How combined aerobic training and pomegranate juice intake affect lipid profile? A clinical trial in men with type 2 diabetes

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Abstract

Study aim: This study aimed to investigate the effect of aerobic training (AT) and pomegranate juice intake (PJI) on the lipid profile in men with type 2 diabetes.

Materials and methods: This randomized clinical trial was performed in middle-aged men (40–50 years old) with type 2 diabetes. Participants were randomly assigned into four groups: AT + PJI (n = 9); AT (n = 10); PJI (n = 9), and control (C) (n = 10). The AT program consisted of 60–75% of HR_{Max} , 40–60 min/day, three days/wk for eight weeks. Participants in the PJI group consumed 240 ml of pomegranate juice (sugar or additive-free) daily for eight weeks. Lipid profile was measured at the beginning and end of the study. The data were analyzed through paired t-test and one-way analysis of variance, as well as Tukey's post hoc test at the significance level of $P < 0.05$.

Results: AT + PJI, PJI, and AT groups demonstrated significant improvements in lipid profile compared to the C group. The results show that the AT + PJI group had significantly lower TC and LDL ($p = 0.001$ and $p = 0.002$, respectively), and significantly higher HDL ($p = 0.023$) compared with the PJI group. There was no significant difference between AT and PJI groups. Also, TG was significantly lower in AT+ PJI compare to the C group.

Conclusions: AT + PJI is more effective than AT or PJI alone in the improvement of lipid profile in patients with type 2 diabetes.

Keywords: Exercise – Pomegranate – Diabetes Mellitus – Anthropometric Indices – Lipid Profile

Introduction

Diabetes, as a metabolic disorder, is mainly the elevation of blood glucose level that is associated with vascular complications leading to increased mortality [6]. Coronary artery disease, myocardial infarction, stroke, congestive heart failure, and peripheral vascular disease are among the cardiovascular complications in patients with diabetes [6, 18]. As one of the main causes of death, the risk of developing these diseases in diabetes is two to four times higher among people with diabetes compared with healthy individuals and reduces life expectancy by five to ten years [15, 20]. Hyperglycemia and lipid disorders are among the main etiology inducing structural heart damage; High levels of glucose lead to lipid disorders and accumulation of reactive oxygen species in myocardiocytes, which eventually leads to apoptosis or necrosis, and cell death [19]. About 11–44% of adults with diabetes have been shown to have lipid profile disorder with a 24–28% prevalence of hypercholesterolemia, and low serum HDL level that is

also common in these patients [32]. It has been reported that improving the immune system via antioxidant therapy significantly reduces the severity of cardiovascular complications in diabetic patients [11]. In recent decades, exercise along with diet has been recommended as a good way to manage diabetes [22]. Aerobic training (AT) is one of the best non-pharmacological interventions in controlling blood glucose and lipid disorders [24]. Moderate-intensity AT has been suggested to improve the metabolic conditions of the diabetic person by strengthening the antioxidant system and reducing oxidative factors [28].

Oxidative stress is the result of an imbalance between the production of free radicals and the antioxidants that increased in diabetes [19]. One of the factors that lead to increased oxidative stress is hyperglycemia [19, 29]. Clinical evidence also supports the antioxidant role of pomegranate in the prevention and treatment of diabetes [4, 13]. The anti-diabetic effects of pomegranate are due to the presence of oleanolic, ursolic, and gallic acids [17]. Pomegranate extract seems to have more polyphenols than many beverages [23]. The most distinctive feature of all flavonoids is acting

as an antioxidant [21, 23]. The antioxidant effects of pomegranate juice (PJ) reduce the ischemic heart disease risk factors by inhibiting the oxidation of low-density lipoprotein, reducing the formation of foam cells, and reducing the oxidation of macrophages [25, 31].

Therefore, using affordable and accessible methods to control and treat diabetes seems to be plausible, with regards to the growing prevalence, subsequent heart complications, and high treatment costs. Some studies have investigated the separate effects of AT and pomegranate juice intake (PJI) on patients with type 2 diabetes. However, to our knowledge, the simultaneous effect of AT and PJI has not been investigated in patients with type 2 diabetes. Considering the effect of PJI as well as AT on improving insulin resistance and lipid disorders, this study was performed to investigate separate and the simultaneous effect of AT and PJI on lipid profile in men with diabetes.

