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Original Article

Acute and long term effects of grape and pomegranate juice consumption on endothelial dysfunction in pediatric metabolic syndrome

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Abstract

BACKGROUND: This study aimed to determine the short- and long-term effects of consumption of grape and pomegranate juices on markers of endothelial function and inflammation in adolescents with metabolic syndrome (MetS).

METHODS: In a non-pharmacologic randomized controlled trial, 30 individuals were randomly assigned to two groups of drinking natural grape or pomegranate juice for 1 month. Measurements of inflammatory factors [Hs-CRP, sE-selectin, sICAM-1, sVCAM, and interleukin 6 (IL-6)] and flow-mediated dilation (FMD) were made at baseline, 4 hours after first juice consumption and after one month of juice consumption.

RESULTS: The percent changes of FMD were significant in both groups in the short- and long-term. Hs-CRP had a non-significant decrease. sE selectin had a significant decrease after 4 hours in total and in the pomegranate juice group, followed by a significant decrease after 1 month in both groups. After 4 hours, sICAM-1 significantly decreased in the pomegranate juice group, and after 1 month it decreased in total and pomegranate juice group. Interleulkin-6 (IL-6) had a significant constant decrease at 4-hour and 1-month measurements after drinking pomegranate juice, and in both groups after 1 month. Significant negative correlations of changes in sICAM-1 and sE-selectin with changes in FMD were found in both periods of follow-up; and at 1 month for IL-6.

CONCLUSIONS: Decline in inflammation was associated with improvement in FMD without changes in conventional risk factors. Daily consumption of natural antioxidants may improve endothelial function in adolescents with MetS.

KEYWORDS: Endothelium function, metabolic syndrome, antioxidants, inflammation, adolescents.

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Obesity in adolescents has been associated with metabolic syndrome (MetS), low-grade inflammation, and endothelial dysfunction. This setting of cardiovascular risks contributes to early atherosclerosis and endothelial dysfunction and has been recognized as a marker for this process.[1] Because weight loss is difficult, alternative low risk strategies for intervention to improve this setting are needed. For example, exercise improves endothelial function in the absence of or only with modest changes in body mass index.[2] It is well documented that some natural antioxidants such as polyphenols have acute beneficial effects on endothelial function. However, all previous studies have confirmed such effects among elderly patients with chronic diseases including atherosclerosis, diabetes mellitus, and hypertension. Studies among children with hyperlipidemia have do-

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cumented improvement of endothelial function by antioxidant therapy using supplementation of vitamins.[3,4] To the best of our knowledge, no previous study has assessed the effects of natural antioxidants on surrogate markers of early stages of atherosclerosis in children. This study was designed to determine the acute and chronic effects of fruit juices, rich in anti-oxidants (grape juice and pomegranate juice), on endothelial dysfunction as well as circulating markers of inflammation and endothelial function in adolescents with MetS.

Methods

The trial was conducted according to the Declaration of Helsinki, and was approved by the Research &Ethics Council of the Faculty of Medicine, Isfahan University of Medical Sciences. After full explanation of the study protocol, the whole program was offered free of charge. We have previously reported the results of this trial on vascular reactivity,[5] and here we report the effects on biochemical markers of inflammation and endothelial dysfunction.

Subjects and design

This non-pharmacologic randomized controlled clinical trial was conducted among 30 adolescents, aged 12-15 years, with MetS as defined by the International Diabetes Federation for the pediatric age group. Accordingly, MetS was diagnosed in the case of central adiposity ≥ 90th percentile waist circumference (WC) or adult threshold if lower than the 90th percentile plus at least two of the following criteria: 1) triglycerides (TG) ≥ 150 mg/dL, 2) HDL-cholesterol (HDL-C) <40 mg/dL, 3) blood pressure (BP) ≥130 mmHg systolic or ≥ 85 mmHg diastolic, and 4) fasting plasma glucose (FPG) ≥ 100 mg/dl or previously diagnosed type 2 diabetes.[6]

Participants were randomly selected from those who met the diagnostic criteria, and were referred from health care centers, schools, public and private clinics to the Farhanguian Medical Clinic,Isfahan,Iranclinic. Those children with signs or symptoms of secondary obesity, endocrine disorders, presence of any physical disability, and history of chronic medication use, smoking, or chronic infection during the two weeks before the study were not included to the trial. The allocation was conducted from computer generated random numbers using the children's record numbers in the clinic. All measurements were made by a trained team of general physicians and nurses under the supervision of the same pediatrician, and used calibrated instruments and standard protocols. Height, weight, and WC were measured, and body mass index (BMI) was calculated as weight (Kg) divided by height in meters squared (m^2). BP was measured after 5 minutes of rest in the sitting position. All measurements were taken in triplicate using appropriate size cuffs on the right arm. The readings at the first and the fifth Korotkoff phase were considered as systolic and diastolic BP, respectively. The average of the last two BP measurements was recorded and included in the analysis.[7]

