ORIGINAL ARTICLE

Effect of pomegranate juice supplementation on matrix metalloproteinases 2 and 9 following exhaustive exercise in young healthy males

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Abstract

Objectives: To evaluate the efficacy of pomegranate juice supplementation on matrix metalloproteinases2 and 9 serum levels and improving antioxidant function in young healthy males during exhaustive exercise.

Methods: The study was conducted at Ardabil University of Medical Sciences, Iran, in 2010-11 and comprised 28 healthy subjects in 18-24 age bracket. They were randomly divided into control and supplemented groups. One cup of pomegranate juice and one cup of tap water were given to supplemented and control groups daily for two weeks respectively. Fasting blood samples were taken at baseline and at the end of two weeks of intervention. The subjects were given one exhaustive exercise and then fasting blood samples were taken for testing blood glutathione peroxidase and superoxide dismutase and serum levels of high sensitivity C-reactive protein, zinc, ceruloplasmin, matrix metalloproteinases 2 and 9, malondialdehyde and total antioxidant capacity. Data was analysed using descriptive statistical tests, paired and independent sample t-test.

Results: The blood levels of glutathione peroxidase and superoxide dismutase and serum levels of total antioxidant capacity after exhaustive exercise in the supplemented group were significantly increased ($p<0.05$), while the content of matrix metalloproteinases 2 and 9, ceruloplasmin and malondialdehyde showed a significant decrease in comparison to the control group ($p<0.05$). Besides, there were no significant changes in other biochemical factors.

Conclusion: Regular intake of pomegranate juice significantly modulates matrix metalloproteinases 2 and 9and serum levels of some inflammatory factors and thus protects against exhaustive exercise-induced oxidative injury in young healthy males.

Keywords: Pomegranate juice, MMPs, Exhaustive exercise, Healthy males. (JPMA 64: 785; 2014)

Introduction

Contraction of skeletal muscle after intense physical activity may be the cause of damage to muscles such as protein breakdown and muscle dysfunction.[1] Certain stimuli, particularly those that induce high levels of mechanical stress such as eccentric or high-impact exercise, activate the local production of matrix metalloproteinase (MMPs) in skeletal muscle.[2] MMPs are a family of zinc-dependent enzymes that play important roles in changes in physiological situation during muscle regeneration[3] and facilitating physiological adaptations to exercise training.[4] Of the several MMPs, MMP2 (gelatinase A) and MMP9 (gelatinase B) play critical roles in cleaving muscle-specific proteins and assisting in extracellular matrix formation, remodelling and regeneration of skeletal muscles.[5] Serum concentrations of MMP2 and MMP9 are reported to peak within a relatively short time period following one exhaustive aerobic activity session.[6] Previous investigation has shown that MMP2 and MMP9 may play inflammatory myopathies in skeletal muscle[7] and consumption of natural antioxidants from fruits such as pomegranate juice provide powerful antioxidant effects.[8,9] Fresh pomegranate juice includes 85% water, 10% total sugars, and 1.5% pectin, ascorbic acid and polyphenolic flavonoids.[10,11] Pomegranate extracts, as a potential natural antioxidant, contribute to free radicals scavenging and reducing damage induced by oxidative stress (OS) and lipid peroxidation in macrophage,[12] and lead to improvements in plasma antioxidant status.[13] It has been shown that the intake of pomegranate juice reduces MMPs and results in improving antioxidant function in elderly subjects.[13] The major purpose of the present study was to evaluate the efficacy of pomegranate juice supplementation on matrix MMP2 and MMP9, and on improving antioxidant function in young healthy males during exhaustive exercise.

