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Evidence that cranberry juice may improve augmentation index in overweight men

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ABSTRACT

The stiffening of arteries is a key step in atherogenesis leading to cardiovascular disease. It has been suggested that dietary polyphenols may be cardioprotective through possible favorable effects on oxidative stress and vascular function. The present study was undertaken in order to examine the effect of consuming low-calorie cranberry juice cocktail (CJC), a source of polyphenols, on arterial stiffness in abdominally obese men. We hypothesize that regular CJC consumption will reduce circulating oxidized low-density lipoproteins concentrations and have a beneficial impact on endothelial function. Thirty-five men (mean age ± SD: 45 ± 10 years) were randomly assigned to drink 500 mL CJC/day (27% juice) or 500 mL placebo juice (PJ)/day for 4 weeks in a double-blind crossover design. Augmentation index (AIx), an index of arterial stiffness, was measured by applanation tonometry of the radial artery and the cardiometabolic profile was assessed in each participant before and after each phase of the study. We found no significant difference in AIx changes between men who consumed CJC or PJ for 4 weeks ($P = .5820$). Furthermore, there was no between-treatment difference in changes in AIx responses to salbutamol ($P = .6303$) and glyceryl trinitrate ($P = .4224$). No significant difference was noted in other cardiometabolic variables between men consuming PJ or CJC. However, a significant within group decrease in AIx (mean decrease ± SE; -14.0 ± 5.8%, $P = .019$) was noted following the consumption of 500 mL CJC/day for 4 weeks. Our results indicate that the effect of chronic consumption of CJC on AIx was not significantly different from changes associated with the consumption of PJ. However, the significant within-group decrease in AIx following CJC consumption in abdominally obese men may deserve further investigation.

1. Introduction

Endothelial dysfunction (ED), ie, the impairment of the normal functions of the vascular endothelium, develops in an individual when vasoconstrictive, growth-promoting, procoagulant and proinflammatory conditions prevail [1]. ED is associated with cardiovascular disease (CVD) risk factors [2] that can also affect the mechanical properties of arteries leading to arterial stiffness [3,4]. Peripheral pulse wave analysis is frequently used to measure augmentation index

Abbreviations: AIx, augmentation index; BMI, body-mass index; CJC, cranberry-juice cocktail; CVD, cardiovascular disease; ED, endothelial dysfunction; FAV, fruits and vegetables; GTN, glyceryl trinitrate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MAP, mean arterial pressure; MetS, metabolic syndrome; NO, nitric oxide; NOS, nitric oxide synthase; NOx, total nitrates/nitrites; PJ, placebo juice; Salb, salbutamol; TG, triglycerides.

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(AIx), an index of arterial stiffness [5] which depends largely on the proportion of collagen and elastin present in the artery wall [6]. AIx can be reduced by β2-adrenoceptor agonists (eg, salbutamol) through the stimulation of endothelial nitric oxide (NO) production and is used as an index of endothelium-dependent function. On the other hand, endothelium-independent function can be assessed by the administration of glyceryl trinitrate (GTN), an NO donor which is also known to reduce AIx by relaxing arterial smooth muscle cells [7,8].

Adoption of healthy nutritional habits is now considered an important and relevant way to help prevent and treat CVD [9] through improvements in circulating lipoprotein-lipid and inflammatory profiles as well as in endothelial function [10,11]. Whereas a lot of emphasis has been put over the years on the reduction of dietary fat intake and a shift from saturated towards mono and polyunsaturated fatty acid consumption [12], recent epidemiological observations also suggest that increasing fruits and vegetables (FAV) consumption may be helpful in lowering CVD risk [13–15].

A commonly proposed explanation for the cardioprotective potential of FAV is their high content in polyphenolic compounds like flavonoids which have been identified as mediators of beneficial effects against inflammation and oxidative stress [16]. Furthermore, in support of the favorable vascular effects of dietary polyphenols, consumption of flavonoid-rich foods such as dark chocolate, red wine or tea has been shown to acutely [17–19] and chronically [20] reduce arterial stiffness likely through the regulation of the synthesis of NO [21] and endothelin-1 [22].

Cranberries are also a potent source of different polyphenols such as phenolic acids, flavonols, anthocyanins, proanthocyanidins [23]. Low-calorie cranberry juice cocktail (CJC) consumption has been associated with favorable cardiometabolic adaptations such as an increase in circulating high-density lipoprotein (HDL) cholesterol concentrations [24] and decreases in plasma oxidized low-density lipoproteins (OxLDL) levels [25–27] as well as in circulating adhesion molecule [26] and matrix metalloproteinase-9 [28] concentrations, the latter observations suggesting some beneficial effects of cranberries on vascular health.

The present short-term placebo-controlled double-blind crossover study examines the effect of consuming low-calorie CJC on a daily basis for a period of 4 consecutive weeks on AIx and cardiometabolic profile of overweight men. We hypothesize that regular CJC consumption will reduce AIx and improve the cardiometabolic profile.

2. Methods and materials

2.1. Subjects

Thirty-five sedentary and healthy overweight men were recruited through the media to participate in the present intervention. To be eligible, subjects had to have a body-mass index (BMI) ≥25 kg/m^2 and a waist circumference ≥90 cm. Participants also had to be free from diabetes and CVD as well as renal, hepatic and endocrine disorders. Furthermore, they had to be nonsmokers and not be using medications known to affect lipid and insulin metabolism or blood pressure. Subjects gave their written consent to participate in the study which was approved by the Medical Ethics Committee of Université Laval.