Blood analyses

Participants were instructed to fast for 12 hours before screening. The compliance with fasting was determined by interview on the morning of examination. While one of the parents accompanied his/her child, blood samples were taken from the left antecubital vein between 8:00 and 9:30 am for measurement of FPG, lipids and adhesion molecules. The blood samples were centrifuged for 10 minutes at 3000 rpm within 30 minutes of venipuncture. FPG, total cholesterol (TC), HDL-C, LDL-C, TG and high-sensitive C-reactive protein (hs-CRP) were measured by autoanalyzer. Serum adhesion molecules (sICAM-1, sVCAM-1 and sE-selectin) and interleukin-6 (IL-6) were measured by enzyme-linked immunosorbent assay (ELISA) method using standard kits (Bender Med Systems, GmbH, Vienna, Austria).

The same cardiologist conducted all studies for measurement of brachial arterial reactivity in the abovementioned clinic. Using the method previously described,[2,5] the diameter of

the brachial artery was measured from high-resolution B-mode ultrasound images (ALOKA 5000 system, 7.5 megahertz transducer) at rest as basal brachial dimension, 90 seconds after cuff deflation to assess reactive hyperemia (endothelium-dependent dilation or flow-mediated dilation), again at rest, and 3 to 4 minutes after administration of 400 micrograms sublingual nitroglycerin which led to endothelium independent dilation. The percent change of flow-mediated dilation was calculated as the ratio of the brachial artery diameter after reactive hyperemia to the baseline diameter; a similar calculation was done for nitroglycerin-mediated vasodilatation.[5]

Intervention

After baseline measurements, adolescents were assigned into two groups of equal number using computer-based randomization. For the next month, participants of one group were asked to drink 18 ml/kg/day of natural grape juice;[8] the second group was asked to drink 240 ml/day of natural pomegranate juice.[9] We emphasized that children and their parents must only use home-made juice without adding any sweetener**,** not concentrated juices that usually have higher-calorie content. Compliance with regular drinking of the recommended type and amount of juice was determined by weekly phone calls to participants, and visiting participants at 2-week intervals.

The baseline survey measurements including physical examination, measurement of flow-mediated dilation (FMD) of the brachial artery, and biochemical analyses were repeated 4 hours after initial juice consumption and 1 month later.

Statistical Analyses

Statistical analyses were performed using the SPSS for Windows software (version 15; SPSS, Chicago, IL). Descriptive data are expressed as mean ± standard deviation (SD). We verified the normality of the distribution of variables with a Kolmogorov-Smirnov test. Statistical analyses of BMI, WC, TG and hs-CRP were performed using log-transformed values because the distribution was skewed. To compare continuous at different stages of the trial between the two groups under study, age- and sex-adjusted analysis of variance (ANOVA) with *post hoc* Bonferoni test, as well as chi square tests for categorical variables were used. Spearman correlation was used to find the association of continues variables. Age- and gender-adjusted linear regression analysis was used to assess the association between changes in biochemical parameters and changes in FMD90 after 4 hours and 1 month. P-value < 0.05 considered as statistically significant level.

Results

This trial was comprised of 30 adolescents (46.7% girls) with a mean (SD) age of 13.4 (1.1) years. They had a mean (SD) body mass index of 27.1 (1.1) kg/m^2 (corresponding to more than the 95th percentile), and mean (SD) WC of 93.5 (9.8) cm (corresponding to the 95th percentile), without significant difference between the two groups studied and no significant changes after the trial ($p > 0.05$). The mean (SD) systolic and diastolic blood pressures were 115.14 (2.11) and 64.1 (1.4) mmHg, respectively; and did not change significantly during the trial ($p > 0.05$). Table 1 represents lipids and inflammatory markers at the baseline and the changes at 4-hours and at 1-month after drinking grape and pomegranate juices. Fasting lipids and glucose were also similar throughout the study. When both groups combined, a significant decline in sE-selectin, sICAM-1, and IL-6 at one month follow up was seen. Moreover, in the group drinking pomegranate juice, significant declines in these three inflammatory markers were observed from baseline to the 4-hour-measurements and after one month follow up. Moreover, sE-selectin and sICAM-1 showed a significant decrease in grape juice group after one month.