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Subjects and Methods

The study was conducted at the Ardabil University of Medical Sciences, Iran, in 2010-11 after approval by the institutional ethics committee. Sample size was determined according to the following formula for each group: $n= [(Z_{1-\alpha/2}) + (Z_{1-\beta})]^2 \times (\delta_1^2 + \delta_2^2) :(\mu_2 - \mu_1)^2$. On the basis of the calculation, 28 healthy male athletes of normal body mass index (BMI) within the 18-24 age group living in the dormitories of the university were recruited and randomly divided into two equal groups of supplemented and control Subjects. The aims of the investigation were explained to the subjects and a written consent was obtained prior to the filling out of the questionnaire. People with diseases involving the heart, lungs, kidney and other vital organs were excluded, and so were non-athletes, smokers and consumers of other supplements like vitamins, antioxidants etc. General information, anthropometric factors (height and weight), and fasting blood samples were collected and then one cup (240ml) of pomegranate juice and one cup (240ml) of tap water were given to the supplemented and control groups respectively every day for two weeks. Fasting blood samples were drawn from the subjects at the beginning and at the end of two weeks of intervention by venipuncture. Sera were separated immediately and were kept frozen at -80°C until analysed. The subjects were given once exhaustive exercise running on treadmill at 70% maximal heart rate and then fasting blood samples were taken immediately after exhaustion.

Serum MMP2 and MMP9 levels were assayed by standard enzyme-linked immunosorbent assay (ELISA) kits (Boster, Wuhan, China). Serum samples were diluted 1:100 according to the manufacturer's specifications for MMP9 and MMP2 assay. Serum total antioxidant capacity (TAC) was measured by colorimetric method using standard kit (Randox Laboratories Ltd., UK). Serummalondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances (TBARS)method. Samples were heated with 0.6% thriobarbituric acid under acidic condition and after cooling, the coloured product was extracted into n-butanol. The pink colour absorbance was measured at 530nm. MDA standards were prepared with 1, 1, 3, 3-tetraethoxypropane. Antioxidant enzymes activity of superoxide dismutase (SOD) and glutathione peroxidase (GPX) were determined by spectrometric method using Ransod and Ransel kit, respectively (Randox Laboratories Ltd., UK). For the accuracy of assessment, duplicate assay were performed. Serum biochemical factors as well as high sensitivity C-reactive protein (hs-CRP), ceruloplasmin and zinc (Zn) serum levels were measured by standard photometric methods.

The food intake was estimated for energy and other nutrients by 24 hour recall method for three days in a week during the study. Mean daily dietary intake and food composition were estimated using Nutritionist IV software.

Data was analysed using descriptive statistical tests, paired and independent sample t-test. Statistical analysis was performed with SPSS 16. Statistically significant level for all tests was considered as $p<0.05$.

Results

There was no significant relationship in terms of age, weight, calorie and nutrient intake in both groups during the study (Table-1, 2). The serum levels of hs-CRP, MMP2 and MMP9 were significantly decreased in the supplemented group after two weeks of pomegranate juice supplementation ($p<0.05$) (Table-3). The blood levels of GPX and SOD and serum levels of TAC after the intervention in the supplemented group were significantly increased ($p<0.05$), while the content of MDA significantly decreased in comparison to the control group ($p<0.05$). The blood levels of GPX and SOD were increased in both groups after exhaustive exercise, but the increase was significantly higher in the supplemented group than the control group ($p<0.05$). In the control group, the increase in the serum levels of MDA, MMP2, MMP9 and ceruloplasmin was significantly higher than in the supplemented group after exhaustive exercise ($p<0.05$). Although serum levels of hs-CRP were increased in both groups after exhaustive exercise, but there was no significant differences between the groups.

Table-1: The comparison of variables in the two groups before the study.

Variables	Groups		P value
	Supplemented M±SD %95 CI	Control M±SD %95 CI*	
Age (year)	19.07±1.07 18.45 to 19.69	19.78±0.89 19.27 to 20.30	0.06
Weight (kg)	68.28±9.18 62.98 to 73.59	72.50±7.84 67.97 to 77.03	0.2
Height (cm)	177.50±4.94 174.65 to 180.35	176.79±6.29 173.15 to 180.42	0.74
BMI (body mass index) (kg/m^2)	22.25±2.92 20.56 to 23.94	23.17±1.89 22.08 to 24.26	0.33

*Confidence Interval.

