</sup> p-value between groups after treatment. Calculated by student t-test.

<sup>d</sup> P-value within intervention group. Calculated by paired t-test.

<sup>e</sup> p-value within control group. Calculated by paired t-test.

**Table 3**

The change of glycemic indices among patients with type 2 diabetes and dyslipidemia in both intervention and control groups at baseline and after 8 weeks.

Variables <sup>e</sup>	Apple vinegar group (n = 32)	Control group (n = 30)	p-value <sup>f</sup>	p-value <sup>h</sup>
<b>FBS<sup>a</sup></b>				
Before	138.62 ± 31.82	137.63 ± 40.04	0.774	
After	128.46 ± 29.74	153.70 ± 52.79	0.033	
Change	-10.16 ± 19.48	16.07 ± 32.24	<0.001	0.001
p-value <sup>g</sup>	0.006	0.018		
<b>Insulin</b>				
Before	17.55 ± 8.23	16.36 ± 6.91	0.731	
After	10.20 ± 6.29	9.06 ± 4.22	0.710	
Change	-7.34 ± 8.45	-7.29 ± 6.08	0.669	0.797
p-value	<0.001	<0.001		
<b>HOMA-IR<sup>b</sup></b>				
Before	5.95 ± 3.05	5.68 ± 3.29	0.670	
After	3.17 ± 1.99	3.39 ± 1.92	0.455	
Change	-2.77 ± 2.98	-2.28 ± 2.56	0.469	0.337
p-value	<0.001	<0.001		
<b>HOMA-B<sup>c</sup></b>				
Before	45.12 ± 27.44	41.39 ± 21.49	0.884	
After	26.96 ± 19.86	20.10 ± 13.39	0.210	
Change	-18.16 ± 25.02	-21.29 ± 16.27	0.564	0.178
p-value	<0.001	<0.001		
<b>QUICKI<sup>d</sup></b>				
Before	0.30 ± 0.02	0.30 ± 0.02	0.651	
After	0.33 ± 0.03	0.32 ± 0.02	0.407	
Change	0.03 ± 0.028	0.02 ± 0.028	0.267	0.301
p-value	<0.001	<0.001		

<sup>a</sup> Fasting blood sugar.

<sup>b</sup> Homeostasis model assessment for insulin resistance.

<sup>c</sup> Homeostasis model assessment for b-cell function.

<sup>d</sup> Quantitative insulin sensitivity checks index.

<sup>e</sup> All values are presented as Mean ± SD.

<sup>f</sup> p-value presents between two groups, calculated by student t-test.

<sup>g</sup> P-value within group, calculated by paired t-test.

<sup>h</sup> Adjusted p-value presents between two groups.

of several enzymes involved in glucose metabolism. Some studies demonstrated that the stated acid may decline the accumulation of xylulose-5-phosphate in the liver and change the activity of phosphofructokinase-1 in skeletal muscles. These changes lead to the reduction of glycolysis and the increase of glycogen synthesis. These effects have impressive roles in the improvement of glycemic indices [49–51].

We found that consuming apple vinegar causes less increase in MDA level, albeit in participants who didn't consume apple vinegar, MDA level increase significantly. Several studies have shown the oxidative stress improvement effects of foods rich in polyphenols and vinegar, and our results confirm them. An experimental study was done by Zhao and et al. in order to assess the effect of a type of tea rich in polyphenols on the mouse, and this study concluded that several enzymes involving in the improvement of oxidative stress were increased and the level of MDA was decreased in the tea group [52]. Also, an inverse association between flavonoid-rich drinks and MDA concentration was reported [53]. Additionally, another study on rabbits indicated the beneficial effect of vinegar on oxidative stress, specifically MDA [54]. This effect can be considered by some mechanisms. The excessive production of reactive oxygen species (ROS) induces oxidative stress, causing several chronic diseases [55]. In order to balance the redox system, our body tries to counteract ROS production [56]. Several studies subscribed that polyphenols, which are defined as antioxidants, have a protective effect against oxidative stress [57] and can prevent the production of destructive elements such as reactive oxygen species [58,59]. The reduction of the most common lipid peroxidation index, MDA, can be related to the ability of a food or supplement to savaging free oxygen radicals. It has been reported that fruits and vegetables

containing flavonoids can scavenge H<sub>2</sub>O<sub>2</sub> induced accumulation of reactive oxygen [60]. Additionally, in the present clinical trial, we assessed DPPH as a method to evaluate the total antioxidant capacity (TAC), consequently, it was found that consumption of apple vinegar can considerably increase DPPH and undoubtedly increase TAC in apple vinegar group. This result confirms the antioxidant ability of the mentioned juice, hence, consuming apple vinegar may lead to a reduction in oxidative damage.

