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Grape Seed Extract Supplementation and the Effects on the Biomarkers of Oxidative Stress and Metabolic Profiles in Female Volleyball Players: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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Abstract

Background: Only limited data are available for evaluating the effects of the administration of grape seed extract (GSE) on the metabolic status of female volleyball players.
Objectives: This study was conducted to determine the effects of GSE administration on the metabolic status of female volleyball players.
Methods: This randomized, double-blind, placebo-controlled clinical trial was performed among 40 female volleyball players. The subjects were randomly divided into two groups, with members of the test group (n = 20) taking 300 mg of GSE twice a day for eight weeks and members of the control group (n = 20) taking a placebo pearl for the same period. Fasting blood samples were taken before and after the eight-week intervention period in order to determine the related variables.
Results: Supplementation with GSE resulted in a significant rise in the plasma glutathione (GSH) level (+265.5 $\pm$ 344.2 vs. +2.2 $\pm$ 378.2 μmol/L, P = 0.02), as well as a significant decrease in the malondialdehyde (MDA) level (-1.4 $\pm$ 2.0 vs. -0.2 $\pm$ 1.2 μmol/L, P = 0.01) when compared to the placebo group. In addition, when compared to the group that received the placebo, the subjects who received GSE had significantly decreased serum insulin concentrations (-23.4 $\pm$ 23.4 vs. +1.8 $\pm$ 25.2 pmol/L, P = 0.002), a decreased homeostasis model of assessment for insulin resistance (HOMA-IR) (-0.7 $\pm$ 0.7 vs. +0.2 $\pm$ 0.9, P = 0.002), and an increased quantitative insulin sensitivity check index (QUICKI) (+0.01 $\pm$ 0.01 vs. -0.01 $\pm$ 0.02, P = 0.03). The administration of GSE had no significant effects on creatine phosphokinase (CPK), total antioxidant capacity (TAC), nitric oxide (NO), fasting plasma glucose (FPG), and lipid concentrations when compared with the administration of the placebo. However, after controlling for baseline NO levels, age, and baseline BMI, the changes in the plasma NO concentrations were significantly different between the two groups.
Conclusions: In conclusion, taking GSE for eight weeks had beneficial effects on the plasma GSH, MDA levels, and markers of insulin metabolism of female volleyball players.

Keywords: Grape Seed Extract, Oxidative Stress, Athletes

1. Background

Due to the increased energy demands and oxygen consumption, chronic exercise is accompanied by the increased production of free radicals, enhanced generation of lipid hydroperoxides, oxidative stress, and changes in blood antioxidant concentrations (1, 2). Increased markers of oxidative stress would result in oxidative damage and inflammation, and such an increase has been reported to play a primary or secondary role in the development of metabolic diseases (3). In addition, in the context of sports, it has been suggested that increased oxidative stress biomarkers can cause the onset of symptoms of overtraining, including enhanced exhaustion, decreased immune action, and decreased efficiency (4).

Various strategies for the management of oxidative stress in athletes, for example, supplementing micronutrients rich in vitamins such as vitamin A, vitamin E, and minerals, have been suggested (5). However, although the administration of such supplements is increasingly prac-

ticed by elite athletes, very little information is currently available regarding their effects on the biomarkers of oxidative stress, markers of insulin metabolism, lipid profiles, muscle recovery, and physical performance. Recently, there has been an increased demand for the use of herbal medications such as grape seed extract (GSE) for decreasing oxidative stress in athletes. In addition, traditional medicinal plants are often cheaper, more locally available, and more easily consumable than manufactured drugs. GSE contains a high concentration of flavonoids, linoleic acid fatty acids, and phenolic procyanidins (6). It has been said that the oxidant-lowering effect of GSE is approximately fifty times higher than that of vitamins C and E (7). In a study by Prior et al. (8), a dramatically increased postprandial antioxidant capacity was observed following the consumption of mixed grape powder. In addition, diabetic rats treated with GSE demonstrated decreased serum insulin and HbA1c levels (9). However, in another study GSE did not influence the enzyme activities related to oxidative stress in the cell line (10).