2.2. Intervention

Upon their entry into the study, subjects were instructed by a dietician to maintain their usual nutritional habits, limit their alcohol consumption to a maximum of 1 drink per day (equivalent to 15 g alcohol/day) as well as restrain themselves from consuming any vitamin, antioxidant or mineral supplements. Following a run-in period of 4 weeks during which participants were asked to drink 500 mL of water a day in order to get the subjects acquainted with the introduction of such an amount of liquid into their usual diet, subjects were then randomly assigned to drink 500 mL/d of either low-calorie CJC (27% juice) or placebo juice (PJ) for 4 weeks. After a 4 week washout period (500 mL water a day), treatments were crossed over for another 4 weeks. All subjects had to complete the entire protocol to be considered for the statistical analyses. The CJC and PJ used in the present study (Ocean Spray Cranberries Inc, Lakeville-Middleboro, MA, USA) had similar organoleptic properties (taste, color and texture) and vitamin C contents but no cranberries entered in the preparation of the PJ. Both juices were packaged at Université Laval in 125 mL ready-to-drink TetraBrik boxes (Tetra-Pak, Richmond Hill, Ontario, Canada) under the close monitoring of Ocean Spray to ensure adequate reconstitution and quality of the juices. Subjects had to drink 4 (4) 125 mL boxes a day of either PJ or CJC and were instructed to consume 2 boxes of juice in the morning and 2 in the evening in order to maximize exposition to the potentially bioactive compounds of CJC. Each daily serving of CJC (500 mL) provided 87 kcal of energy and contained 400 mg total polyphenols, 20.8 mg anthocyanins, and 21.84 g carbohydrates. A more detailed description of the PJ and CJC has been previously published [24]. In an effort to keep the subjects' sugar consumption to a minimum and limit possible detrimental health effects, we provided subjects with CJC and PJ sweetened with sucralose. All CJC used in the present study was reconstituted from concentrate of the same processed batch of cranberries, in order to avoid any variations in the composition of the juice throughout the intervention.

2.3. Anthropometry

Body weight and height as well as waist and hip circumferences were measured following standardized procedures [29] upon each visit of the subjects to the investigation unit. BMI and waist-to-hip ratio values were calculated.

2.4. Plasma measurements

Blood samples were collected in the morning after a 12-hour fast. Upon collection, cholesterol and triglyceride (TG) concentrations were determined in plasma by enzymatic methods using a Technicon RA-1000 analyzer (Bayer Corporation Inc, Tarrytown, NY, USA), as previously described [30]. Plasma very low-density lipoproteins (d<1.006 g/mL) were isolated by ultracentrifugation and the HDL fraction was

obtained after precipitation of LDL from the infranatant (d>1.006 g/mL) with heparin and $MnCl_2$ [31]. The cholesterol and TG contents of the infranatant fraction were measured before and after the precipitation step. Apolipoproteins (apo) AI and B concentrations were measured by nephelometry (Dade Behring, Mississauga, ON, Canada). The lyophilized serum standards for apo measurements were prepared at the Lipid Research Center of Laval University Medical Center and calibrated with reference standards obtained from the Centers for Disease Control (Atlanta, GA, USA). Plasma glucose concentration was determined with a glucose oxidase assay (Sigma, St-Louis, MO, USA) as previously described [32] while insulin level was measured in plasma using a commercial double-antibody radio immunoassay (LINCO Research, St-Louis, MO, USA) that shows little cross-reactivity (<0.02%) with pro-insulin. Plasma uric acid (BioAssay Systems, Hayward, CA, USA) as well as total nitrate/nitrite (NOx, Cayman Chemical, Ann Arbor, MI, USA) concentrations were measured by colorimetry.

2.5. *Blood pressure and arterial stiffness*

Blood pressure was measured using a sphygmomanometer with the subject in a supine position after a 5 minute rest. Mean arterial pressure (MAP) was calculated as the sum of diastolic blood pressure and one-third of the difference between systolic and diastolic blood pressure as previously reported [33]. Arterial stiffness (expressed as the augmentation index, AIx) was obtained by applanation tonometry using the SphygmoCor System (AtCor Medical, Sydney, Australia) as previously described [34]. Briefly, after a 30-minute resting period, peripheral artery waveforms were recorded on the subjects' radial artery. Pulse waveforms were also recorded at 5, 10, 15, and 20 minutes after inhalation of 400 μg of salbutamol (a short-acting β2-adrenergic receptor agonist) which elicits the synthesis of nitric oxide (NO) and a vascular response that can be used as a proxy measure of endothelium-dependent vasodilation. The same tonometry technique was used to assess arterial stiffness during endothelium-independent radial artery vasodilation by performing measurements at 3, 5, 10, 15, 20, and 30 minutes following sublingual administration of 400 μg of GTN, an NO donor [6,7]. Global endothelial function was calculated as the ratio of the changes in AIx in response to salbutamol relative to GTN as previously described [35].

2.6. *Nutritional habits assessment*

A 91-item validated food frequency questionnaire [36] was administered by a nutritionist during each of the subjects' visit to the investigation unit. During the interview, participants were questioned about frequency of intake for different foods during the last month and were asked to report the frequency of daily, weekly, and monthly consumption. The nutritionist used food models for a better estimation of the real portion consumed by the subjects. Analysis of data derived from the food frequency questionnaire was performed using the following food composition databases: Nutrition Data System for Research (software version 4.03, Food and Nutrient Database 31, Minneapolis, MN, USA) [37] and the Canadian Nutrient File (CNF, version 2007b, Ottawa, ON, Canada) [38].

2.7. *Statistical analyses*

Data are presented as means ± SD unless stated otherwise. Men were separated in those with (MetS+, n = 13) and without (MetS–, n = 22) the metabolic syndrome (MetS) according to the definition of the National Cholesterol Education Program [9], ie, the presence of at least three of the following criteria: waist circumference ≥102 cm; fasting triglycerides ≥1.7 mmol/L; fasting HDL cholesterol ≤1.03 mmol/L; fasting glucose ≥5.6 mmol/L and blood pressure ≥130/85 mm Hg. In MetS+ men, 46% had a waist circumference ≥ 102 cm, 85% had TG ≥1.70 mmol/L, 77% had HDL cholesterol ≤1.03 mmol/L, 46% had TG ≥1.70 mmol/L and all of them had hyperglycemia (>5.6 mmol/L). Furthermore, the most common combination of features in the 13 men with the MetS of the present study was high TG/low HDL cholesterol/hyperglycemia (n = 9). The MIXED model procedure was used to test the difference between treatments (PJ vs CJC) as well as within-group differences with an adjustment for sequence of treatments. Adjustment for baseline value was also used when comparing changes in the variables of interest. Plasma triglycerides levels were log10 transformed for the analysis but raw data are presented in Tables. All analyses were performed with the SAS statistical package (version 9.2; SAS Institute, Cary, NC, USA) and $P \leq .05$ was considered significant.