Table-2: The comparison of nutrient variables in the two groups before the study.

Variables	Groups		P value	Variables	Groups		P value
	Supplemented M±SD %95 CI	Control M±SD %95 CI*			Supplemented M±SD %95 CI	Control M±SD %95 CI*	
Calorie (Kcal/day)	2418.01±192.09 2307.09 to 2528.91	2575.21±391.22 2349.31 to 2801.10	0.19	VitaminB1 (mg)	1.49±0.22 1.37 to 1.62	1.56±0.39 1.33 to 1.79	0.58
Protein (g)	93.85±11.62 87.15 to 100.57	96.07±13.43 88.31 to 103.83	0.64	VitaminB2 (mg)	1.33±0.28 1.17 to 1.49	1.40±0.36 1.20 to 1.61	0.54
Carbohydrate (g)	325.28±2.45 306.55 to 344.03	249.62±1.86 308.15 to 391.13	0.26	VitaminB3 (mg)	23.67±7.84 19.14 to 28.20	23.65±3.93 21.38 to 25.93	0.99
Fibre (g)	12.50±4.51 9.89 to 15.11	12.07±2.87 10.42 to 13.73	0.77	Vitamin B6 (mg)	1.33±0.48 1.06 to 1.61	1.05±0.30 0.88 to 1.23	0.07
Total fat (g)	84.14±11.83 77.31 to 90.98	87.92±15.60 78.92 to 96.94	0.47	Folic acid (µg)	115.57±69.22 75.60 to 155.54	120.64±48.76 92.48 to 148.80	0.82
Saturated fat (g)	16.07±3.07 14.29 to 17.85	19.29±5.97 15.84 to 22.73	0.08	Vitamin C (mg)	51.04±36.61 29.90 to 72.18	57.35±25.49 42.64 to 72.08	0.6
Poly unsaturated fat (g)	13.21±4.77 10.46 to 15.97	13.92±4.04 11.59 to 16.26	0.67	Calcium (mg)	589.01±215.51 464.56 to 713.44	647.36±279.21 486.15 to 808.57	0.54
Mono unsaturated fat (g)	20.57±3.22 18.71 to 22.44	23.21±5.57 19.99 to 26.43	0.14	Iron (mg)	20.14±5.02 17.24 to 23.04	24.29±7.53 19.94 to 28.13	0.1
Cholesterol(mg)	306.21±124.56 234.29 to 378.13	341.78±148.37 256.12 to 427.45	0.49	Zinc (mg)	4.20±1.06 3.60 to 4.82	3.49±1.13 2.84 to 4.15	0.09
Vitamin E(mg)	13.67±2.85 12.03 to 15.33	15.50±2.95 13.79 to 17.21	0.11	Copper (mg)	0.93±0.26 0.77 to 1.08	0.87±0.14 0.79 to 0.95	0.47
Vitamin B6(mg)	3.34±0.89 2.82 to 3.86	3.25±0.68 2.86 to 3.65	0.79	Magnesium (mg)	156.07±59.80 121.54 to 190.60	146.86±32.66 128.01 to 165.72	0.61
Potassium(mg)	2054.12±655.18 1675.80 to 2432.40	1862.90±271.99 1705.90 to 2020.01	0.32	Selenium(µg)	42.42±8.41 37.57 to 47.29	42.64±10.30 36.69 to 48.59	0.95
Vitamin B12 (µg)	3.07±1.28 2.96 to 4.44	4.36±1.38 3.56 to 5.16	0.2	Phosphorous (mg)	913.93±185.30 806.94 to 1020.90	916.50±128.15 842.51 to 990.49	0.97

Table-3: The comparison of biochemical parameters before and after exhaustive exercise in both groups.