The active polyphenolic flavonoids in GSE, including oligomeric proanthocyanidin, may act as scavengers of free radicals (11) and hence as improved biomarkers of oxidative stress. In addition, the reduction in the mRNA expression of glycogen synthase kinase-3-α following the intake of GSE may result in decreased insulin resistance (12). To the best of our knowledge, no previous study has evaluated the favorable effects of GSE administration on the oxidative stress, insulin metabolism, and lipid profiles of female volleyball players.

2. Objectives

We hypothesized that GSE supplementation might help female volleyball players to control their oxidative stress and metabolic profiles. This study was therefore carried out to evaluate the beneficial effects of GSE administration on the oxidative stress, insulin metabolism, and lipid profiles of female volleyball players.

3. Methods

3.1. Participants

This study was a randomized double-blind placebo-controlled clinical trial in which 40 female volleyball athletes from sport club of Barij Essence between July 2014 and September 2014 in Kashan, Iran were included. To calculate the sample size, we used a randomized clinical trial sample size formula that considered the type one (α) and type two errors (β) to be 0.05 and 0.20 (Power = 80%), respectively. Based on the approach of a previous study (13), we applied a standard deviation (SD) of 0.88 nmol/mL and a difference in mean (d) of 0.90 nmol/mL, considering malondialdehyde (MDA) to be the key variable. The calculation demonstrated that 17 subjects were needed in each group. Assuming a dropout rate of three participants per group, the final sample size was determined to be 20 persons per group.

In the current study, the inclusion criteria were female volleyball players aged 14 - 42 years. We excluded individuals with neoplastic, hepatic, renal, or cardiovascular disorders, individuals with malabsorptive disorders, those who consumed antibiotics or supplemental vitamins and minerals, those who consumed drugs that impact metabolic profiles, smokers, and vegetarians. The study protocol was confirmed by the ethics committee of Kashan University of Medical Sciences (KUMS), and all the subjects provided written informed consent to participate. The trial was recorded on the Iranian website (www.irct.ir) for the registration of clinical trials (IRCT code: IRCT2014043012438N9).

3.2. Study Design

At the beginning of the study, after stratification for pre-intervention BMI (< 20 and ≥ 20 kg/m^2) and age ($<$ 18 and ≥ 18 years), the participants were randomized into two groups. Group one received 300 mg GSE (n = 20) and group two received placebo pearls (n = 20) twice a day for eight weeks along with their lunch and dinner. The GSE and placebo pearls were provided by the formulation department of Barij medicinal plants research center (Barij essence pharmaceutical, Kashan, Iran). In terms of appearance, including color, form, dimensions, and packaging, the placebo pearls were similar to the GSE pearls. The analysis of the GSE pearls was carried out in the laboratory of Barij essence pharmaceutical using the gas chromatography spectrometry (GC) method. Following the analysis of the GSE, it was determined that the major components were flavonoids, linoleic acid, phenolic procyanidins, and vitamins C and E. The randomized allocation sequence was generated using a computerized random number generator by a trained nutritionist at the diet therapy clinic. The randomization and allocation were concealed from the researchers and the participants until the end of the trial. The consumption of the GSE and placebo pearls throughout the study was monitored by requesting that the subjects bring in their medication containers. All participants completed three dietary records (on one weekend day and two weekdays) and three physical activity records on weeks two, four, and six of the intervention. In the present study, we used nutritionist IV software (First Databank, San Bruno, CA) adjusted for Iranian food patterns to

obtain the macro- and micronutrient intakes of the subjects based on their three-day food diaries.

3.3. Assessment of Anthropometric Measures

The weight and height (Seca, Hamburg, Germany) of the participants were measured based on standard protocols at the beginning and end of the study period at the diet therapy clinic. All anthropometric measures were performed by a trained nutritionist. In addition, the nutritionist was blinded to the randomization assignments. The participants' BMI was determined as their weight in kg divided by their height in meters squared. Their systolic blood pressure (SBP) and diastolic blood pressure (DBP) were quantified via a sphygmomanometer (ALPK2, Zhejiang, China).

