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ORIGINAL CONTRIBUTION

Chronic cranberry juice consumption restores cholesterol profiles and improves endothelial function in ovariectomized rats

Lai-Ming Yung · Xiao Yu Tian · Wing Tak Wong · Fung Ping Leung · Lai Hang Yung · Zhen Yu Chen · Chi Wai Lau · Paul M. Vanhoutte · Xiaoqiang Yao · Yu Huang

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Abstract
Purpose Postmenopausal women experience higher risks for cardiovascular diseases than age-matched men and pre-menopausal women. There is a need for better treatment strategy for estrogen-deficient-related cardiovascular complications. We and others have recently reported that activated renin–angiotensin system and the associated oxidative stress impaired endothelium-dependent relaxation in ovariectomized rat, while angiotensin receptor blocker rescues endothelial dysfunction. Dietary supplements and lifestyle modifications provide an alternative way to improve cardiovascular health. The present study tests the hypothesis that chronic cranberry juice consumption improves cholesterol profiles and vascular functions in estrogen-deficient animal model. The effect of cranberry consumption on expression and activity of renin–angiotensin system in the vasculature will be determined.
Methods Ovariectomized rats were treated daily with commercial cranberry juice at 7 mg/kg for 8 weeks, a dosage comparable to recent clinical studies. Serum was collected for measuring cholesterol levels while aorta was isolated for isometric force assay and expression studies.
Results Cranberry juice consumption reduced circulating levels of total cholesterol, triacylglycerols, HDL, nHDL, and nHDL/HDL ratio. Meanwhile, cranberry juice consumption improved endothelium-dependent relaxation in aorta of ovariectomized rats by restoring p-eNOS level (endothelial nitric oxide synthase phosphorylated at ser-1177), reversing the up-regulated levels of renin–angiotensin system markers (angiotensin-converting enzyme, angiotensin II, and angiotensin II type 1 receptor), and normalizing the elevated NAD(P)H oxidase expression and oxidative stress.
Conclusions Our data demonstrate the novel cardiovascular benefits of cranberry juice consumption in improving both vascular functions and cholesterol profiles, providing insight into developing cranberry products into useful dietary supplements for postmenopausal women.

Keywords Cranberry · Estrogen deficiency · Nitric oxide · Oxidative stress · Renin–angiotensin system · Endothelial functions

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L.-M. Yung (✉) · X. Y. Tian · W. T. Wong · F. P. Leung · L. H. Yung · C. W. Lau · X. Yao · Y. Huang (✉)
Institute of Vascular Medicine, Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong, Hong Kong, China
e-mail: lyung@partners.org

Y. Huang
e-mail: yu-huang@cuhk.edu.hk

L.-M. Yung · X. Y. Tian · W. T. Wong · F. P. Leung · L. H. Yung · C. W. Lau · X. Yao · Y. Huang
School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong, China

Present Address:
L.-M. Yung
Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Z. Y. Chen
School of Life Sciences, Chinese University of Hong Kong, Hong Kong, China

P. M. Vanhoutte
Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong, China

Introduction

Declined circulating estrogen level exerts detrimental effects on cardiovascular functions and lipid metabolisms. Before menopause, women experience fewer incidences of cardiovascular events than age-matched men [1–3]. However, endothelial dysfunction, as assessed by impaired flow-mediated dilatation, is commonly observed in postmenopausal women [2, 4–6]. Severity of endothelial dysfunction correlates with the occurrence and progressions of cardiovascular and metabolic diseases, including hypertension and atherosclerosis [2]. In fact, estrogen deficiency-related cardiovascular risks confer a huge health burden, and it is inadequately resolved due to a lack of effective and specific treatments. For instance, safety and efficacy of hormone replacement therapy (HRT) and the usage of selective estrogen receptor modulators (SERMs) have been questioned. Interventional trials reported no overall therapeutic benefit of HRT [7]. Long-term treatment with raloxifene, the second generation of SERMs, did not alter the incidence of primary coronary events, but associated with an increased risk of fetal stroke and venous thromboembolism [8].

Recent advances in the understanding of vascular biology and molecular mechanisms of cardiovascular diseases help identify novel therapeutic targets. For example, renin–angiotensin system (RAS) and the associate oxidative stress reduce endothelial NO bioavailability and impair endothelium-dependent relaxations in postmenopausal women [6, 9] and estrogen-deficient animal models [10, 11]. We and the others have recently reported that angiotensin receptor blockers reduce oxidative stress, ameliorate vascular inflammatory phenotypes, and improve cardiovascular health in estrogen-deficient models [10–12].

Alternatively, non-pharmacological interventions, such as lifestyle and dietary modifications, improve cardiovascular health [13, 14]. For instance, consumption of vegetables and fruits rich in flavonoids reduces the incidence of coronary heart disease [15–18]. Recent clinical studies suggest that chronic cranberry juice (CJ) consumption improves cardiovascular health in patients with coronary artery disease [19] and type 2 diabetics [20]. Notably, CJ consumption (2 cups each day for 8 weeks) reduces lipid oxidation and increases plasma antioxidant capacity in women with metabolic syndrome [21]. Taken together, it is tempting to examine the cardiovascular benefits of chronic CJ consumption in estrogen-deficient models, which will also allow us to dissect the underlying molecular mechanisms.