3. Results

Baseline physical and metabolic characteristics of study participants are presented in Table 1. As a group, subjects were overweight and abdominally obese [39] with plasma lipoprotein lipid levels within recommended guidelines [9]. Although at the high-end for total fat and low-end for carbohydrate intakes, daily energy and nutrient intakes of the subjects at baseline also corresponded to nutritional recommendations for the Canadian adult population, ie, 45% to 65% carbohydrates, 10% to 35% protein and 20% to 35% total fat (Table 2). Furthermore, men with the MetS (MetS+) had a higher BMI as well as higher circulating TG and lower HDL cholesterol concentrations compared to those without the MetS (MetS–). No difference was found in daily energy and nutrient intakes between MetS+ *vs.* MetS– individuals (Table 2).

Fig. 1 illustrates AIx in the 35 participants prior to the start of the intervention. Administration of salbutamol significantly reduced AIx compared to resting values by –10.8% ± 6.4% ($P < .0001$). GTN further decreased AIx values compared to salbutamol (–2.1% ± 6.0%, $P < .05$).

As shown in Table 3, there was no significant difference between changes in AIx following the consumption of CJC or PJ. Furthermore, changes in AIx responses to salbutamol and GTN did not significantly differ between men who consumed CJC or PJ for 4 weeks. However, we noted a statistically significant within-group decrease in AIx ($P < .05$ vs Week 0) in subjects that consumed 500 mL CJC per day for 4 weeks. Furthermore, there were apparent deteriorations of the AIx

Table 1 – Baseline physical and metabolic characteristics of men

Variable	All	MetS-	MetS+	P value
n	35	22	13	-
Age (y)	45 ± 10	47 ± 10	42 ± 11	.2286
Body weight (kg)	86.1 ± 9.7	83.6 ± 9.1	89.7 ± 9.7	.0697
Body mass index (kg/m^2)	28.3 ± 2.4	27.6 ± 2.1	29.3 ± 2.8	.0457
Waist circumference (cm)	98.5 ± 6.0	97.7 ± 4.9	101.4 ± 7.1	.0800
Waist-to-hip ratio	0.96 ± 0.04	0.96 ± 0.03	0.97 ± 0.06	.3365
Visceral AT (cm^2)	130 ± 50	121 ± 27	145 ± 72	.2596
Subcutaneous AT (cm^2)	239 ± 63	221 ± 59	267 ± 60	.0360
Systolic BP (mm Hg)	116.2 ± 12.2	113.6 ± 10.7	120.5 ± 13.6	.1087
Diastolic BP (mm Hg)	74.4 ± 6.7	73.5 ± 6.9	76.1 ± 6.2	.2676
Glucose (mmol/L)	5.82 ± 0.45	5.80 ± 0.45	5.98 ± 0.32	.2340
Insulin (pmol/L)	119 ± 65	102 ± 64	146 ± 61	.0539
Cholesterol (mmol/L)	5.33 ± 0.74	5.19 ± 0.90	5.73 ± 0.62	.0661
LDL cholesterol (mmol/L)	3.51 ± 0.62	3.41 ± 0.72	3.81 ± 0.53	.0893
HDL cholesterol (mmol/L)	1.19 ± 0.31	1.31± 0.29	1.06 ± 0.29	.0206
Total/HDL cholesterol	4.75 ± 1.31	4.14 ± 1.04	5.53 ± 1.19	.0010
Triglycerides (mmol/L)	1.54 ± 0.70	1.26 ± 0.79	2.05 ± 0.46	.0024
Apolipoprotein AI (g/L)	1.24 ± 0.19	1.31 ± 0.19	1.23 ± 0.18	.2006
Apolipoprotein B (g/L)	1.06 ± 0.18	1.00 ± 0.20	1.22 ± 0.18	.0019

Results are presented as means ± SD.
BP, blood pressure
Proc MIXED with adjustment for treatment sequence.

response to salbutamol and global endothelial function within those who consumed CJC ($P < .05$ *vs.* Week 0). Table 3 also shows the changes in different metabolic markers following the consumption of PJ and CJC for 4 weeks. We found no significant between-treatment differences in plasma NOx, uric acid, oxidized LDL and sE-selectin concentrations. There was a tendency ($P = .10$) for a difference between PJ and CJC for changes in plasma sVCAM-1 levels which was mostly due to a significant within-group decrease in sVCAM-1 when men consumed PJ for 4 weeks ($P < .005$ vs Week 0). Furthermore, despite the fact that the changes in circulating sICAM-1 concentrations did not significantly differ between both treatments, we noted a tendency for a decrease in plasma sICAM-1 levels within CJC treatment ($P = .09$ vs Week 0).

In an effort to further explore the vascular impact of CJC supplementation, we compared AIx values in men separated on the basis of the presence ($n = 13$) or absence ($n = 22$) of the MetS [9]. Prior to the intervention, responses to salbutamol and GTN were comparable when MetS– *vs.* MetS+ men were compared (data not shown). After the intervention, we found that there was no significant between-treatment (PJ *vs.* CJC) differences in changes in AIx measured at rest or following salbutamol and GTN administration in men with or without the MetS (Fig. 2). However, we noted a significant within-group decrease in resting AIx values in MetS+ men who consumed CJC for 4 weeks ($P < .05$ vs Week 0). Surprisingly, within MetS+ individuals who consumed CJC, there were also significant increases in AIx responses to salbutamol and GTN ($P < .05$ vs Week 0, Fig. 2).