3.4. Outcomes

In the current study, the serum creatine phosphokinase (CPK) and biomarkers of oxidative stress were the primary outcomes, while the markers of insulin metabolism and lipid concentrations were considered to be secondary outcomes.

3.5. Biochemical Assessment

Fasting blood samples (10 mL) were obtained before and after the intervention at the KUMS reference laboratory in the early morning following a 12-hour fast. The blood samples were immediately centrifuged (Hettich D-78532, Tuttlingen, Germany) at 3500 rpm for 10 minutes to separate the serum and then stored at -70°C prior to analysis at the KUMS reference laboratory. The total antioxidant capacity (TAC) of the plasma was measured according to the ferric reducing antioxidant power (FRAP) method developed by Benzie and Strain (14), while the total glutathione (GSH) was measured using Beutler et al.'s method (15). The MDA levels in the plasma were quantified according to the thiobarbituric acid reactive substance (TBARs) spectrophotometric test (16). The level of nitrite/nitrate (NOx) in the plasma was assessed using the Giess method modified by Tatsh et al. The available auto-analyzer kits (Pars Azmun, Tehran, Iran) were used to determine the serum CPK, fasting plasma glucose (FPG), serum triglycerides, VLDL-, total-, LDL-, and HDL-cholesterol concentrations. All the inter- and intra-assay coefficient variances (CVs) for the CPK, FPG, and lipid profile measurements were less than 5%. To determine the serum insulin concentrations, we used the available ELISA kit (Monobind, California, USA) with an enzyme-linked immunosorbent assay method. The inter- and intra-assay CVs for the serum insulin assays ranged from 3.5% to 4.7%. The homeostatic model of assessment for insulin resistance (HOMA-IR), the homeostatic model of assessment for beta cell function (HOMA-B), and the quantitative insulin sensitivity check index (QUICKI) were calculated according to the suggested formulas (17).

3.6. Statistical Methods

To ensure the normality of the variables, we applied the Kolmogorov-Smirnov test. To detect any differences in the general characteristics and dietary intakes between the two groups, we used the independent samples (or Student's) t-test. To identify any within-group differences (endpoint minus baseline), we used paired samples t-tests. Comparisons of any changes between the two groups after eight weeks of intervention were performed by one-way repeated measures analysis of variance (ANOVA). In this analysis, the treatment (GSE vs. placebo) was regarded as the between-subject factor and the time with two time-points (study baseline and after the eight-week intervention) was considered to be the within-subject factor. To identify any between-group differences for the non-normally distributed variables, we used the Mann-Whitney U test. The results of the normally distributed variables (all variables except CPK) were presented as the mean $\pm$ standard deviations (SDs), while the non-normally distributed variables (CPK) were presented as the median (IQR). To control for any confounding variables, including baseline values, age, and BMI at baseline, an analysis of covariance (ANCOVA) test was applied. P-values < 0.05 were considered to be statistically significant. All statistical analyses were carried out using the Statistical Package for Social Science version 17 (SPSS Inc., Chicago, Illinois, USA).

4. Results

In total, 40 participants (GSE group (n = 20) and placebo group (n = 20) completed the trial (Figure 1). On average, the rate of compliance in this study was high, such that more than 90% of the pearls were consumed throughout the intervention in both groups. No side effects or cytotoxic effects were reported throughout the study following the administration of GSE to the female volleyball players.

The mean age, height, SBP, and DBP of the subjects were not statistically different between the GSE and placebo groups (Table 1). The mean weight and BMI before and after the eight-week intervention period were also not significantly different between the two groups.