An epidemiological observational study with 2573 participants revealed that individuals in the higher tertile of polyphenol consumption have lower SBP and DBP [61]. Additionally, a clinical trial study was demonstrated that vinegar consumption can decrease blood pressure in hypertensive rats. This study showed that vinegar, as well as acetic acid, can affect the renin-angiotensin-aldosterone system and subsequently, regulate blood pressure [62]. In spite of mentioned studies, which reported a significant effect of vinegar on blood pressure, our study showed no significant effect of apple vinegar, as a good source of acetic acid and flavonoids, on systolic and diastolic blood pressure.

Additionally, in the current trial, for the first time, the effect of apple vinegar on homocysteine was examined. We found that the apple vinegar consumption did not change the homocysteine concentration significantly. Although, the results of a cross-sectional study done by Murakami and et al., in 1050 Japanese female indicated that lower intake of green and oolong tea cause lower concentration of homocysteine [63]. Additionally, a clinical trial aimed to assess the effect of tea on fasting and non-fasting plasma total homocysteine concentrations in human. As this study reported, consumption of tea did not change homocysteine in

**Table 4**

The change of oxidative stress markers, homocysteine, and blood pressure among patients with type 2 diabetes and dyslipidemia in both intervention and control groups at baseline and after 8 weeks.

Variables <sup>e</sup>	Apple vinegar group (n = 32)	Control group (n = 30)	p-value <sup>f</sup>	p-value <sup>h</sup>
<b>MDA<sup>d</sup></b>				
Before	6.03 ± 1.33	6.15 ± 1.69	0.813	
After	5.88 ± 1.14	8.08 ± 3.16	0.001	
Change	-0.14 ± 1.20	1.93 ± 2.93	0.001	<0.001
p-value <sup>g</sup>	0.582	0.001		
<b>DPPH<sup>b</sup></b>				
Before	35.15 ± 12.31	35.97 ± 12.49	0.795	
After	51.73 ± 7.85	40.33 ± 11.80	<0.001	
Change	16.58 ± 11.56	4.36 ± 14.79	0.001	<0.001
p-value	<0.001	0.117		
<b>Homocysteine</b>				
Before	16.10 ± 7.45	13.22 ± 5.68	0.094	
After	15.81 ± 7.66	15.33 ± 6.79	0.794	
Change	-0.28 ± 9.44	2.11 ± 6.77	0.294	0.881
p-value	0.865	0.099		
<b>SBP<sup>c</sup></b>				
Before	125.89 ± 10.88	131.70 ± 11.56	0.046	
After	124.65 ± 8.71	126.45 ± 25.16	0.713	
Change	-1.23 ± 7.76	-5.25 ± 26.74	0.419	0.876
p-value	0.375	0.291		
<b>DBP<sup>c</sup></b>				
Before	79.47 ± 7.76	88.18 ± 15.48	0.006	
After	80.39 ± 10.15	85.43 ± 9.15	0.045	
Change	0.92 ± 7.69	-2.75 ± 10.54	0.121	0.798
p-value	0.503	0.164		

<sup>d</sup>Diastolic blood pressure.

<sup>a</sup> Malondialdehyde.

<sup>b</sup> 2,20-Diphenyl-1- picrylhydrazyl.

<sup>c</sup> Systolic blood pressure.

<sup>e</sup> All values are presented as Mean ± SD. p-value presents between two groups, calculated by student t-test.

<sup>g</sup> P-value within group, calculated by paired t-test.

<sup>h</sup> Adjusted p-value presents between two groups.

none of the conditions, furthermore, a negative association between tea intake and homocysteine level were reported [64]. Hence, further studies are needed to assess the effect of vinegar on homocysteine concentration.