Variables	Measurement stage	Supplemented group M±SD %95 CI	Control group M±SD % 95 CI	P value
GPX (U/gHb)	Baseline	42.21 ± 2.77* 40.61 to 43.81	40.58 ±2.41 39.19 to 41.98	0.11
	After supplementation	45.46 ± 2.01 44.29 to 46.62	40.15 ±1.81 39.10 to 41.20	0.001
	After exhaustive exercise	46.05 ± 2.27 44.74 to 47.36	42.40 ± 2.77 40.80 to 43.99	0.001
SOD (U/gHb)	Baseline	1556.14 ± 169.35* 1458.35 to 1653.92	1469.50 ±177.91 1366.77 to 1572.22	0.19
	After supplementation	1720.14 ± 136.52 1641.32 to 1799.97	1486.71 ±161.67 1393.37 to 1580.06	0.001
	After exhaustive exercise	1774.57 ± 140.82 1693.26 to 1855.88	1542.79 ± 161.56 1449.50 to 1636.07	0.001
TAC (mmol/l)	Baseline	0.76 ± 0.14* 0.68 to 0.84	0.67±0.09 0.62 to 0.73	0.07
	After supplementation	0.87 ± 0.12 0.80 to 0.93	0.66±0.07 0.62 to 0.70	0.001
	After exhaustive exercise	0.81 ± 0.09 0.76 to 0.87	0.64 ± 0.10 0.58 to 0.69	0.001
MDA(nmol/ml)	Baseline	0.20 ± 0.04* 0.17 to 0.22	0.19±0.04 0.17 to 0.21	0.78
	After supplementation	0.17 ± 0.03	0.20±0.03	0.002

		0.15 to 0.18	0.18 to 0.22	
	After exhaustive exercise	0.26 ± 0.05	0.34 ± 0.10	0.02
		0.23 to 0.29	0.28 to 0.40	
MMP2(ng/ml)	Baseline	27622.32±3523.33*	27583.33±9202.46	0.99
		25383.70 to 29860.93	22679.68 to 32486.97	
	After supplementation	25217.57±3567.06	28020.42±7199.18	0.23
		22951.17 to 27483.97	24184.24 to 31856.59	
	After exhaustive exercise	28689.84±4990.69	35531.05±10171.70	0.04
		25518.90 to 31860.77	30110.93 to 40951.17	
MMP9(ng/ml)	Baseline	18474.61±2030.50*	18973.61±2134.70	0.73
		17184.49 to 19764.73	17791.45 to 20155.77	
	After supplementation	16870.42±1733.85	18669.98±2508.03	0.07
		15768.78 to 17972.05	17281.08 to 20058.88	
	After exhaustive exercise	19310.24±1997.47	21450.56±2395.67	0.02
		18041.11 to 20579.37	20123.88 to 22777.24	
Ceruloplasmin (mg/dl)	Baseline	30.29±4.79	27.65±5.56	0.19
		27.52 to 33.06	24.44 to 30.87	
	After supplementation	28.35±5.24	29.32±6.39	0.66
		25.34 to 31.37	25.63 to 33.01	
	After exhaustive exercise	29.82±6.23	40.11±8.48	0.001
		26.16 to 33.47	35.22 to 45.00	
Zn (mg/dl)	Baseline	86.65±22.40	79.80±21.29	0.26
		167.62 to 187.38	67.51 to 92.09	
	After supplementation	101.01±17.07	88.12±13.31	0.04
		90.69 to 111.33	80.43 to 95.80	
	After exhaustive exercise	123.44±41.61	78.96±17.37	0.001
		98.29 to 148.58	68.93 to 88.99	
hs-CRP (mg/dl)	Baseline	0.64±0.30*	0.68±0.55	0.19
		0.47 to 0.81	0.36 to 1.00	
	After supplementation	0.48±0.34	0.72±0.43	0.1
		0.28 to 0.67	0.48 to 0.97	
	After exhaustive exercise	1.24±0.46	1.53±0.51	0.15
		0.97 to 1.51	1.23 to 1.83	

Values are mean ±SD, *. ($p \leq 0.05$) Paired-sample t test indicated a significant difference in supplemented group between baseline and after supplementation.
GPX: Glutathione peroxidase
SOD: Superoxide dismutase
TAC: Total antioxidant capacity
MDA: Malondialdehyde
Zn: Zinc
Ha-CRP:high sensitivity C-reactive protein
MMP2: Matrix metalloproteinases 2
MMP9: Matrix metalloproteinases9.
MMP9: Matrix metalloproteinases9.