A comparison of the dietary intakes of the subjects throughout the study demonstrated no significant differences in the dietary macro- and micronutrient intakes between the GSE and placebo groups in terms of energy, carbohydrates, proteins, fats, saturated fatty acids

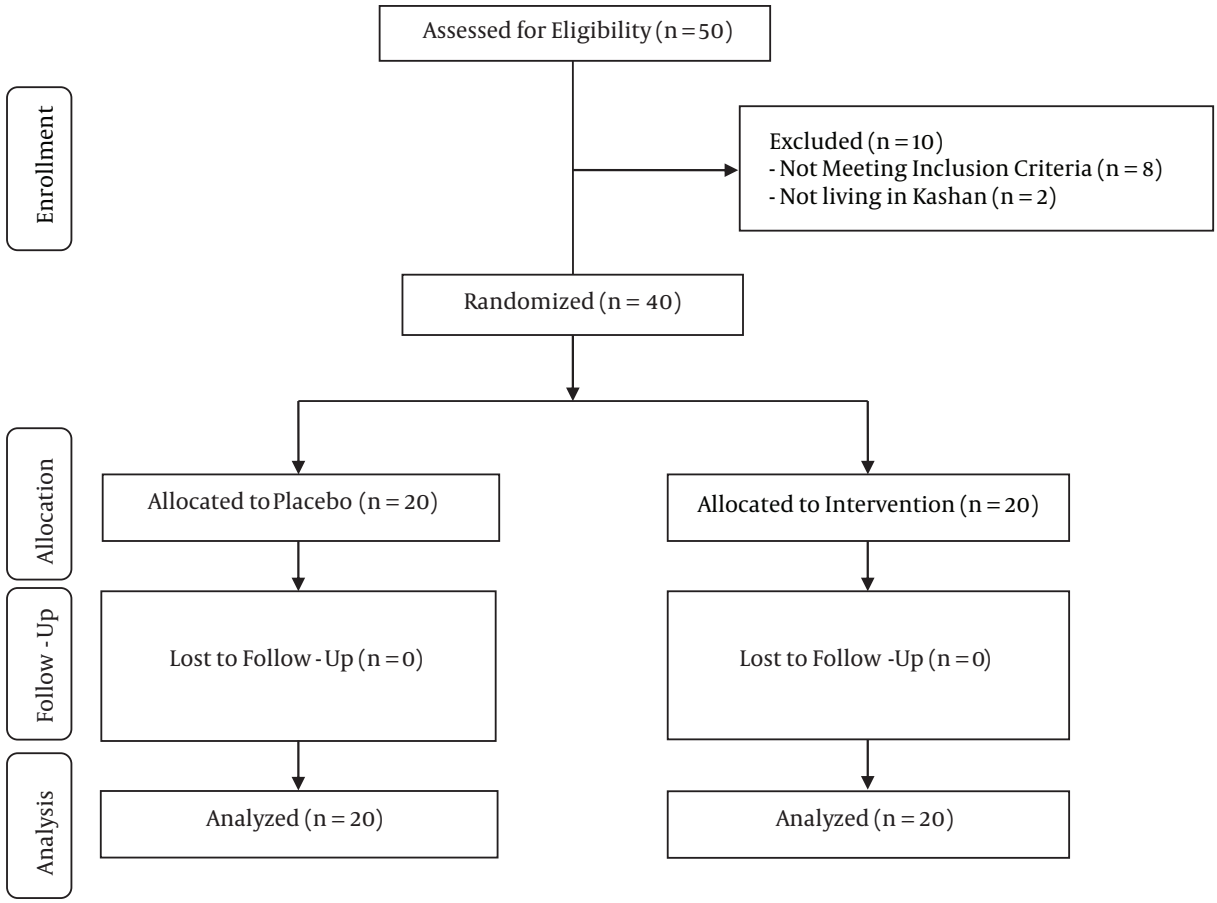

Figure 1. . Flow Diagram Summarizing Participation in the Trial

(SFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), cholesterol, total dietary fiber (TDF), magnesium, zinc, and manganese (Table 2).