Materials and methods

Participants

In this randomized clinical trial study, 40 men with type 2 diabetes were recruited by a voluntary sampling method and by referring to specialized diabetes clinics. The sample size for each group was estimated to be 10, using G*Power software with $\alpha = 0.05$ and statistical power of 80%. Participants were randomly assigned into four groups including AT + PJI ($n = 10$), AT ($n = 10$), PJI ($n = 10$), and control (C) group ($n = 10$). It is worth mentioning, one participant in the AT + PJI group and one subject in the PJI group refused to participate in the intervention program (figure 1). The present study was approved by the Committee of Ethics in Research at Kermanshah University of Medical Sciences and registered

Figure 1. Diagram of the progress through the phases of the parallel randomized trial of four groups (enrolment, intervention allocation, follow-up, and data analysis)

Table 1. Aerobic training protocol

Variables	Week							
	1	2	3	4	5	6	7	8
Intensity [HR_{Max}]	60–65%	60–65%	65–70%	65–70%	65–70%	70–75%	70–75%	70–75%
Time [min]	40	40	50	50	50	60	60	60

in the Iranian Clinical Trial Registration Center under the code IRCT20200907048650N1. Before the study, informed written consent was obtained from all participants after a comprehensive oral and written explanation of the study was provided to them. All patients were allowed to leave the study at any point, voluntarily.

Study design

Questionnaires of cooperation and personal information, physical activity readiness questionnaire (PAR-Q), and informed consent were provided from all participants. Inclusion criteria included: men with type 2 diabetes and no other chronic disease or musculoskeletal problems, aged 40–50 years, fasting blood sugar above 126 mg, taking common diabetes medications such as metformin and glibenclamide, no insulin injection, body mass index above 24.99, no smoking, and lack of regular exercise for at least last six months before the beginning of the study. Participants were fully acquainted with the aims of the study and received the necessary oral and written explanation.

Procedures

After the informed consent was signed, the volunteers were examined by a specialist in endocrinology and metabolism. On the first day, height was measured to the nearest 0.5 cm using a stadiometer (DETECTO, Model 3PHTROD-WM, USA). Bodyweight (BW), Body mass index (BMI), waist-hip ratio (WHR), and body fat percentage (BFP) were obtained by a bioelectric impedance body composition analyzer (Zeus 9.9 PLUS; Jawon Medical Co., Ltd., Kungsang Bukdo, South Korea) in the fasting state.

Aerobic training

The 8-week AT program was consisted of a total of 40–60 min jogging and running at 60–75% of maximum heart rate (HR_{max}) (Table 1). The AT program was based on previous studies and recommendations from the American Diabetes Association (ADA) [33, 34]. AT consists of three phases: warm-up (10 min), AT (20–40 min), and cool down (10 min). At baseline, the training phase commenced with 40 minutes of jogging at 65% of HR_{max} in the first

week and increased to 60-minute of running at 75% of HR_{max} by the final week. To assure that the desired heart rate (exercise intensity) was achieved and maintained during the AT phase, each participant underwent heart-rate monitoring with a polar heart rate monitor (model: FT1) and using the 6–20 rating of perceived exertion (RPE) scale (Table 1) [12]. The HR_{max} formula was used to determine the target heart rate [$HR_{max} = 220 - \text{age}$].

Pomegranate juice intake

Participants in both PJI consumed 240 ml of pure PJ from Orum Narin Company with SHADLEE brand (without sugar and other additives) daily after lunch for eight weeks.

To prepare the PJ, the pomegranates were picked by hand and after washing, the pomegranate seeds were separated from the skin and the meat inside it by a specific machine and stored in the tank. Then the pomegranates were pressed, juiced, and pasteurized at 80°C. Concentrated PJ was produced after enzymatic, fining, and filtration steps. Finally, it was packed and transported to a cool warehouse. The PJ contained 20% PJ concentrate and no additives were added.

Participants in the AT and C groups received a water-based placebo with the same shape, color, and smell as the PJI, during the study [30]. Before attending the training program, the participants filled two questionnaires. First, a questionnaire was determined to assess the readiness of participants, including demographic data, health status, current alcohol intake, and physical activity. A second questionnaire was a detailed semi-quantitative food frequency questionnaire adapted to the Iranian population and composed everyday food items, serving sizes, and meals, as designed to record and analyze 3-day food, recalls, before, and at the end of the intervention. To determine the food intake and the amount of macronutrient consumption (protein, fat, and carbohydrates), the Food Processor nutritionist four software (FPN4) was used. Subjects were asked to consume the same food and macronutrient composition one day before collecting blood samples in the pre and post-test. In general, the subject's diet consisted of 55% carbohydrates, 30% fat, and 15% protein.