The major strengths of the present study are described below. The number of studies doing on the human is restricted, however, this study as a randomized clinical trial was designed in the human population with 88.57% of participants completed the study (dropout rate: 11.43%). Furthermore, daily consumption of the apple vinegar did not have any side-effect and was tolerated by most of the participants. Additionally, to the best of our knowledge, any other randomized clinical trial has not assessed the effect of apple vinegar on homocysteine, as an indicator of cardiovascular disease, and our trial is the first one to assess this association.

The limitation of the current study is the lack of blinding among participants and personnel. Also, Lack of a fat-restricted diet in intervention and control group can be considered as another limitation. Additionally, the content of apple vinegar poly phenols was not measured in the current study. Moreover, we believed that a longer intervention duration may provide more precise results, although several previous studies were designed with an intervention duration the same as the current study.

## 5. Conclusion

To be concluded, apple vinegar consumption may have several beneficial effects in individuals with diabetes and dyslipidemia. Daily consumption of this juice (20 ml/day) may decrease glycemic indices, specifically FBS, and may increase the total antioxidant capacity.

## Contributors and authorship

Ms. Bashiri and Dr. Nadjarzadeh designed, performed and conducted the design theory of the study. Mr. Gheflati supported in statistical analyses and wrote this article. Dr. Nadjarzadeh revised and approved the final version of the article. All authors have approved the final article. Dr Akram Ghadiri designed the proposal and conducted the study.

## Conflict of interest

Authors declared no conflict of interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clnesp.2019.06.006>.