When compared with the placebo group, the administration of GSE resulted in a significant rise in plasma GSH (changes from baseline in the GSE group: +265.5 ± 344.2 vs. in the placebo group: +2.2 ± 378.2 μmol/L, P = 0.02) and a significant decrease in MDA (-1.4 ± 2.0 vs. -0.2 ± 1.2 μmol/L, P = 0.01) (Table 3). Moreover, the subjects who received GSE exhibited significantly decreased serum insulin concentrations (-23.4 ± 23.4 vs. +1.8 ± 25.2 pmol/L, P = 0.002), HOMA-IR (-0.7 ± 0.7 vs. +0.2 ± 0.9, P = 0.002), and HOMA-B (-18.3 ± 17.2 vs. +1.2 ± 17.7, P = 0.001), as well as increased QUICKI (+0.01 ± 0.01 vs. -0.01 ± 0.02, P = 0.03), when compared with the placebo group. The administration of GSE had no significant effects on the CPK, TAC, NO, FPG, and lipid concentrations compared with the administration of the placebo. The within-group changes demonstrated a significant rise in the plasma GSH (P = 0.003) and QUICKI (P < 0.001) and a significant decrease in the plasma MDA (P = 0.004), serum insulin (P < 0.001), HOMA-IR (P < 0.001), HOMA-B (P < 0.001), serum triglycerides (P = 0.04), VLDL-C concentrations (P = 0.04), and total-/HDL-C (P = 0.04) in the GSE group. In addition, the within-group differences demonstrated a significant decrease in the total-/HDL-C (P = 0.02) in the placebo group.

When we adjusted the analysis for the baseline values, the abovementioned findings remained unchanged, except for the plasma NO level (P = 0.02) (Table 4). The adjustment for the baseline values, age, and baseline BMI did not affect our findings, except those regarding the plasma NO level (P = 0.04).

5. Discussion

We assessed the effects of the administration of GSE on the biomarkers of oxidative stress and metabolic profiles among female volleyball athletes. The major finding

Table 1. General Characteristics of the Study Participants[a]

	Placebo, n = 20	Grape Extract, n = 20	P[b]
Age, y	21.6 ± 7.0	19.8 ± 5.0	0.35
Height, cm	168.7 ± 6.3	168.6 ± 6.2	0.98
Weight at study baseline, kg	60.5 ± 6.3	61.7 ± 12.4	0.70
Weight at end of trial, kg	72.1 ± 10.7	75.8 ± 9.5	0.16
Weight change, kg	0.4 ± 1.5	1.0 ± 2.3	0.30
BMI at study baseline, kg/m^2	21.2 ± 1.5	21.6 ± 3.6	0.68
BMI at end of trial, kg/m^2	21.4 ± 1.7	21.9 ± 3.4	0.50
BMI change, kg/m^2	0.2 ± 0.5	0.3 ± 0.8	0.34
SBP, mmHg	112.6 ± 2.3	113.1 ± 2.1	0.47
DBP, mmHg	77.4 ± 2.9	78.1 ± 1.9	0.34

[a] Data are given as means ± SDs.
[b] Obtained from an independent t-test.

Table 2. Dietary Intakes of Study Participants Throughout the Trail[a]

	Placebo, n = 20	Grape Extract, n = 20	P[b]
Energy, kcal/d	2545 ± 185	2522 ± 147	0.59
Carbohydrates, g/d	348.9 ± 39.1	337.1 ± 47.5	0.39
Protein, g/d	90.4 ± 10.4	89.3 ± 13.2	0.76
Fat, g/d	92.2 ± 12.5	94.7 ± 10.7	0.49
SFA, g/d	27.1 ± 4.9	27.8 ± 5.6	0.67
PUFA, g/d	29.1 ± 8.3	28.9 ± 7.6	0.95
MUFA, g/d	24.5 ± 4.4	26.8 ± 6.5	0.19
Cholesterol, mg/d	216.6 ± 97.9	252.2 ± 122.2	0.31
TDF, g/d	20.5 ± 4.4	18.4 ± 5.4	0.18
Magnesium, mg/d	317.6 ± 62.2	300.3 ± 88.6	0.48
Zinc, mg/d	11.3 ± 2.2	11.4 ± 3.6	0.88
Manganese, mg/d	2.4 ± 0.7	2.3 ± 0.6	0.49

[a] Data are given as means ± SDs.
[b] Obtained from an independent t-test.

of this study was that the administration of GSE increased the plasma GSH, decreased MDA, prevented increased CPK levels, and improved insulin metabolism parameters in female volleyball players. To the best of our knowledge, the current study is the first to report the effects of GSE intake on the oxidative stress and metabolic profiles of female volleyball players.