Other variables did not change significantly. In separate group analyses, the effect was confined to the pomegranate juice group only for sICAM-1 but was seen in both groups for the other variables. For pomegranate juice, a bene

Table 1. Mean (SD) of investigated factors at baseline, 4 hours and 1month after daily drinking juices in adolescents with metabolic syndrome

	Total	Grape juice	Pomegranate juice
Fasting plasma glucose (mg/dl)			
Baseline	90.4(8.3)	91.8(8.3)	89.0 (8.7)
At 4 hours	91.7(8.7)	93.1(7.6)	90.7 (8.1)
At 1 month	90.8(8.7)	92.1(8.7)	90.0 (8.2)
Total cholesterol (mg/dl)			
Baseline	181.7(32.7)	184.3 (38.6)	179.2 (34.0)
At 4 hours	181.8(32.5)	184.7 (38.1)	179.7 (34.5)
At 1 month	182.1(31.7)	183.1 (38.7)	180.2 (33.5)
LDL- cholesterol (mg/dl)			
Baseline	108.6 (30.1)	110.2(36.7)	106.0 (30.2)
At 4 hours	108.6 (30.1)	110.7(35.9)	106.4 (31.1)
At 1 month	108.6 (30.1)	110.9(36.8)	106.8 (31.4)
HDL- cholesterol (mg/dl)			
Baseline	36.0(7.9)	36.2(6.7)	35.8 (9.2)
At 4 hours	36.2(7.8)	36.4(6.8)	35.4 (9.1)
At 1 month	36.7(8.4)	36.8(7.2)	36.1 (9.5)
Triglycerides (mg/dl)			
Baseline	186.8 (46.8)	185.0(47.0)	188.6(47.4)
At 4 hours	187.4 (46.5)	185.4(47.2)	188.1(48.1)
At 1 month	185.2 (46.1)	186.1(48.1)	189.1(47.4)
s-ICAM-1 (ng/ml)			
Baseline	227.1(45.5)	226.3(47.5)	229.1(40.4)
At 4 hours	224.3(45.5)	225.0 (45.1)	224.2 (45.7)*
At 1 month	222.5 (56.5) †¶	224.9(59.5)	221.5(55.9) †¶
s-VCAM-1 (ng/ml)			
Baseline	626.1 (159.5)	629.3 (162.5)	622.6(157.4)
At 4 hours	627.2 (172.7)	628.3 (172.6)	622.8(173.2)
At 1 month	627.5(179.1)	627.5(189.5)	623.6(155.9)
sE-selectin (ng/ml)			
Baseline	96.1(27.7)	97.3 (27.4)	95.5(28.7)
At 4 hours	92.7 (27.5)*	96.8 (27.7)	87.2 (28.1)*
At 1 month	86.7 (26.1) †¶	87.7 (27.5) †¶	83.7(24.1) †¶
Interleulin-6(pg/ml)			
Baseline	9.2(2.1)	10.2 (2.3)	9.2(2.7)
At 4 hours	9.6(2.2)	10.1 (2.1)	8.1(2.4)*
At 1 month	7.7(2.4) †¶	7.5 (3.5) †¶	7.1(2.0) †¶
C-reactive protein (mg/dl)			
Baseline	1.04(0.02)	1.04(0.04)	1.05(0.03)
At 4 hours	1.04 (0.04)	1.04 (0.05)	1.05(0.02)
At 1 month	1.01(0.01)	1.01(0.06)	1.04 (0.02)

*****: $p < 0.05$ at 4 hours vs. baseline
†: $p < 0.05$ at 1 month vs. baseline
¶: $p < 0.05$ at 1 month vs. 4 hours

Table 2. Percent change of brachial artery diameter from baseline to 4 hours and 1month after daily drinking juices in adolescents with metabolic syndrome

	Grape Juice			Pomegranate Juice		
	Baseline Diameter (mm) Mean(SD)	**Post-ischemic (% change)**	**Post TNG (% change)**	**Baseline Diameter (mm) Mean(SD)**	**Post - ischemic (%change)**	**Post TNG (%change)**
Baseline	3.21(0.51)	2.18	18.69	3.33(0.51)	6.31	19.52
At 4 hours	3.17(0.51)	10.73*	21.45*	3.33(0.51)	10.81*	21.02*
At 1 month	3.23(0.43)	10.84†	22.91†	3.40(0.67)	10.59	20.29