We hypothesize that chronic CJ consumption improves cholesterol profiles and vascular functions in ovariectomized (OVX) rats, by opposing the elevated expression and activity of RAS makers in the vasculature. OVX rats will be treated with commercial CJ for 8 weeks at 7 mL/kg [22], a protocol similar to that previously used. Estrogen treatment will be used as positive control. At the end of treatment, serum will be collected to examine the impact of CJ consumption on cholesterol profiles, while aorta will be isolated for isometric force measurement to compare changes in vascular reactivity. Immunohistochemical staining and Western blot will be performed to examine the changes in expressions of eNOS, RAS markers, NAD(P)H oxidase, and oxidative stress in the vasculature of OVX rats.

Methods and materials

The experimental protocol was approved by the institutional animal care and use committee and were consistent with the Guide for the Care and Use of laboratory Animals published by the National Institutes of Health. Adult female Sprague–Dawley rats (three-month old, weighing 200–230 g) were purchased from the Laboratory Animal Service Center, the Chinese University of Hong Kong. Rats were anesthetized using sodium pentobarbital (40 mg/kg body weight, intraperitoneal injection), ovariectomized via a mid-abdominal route, and divided into four groups: (1) OVX, ovariectomized rats receiving sham operation (without estrogen pellet placement); (2) OVX + CJ, ovariectomized rats receiving daily consumption of cranberry juice (Sunraysia, composition in Table 1) at 7 mL/kg [22] by gastric gavage for 8 weeks; (3) OVX + E_2, ovariectomized rats with estrogen treatment, by inserting a 17β-estradiol pellet (0.5 mg/pellet, Innovative Research of America) into the dorsal subcutaneous pockets for 3 weeks [23, 24]; and (4) Sham-operated control rats. Cranberry administration was initiated at the fourth week after ovariectomy. At the end of treatments, blood pressure was measured by a tail-cuff method.

Blood collection and measuring cholesterol profiles and estrogen

Rats were killed by CO_2 suffocation and sera were collected for later analysis of levels of estrogen and lipids. Hearts and uteri were dissected free of surrounding fat pads and then weighed. Serum levels of total cholesterol (Sigma 352-20), triacylglycerols (Sigma 336-20), and HDL cholesterol were measured using enzymatic kits as described previously. Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol [25]. Triacylglycerols are converted to glycerol and fatty acids, and finally into NADH. Colored formazan formed upon addition of 2-(p-iodophenyl)-3-p-nitrophenyl-5-phenyltetrazolium. Absorbance at 500 nm was measured. For

Table 1 Basic parameters of control, ovariectomized (OVX) rats, and OVX rats receiving administration of cranberry juice (OVX + CJ) or estrogen treatment (OVX + E_2)

Parameters	Control	OVX	OVX + CJ	OVX + E_2
Number	6	6	6	6
BW (g)	237.5 ± 3.1	353.3 ± 6.2^{a,***}	342.5 ± 1.7	283.3 ± 7.7^{b,*}
BP (mmHg)	100.1 ± 2.9	105.6 ± 2.1	101.3 ± 2.1	100.5 ± 2.9
HW (g)	0.96 ± 0.01	1.37 ± 0.10^{a,**}	1.11 ± 0.03^{b,*}	1.04 ± 0.05^{b,*}
% HW/BW	0.41 ± 0.01	0.39 ± 0.03	0.32 ± 0.01	0.37 ± 0.04
UW (g)	0.49 ± 0.02	0.08 ± 0.02^{a,***}	0.12 ± 0.02	0.59 ± 0.01^{b,***}
% UW/BW	0.21 ± 0.01	0.02 ± 0.01^{a,***}	0.04 ± 0.01	0.21 ± 0.01^{b,***}

Basic parameters measured in different groups of rats included body weight (BW), blood pressure (BP), heart weight (HW), and uterine weight (UW). Results are means ± SEM of 6 rats. Statistical significance between control and OVX (a) or between OVX and treated OVX rats (b) is indicated by * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

the measurement of HDL cholesterol level, LDL and very low-density lipoprotein (VLDL) fractions were removed by the addition of HDL cholesterol assay reagent (Sigma 352-4) [26]. The remaining level of cholesterol, that is HDL, was measured. Serum level of estrogen was measured as described previously [27, 28].

Artery preparation

The thoracic aorta was dissected out and cleaned of adhering connective tissue in ice-cold and oxygenated Krebs-Henseleit solution containing (mmol/L): 119 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$, 1.2 KH_2PO_4, and 11 D-glucose. Each aorta was cut into several ring segments (~3 mm in length) for parallel studies, and each experiment was performed on rings obtained from different rats. The aortic ring was suspended between two stainless steel hooks in a 10-ml organ bath filled with Krebs solution. Bathing solution was continuously bubbled with 95 % O_2 and 5 % CO_2 and maintained at 37 °C (pH of 7.3–7.5). An optimal baseline tone of 2 g was applied to all rings [10, 11].