Finally, significant decreases in global endothelial function and circulating sICAM-1 concentrations in MetS– individuals who consumed CJC (Table 4) whereas MetS– individuals supplemented with PJ for 4 weeks showed a significant reduction in plasma sVCAM-1 levels. We found no other

Table 2 – Baseline daily nutritional profiles of men

Variable	All	MetS-	MetS+	P value
Energy (kcal)	2262 ± 439	2284 ± 440	2224 ± 452	.7026
Carbohydrates (% of energy)	47.4 ± 6.1	47.9 ± 5.7	46.5 ± 6.8	.5313
Proteins (% of energy)	17.3 ± 2.1	17.3 ± 1.9	17.3 ± 2.4	.9612
Fat (% of energy)	33.4 ± 5.1	34.0 ± 4.9	32.3 ± 5.4	.3541
Alcohol (% of energy)	4.1 ± 5.8	3.0 ± 2.3	6.0 ± 9.0	.2604
Saturated fat (% of energy)	11.6 ± 2.4	12.0 ± 2.8	11.0 ± 1.4	.1462
Monounsaturated fat (% of energy)	13.0 ± 2.0	13.3 ± 1.7	12.4 ± 2.4	.1799
Polyunsaturated fat (% of energy)	5.9 ± 1.8	5.6 ± 1.4	6.4 ± 2.3	.2257
Cholesterol (mg)	306.6 ± 97.1	323.5 ± 109.2	278.0 ± 66.9	.1849
Vitamin A (retinol equivalents, μg)	1735 ± 851	1751 ± 675	1708 ± 1119	.9010
Vitamin C (mg)	175.4 ± 77.3	181.5 ± 78.4	165.0 ± 76.8	.5479
Vitamin E (mg)	10.7 ± 3.4	12.0 ± 3.7	11.8 ± 4.1	.8499

Results are presented as means ± SD.
Proc MIXED with adjustment for treatment sequence.

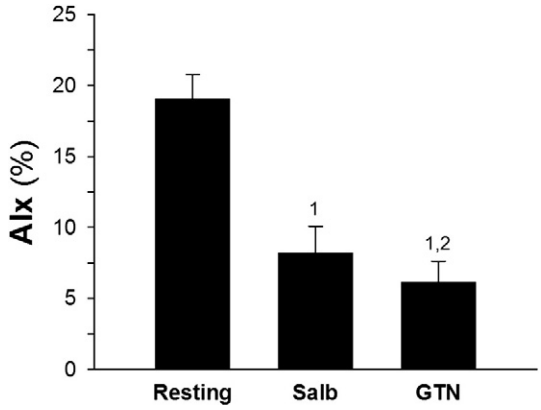

Fig. 1 – Baseline augmentation index (AIx) of the 35 men measured at rest as well as following oral administration of Salb and glyceryl trinitrate (GTN). Data are presented as means ± SEM. [1] Significantly different from resting values ($P < .0001$) [2] Significantly different from salbutamol values ($P < .05$).

significant differences in the effects of the intervention (PJ *vs.* CJC) on the metabolic markers of MetS– or MetS+ individuals.

4. Discussion

Dietary FAV intake is negatively associated with the risk of CVD [13–15], an observation that is explained, at least partly, by the polyphenolic compounds present in FAV that are known to regulate antioxidant and anti-inflammatory mechanisms [16,40–44] improving the vascular function. Cranberries are rich in polyphenols [23] and results from the present 4-week placebo-controlled double-blind crossover trial in healthy overweight men indicate that although CJC consumption did reduce resting AIx in overweight men, this decrease in AIx was not found to significantly differ from the placebo group. Therefore, we rejected our original study hypothesis that, compared to a placebo, regular CJC consumption reduces AIx. However, we feel that the significant within-group decrease in AIx following CJC consumption cannot be overlooked and may need to be further investigated.

Our results are in apparent contradiction with recent observations in coronary patients showing that chronic CJC consumption reduced carotid-femoral pulse wave velocity which is recognized as a clinically relevant measure of arterial stiffness [45]. Daily consumption of 400 mL of polyphenol-rich dealcoholized red wine for a period of 6 weeks has also been associated with a decrease in AIx in postmenopausal women [20]. Differences in the populations under study as well as in the design of these previous studies and the one we describe herein may account for these apparent discrepancies. For instance, Dohadwala et al [45] used a CJC containing 54% juice while we provided our subjects with CJC containing 27% juice. Our choice to use the 27% juice was based on the fact that when the study was conducted, the 54% CJC was not marketed in Canada. Further, Naissides et al [20] tested the effects of dealcoholized red wine over a period of 6 weeks compared to our study which lasted only 4 weeks.

Studies have suggested a cause/effect relationship between endothelial NO production and arterial stiffness [46,47]. Salb, a α-adrenoceptor agonist, has been shown to reduce AIx through the stimulation of NO release by endothelial cells and thus, the vascular response to salbutamol is considered as a marker of endothelium-dependent vasodilation. In addition, endothelium-independent vasodilation can be measured through the vasodilatory response to NO donors like GTN, leading to relaxation of smooth muscle cells [7,8]. As presented above, although chronic consumption of CJC has been reported to beneficially impact carotid-femoral pulse wave velocity [45], no chronic effect of CJC on flow-mediated dilation measures was reported in the same study suggesting no improvement of arterial vasodilatory function. Furthermore, a 4-month daily