*: $p < 0.05$ at 4 hours vs. baseline
†: $p < 0.05$ at 1 month vs. baseline
No significant difference was documented at 1month vs.4 hours
No significant difference was found between two groups

ficial effect was apparent at 4 hours and even increased over one month follow up. No significant differences was seen for CRP or sVCAM. Considering baseline diameter, The percent changes of FMD of the brachial artery at 90 seconds after ischemia and after receiving nitroglycerin were significant at short-term (at 4 hours) in both groups (Table 2). The improvement in FMD persisted at long-term (at 1 month), but the percent change at 1 month versus 4 hours was significant only in the grape juice group. The percent changes were not significantly different between the two groups receiving grape juice and pomegranate juice.

At baseline there were significant correlations between components of the MetS and inflammatory markers. Hs-CRP had significant correlation with BMI ($r = 0.5$, $p = 0.03$), WC ($r = 0.7$, $p = 0.01$), and TG ($r = 0.7$, $p = 0.005$). sE-selectin was also significantly correlated with BMI ($r = 0.5$, $p = 0.02$), WC ($r = 0.6$, $p = 0.01$) , TC ($r = 0.5$, $p = 0.04$), and TG ($r = 0.7$, $p = 0.01$). Also, sICAM-1 had significant correlation with FPG ($r = 0.6$, $p = 0.01$), TG ($r = 0.5$, $p = 0.04$), and HDL-C ($r = -0.7$, $p = 0.01$). IL-6 was significantly correlated with TC ($r = 0.5$, $p = 0.04$).

Table 3. Correlations[a] between changes in mean flow mediated dilation (FMD) of the brachial artery with changes in biochemical parameters after 4 hours and 1 month drinking grape and/or pomegranate juices in adolescents with metabolic syndrome

	FMD90 at 4 hours	**FMD90 at 1 month**
	Correlation coefficient	**Correlation coefficient**
Δ Fasting plasma glucose (mg/dl)	-0.01	-0.01
ΔTotal cholesterol (mg/dl)	-0.02	-0.01
Δ LDL- cholesterol (mg/dl)	-0.02	-0.02
ΔHDL cholesterol (mg/dl)	0.01	0.01
ΔTriglycerides (mg/dl)	-0.03	-0.04
Δ s-ICAM-1 (ng/ml)	-0.44*	-0.47*
Δ s-VCAM-1(ng/ml)	-0.08	-0.07
Δ E-selectin (ng/ml)	-0.31*	- 0.37*
Δ Interleulin-6(pg/ml)	-0.22	-0.41*
Δ hs-C-reactive protein (mg/dl)	-0.11	-0.14

a: age and sex-adjusted regression analysis
Δ: change between before and after intervention
*: $p < 0.05$

Age- and gender-adjusted regression analysis of changes in FMD90 at 4 hours and 1 month with changes in biochemical parameters showed significant negative association of sICAM-1 and sE-selectin with FMD at both follow up times. The corresponding figure for IL-6 was significant at 1 month (Table 3). These results suggest that the decline in inflammation was associated with improvement in FMD.

Discussion

This study has shown that consumption of natural grape and pomegranate juice have short and one month benefits on endothelial function, soluble intercellular adhesion molecules and some markers of inflammation among obese adolescents with metabolic syndrome. These changes occur in the absence of changes in conventional risk factors. We found weak but significant inverse associations between changes in sICAM-1, sE-selectin and IL-6 and changes in FMD90 after drinking both types of juices.

These sustained effects on markers of endothelium function are consistent with the findings of a recent trial among hypertensive adults, which documented a dose-response relationship between increasing fruit and vegetable consumption and improved FMD.[10] MetS may result from interactions of vascular abnormalities, oxidative stress, visceral fat, inflammation, adipocytokines, and cortisol, as part of the larger environment of obesity and insulin resistance, and under the influence of genetic and ethnic predispositions.[11] In adults it has been documented that MetS is associated with endothelial dysfunction as assessed by FMD of brachial artery.[12,13] Recent studies confirmed this association in the pediatric age group.[1,14,15] Up-regulation of endothelial adhesion molecules, including endothelial-leukocyte adhesion molecule (sE-selectin), intercellular cell adhesion molecule-1 (sICAM-1), and vascular cell adhesion molecule-1 (sVCAM-1), play a crucial role in the earliest phases of atherosclerosis.[16,17] Inflammation markers and soluble adhesion molecules concentrations have been found to be higher in the obese than in the lean children.[18,19] Higher levels of markers of inflammation and oxidative stress in children with MetS and obesity[20,21] suggest early stages of endothelial dysfunction in obese children.[22-24] In this study, juices with anti-oxidant properties improved these markers and they might be beneficial for prevention and control of atherosclerotic diseases.