Western blot analysis

Aortae were homogenized in tissue homogenizer on ice and centrifuged to collect supernatants. Protein samples (50 μg) were separated with 10 % SDS–PAGE and transferred to a nitrocellulose immobilon-P polyvinylidene difluoride membrane. Membranes were blocked with 1 % bovine serum albumin. Primary antibodies against eNOS (1:500, BD Transduction Laboratories), eNOS phosphorylated at ser^{1177} (1:1,000, Upstate Biotechnology), ACE (1:1,000, Santa Cruz), AT_1R (1:1,000, Abcam), $gp91^{phox}$ (1:500, Santa Cruz), $p22^{phox}$ (1:500, Santa Cruz), nitrotyrosine (1:1,000, Upstate Biotechnology), and GAPDH (1:3,000, Ambion) were used. Corresponding secondary antibody conjugated to horseradish peroxidase (Dako) was used. The membranes were developed with an enhanced chemiluminescence detection system and exposed on X-ray films.

Immunohistochemical staining

Aortic rings were fixed in 4 % paraformaldehyde overnight at 4 °C and embedded in paraffin. Cross-sections in 5-μm thickness were prepared on microtome (Leica). Sections were rehydrated and microwave boiled in citrate buffer (pH 6.0) and then incubated with H_2O_2. Sections were blocked in 5 % normal goat (for Ang II) or donkey (for ACE) serum. Primary antibodies (Ang II 1:500, Peninsula laboratory; ACE 1:200, Santa Cruz) were incubated overnight at 4 °C. Biotin-SP-conjugated goat anti-rabbit (for Ang II) or donkey anti-goat (for ACE) secondary antibodies (Jackson Immunoresearch) at 1:200 were added. Streptavidin-HRP conjugate (Invitrogen) at 1:200 dilution was used. Slides were incubated with DAB chromogen substrate (Vector Laboratory) and then counterstained with hematoxylin. Photograph was taken under Leica DMRBE microscope and analyzed by ImageJ (NIH).

Dihydroethidium (DHE) staining

Aortic rings were embedded in OCT compound (Tissue-Tek). Sections were cut in 10-μm thickness on cryostat and incubated for 30 min with 5 μmol/L dihydroethidium (DHE; Molecular Probes)-containing phosphate-buffered saline at 37 °C. Fluorescence was observed by confocal microscopy (515-nm excitation; 585-nm long pass filter; Olympus Fluoview). DHE fluorescence intensity was analyzed by Fluoview (version 1.5; FV10-ASW1.5). For each section, a square region with an area of 80 μm × 80 μm was selected for analysis. The summarized data represent the fold change in fluorescence intensity relative to that in control rat aortae.

HPLC analysis of cranberry juice extract

Potential bioactive ingredients present in CJ extract were examined and identified using HPLC using a similar method described before [29]. The individual flavonoid was monitored at 280 nm and quantified using catechin as an internal standard. In brief, 10 μL CJ extract (100 mg/mL) was mixed with 250 μL catechin/ethanol solvent (0.4 mg/mL). After flushed with N_2, the mixture was resolved in 1 mL ethanol again. The sample was then injected into an HPLC column (Hypersil ODS, 250 × 4.6 mm i.d., 5 μm, Attech, Deerfield, IL, USA) using HP-1100 HPLC system equipped with a UV detector. Solvent gradients were formed by the dual pumping system by varying the proportion of solvent A (acetonitrile) to solvent B (2 % acetic acid). After the injection of the sample, solvent B was decreased from 90 to 75 % over 10 min, to 25 % over an additional 6 min, to 20 % over an additional 6 min again, and then back to the starting ratio over an additional 2 min. The flow rate was maintained at 1 mL/min.

Statistical analysis

Results represent mean ± SEM on aortic rings from n different rats. The contraction was expressed in active tension as g (absolute tension developed)/mg dry weight. The cumulative concentration–response relationship was analyzed with a nonlinear curve fitting (GraphPad Prism, version 4.0). The pD_2 was calculated as the negative logarithm of the dilator concentration that induced 50 % of the maximum relaxation (E_{max}). Protein expression was normalized to GAPDH and then expressed relative to control. Student's *t* test (unpaired two-tailed) was used, and concentration–response curves were analyzed by two-way ANOVA followed by Bonferroni post hoc tests. $p < 0.05$ indicates significant difference.

Results

Chronic CJ consumption improved cholesterol profiles in OVX rats

Body weight gain in OVX rats was unaffected with CJ consumption, but significantly reduced by estrogen treatment (Table 1). Systolic blood pressure was comparable among all groups (Table 1). The ratio of heart weight over body weight was similar in all groups (Table 1). Uterine weight decreased markedly in OVX rats, which was restored by estrogen treatment while CJ consumption had no effects (Table 1).

A lowered serum level of estrogen in OVX rats was restored by estrogen treatment, but unaffected following chronic CJ consumption (Table 2). The OVX rats had higher serum levels of total cholesterol, triacylglycerols, HDL, nHDL, and the nHDL/HDL ratio than age-matched control rats (Table 2). CJ consumption and estrogen treatment favorably modified the lipid profile in OVX rats through lowering total cholesterol and non-HDL cholesterol with CJ consumption being more effective (Table 2). CJ consumption, but not estrogen treatment, decreased the nHDL/HDL ratio in OVX rats (Table 2).