Increased oxidative stress during sport may result in several complications, including impaired immune function and decreased performance (4). The current study demonstrated that taking 600 mg of GSE per day for eight weeks significantly increased the plasma GSH, decreased MDA, and led to a significant difference in CPK levels among female volleyball athletes when compared to the administration of a placebo, although it did not affect the TAC and NO levels. However, after controlling for the baseline NO levels, age, and baseline BMI, the changes in the plasma NO concentrations were significantly different between the groups. Similar to the findings of the current study, Prior et al. (8) found that taking mixed grape powder significantly increased the post-prandial antioxidant capacity. In addition, the administration of GSE prevented oxidative stress by preventing lipid peroxidation in acutely and chronically exercised rats for six weeks (6). Similar

Table 3. Biomarkers of Oxidative Stress and Metabolic Profiles at Baseline and After the Intervention[a]

	Placebo, n = 20			Grape Extract, n = 20			P[b]		
	Wk. 0	Wk. 8	Change	Wk. 0	Wk. 8	Change	Time	Group	Time x Group
TAC, mmol/L	826.5 ± 128.9	792.3 ± 128.2	-34.2 ± 140.1	811.4 ± 122.9	787.9 ± 138.9	-23.5 ± 133.6	0.19	0.78	0.80
GSH, μmol/L	952.5 ± 310.1	954.7 ± 265.1	2.2 ± 378.2	824.0 ± 176.8	1089.5 ± 362.8[c]	265.5 ± 344.2	0.02	0.96	0.02
MDA, μmol/L	3.9 ± 1.1	3.7 ± 1.4	-0.2 ± 1.2	4.7 ± 2.1	3.3 ± 0.8[c]	-1.4 ± 2.0	0.003	0.57	0.01
CPK, U/L	112.1 (70.0)	102.1 (328.1)	3.0 (195.7)	96.9 (104.6)	101.8 (101.2)	0.1 (84.2)	-	-	0.22[d]
NO, μmol/L	62.4 ± 11.2	53.3 ± 13.5	-9.1 ± 19.3	65.2 ± 7.1	61.9 ± 8.2	-3.3 ± 9.1	0.01	0.01	0.23
FPG, mmol/L	4.4 ± 0.7	4.6 ± 0.6	0.2 ± 0.7	4.5 ± 0.6	4.4 ± 0.9	-0.1 ± 0.9	0.55	0.80	0.28
Insulin, pmol/L	64.2 ± 21.0	66.0 ± 19.2	1.8 ± 25.2	84.0 ± 29.4	60.6 ± 19.8[c]	-23.4 ± 23.4	0.008	0.26	0.002
HOMA-IR	2.2 ± 0.6	2.4 ± 0.7	0.2 ± 0.9	2.7 ± 0.8	2.0 ± 0.8[c]	-0.7 ± 0.7	0.03	0.73	0.002
HOMA-B	46.4 ± 16.0	47.6 ± 14.5	1.2 ± 17.7	59.9 ± 26.0	41.6 ± 16.8[c]	-18.3 ± 17.2	0.004	0.47	0.001
QUICKI	0.34 ± 0.01	0.33 ± 0.01	-0.01 ± 0.02	0.33 ± 0.01	0.34 ± 0.01[c]	0.01 ± 0.01	0.08	0.91	0.03
Triglycerides, mmol/L	0.8 ± 0.3	0.9 ± 0.3	0.1 ± 0.4	0.8 ± 0.3	0.7 ± 0.3[c]	-0.1 ± 0.2	0.95	0.19	0.12
VLDL cholesterol, mmol/L	0.4 ± 0.2	0.4 ± 0.2	0.0 ± 0.2	0.4 ± 0.1	0.3 ± 0.1[c]	-0.1 ± 0.1	0.95	0.19	0.12
Total cholesterol, mmol/L	3.8 ± 0.6	3.8 ± 0.7	0.0 ± 0.6	3.9 ± 0.9	3.8 ± 0.8	-0.1 ± 0.7	0.54	0.89	0.54
LDL cholesterol, mmol/L	1.9 ± 0.5	1.8 ± 0.5	-0.1 ± 0.4	2.1 ± 0.7	1.9 ± 0.5	-0.2 ± 0.5	0.06	0.40	0.89
HDL cholesterol, mmol/L	1.5 ± 0.3	1.6 ± 0.4	0.1 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	0.0 ± 0.3	0.12	0.45	0.46
Total: HDL cholesterol ratio	2.6 ± 0.4	2.4 ± 0.4[c]	-0.2 ± 0.2	2.6 ± 0.5	2.5 ± 0.3[c]	-0.1 ± 0.3	0.003	0.56	0.81