Daily consumption of grape juice [8] and pomegranate juice[9] improve endothelial function and myocardial perfusion in patients with ischemic coronary heart disease. It is suggested that certain natural antioxidants or flavonoids are responsible for these effects on endothelial function, and ingesting moderate amounts of grape juice each day might supply these nutrients.[25] In animal and human studies, grape products have been shown to produce hypotensive, hypolipidemic and anti-atherosclerotic effects, and also to improve antioxidant status as measured in terms of plasma antioxidant capacity, oxidation biomarkers, antioxidant compounds or antioxidant enzymes.[10,26] The anti-atherosclerotic effects of grape juice are suggested to be mediated by its antioxidant content and influence on intracellular production of reactive oxygen species[27] through possible indirect mechanisms such as changes in HDL paraoxonase 1 and 2 activity.[28]

A few adult trials have assessed the effect of juices on plasma intracellular cell adhesion levels and revealed both positive and negative findings. One type of antioxidant-rich juice (sea buckthorn) had no effect on plasma sICAM-1 level.[29] Grape juice could improve FMD and reduce sICAM-1 of hypercholesterolemic individuals but had no effect on sVCAM-1.[30] In a 2-week trial in patients undergoing hemodialysis, grape juice was not effective in reducing the concentration of markers of inflammation and adhesion molecules.[10] In the current trial, both types of juices had beneficial effects on vascular reactivity, some adhesion molecules and markers of inflammation. Some of these beneficial effects on biochemical parameters were significantly greater in the

group consuming pomegranate juice than in the group consuming grape juice.

The pomegranate (*Punica granatum*) is a fruit native of Iran,[31] and now it is cultivated in many countries. The antioxidant capacity of pomegranate juice is reported to be three times higher than that of red wine and green tea[32] and higher than other juices including grape juice.[33,34] Several studies confirmed its antioxidant and anti-inflammatory properties.[35,36] Pomegranate juice may increase serum antioxidant capacity, decrease plasma lipids and lipid peroxidation, diminish oxidized-LDL uptake by macrophages, reduce intima media thickness, decrease atherosclerotic lesion areas, enhance biological actions of nitric oxide, lessen inflammation, decrease angiotensin converting enzyme activity, and lower systolic blood pressure.[35-37] In these adult trials of antioxidant juices, the process of aging and the presence of underlying chronic disease may have masked the effects of juices on early atherosclerosis. The findings of the current trial supplement the existing knowledge about antiatherogenic properties of pomegranate and grape juices. Given that dietary intake of fruits and vegetables is found to improve microvascular function in hypertensive subjects in a dose-dependent manner,[38] trials with longer duration than the current one might show better results over time.

In children, studies have shown that many factors including acute infections, inflammation, trauma, active and passive smoking, postprandial lipemia, and mental stress affect endothelial function.[27] Changes in dietary and physical activity habits[2,39] and zinc supplementation[40] have shown beneficial improvements in components of MetS, markers of inflammation and endothelial dysfunction. Interestingly, as in this study was shown, these improvements are often with minimal or no change in body mass index. This suggests that at least for the short term, modest lifestyle changes alone may confer beneficial health effects. In the current trial, natural home-made juices without any added sweetener were consumed. Excessive consumption of sugar supplemented beverages, including fruit juices have been implicated in the obesity epidemic.

This trial emphasizes the importance of considering the overall nutrition quality of the diet with attention paid to the food quality and overall food intake in relation to energy expenditure.[41] Daily consumption of diets rich in natural antioxidants may improve endothelial function in adolescents with metabolic syndrome. The beneficial effects of natural juices, particularly pomegranate juice should be considered in additional clinical research on lifestyle interventions in the pediatric age group to prevent atherosclerosis-related heart disease. We should acknowledge that one of the limitations of this trial was lack of control group.

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Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

RK participated in the design and conducting the study as well as drafting and editing the manuscript; SSG participated in the design and helped to edit the manuscript; MH participated in the design and conducting the study; MH participated in the design and conducting the study;AZ participated in the design and conducting the study; PP helped to draft and edit the manuscript. All authors read and approved the final manuscript.

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