## References

- [1] Funnell MM, Brown TL, Childs BP, Haas LB, Hoseney GM, Jensen B, et al. National standards for diabetes self-management education. *Diabetes Care* 2009;32(Suppl. 1):S87–94.
- [2] WHO. World Health Organisation (WHO). Global report on diabetes. 2016. [http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257\\_eng.pdf?ua=1](http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf?ua=1) 2016.
- [3] Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract* 2011;94(3):311–21.
- [4] Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev* 2013;93(1):137–88.
- [5] Roper NA, Bilous RW, Kelly WF, Unwin NC, Connolly VM. Excess mortality in a population with diabetes and the impact of material deprivation: longitudinal, population based study. *BMJ* 2001;322(7299):1389–93.
- [6] Bommer C, Heesemann E, Sagalova V, Manne-Goehler J, Atun R, Barnighausen T, et al. The global economic burden of diabetes in adults aged 20–79 years: a cost-of-illness study. *Lancet Diabetes Endocrinol* 2017;5(6):423–30.
- [7] Danaei G, Lu Y, Singh G, Stevens G, Cowan M, Farzadfar F, et al. Cardiovascular disease, chronic kidney disease, and diabetes mortality burden of cardiometabolic risk factors from 1980 to 2010: a comparative risk assessment. *The Lancet* 2014;2(2):634–47.
- [8] Campbell PT, Deka A, Jacobs EJ, Newton CC, Hildebrand JS, McCullough ML, et al. Prospective study reveals associations between colorectal cancer and type 2 diabetes mellitus or insulin use in men. *Gastroenterology* 2010;139(4):1138–46.
- [9] Lee M-S, Hsu C-C, Wahlqvist ML, Tsai H-N, Chang Y-H, Huang Y-C. Type 2 diabetes increases and metformin reduces total, colorectal, liver and pancreatic cancer incidences in Taiwanese: a representative population prospective cohort study of 800,000 individuals. *BMC Canc* 2011;11(1):20.
- [10] Ettaro L, Songer TJ, Zhang P, Engelgau MM. Cost-of-illness studies in diabetes mellitus. *Pharmacoeconomics* 2004;22(3):149–64.
- [11] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, et al. The role of oxidative stress in the pathogenesis of type 2 diabetes mellitus micro-and macrovascular complications: avenues for a mechanistic-based therapeutic approach. *Curr Diabetes Rev* 2011;7(5):313–24.
- [12] Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res* 2010;107(9):1058–70.
- [13] Houston N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* 2006;440(7086):944.
- [14] Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999;340(2):115–26.
- [15] Hoogeveen EK, Kostense PJ, Beks PJ, Mackaay AJ, Jakobs C, Bouter LM, et al. Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non-insulin-dependent diabetes mellitus: a population-based study. *Arterioscler Thromb Vasc Biol* 1998;18(1):133–8.
- [16] Zanella MT, Kohlmann Jr O, Ribeiro AB. Treatment of obesity hypertension and diabetes syndrome. *Hypertension* 2001;38(3):705–8.
- [17] Martini LA, Catania AS, Ferreira SR. Role of vitamins and minerals in prevention and management of type 2 diabetes mellitus. *Nutr Rev* 2010;68(6):341–54.
- [18] Group UPDS. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *The Lancet* 1998;352(9131):837–53.
- [19] Georgoulis M, Kontogianni M, Yiannakouris N. Mediterranean diet and diabetes: prevention and treatment. *Nutrients* 2014;6(4):1406–23.
- [20] Lindström J, Eriksson JG, Valle TT, Aunola S, Cepaitis Z, Hakumäki M, et al. Prevention of diabetes mellitus in subjects with impaired glucose tolerance in the Finnish Diabetes Prevention Study: results from a randomized clinical trial. *J Am Soc Nephrol* 2003;14(Suppl. 2):S108–13.
- [21] Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. *Phytomedicine* 1995;2(2):137–89.
- [22] Natera R, Castro R, de Valme García-Moreno M, Hernández MJ, García-Barroso C. Chemometric studies of vinegars from different raw materials and processes of production. *J Agric Food Chem* 2003;51(11):3345–51.
- [23] Grosso G, Stepaniak U, Micek A, Stefler D, Bobak M, Pająk A. Dietary polyphenols are inversely associated with metabolic syndrome in Polish adults of the HAPIEE study. *Eur J Nutr* 2017;56(4):1409–20.
- [24] Pounis G, Bonaccio M, Di Castelnuovo A, Costanzo S, De Curtis A, Persichillo M, et al. Polyphenol intake is associated with low-grade inflammation, using a novel data analysis from the Moli-sani study. *Thromb Haemostasis* 2016;115(02):344–52.
- [25] Zamora-Ros R, Forouhi NG, Sharp SJ, González CA, Buijsse B, Guevara M, et al. Dietary intakes of individual flavanols and flavonols are inversely associated with incident type 2 diabetes in European populations—3. *J Nutr* 2013;144(3):335–43.
- [26] Sakakibara S, Yamauchi T, Oshima Y, Tsukamoto Y, Kadowaki T. Acetic acid activates hepatic AMPK and reduces hyperglycemia in diabetic KK-A (y) mice. *Biochem Biophys Res Commun* 2006;344(2):597–604.
- [27] Halima BH, Sonia G, Sarra K, Houda BJ, Fethi BS, Abdallah A. Apple cider vinegar attenuates oxidative stress and reduces the risk of obesity in high-fat-fed male Wistar rats. *J Med Food* 2018;21(1):70–80.