CJ consumption improved endothelial functions in OVX rats

Representative traces showed the impaired endothelium-dependent relaxations in response to acetylcholine (ACh) in aortae of OVX rats (Fig. 1a). CJ consumption restored the blunted relaxations in OVX rats (Fig. 1b). The maximum response to ACh was reduced in aortae of OVX rats (91.4 ± 1.9 vs. 55.0 ± 1.9 %, $p < 0.001$ vs. control), whereas CJ consumption largely restored the maximum relaxation (83.5 ± 1.7, $p < 0.001$ vs. OVX, Table 3). Estrogen treatment used as the positive control also augmented the relaxations (Fig. 1c). Estrogen treatment also enhanced the maximum relaxation to ACh (76.4 ± 2.7 %, $p < 0.001$ vs. OVX, Table 3). The initial tension developed by the addition of 1 μmol/L phenylephrine was increased in aortae of OVX rats, whereas CJ consumption and estrogen treatment reversed the increased initial tension (Table 3).

Western blotting showed that the reduced phosphorylation of eNOS at Ser-1177 (p-eNOS) in aortae of OVX rats was reversed after CJ consumption (Fig. 2a), while the total protein level for eNOS was unaffected (Fig. 2b).

CJ consumption reversed the up-regulation of RAS components

ACE plays a major role in catalyzing Ang II production in aortae of estrogen-deficient rats since chymase was present at very low levels [11]. Western blotting (Fig. 3a) and immunohistochemical staining (Fig. 3b) showed that CJ consumption attenuated the up-regulated ACE protein expression in aortae of OVX rats and this effect was comparable with that in estrogen-treated OVX rats. Immunohistochemical staining also revealed that the elevated Ang II level was normalized by CJ consumption, while it was unaffected by estrogen treatment (Fig. 3c).

Acute treatment (30 min) with 3 μmol/L losartan (AT_1R blocker) significantly augmented endothelium-dependent relaxations in aortae of OVX rats (Fig. 4a). The maximum relaxation to ACh was restored by losartan, while phenylephrine-induced initial tension was unaffected (Table 3). By contrast, acute treatment with losartan did not affect ACh-induced relaxations in aortae of CJ-treated OVX rats (Fig. 4b).

Table 2 Estrogen level and lipid profiles in control, ovariectomized (OVX) rats, and OVX rats receiving administration of cranberry juice (OVX + CJ) or estrogen treatment (OVX + E_2)

Concentration	Control	OVX	OVX + CJ	OVX + E_2
Number	6	6	6	6
Estrogen (pg/mL)	22.3 ± 1.2	5.5 ± 0.3[a,***]	6.0 ± 0.3	38.6 ± 1.7[b,***]
Total cholesterol (mg/dL)	84.1 ± 3.4	121.2 ± 0.9[a,***]	83.3 ± 2.2[b,***]	106.1 ± 4.3[b,**]
Triglyceride (mg/dL)	82.3 ± 0.3	113.5 ± 4.6[a,***]	89.9 ± 9.0[b,**]	77.4 ± 6.1[b,***]
HDL (mg/dL)	50.7 ± 0.5	60.5 ± 1.0[a,***]	47.5 ± 3.4[b,***]	54.9 ± 1.3[b,**]
nHDL (mg/dL)	33.4 ± 3.4	60.7 ± 1.6[a,***]	35.8 ± 1.1[b,***]	51.2 ± 4.9
nHDL/HDL ratio	0.66 ± 0.08	1.00 ± 0.04[a,**]	0.79 ± 0.08[b,**]	0.94 ± 0.10

Serum levels of estrogen and lipids. Results are means ± SEM of 6 rats. Statistical significance between control and OVX (a) and OVX and treated OVX rats (b) is indicated by ** $p < 0.01$ and *** $p < 0.001$

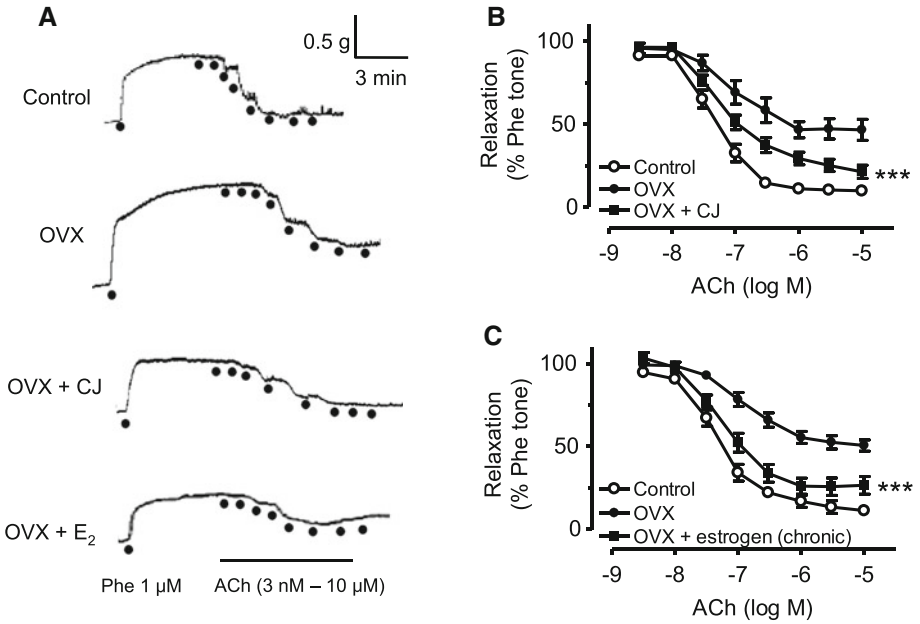

Fig. 1 Relaxation in isolated aortae in isometric force measurement. Representative traces of acetylcholine (ACh)-induced endothelium-dependent relaxations in aortae (**a**). Phenylephrine (Phe, 1 µM) was added to induce vasoconstriction, and cumulative doses of ACh (3 nM–10 µM) were added to induce endothelial nitric oxide-dependent relaxation as shown. ACh-induced concentration-dependent relaxations in aortae from control and OVX, and OVX rats receiving CJ consumption (OVX + CJ, **b**) and estrogen treatment (OVX + E_2, **c**). Results are means ± SEM of 6–8 experiments. Statistical significance is indicated by ***$p < 0.001$ between OVX and treated OVX rats