Table 2. Mean $\pm$ SD demographic information and anthropometric indices before the intervention among the groups

Variables	AT + PJI (n = 9)	AT (n = 10)	PJI (n = 9)	C (n = 10)	P-value ^a
Age [years]	43.22 $\pm$ 2.48	42.60 $\pm$ 1.89	41.33 $\pm$ 1.50	41.81 $\pm$ 1.88	0.336
Height [cm]	162 $\pm$ 54.18	166 $\pm$ 5.45	164.55 $\pm$ 4.79	164.45 $\pm$ 5.78	0.393
Body weight [kg]	90.44 $\pm$ 4.92	88.80 $\pm$ 3.35	88.44 $\pm$ 2.50	90.09 $\pm$ 4.20	0.537
BMI [kg/m ²]	34.55 $\pm$ 3.14	32.28 $\pm$ 1.78	32.69 $\pm$ 1.30	33.34 $\pm$ 1.51	0.112
FBS [mg/dl]	144.44 $\pm$ 3.12	145.10 $\pm$ 3.84	146.88 $\pm$ 7.42	144.90 $\pm$ 7.24	0.802
Insulin [μ U/ml]	7.30 $\pm$ 0.70	7.31 $\pm$ 0.86	7.06 $\pm$ 0.75	7.30 $\pm$ 0.78	0.832

AT + PJI – Aerobic training + pomegranate juice intake group; AT – Aerobic training group; PJI – pomegranate juice intake group; C – control group; P values with superscript “a” are calculated using a one-way analysis of variance test.

Blood sampling

Blood samples were taken twice, a day before the first training session (pre-test) and all subjects arrived at 8:00 a.m. on the day of the test in the clinical laboratory after more than 12 hours fasting. After maintaining the subjects in a stable state for 30 minutes, 20ml of venous blood was sampled from the antecubital vein using an anticoagulant-treated syringe. The sampled blood was placed in a tube that was not treated for anticoagulation and was then centrifuged at 3,000 rpm using a centrifugal separator for 10 minutes. After extracting the serum from the cellular components, the serum was put in a storage tube and stored in the refrigerator at -70°C until analysis. Blood lipid profile (Triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL)) were measured enzymatically with Hitachi Kit, Tokyo, Japan, and Glucose with the enzymatic method (Pars Azmoon kit made in Iran). Fasting blood sugar was calculated by enzymatic method using Pars Azmoon test kits (Iran), insulin by ELISA method using Mercoda Company kit (Sweden), and Insulin resistance index based on the following formula [17].

Statistical analysis

The results obtained in this study were analyzed using IBM SPSS (version 24.0) at the significance level of $\alpha = 0.05$. The descriptive statistics quantity was presented as the mean and the standard error of the mean (SE). The Shapiro-Wilk's test was used for evaluating the normality of distribution. To compare the mean hepatic risk factors between and within groups, one-way ANOVA and t-test were used, respectively; Tukey's post hoc test was used if significant differences were found.

Results

The findings on some demographic information, fasting blood sugar (FBS), and insulin of the participants and their between-group comparison are presented in Table 2.

The results of the one-way ANOVA test showed no significant difference in demographic information, FBS, and insulin between the groups.

TC, TG, LDL, and HDL levels improved significantly from pre-test to post-test within all experimental groups, as detailed in Figures 2, 3, 4, and 5. The results of one-way ANOVA showed no significant difference in TC, TG, LDL, and HDL between the groups in the pre-test, however in the post-test, there was a significant difference in the above variables between the groups. Lipid Profile changes were significant in AT + PJI, AT, and PJI compared to C.

Figures 2, 3, 4, and 5 show that the AT + PJI had significantly lower TC, LDL ($p = 0.001$, and $p = 0.002$, respectively), and significantly higher HDL ($p = 0.023$) compared with PJI. Also, the results of this study showed no significant difference between AT and PJI groups. Also, TG was significantly lower in AT + PJI compared to C, but not in AT and PJI alone.

Discussion

Findings of the present study showed that after 8 weeks of the interventional program AT+PJI the lowest levels of TC, TG, LDL, and the highest levels of HDL were observed in the AT+PJI; Therefore, AT+PJI had been more effective in improving blood lipid profile compared to

Figure 2. Comparison between mean $\pm$ SD of TC between groups

AT+PJI – Aerobic training + pomegranate juice intake group; AT – Aerobic training group; PJI – pomegranate juice intake group; C – control group; TC – Total Cholesterol; *: Significantly different compared to pre-test within the groups; £: Significantly different compared to C; €: Significantly different compared to PJI.