Table 3 – Changes in hemodynamic variables and AIx values in the 35 men

	PJ			*CJC*			*Between-treatment effect Placebo vs. CJC*
	Week 0	Week 4	*P value*	Week 0	Week 4	*P value*	
Heart rate (beats/min)	69 ± 10	68 ± 10	.5781	66 ± 10	68 ± 9	.3539	0.2968
Systolic BP (mm Hg)	117 ± 13	115 ± 12	.2298	115 ± 13	114 ± 10	.6542	0.6053
Diastolic BP (mm Hg)	75 ± 7	74 ± 8	.8324	74 ± 7	74 ± 6	.7477	0.9334
MAP (mm Hg)	89 ± 8	88 ± 9	.4900	87 ± 9	87 ± 7	.6853	0.8496
Resting AIx (%)	18.7 ± 9.1	17.4 ± 9.5	.1442	19.8 ± 9.7	17.8 ± 10.9	.0271	0.5820
Δ AIx salbutamol (%)	–12.3 ± 7.2	–10.9 ± 6.2	.1562	–12.7 ± 5.7	–10.3 ± 7.2	.0379	0.6303
Δ AIx GTN (%)	–13.4 ± 5.0	–13.1 ± 4.9	.7343	–13.4 ± 4.2	–12.0 ± 7.0	.1429	0.4224
Global endothelial function	0.90 ± 0.71	0.89 ± 0.55	.8726	1.02 ± 0.56	0.42 ± 1.93	.0205	0.1236
NOx (μmol/L)	6.94 ± 3.12	6.97 ± 3.15	.9566	6.69 ± 2.61	6.91 ± 3.15	.8160	0.9009
Uric acid (mg/dL)	6.24 ± 1.10	6.30 ± 1.01	.6843	6.33 ± 1.16	6.23 ± 1.15	.5083	0.4508
Oxidized LDL (U/L)	63.2 ± 16.2	64.1 ± 17.1	.7185	64.3 ± 17.9	64.8 ± 19.2	.7692	0.9622
sICAM-1 (ng/mL)	221.3 ± 52.2	213.1 ± 50.1	.1316	221.8 ± 51.2	212.3 ± 47.0	.0942	0.9040
sVCAM-1 (ng/mL)	452.1 ± 140.8	394.5 ± 102.8	.0017	407.9 ± 134.4	407.7 ± 107.3	.3723	0.1003
sE-selectin (ng/mL)	33.6 ± 20.2	32.0 ± 20.1	.6931	32.6 ± 18.4	38.0 ± 34.2	.1862	0.2252

Results are presented as means ± SD.
BP, blood pressure.
Proc MIXED with adjustments for treatment sequence and baseline value.

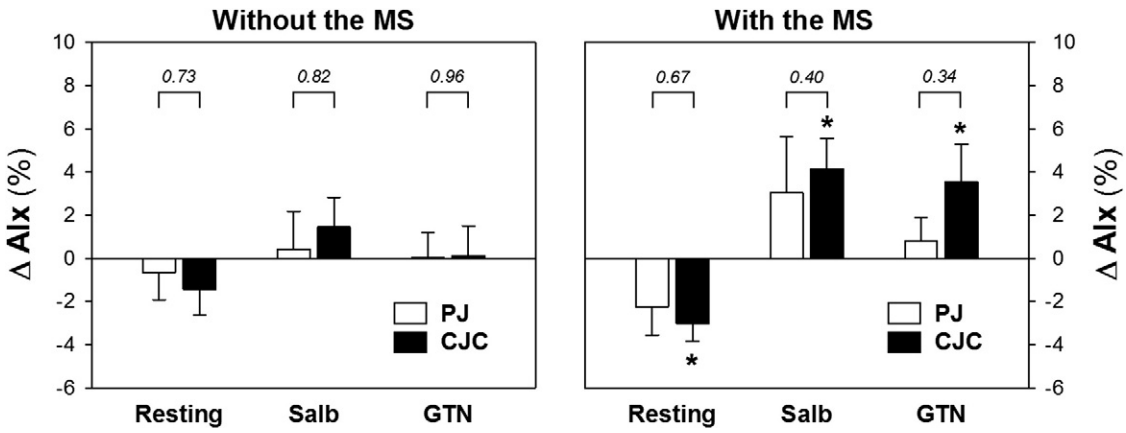

Fig. 2 – Changes in arterial stiffness following the consumption of 500 mL PJ/day (white bars) and 500 mL CJC/day (black bars) for 4 weeks in men characterized with (right panel) or without (left panel) the metabolic syndrome (MS) [9]. Augmentation index (AIx) was measured at rest as well as after oral administration of Salb and glyceryl trinitrate (GTN). Data are presented as means ± SEM. *p* values for between-treatment effects are shown above the square brackets * Significant within-group difference (*P* < .05 *vs.* Week 0) Proc MIXED with adjustment for treatment sequence and baseline value.

supplementation with 54% CJC had a neutral effect on endothelial function assessed by digital peripheral arterial tonometry [48]. These observations are in accordance with results of the present study as we found no significant difference between the effects of consuming CJC or PJ for 4 weeks on AIx responses to salbutamol. Within-group analyses rather suggest a less important AIx response to salbutamol after consuming CJC for 4 weeks.

We previously reported that CJC supplementation was associated with reductions in circulating OxLDL [25,26] and adhesion molecule [26] concentrations in men. In the present study, we found no significant variation in plasma OxLDL or adhesion molecule concentrations in response to CJC supplementation for 4 weeks. Differences in the design of our studies (duration and dose of CJC) are likely to explain the discrepancies between the present study and our previous observations. However, the lack of effect of CJC on plasma OxLDL and adhesion molecule levels we report herein are in line with previous reports showing that CJC consumption for 2 weeks had no effect on oxidative stress markers [49] while supplementing men and women with 54% CJC for 4 consecutive weeks failed reduce circulating sICAM-1 concentrations [45].

When men were separated on the basis of the presence or absence of the MetS, differences between the effects of CJC and PJ on AIx also failed to reach statistical significance. However, a significant within-group decrease in AIx measured at rest was noted in men with the MetS consuming CJC while significant increases in AIx responses to salbutamol and GTN were observed suggesting a probable deterioration of the endothelium-dependent and independent vasodilation processes. These results may appear contradictory but it has been shown that vascular tone is positively correlated with flow-mediated dilation in healthy individuals [50], and thus, a more relaxed (and possibly wider) artery at rest has a lower

Table 4 – Changes in hemodynamic variables in the men with and without the metabolic syndrome