Table 3 pD_2 and E_{max} for acetylcholine-induced relaxations

	Initial tone (g)	pD_2	E_{max} (%)	*n*
Control	0.64 ± 0.04	7.35 ± 0.07	91.4 ± 1.9	8
OVX	1.00 ± 0.06	7.04 ± 0.18	55.0 ± 1.9	8
OVX + CJ	0.75 ± 0.06*	7.31 ± 0.07*	83.5 ± 1.7***	8
OVX + E_2	0.82 ± 0.07*	7.30 ± 0.10*	76.4 ± 2.7***	6

Initial tension developed by 1 µmol/L phenylephrine, cumulative doses of acetylcholine were added to induce relaxations in aortae from control, ovariectomized (OVX) rats, and ovariectomized rats receiving daily consumption of cranberry juice (OVX + CJ) or estrogen treatment (OVX + E_2). Cumulative concentration–response relationship was analyzed with a nonlinear curve fitting (GraphPad Prism 5.0). The pD_2 was calculated as the negative logarithm of the dilator concentration that induced 50 % of the maximum relaxation (E_{max}). Representative original traces were shown in Fig. 1a. Results are means ± SEM. Statistical significance is indicated as * $p < 0.05$ and *** $p < 0.001$

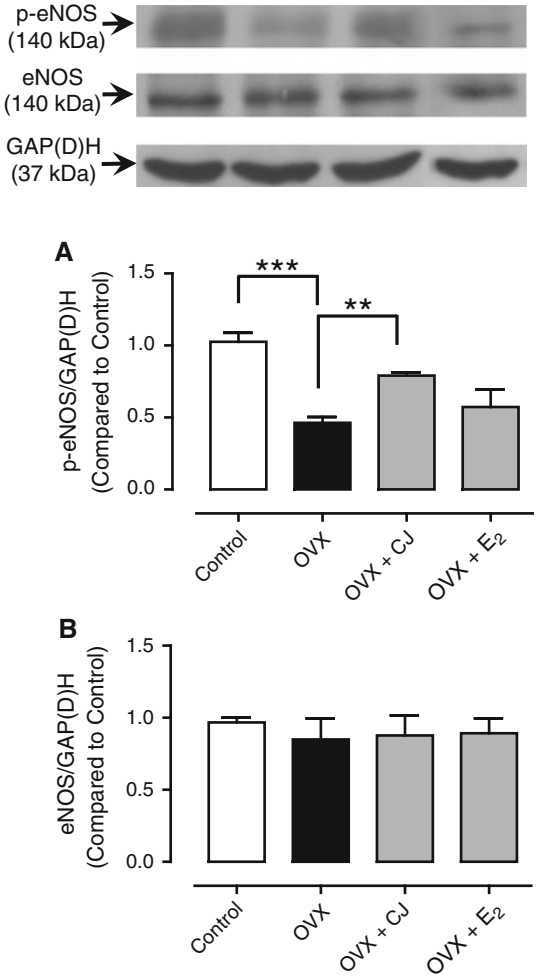

Fig. 2 Effects of chronic CJ consumption on the levels of phosphorylated and total eNOS. Western blot analysis of **a** eNOS phosphorylated at Ser-1177 (p-eNOS) and **b** the total eNOS. Results are means ± SEM of 4–5 experiments. Intensities were normalized to GAP(D)H and expressed relative to control. Statistical significance between groups is indicated by $^{**}p < 0.01$ and $^{***}p < 0.001$

The elevated AT_1R expression was reduced in OVX + CJ rats (Fig. 4c), while AT_2R level was similar in OVX rats with and without CJ treatment (data not shown). In the presence of eNOS inhibitor (100 μmol/L L-NAME), Ang II (100 nmol/L)-induced aortic contraction was larger in OVX rats than control rats and reduced in OVX rats receiving CJ (Fig. 4d). Estrogen treatment neither affected the AT_1R expression (Fig. 4c) nor Ang II-induced contraction in aortae of OVX rats (Fig. 4d).

CJ consumption prevented NAD(P)H oxidase-mediated oxidative stress

Acute treatment (30 min) with 100 μmol/L apocynin [putative inhibitor of NAD(P)H oxidase] restored the impaired relaxations of aortae of OVX rats, while it did not cause further improvement in aortae from OVX + CJ rats (Fig. 5a). Western blotting showed that both CJ consumption and estrogen treatment reversed the elevated protein levels of membrane-bound NAD(P)H oxidase subunits, $gp91^{phox}$ and $p22^{phox}$, in aortae of OVX rats (Fig. 5b).

DHE staining (Fig. 6a) and Western blotting (Fig. 6b) showed an elevated oxidative stress in OVX rat aortae and this increase was inhibited by CJ consumption or estrogen treatment.