Discussion

The study demonstrated that intake of pomegranate juice is an efficient oral supplementation before and after exhaustive exercise. Intake of the pomegranate juice supplementation for two weeks significantly reduced serum levels of MMP2, MMP9, hs-CRP and MDA, and increased antioxidant enzymes activity. Similar to our findings, a 2008 study showed that 250ml of pomegranate juice given daily increased plasma antioxidant capacity.[13] Pomegranate juice is a polyphenol-rich juice with high antioxidant capacity. It has been shown that pomegranate juice has exerted significant anti-inflammatory effects in colon cancer cells.[14] The hs-CRP, ceruloplasmin and MMPs are biomarkers of inflammation.[15] Our study showed that the consumption of 250ml pomegranate juice for two week significantly reduced hs-CRP levels. Although, the serum levels of MDA, MMP2, MMP9 and ceruloplasmin were increased in both groups after exhaustive exercise, but increases in their levels were significantly higher in control group. It is shown that exercise induces increases in MMP9 messenger ribonucleic acid (mRNA) levels and its activity in the skeletal muscle.[16] Physical exercise is a complex process constituting several

factors affecting the expression levels of MMP2 and MMP9 in skeletal muscle, in a situation such as local ischemia and increases in muscle stretching; in shear stress; and in the wall tension of blood vessels.[17] It has been reported that the administration of antioxidant supplementation may play a positive role on metabolism in exercise.[18] A 2005in vitro study demonstrated that pomegranate fruit extract has a significant and broad inhibitory activity on MMPs,[19] which is consistent with this study. There is evidence that a plant antioxidant such as pomegranate inhibits MMP2 expression induced by a tumour necrosis factor (TNF) in smooth muscle cells of human aorta. The inhibition depended on the species of reactive oxygen generated by nicotinamide adenine dinucleotide phosphate-oxidase (NAD(P)H).[19,20] Pomegranate juice has been extensively used in ancient cultures for various medical features.[21] Our results reveal significantly elevated serum levels of SOD and GPX in the supplemented group in comparison with the controls at the end of two weeks of intervention. In the supplemented group the increase of MMP2, MMP9, ceruloplasmin and MDA were less than the control group following exhaustive exercise. Consistent with our study, a 2011 trial showed that the pomegranate juice has positive effects on lipid peroxidation levels due to exercise-induced oxidative stress.[22] Another study showed the substantial antagonizing effects of pomegranate extract, as a polyphenol-rich antioxidant supplement, on oxidative stress and lipid peroxidation.[23] Trial results of another 2010 study confirmed that pomegranate consumption improved muscular strength recovery after two days of sports activity.[24] Previous work in animal models has shown that the reduction in post-exercise inflammation[25] and oxidative stress attenuate myofiber damage.[26] It has been shown that the pomegranate juice has significant antioxidant and anti-inflammatory effects.[27] We found that ceruloplasmin, MMP2, MMP9 and MDA levels increased in both the groups after exhaustive exercise, but the increase was significantly less in the supplemented group than the control group. Antioxidant rise in supplemented group may have inhibition on inflammatory factors. Polyphenolic compounds originating in pomegranate juice have antioxidant activity and inhibit pro-inflammatory enzymes including the cyclooxygenases and lipoxygenases.[8]

Conclusion

Regular intake of pomegranate juice significantly modulates MMP9, MMP2 and some inflammatory factors, and thus protects against exhaustive exercise-induced oxidative injury in young healthy males. Pomegranate juice seems to exert beneficial effects in reducing MMP2, MMP9 serum levels and increasing antioxidant enzyme activities before and after exhaustive exercise. Further large-scale studies in this area are recommended.

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