Figure 3. Comparison between mean $\pm$ SD of TG between groups

AT + PJI – Aerobic training + pomegranate juice intake group; AT – Aerobic training group; PJI – pomegranate juice intake group; C – control group; TG – Triglycerides; *: Significantly different compared to pre-test within the groups; £: Significantly different compared to C.

Figure 4. Comparison between mean $\pm$ SD of LDL between groups

AT+PJI – Aerobic training + pomegranate juice intake group; AT – Aerobic training group; PJI – pomegranate juice intake group; C – control group; LDL – Low-Density Lipoprotein; *: Significantly different compared to pre-test within the groups; £: Significantly different compared to C; €: Significantly different compared to PJI.

Figure 5. Comparison between mean $\pm$ SD of HDL between groups

AT + PJI – Aerobic training + pomegranate juice intake group; AT – Aerobic training group; PJI – pomegranate juice intake group; C – control group; HDL – High-Density Lipoprotein; *: Significantly different compared to pre-test within the groups; £: Significantly different compared to C; €: Significantly different compared to PJI.

PJI or AT alone. In Contrast, Kaplan et al. [14] found that two months of PJI did not affect TC levels significantly in rats [14]. Aviram et al. [1] investigated the anti-atherogenic effect of various pomegranate-based supplements in atherosclerotic mice with impaired apolipoprotein E production and concluded that PJ and pomegranate

flower extract had the most reducing effect on atherosclerotic plaque formation by 34% and 70%, respectively [1]. The results of Rajabian et al. [27] study showed that the PJ or seed oil was not effective in reducing TC, TG, LDL, and VLDL; however, it significantly prevented the development of aorta atherosclerotic plaques formation

in treated hypercholesterolemic rabbits [27]. Rosenblatt et al (2006) found no significant effect on TC and TG levels in diabetic patients after consuming 50 ml/day of PJ for 3 months [29]. Esmailzadeh et al. [7] Showed that consumption of 40 g of concentrated PJ per day for 8 weeks in diabetic patients, significantly reduced TC and LDL, while was not significantly affecting serum TG and HDL [7]. Several studies have shown that PJ contains flavonoids compounds that could improve the lipid profile due to its antioxidant feature [7; 37]. Human and cellular studies have shown that PJ can increase the expression and activity of paraoxonase-1. This enzyme has antioxidant properties that prevent the oxidation of LDL-C and HDL-C [2, 35]. PJ increases paraoxonase-1 activity by reducing fat peroxides or directly affecting the enzyme [8]. Increased serum activity of this enzyme in diabetics, decreases hypercholesterolemia, and cardiovascular complications [2, 8]. On the other hand, reducing cholesterol absorption, increasing cholesterol excretion, and modulating HMG-CoA reductase and acyltransferase (two key enzymes in cholesterol metabolism) are the other impacts of PJ [7, 35]. The PJ also inhibits HDL depletion by stimulating the release of HDL from macrophage cells [14]. Numerous studies have shown the beneficial effects of AT in improving lipid profile [9, 16, 38]. For example, Kasterswannack et al. (2015) observed that AT decreased TC, TG, and LDL, and increased HDL [16]. Increased fat oxidation as an energy substrate, both during exercise and recovery, are the main mechanisms of AT in improving the lipid profile. Other studies also showed that AT increases the amount of type A lipoprotein and the enzyme lipoprotein lipase (LPL) and ultimately decreases LDL [3, 16]. Therefore, the possible mechanism by which AT + PJI reduces TC and TG levels might be explained by the increased lipoprotein lipase response and activity. Gordon et al. (2016) observed that subjects with regular physical activity have significantly higher HDL concentrations than non-physically active subjects [10]. According to the results of studies, AT increases HDL by increasing the activity of lipoprotein lipase enzyme and hydrolyzing plasma triglycerides, which have protective effects against heart disease [10, 26].

Conclusions

Based on the findings of the present study, eight weeks of AT or PJI has positive effects on the lipid profile in patients with type 2 diabetes. However, consumption of PJ in conjunction with AT would have more positive effects on lipid profile in these patients.

Conflict of interest: Authors state no conflict of interest.

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