	MetS-			*MetS+*		
	PJ	CJC	*P value* [1]	PJ	CJC	*P value* [1]
Heart rate (beats/min)	−1.0 ± 8.1	0.9 ± 7.7	.5782	−1.2 ± 6.8	2.7 ± 6.7	.1049
Systolic BP (mm Hg)	−3.2 ± 8.5	2.1 ± 8.3	.1120	0.4 ± 13.2	−4.8 ± 10.1	.6729
Diastolic BP (mm Hg)	−1.1 ± 5.2	0.3 ± 6.6	.6668	0.9 ± 9.1	−1.3 ± 7.2	.8611
MAP (mm Hg)	−1.8 ± 4.7	0.9 ± 6.6	.2519	0.75 ± 10.0	−2.5 ± 7.8	.7818
Global endothelial function	0.11 ± 0.82	−0.93 ± 2.33 *	.1284	−0.21 ± 0.50	−0.03 ± 0.90	.8413
NOx (μmol/L)	−0.32 ± 2.79	0.13 ± 2.51	.8563	0.36 ± 5.36	0.38 ± 3.16	.9664
Uric acid (mg/dL)	0.11 ± 0.67	−0.15 ± 0.51	.1336	−0.02 ± 0.98	0.01 ± 1.03	.7462
Oxidized LDL (U/L)	2.3 ± 15.4	2.9 ± 10.9	.7216	−1.54 ± 14.2	−3.4 ± 9.8	.9572
sICAM-1 (ng/mL)	−11.5 ± 44.6	−14.4 ± 22.3 *	.5056	−2.7 ± 28.8	−1.26 ± 43.0	.6984
sVCAM-1 (ng/mL)	−45.1 ± 119.2 *	−14.4 ± 80.6	.3297	−78.7 ± 149.1	23.6 ± 110.6	.3291
sE-selectin (ng/mL)	−3.7 ± 7.8	8.3 ± 40.2	.2122	1.9 ± 16.7	0.54 ± 6.7	.9967

Results are presented as means ± SD.
BP, blood pressure.
Proc MIXED with adjustments for treatment sequence and baseline value
[1] *P* value for between-treatment comparisons
* *P* < .05 for within-group comparisons.

endothelium-dependent vasodilation response as previously reported [51–54]. This suggestion of abnormal dilation in individuals with larger arteries that in reality have a normal endothelial function has already been suggested [51].

The significant within-group improvement in AIx in men consuming CJC could result from different physiological mechanisms. Arterial stiffness has been shown to decrease after angiotensin-converting enzyme inhibitor therapy [55] and consumption of flavonoid-rich foods has been shown to inhibit angiotensin-converting enzyme activity in rodents [56]. Interestingly, a cranberry water-soluble phytochemical extract has also been associated with angiotensin-converting enzyme activity inhibition [57]. Cyanidin-3-glucoside, a polyphenol found in cranberries, has been also shown to increase endothelial nitric oxide synthase (eNOS) expression [58] and decrease inducible nitric oxide synthase (iNOS) expression [59]. Such changes in the balance between eNOS and iNOS expression/abundance/activity would favor the bioavailability of the vasoactive NO. However, as we did not measure enzyme activities/expressions in the present study, we were not able to establish whether our intervention was able to modulate these parameters. On the other hand, we did measure total plasma nitrate/nitrite concentration (NOx), which is often used as a marker of eNOS activity [60], and found that the intervention had no effect on the plasma NOx. It must be stressed that other more sensible techniques that the one we used to determine NOx are available and may have provided more insights on the modulation of NO bioavailability in response to CJC supplementation.

Some factors may also have contributed to limit the extent of the change in AIx following CJC supplementation. Although we recruited overweight men with slightly elevated waist circumference, they were still healthy individuals which may have limited our capacity to observe significant cardiometabolic improvements following the intervention. Duration of the study as well as the daily dose of CJC may have been too short and low to render a large effect on AIx and other cardiometabolic variables. It has been shown that after polyphenol consumption, bioabsorption occurs very rapidly and bioavailability remains very low as polyphenolic compounds are quickly removed from the circulation [61]. As we studied the effect of CJC on AIx measured in the fasting state, polyphenol concentrations in circulation may have been at their lowest. Furthermore, our study does not allow us to measure bioavailability and short-term action of polyphenols from CJC in the present study. Acute [62] and chronic [63–65] vitamin C consumption has been shown to reduce arterial stiffness. The PJ and CJC used in the present study provided similar daily vitamin C quantities to the participants. Thus, a potential slight beneficial impact of vitamin C on AIx in the PJ group cannot be excluded and may have prevented the significance of the differences in AIx changes between both treatments. Finally, chronic hyperuricemia is detrimental for the cardiometabolic risk profile [66,67]. However, recent observations also suggest that food-induced moderate elevation of uric acid can protect against arterial stiffening in healthy humans. Indeed, consumption of fructose was shown to prevent the increase in hypoxia-induced arterial stiffness [68], an effect partially explained by an increase in plasma uric acid concentrations. In the present study, we used CJC and PJ containing similar quantities of fructose and no significant variation in circulating uric acid concentrations following either CJC or PJ.

In summary, results of the present study indicate no statistically significant difference between the effects of a 4-week low-calorie CJC and PJ supplementation on arterial stiffness. However, the significant within-group decrease in AIx following CJC consumption in overweight men as well as in those with the MetS remains intriguing and surely deserves to be further investigated with regards to its clinical and physiological relevance.

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REFERENCES

[1] McVeigh GE, Cohn JN. Endothelial dysfunction and the metabolic syndrome. Curr Diab Rep 2003;3:87–92.
[2] Verma S, Anderson TJ. Fundamentals of endothelial function for the clinical cardiologist. Circulation 2002;105:546–9.
[3] McEniery CM, Cockcroft JR. Does arterial stiffness predict atherosclerotic coronary events? Adv Cardiol 2007;44:160–72.
[4] Stehouwer CD, Henry RM, Ferreira I. Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. Diabetologia 2008;51:527–39.
[5] Wilkinson IB, Fuchs SA, Jansen IM, Spratt JC, Murray GD, Cockcroft JR, et al. Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. J Hypertens 1998;16:2079–84.
[6] Wilkinson IB, Hall IR, MacCallum H, Mackenzie IS, McEniery CM, van der Arend BJ, et al. Pulse-wave analysis: clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. Arterioscler Thromb Vasc Biol 2002;22:147–52.
[7] Chowienczyk PJ, Kelly RP, MacCallum H, Millasseau SC, Andersson TL, Gosling RG, et al. Photoplethysmographic assessment of pulse wave reflection: blunted response to endothelium-dependent beta2-adrenergic vasodilation in type II diabetes mellitus. J Am Coll Cardiol 1999;34:2007–14.
[8] Hayward CS, Kraidly M, Webb CM, Collins P. Assessment of endothelial function using peripheral waveform analysis: a clinical application. J Am Coll Cardiol 2002;40:521–8.