[a] Values are given as means ± SDs for normally distributed variables and median (IQR) for non-normally distributed variables.
[b] Obtained from a repeated measures ANOVA test.
[c] Different from wk. 0, $P < 0.05$.
[d] Obtained from a Mann-Whitney U test.

Table 4. Adjusted Changes in Metabolic Variables in Female Volleyball Players[a]

	Placebo, n = 20	Grape Extract, n = 20	P[b]
TAC, mmol/L	-23.28 ± 26.78	-34.34 ± 26.78	0.77
GSH, μmol/L	69.67 ± 70.46	197.93 ± 70.46	0.21
MDA, μmol/L	-0.46 ± 0.25	-1.15 ± 0.25	0.07
NO, μmol/L	-9.99 ± 2.52	-2.30 ± 2.52	0.04
FPG, mmol/L	0.17 ± 0.16	-0.02 ± 0.16	0.39
Insulin, pmol/L	-4.32 ± 4.20	-16.98 ± 4.20	0.05
HOMA-IR	0.01 ± 0.16	-0.56 ± 0.16	0.02
HOMA-B	-2.63 ± 3.00	-14.48 ± 3.00	0.01
QUICKI	0.0001 ± 0.004	0.01 ± 0.004	0.02
Triglycerides, mmol/L	0.08 ± 0.06	-0.09 ± 0.06	0.07
VLDL cholesterol, mmol/L	0.03 ± 0.03	-0.04 ± 0.03	0.07
Total cholesterol, mmol/L	-0.03 ± 0.13	-0.09 ± 0.13	0.74
LDL cholesterol, mmol/L	-0.17 ± 0.08	-0.08 ± 0.08	0.43
HDL cholesterol, mmol/L	0.10 ± 0.06	0.03 ± 0.06	0.44
Total: HDL cholesterol ratio	-0.16 ± 0.05	-0.14 ± 0.05	0.84

[a] All values are given as means ± SDs.
[b] Obtained from an ANCOVA adjusted for the baseline values, age, and baseline BMI.

findings were observed following GSE supplementation in animal models (18, 19). An improved antioxidant capacity was also seen following the intake of organic grape juice among triathletes (20). A human model study conducted by Zern et al. (21) demonstrated that supplementation with grape polyphenols led to decreased oxidative stress through lower levels of F(2)-isoprostanes and plasma tumor necrosis factor-α in pre- and postmenopausal women.