- [28] Kajimoto O. Hypotensive effects of drinks containing vinegar on high normal blood pressure and mild hypertensive subjects. *J Nutr Food* 2003;6(1):51–68.
- [29] Shishebor F, Mansoori A, Sarkaki A, Jalali M, Latifi S. Apple cider vinegar attenuates lipid profile in normal and diabetic rats. *Pakistan J Biol Sci* 2008;11(23):2634.
- [30] Johnston CS, Steplewska I, Long CA, Harris LN, Ryals RH. Examination of the antihyperglycemic properties of vinegar in healthy adults. *Ann Nutr Metabol* 2010;56(1):74–9.
- [31] Leeman M, Östman E, Björck I. Vinegar dressing and cold storage of potatoes lowers postprandial glycaemic and insulinaemic responses in healthy subjects. *Eur J Clin Nutr* 2005;59(11):1266.
- [32] White AM, Johnston CS. Vinegar ingestion at bedtime moderates waking glucose concentrations in adults with well-controlled type 2 diabetes. *Diabetes Care* 2007;30(11):2814–5.
- [33] Kondo T, Kishi M, Fushimi T, Ugajin S, Kaga T. Vinegar intake reduces body weight, body fat mass, and serum triglyceride levels in obese Japanese subjects. *Biosci Biotech Biochem* 2009;73(8):1837–43.
- [34] Panetta C, Menk J, Jonk Y, Brown A, Powers M, Shapiro A. Prospective randomized clinical trial evaluating the impact of vinegar on high density lipoprotein. *J Am Diet Assoc* 2010;110(9):A87.
- [35] Siddiqui FJ, Assam PN, de Souza NN, Sultana R, Dalan R, Chan ES-Y. Diabetes control: is vinegar a promising candidate to help achieve targets? *J Evid Based Integr Med* 2018;23. 2156587217753004.
- [36] Rafiei M, Sadr S, Nasirian M, Namayandeh S, Abdoli A. Dyslipidemia in diabetic patients: a cross sectional study in urban population of Yazd, Iran. *Tehran Univ Med J* 2008;66(7):503–7.
- [37] Hallal PC, Victora CG. Reliability and validity of the international physical activity questionnaire (IPAQ). *Med Sci Sport Exerc* 2004;36(3):556.
- [38] Gosse P. Blood pressure should be measured in both arms on the first consultation. *J Hypertens* 2002;20(6):1045–6.
- [39] Pisprasert V, Ingram KH, Lopez-Davila MF, Munoz AJ, Garvey WT. Limitations in the use of indices using glucose and insulin levels to predict insulin sensitivity: impact of race and gender and superiority of the indices derived from oral glucose tolerance test in African Americans. *Diabetes Care* 2013;36(4):845–53.
- [40] Janaszewska A, Bartosz G. Assay of total antioxidant capacity: comparison of four methods as applied to human blood plasma. *Scand J Clin Lab Invest* 2002;62(3):231–6.
- [41] Enderlein G, Fleiss JL. The design and analysis of clinical experiments, vol. 432. New York–Chichester–Brisbane–Toronto–Singapore: Wiley; 1986. S.,t 38.35. *Biometrical Journal*. 1988;30(3):304.
- [42] Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, et al. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2009;32(1):193–203.
- [43] Ebrahimi-Mamaghani M, Arefhosseini S, Golzarand M, Aliasgarzadeh A, Vahed-Jabbary M. Long-term effects of processed *Berberis vulgaris* on some metabolic syndrome components. *Iran J Endocrinol Metabol* 2009;11(1).
- [44] Mahmoodi M, Hosseini-zijoud S-M, Nabati S, Modarresi M, Mehrabian M, Sayyadi A, et al. The effect of white vinegar on some blood biochemical factors in type 2 diabetic patients. *J Diabetes Endocrinol* 2013;4(1):1–5.
- [45] Yoon JW, Kang SM, Vassy JL, Shin H, Lee YH, Ahn HY, et al. Efficacy and safety of ginseng, a vinegar extract from *Panax ginseng*, in type 2 diabetic patients: results of a double-blind, placebo-controlled study. *J Diabetes Invest* 2012;3(3):309–17.
- [46] Mitrou P, Petsiou E, Papakonstantinou E, Maratou E, Lambadiari V, Dimitriadis P, et al. Vinegar consumption increases insulin-stimulated glucose uptake by the forearm muscle in humans with type 2 diabetes. *J Diabetes Res* 2015;2015.