Discussion

The present study provides novel findings about chronic health benefits of cranberry juice consumption in restoring serum cholesterol profiles and improving endothelial function. Specifically, CJ consumption reduces the elevated serum levels of total cholesterol, triacylglycerols, HDL cholesterol, nHDL cholesterol, and nHDL/HDL ratio. Meanwhile, CJ consumption improves endothelium-dependent relaxation. Expression studies suggest CJ consumption (1) augmented eNOS activity, (2) inhibits ACE up-regulation and Ang II over-production, (3) inhibits AT_1R up-regulation, and (4) suppresses NAD(P)H oxidase up-regulation and associated ROS over-production.

Numerous reports support the notion that daily consumption of cranberry could reduce the risk of urinary tract infection in women [30–35]. However, there is only limited information about its potential cardiovascular health benefits [36]. Chronic CJ consumption (one cup per day of 54 % CJ for 4 weeks) reduced the carotid femoral pulse wave velocity (a measure of arterial stiffness) in patients with coronary artery diseases [19]. Besides, CJ consumption (2 cup per day for 8 weeks) reduced lipid oxidation and increased plasma antioxidant capacity in women with metabolic syndrome [21]. Lee et al. [20] showed that CJ consumption modified serum cholesterol levels in type 2 diabetic patients. It should be noted that only serum biomarkers (glucose and lipid), physical (body mass index), or physiological parameters (blood pressure and flow-mediated dilatation) were measured in these clinical studies, limiting our knowledge on the possible mechanistic basis of health benefits of CJ consumption. To the best of our knowledge, our findings are the first providing mechanistic insights into how chronic CJ consumption improves cardiovascular health.

CJ consumption preserves endothelial NO bioavailability via multiple cellular mechanisms. CJ consumption not only maintains the NO production, but also favorably modulates the expression and activity of ACE, Ang II, and AT_1R of the RAS axis, as well as curtails oxidative stress in OVX rats. More importantly, CJ consumption also

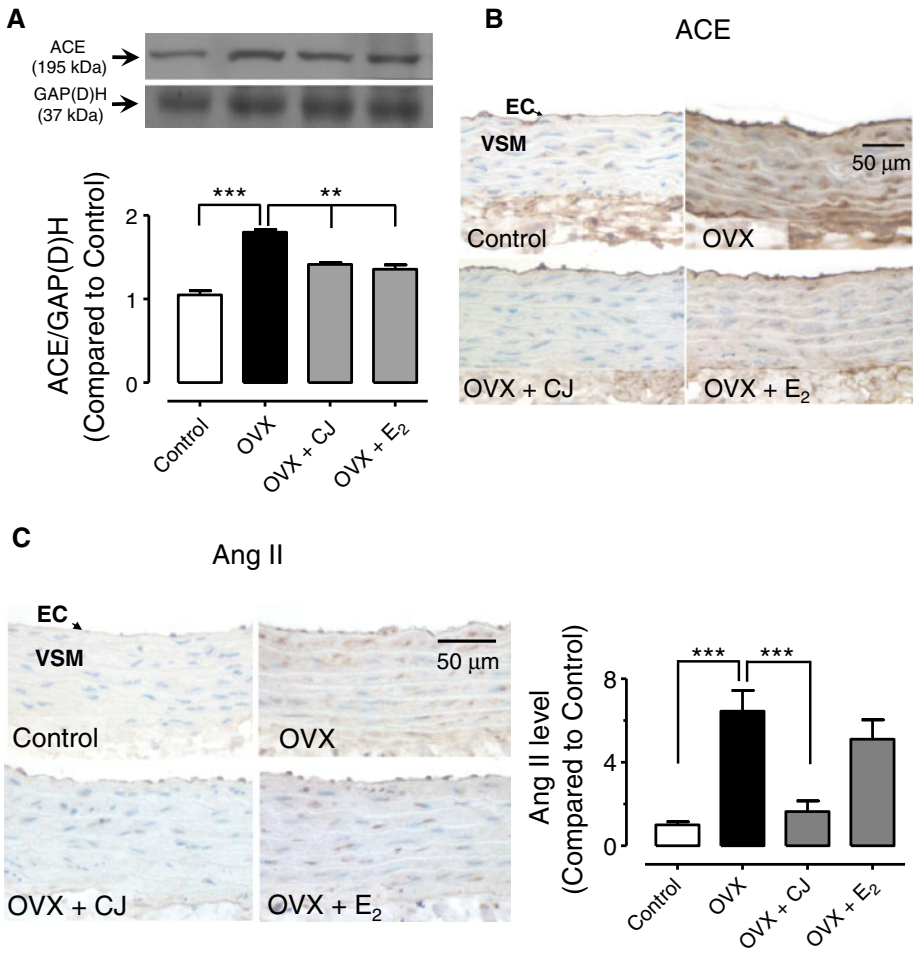

Fig. 3 Effects of CJ consumption on the expression of angiotensin-converting enzyme (ACE) revealed by Western blotting (**a**) and immunohistochemical staining (**b**). Angiotensin II (Ang II) in the aortic vascular wall was detected by immunohistochemistry (**c**). Results are means ± SEM of 4–5 experiments. Statistical significance indicated by $^{**}p < 0.01$ and $^{***}p < 0.001$. *EC* endothelial cells, *VSM* vascular smooth muscle

reduces the expression of NAD(P)H oxidase subunits ($gp91^{phox}$ and $p22^{phox}$) and nitrotyrosine (a oxidative stress marker). The vascular benefit of CJ consumption is similar to that observed in OVX rats treated with angiotensin receptor blocker [11]. Considering the established causative role of RAS and ROS in endothelial dysfunction in diabetics and hypertension [37–40], it is reasonable to postulate that the consumption of CJ or cranberry products could be useful in retarding the cardiovascular complications under these conditions through reducing oxidative stress.