In another study, Yuan et al. (13) demonstrated that two weeks of grape juice consumption in healthy subjects increased the plasma TAC levels and decreased the circulating concentrations of MDA. However, such beneficial effects were not seen in other studies. For example, several GSE doses (between 0 and 100 mg/L for 24 hours) did not affect the GSH content and glutathione peroxidase (GPx) and glutathione reductase (GR) enzyme activities in the cell line (10). Previous studies have indicated that oxidative stress could result in insulin resistance, hypertension, hyperlipidemia (22), altered membrane fluidity, and type 2 diabetes mellitus (T2DM) (23). GSE is rich in active polyphenolic flavonoids, including oligomeric proanthocyanidin (24), which may act as scavengers of free radicals (11) and thus result in increased plasma GSH and decreased MDA levels. In addition, diets rich in phenolic compounds may be used to increase the levels of polyphenols bound to lipoproteins, thereby achieving prolonged protection for the serum lipid fraction against oxidative stress (25).

Our study revealed that among female volleyball players, the administration of GSE for eight weeks decreased the serum insulin levels, HOMA-IR, and HOMA-B, as well as increasing the QUICKI score, when compared with placebo administration; however, it did not affect the FPG and lipid profiles. Similar to the findings of our study, diabetic rats treated with GSE demonstrated decreased serum insulin and HbA1c levels (9). In addition, GSE administration combined with the cafeteria diet (CD) significantly decreased insulinemia and the HOMA-IR score (26). However, taking 400 mg of GSE did not influence the lipid profiles of elite male athletes (27). GSE consumption also decreased the Ox-LDL-cholesterol and lipid profiles of patients with mild hyperlipidemia after eight weeks (28). Significant decreases in the insulin levels and HOMA-IR were also observed following grape seed treatment in db/db mice; however, it did not affect the glucose levels (29). Furthermore, organic grape juice intake (300 mL/day) has resulted in improved glucose homeostasis parameters among triathletes after 20 days (20). However, some researchers did not observe any beneficial effects of GSE supplementation on glucose homeostasis parameters and lipid profiles. For instance, no statistically significant changes were shown in the HOMA-IR scores among high-risk cardiovascular subjects with type 2 diabetes mellitus (T2DM) after taking 600 mg of GSE per day for four weeks (30). A daily intake of 150 mL of muscadine grape wine did not influence the lipid profiles among patients with T2DM after 28 days (31). The results of the current study may be mediated by different mechanisms, for example, the insulin-mimetic effects of the polyphenolic compounds found in GSE, including the enhanced expression of glucose transporter type 4 (GLUT4), the increased mRNA levels of glycogen synthase, and the suppression of the mRNA expression of glycogen synthase kinase-3-α after GSE supplementation, may be explained by the beneficial effects of GSE on insulin metabolism parameters (12). Furthermore, previous studies have shown that the phenolic compounds in GSE may result in improved insulin function by increasing adiponectin secretion and expression (32, 33). The discrepancies between our findings and those of previous reports concerning the effects of GSE supplementation on lipid profiles might be explained by the dosage of the GSE supplements used, the intervention time, or the study participants.

The main strengths of our study were the assessment of the markers of insulin metabolism and lipid profiles, as well as its randomized design. The current study did, however, have some limitations. Due to budgetary restrictions, we could not assess the beneficial effects of GSE on inflammatory cytokines, including high sensitivity C-reactive protein (hs-CRP), interleukin 1 (IL-1), IL-6, and tumor necrosis factor alpha (TNF-α), as well as other biomarkers of oxidative stress such as superoxide dismutase (SOD). Moreover, the current study featured an intervention of a relatively short duration. Long-term interventions might lead to greater changes in other lipid profiles.

In conclusion, our findings show that the administration GSE for eight weeks in female volleyball players had beneficial effects on some biomarkers of oxidative stress and insulin metabolism parameters; however, it did not influence the plasma TAC, NO, FPG, and serum lipid profile levels.

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Footnotes

Authors' Contribution: Zatollah Asemi helped in designing the study, conducting the statistical analysis, and drafting the manuscript. Elaheh Malekian, Mohsen Taghizadeh, Mohammad Reza Memarzadeh and Ali Akbar Mohammadi contributed to data collection. Zatollah Asemi supervised the study. All authors approved the final paper for submission.

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