- [47] Liljeberg H, Björck I. Delayed gastric emptying rate may explain improved glycaemia in healthy subjects to a starchy meal with added vinegar. *Eur J Clin Nutr* 1998;52(5):368–71.
- [48] Ogawa N, Satsu H, Watanabe H, Fukaya M, Tsukamoto Y, Miyamoto Y, et al. Acetic acid suppresses the increase in disaccharidase activity that occurs during culture of caco-2 cells. *J Nutr* 2000;130(3):507–13.
- [49] Fushimi T, Tayama K, Fukaya M, Kitakoshi K, Nakai N, Tsukamoto Y, et al. Acetic acid feeding enhances glycogen repletion in liver and skeletal muscle of rats. *J Nutr* 2001;131(7):1973–7.
- [50] Fushimi T, Tayama K, Fukaya M, Kitakoshi K, Nakai N, Tsukamoto Y, et al. The efficacy of acetic acid for glycogen repletion in rat skeletal muscle after exercise. *Int J Sports Med* 2002;23(3):218–22.
- [51] Monnier L, Colette C, Dunseath GJ, Owens DR. The loss of postprandial glycaemic control precedes stepwise deterioration of fasting with worsening diabetes. *Diabetes Care* 2007;30(2):263–9.
- [52] Qian Y, Zhang J, Fu X, Yi R, Sun P, Zou M, et al. Preventive effect of raw liubao tea polyphenols on mouse gastric injuries induced by HCl/Ethanol via anti-oxidative stress. *Molecules* 2018;23(11).
- [53] Pan Y, Long X, Yi R, Zhao X. Polyphenols in liubao tea can prevent CCl<sub>4</sub>-induced hepatic damage in mice through its antioxidant capacities. *Nutrients* 2018;10(9).
- [54] Setorki M, Asgary S, Eidi A, Rohani AH, Khazaei M. Acute effects of vinegar intake on some biochemical risk factors of atherosclerosis in hypercholesterolemic rabbits. *Lipids Health Dis* 2010;9:10.
- [55] Dennery PA. Introduction to serial review on the role of oxidative stress in diabetes mellitus. *Free Radic Biol Med* 2006;40(1):1–2.
- [56] Mittal SPK, Khole S, Jagadish N, Ghosh D, Gadgil V, Sinkar V, et al. Andrographolide protects liver cells from H<sub>2</sub>O<sub>2</sub> induced cell death by upregulation of Nrf-2/HO-1 mediated via adenosine A<sub>2</sub>A receptor signalling. *Biochim Biophys Acta* 2016;1860(11 Pt A):2377–90.
- [57] Martin MA, Serrano AB, Ramos S, Pulido MI, Bravo L, Goya L. Cocoa flavonoids up-regulate antioxidant enzyme activity via the ERK1/2 pathway to protect against oxidative stress-induced apoptosis in HepG2 cells. *J Nutr Biochem* 2010;21(3):196–205.
- [58] Choi HY, Lee JH, Jegal KH, Cho IJ, Kim YW, Kim SC. Oxyresveratrol abrogates oxidative stress by activating ERK-Nrf2 pathway in the liver. *Chem Biol Interact* 2016;245:110–21.
- [59] Pareek A, Godavarthi A, Issarani R, Nagori BP. Antioxidant and hepatoprotective activity of *Fagonia schweinfurthii* (Hadidi) Hadidi extract in carbon tetrachloride induced hepatotoxicity in HepG2 cell line and rats. *J Ethnopharmacol* 2013;150(3):973–81.
- [60] Xing H-Y, Liu Y, Chen J-H, Sun F-J, Shi H-Q, Xia P-Y. Hyperoside attenuates hydrogen peroxide-induced L02 cell damage via MAPK-dependent Keap1–Nrf2–ARE signaling pathway. *Biochem Biophys Res Commun* 2011;410(4):759–65.
- [61] Vitale M, Vaccaro O, Masulli M, Bonora E, Del Prato S, Giorda CB, et al. Polyphenol intake and cardiovascular risk factors in a population with type 2 diabetes: the TOSCA.IT study. *Clin Nutr (Edinb, Scotl)* 2017;36(6):1686–92.
- [62] Na L, Chu X, Jiang S, Li C, Li G, He Y, et al. Vinegar decreases blood pressure by down-regulating AT1R expression via the AMPK/PGC-1 $\alpha$ /PPAR $\gamma$  pathway in spontaneously hypertensive rats. *Eur J Nutr* 2016;55(3):1245–53.
- [63] Murakami K, Sasaki S, Uenishi K. Higher intake of vitamin B-6 and dairy products and lower intake of green and oolong tea are independently associated with lower serum homocysteine concentration in young Japanese women. *Nutr Res (NY)* 2013;33(8):653–60.
- [64] Hodgson JM, Puddey IB, van Bockxmeer FM, Burke V. Acute effects of tea on fasting and non-fasting plasma total homocysteine concentrations in human subjects. *Br J Nutr* 2007;97(5):842–6.