Moreover, the present data showed that chronic CJ consumption reduced the elevated serum level of total cholesterol, in a similar manner to that in postmenopausal women treated with raloxifene or estrogen [41]. Notably, the lack of obvious side effects for the intake of cranberry products may offer an important advantage over pharmacological therapies in postmenopausal women [42]. The present results shed some light on the search for dietary products that can correct the dysregulation of lipid metabolism after menopause. HPLC analysis suggested that CJ contains high levels of gallic acid, epicatechin, and p-anisic acid (Supplementary table 2–3). The lipid-lowering effects of some active ingredients are summarized in supplementary table 3. Gallic acid restores the serum cholesterol level and inhibits body weight gain in rats fed with high-fat diet [43], while epicatechin reduces serum cholesterol and triacylglycerols levels [44]. Further investigation is warranted to confirm which specific active ingredient(s) contribute to the cardiovascular and metabolic benefits of chronic CJ consumption in estrogen-deficient rats. But one may expect the cardiovascular and cholesterol benefit is a result of long-term synergetic effect of various CJ active ingredients. On the other hand, it is tempting to determine whether the cardiovascular benefit is secondary to the lipid-lowering effect. However, considering a relatively long-term (8 weeks) treatment protocol and ovariectomized rats used in the present study, it is difficult to dissect the cross-talks between the vascular and cholesterol benefits of chronic CJ consumption. A recent clinical study showed that fluvastatin lowered LDL levels without affecting endothelial function in hypertensive patients, suggesting that lipid-lowering effect may not necessarily link to improved vascular function [45]. Taken together, other models of cardiovascular risk factors, such as hypertensive rats, in which baseline cholesterol profiles

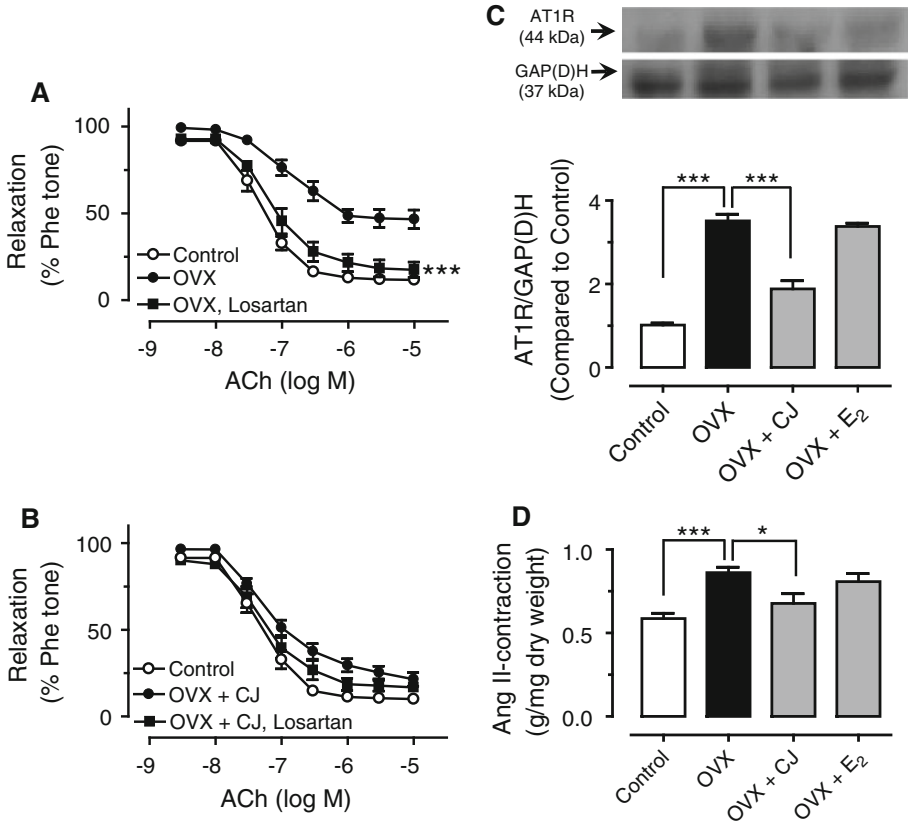

Fig. 4 Effect of losartan on relaxations in aortae from OVX (**a**) and OVX + CJ (**b**) rats. Results are means ± SEM of 6–8 experiments. Statistical significance between OVX and losartan-treated OVX rats is indicated by $***p < 0.001$. Effect of CJ consumption on the level of AT_1R (**c**). Results are means ± SEM of 4–5 experiments. Intensities were normalized to GAP(D)H and expressed relative to control. In the presence of 100 μmol/L L-NAME, angiotensin II (Ang II, 100 nmol/L) induced contraction in aortae from control, OVX, OVX + CJ, and OVX + E_2 rats (**d**). The contraction was expressed as active tension (absolute tension developed divided by the dried weight of each aortic ring). Results are means ± SEM of 6–8 experiments. Statistical significance between groups is indicated by $*p < 0.05$ and $***p < 0.001$