[9] Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143–421.
[10] Jenkins DJ, Kendall CW, Marchie A, Faulkner DA, Wong JM, de Souza R, et al. Effects of a dietary portfolio of cholesterol-lowering foods vs lovastatin on serum lipids and C-reactive protein. J Am Med Assoc 2003;290:502–10.
[11] Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, et al. Effects on blood pressure of reduced dietary sodium and the Dietary
Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. N Engl J Med 2001;344:3–10.
[12] Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA, et al. Dietary fat intake and the risk of coronary heart disease in women. N Engl J Med 1997;337:1491–9.
[13] Bazzano LA, He J, Ogden LG, Loria CM, Vupputuri S, Myers L, et al. Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. Am J Clin Nutr 2002;76:93–9.
[14] Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case–control study. Lancet 2004;364:937–52.
[15] Crowe FL, Roddam AW, Key TJ, Appleby PN, Overvad K, Jakobsen MU, et al. Fruit and vegetable intake and mortality from ischaemic heart disease: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heart study. Eur Heart J 2011;32:1235–43.
[16] Rahman I, Biswas SK, Kirkham PA. Regulation of inflammation and redox signaling by dietary polyphenols. Biochem Pharmacol 2006;72:1439–52.
[17] Karatzi KN, Papamichael CM, Karatzis EN, Papaioannou TG, Aznaouridis KA, Katsichti PP, et al. Red wine acutely induces favorable effects on wave reflections and central pressures in coronary artery disease patients. Am J Hypertens 2005;18: 1161–7.
[18] Vlachopoulos C, Aznaouridis K, Alexopoulos N, Economou E, Andreadou I, Stefanadis C. Effect of dark chocolate on arterial function in healthy individuals. Am J Hypertens 2005;18: 785–91.
[19] Vlachopoulos C, Alexopoulos N, Dima I, Aznaouridis K, Andreadou I, Stefanadis C. Acute effect of black and green tea on aortic stiffness and wave reflections. J Am Coll Nutr 2006;25:216–23.
[20] Naissides M, Pal S, Mamo JC, James AP, Dhaliwal S. The effect of chronic consumption of red wine polyphenols on vascular function in postmenopausal women. Eur J Clin Nutr 2006;60: 740–5.
[21] Leikert JF, Rathel TR, Wohlfart P, Cheynier V, Vollmar AM, Dirsch VM. Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells. Circulation 2002;106: 1614–1617.
[22] Khan NQ, Lees DM, Douthwaite JA, Carrier MJ, Corder R. Comparison of red wine extract and polyphenol constituents on endothelin-1 synthesis by cultured endothelial cells. Clin Sci (Lond) 2002;103(Suppl. 48):72S–5S.
[23] Reed J. Cranberry flavonoids, atherosclerosis and cardiovascular health. Crit Rev Food Sci Nutr 2002;42:301–16.
[24] Ruel G, Pomerleau S, Couture P, Lemieux S, Lamarche B, Couillard C. Favourable impact of low-calorie cranberry juice consumption on plasma HDL-cholesterol concentrations in men. Br J Nutr 2006;96:357–64.
[25] Ruel G, Pomerleau S, Couture P, Lamarche B, Couillard C. Changes in plasma antioxidant capacity and oxidized low-density lipoprotein levels in men after short-term cranberry juice consumption. Metabolism 2005;54:856–61.
[26] Ruel G, Pomerleau S, Couture P, Lemieux S, Lamarche B, Couillard C. Low-calorie cranberry juice supplementation reduces plasma oxidized LDL and cell adhesion molecule concentrations in men. Br J Nutr 2008;99:352–9.
[27] Basu A, Betts NM, Ortiz J, Simmons B, Wu M, Lyons TJ. Low-energy cranberry juice decreases lipid oxidation and increases plasma antioxidant capacity in women with metabolic syndrome. Nutr Res 2011;31:190–6.
[28] Ruel G, Pomerleau S, Couture P, Lemieux S, Lamarche B, Couillard C. Plasma matrix metalloproteinase (MMP)-9 levels are reduced following low-calorie cranberry juice supplementation in men. J Am Coll Nutr 2009;28:694–701.
[29] van der Kooy K, Seidell JC. Techniques for the measurement of visceral fat: a practical guide. Int J Obes 1993;17:187–96.
[30] Moorjani S, Dupont A, Labrie F, Lupien PJ, Brun D, Gagné C, Giguère M, Bélanger A. Increase in plasma high-density lipoprotein concentration following complete androgen blockage in men with prostatic carcinoma. Metabolism 1987;36:244–50.
[31] Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res 1970;11:583–95.
[32] Raabo E, Terkildsen TC. On the enzymatic determination of blood glucose. Scand J Clin Lab Invest 1960;12:402–7.
[33] Sesso HD, Stampfer MJ, Rosner B, Hennekens CH, Gaziano JM, Manson JE, et al. Systolic and diastolic blood pressure, pulse pressure, and mean arterial pressure as predictors of cardiovascular disease risk in men. Hypertension 2000;36: 801–7.
[34] Vlachopoulos C, Dima I, Aznaouridis K, Vasiliadou C, Ioakeimidis N, Aggeli C, et al. Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals. Circulation 2005;112:2193–200.
[35] McEniery CM, Wallace S, Mackenzie IS, McDonnell B. Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. Hypertension 2006;48:602–8.
[36] Goulet J, Nadeau G, Lapointe A, Lamarche B, Lemieux S. Validity and reproducibility of an interviewer-administered food frequency questionnaire for healthy French-Canadian men and women. Nutr J 2004;3:13.
[37] Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. J Am Diet Assoc 1988;88:1268–71.
[38] Health Canada (2007). Canadian Nutrient File (electronic version at www.healthcanada.gc.ca/cnf).
[39] The IDF consensus worldwide definition of the metabolic syndrome. 2006: http://www.idf.org/webdata/docs/IDF_Meta_def_final.pdf.
[40] Scalbert A, Manach C, Morand C, Remesy C, Jimenez L. Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr 2005;45:287–306.
[41] Zern TL, Fernandez ML. Cardioprotective effects of dietary polyphenols. J Nutr 2005;135:2291–4.
[42] Kaliora AC, Dedoussis GV, Schmidt H. Dietary antioxidants in preventing atherogenesis. Atherosclerosis 2006;187:1–17.
[43] Del Rio D, Borges G, Crozier A. Berry flavonoids and phenolics: bioavailability and evidence of protective effects. Br J Nutr 2010;104(Suppl. 3):S67–90.
[44] Curin Y, Andriantsitohaina R. Polyphenols as potential therapeutical agents against cardiovascular diseases. Pharmacol Rep 2005;57(Suppl.):97–107.
[45] Dohadwala MM, Holbrook M, Hamburg NM, Shenouda SM, Chung WB, Titas M, et al. Effects of cranberry juice consumption on vascular function in patients with coronary artery disease. Am J Clin Nutr 2011;93:934–40.
[46] Stewart AD, Millasseau SC, Kearney MT, Ritter JM, Chowienczyk PJ. Effects of inhibition of basal nitric oxide synthesis on carotid-femoral pulse wave velocity and augmentation index in humans. Hypertension 2003;42:915–8.

[47] Rashid P, Weaver C, Leonardi-Bee J, Bath F, Fletcher S, Bath P. The effects of transdermal glyceryl trinitrate, a nitric oxide donor, on blood pressure, cerebral and cardiac hemodynamics, and plasma nitric oxide levels in acute stroke. J Stroke Cerebrovasc Dis 2003;12:143–51.
[48] Flammer, AJ, Martin, EA, Gossl, M, Widmer, RJ, Lennon, RJ, Sexton, JA, et al. A. Polyphenol-rich cranberry juice has a neutral effect on endothelial function but decreases the fraction of osteocalcin-expressing endothelial progenitor cells. Eur J Nutr. 2012. [Epub ahead of print].
[49] Duthie SJ, Jenkinson AM, Crozier A, Mullen W, Pirie L, Kyle JA, et al. The effects of cranberry juice consumption on antioxidant status and biomarkers relating to heart disease and cancer in healthy human volunteers. Eur J Nutr 2006;45: 113–22.
[50] Lauer T, Heiss C, Balzer J, Keymel S, Kelm M, Preik M, et al. Resting microvascular resistance and conduit artery tone: relevance to endothelium-dependent flow-mediated dilation. Eur J Cardiovasc Prev Rehabil 2008;15:677–82.
[51] Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257–65.
[52] Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, et al. Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995;26:1235–41.
[53] Adams MR, Robinson J, McCredie R, Seale JP, Sorensen KE, Deanfield JE, et al. Smooth muscle dysfunction occurs independently of impaired endothelium-dependent dilation in adults at risk of atherosclerosis. J Am Coll Cardiol 1998;32: 123–7.
[54] Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992;340:1111–5.
[55] Mallareddy M, Parikh CR, Peixoto AJ. Effect of angiotensin-converting enzyme inhibitors on arterial stiffness in hypertension: systematic review and meta-analysis. J Clin Hypertens (Greenwich) 2006;8:398–403.
[56] Actis-Goretta L, Ottaviani JI, Fraga CG. Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. J Agric Food Chem 2006;54:229–34.
[57] Apostolidis E, Kwon YI, Shetty K. Potential of cranberry-based herbal synergies for diabetes and hypertension management. Asia Pac J Clin Nutr 2006;15:433–41.
[58] Xu JW, Ikeda K, Yamori Y. Upregulation of endothelial nitric oxide synthase by cyanidin-3-glucoside, a typical anthocyanin pigment. Hypertension 2004;44:217–22.
[59] Wang Q, Xia M, Liu C, Guo H, Ye Q, Hu Y, et al. Cyanidin-3-O-beta-glucoside inhibits iNOS and COX-2 expression by inducing liver X receptor alpha activation in THP-1 macrophages. Life Sci 2008;83:176–84.
[60] Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991;43: 109–42.
[61] McGhie TK, Walton MC. The bioavailability and absorption of anthocyanins: towards a better understanding. Mol Nutr Food Res 2007;51:702–13.
[62] Mullan BA, Ennis CN, Fee HJ, Young IS, McCance DR. Protective effects of ascorbic acid on arterial hemodynamics during acute hyperglycemia. Am J Physiol Heart Circ Physiol 2004;287:H1262–8.
[63] Wilkinson IB, Megson IL, MacCallum H, Sogo N, Cockcroft JR, Webb DJ. Oral vitamin C reduces arterial stiffness and platelet aggregation in humans. J Cardiovasc Pharmacol 1999;34:690–3.
[64] Plantinga Y, Ghiadoni L, Magagna A, Giannarelli C, Franzoni F, Taddei S, et al. Supplementation with vitamins C and E improves arterial stiffness and endothelial function in essential hypertensive patients. Am J Hypertens 2007;20:392–7.
[65] Mullan BA, Young IS, Fee H, McCance DR. Ascorbic acid reduces blood pressure and arterial stiffness in type 2 diabetes. Hypertension 2002;40:804–9.
[66] Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. N Engl J Med 2008;359:1811–21.
[67] Gagliardi AC, Miname MH, Santos RD. Uric acid: a marker of increased cardiovascular risk. Atherosclerosis 2009;202:11–7.
[68] Vukovic J, Modun D, Budimir D, Sutlovic D, Salamunic I, Zaja I, et al. Acute, food-induced moderate elevation of plasma uric acid protects against hyperoxia-induced oxidative stress and increase in arterial stiffness in healthy humans. Atherosclerosis 2009;207:255–60.