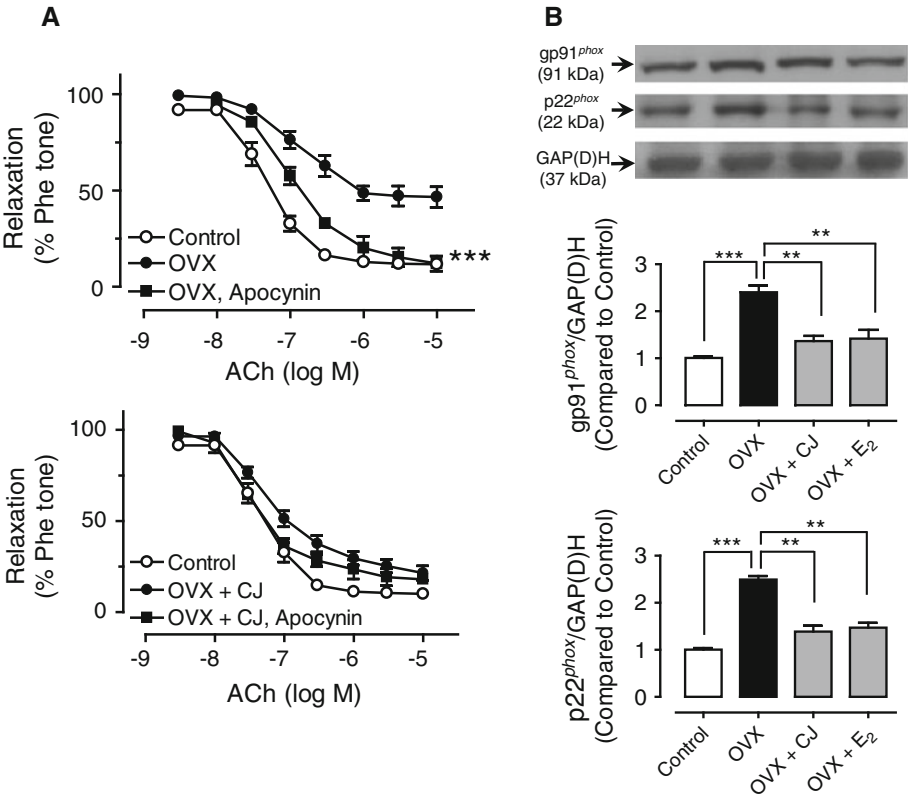

Fig. 5 Effects of apocynin on relaxations of aortae from OVX and OVX + CJ rats (**a**). Results are means ± SEM of 6–8 experiments. Statistical significance between OVX and apocynin-treated OVX is indicated by $***p < 0.001$. **b** Effects of CJ consumption on the protein levels of NAD(P)H oxidase subunits ($gp91^{phox}$ and $p22^{phox}$) in aortae from OVX rats revealed by Western blotting. Results are means ± SEM of 4–5 experiments. Statistical significance is indicated by $**p < 0.01$ and $***p < 0.001$

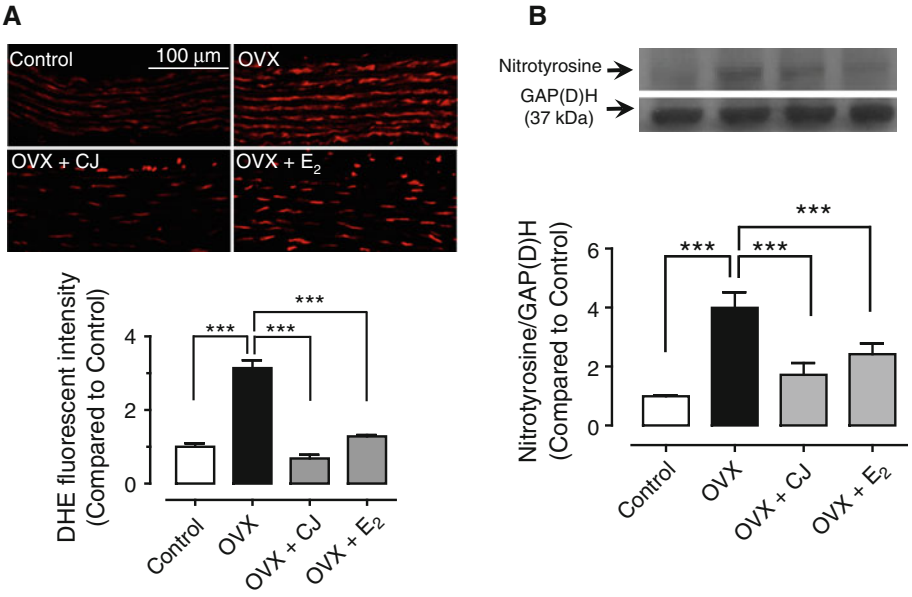

Fig. 6 Effects of CJ consumption on ROS level in vascular wall as stained by DHE (**a**) and protein level of nitrotyrosine (**b**). Results are means ± SEM of 4–5 experiments. Statistical significance is indicated by ***$p < 0.001$

are similar to normotensive controls, will be useful in addressing whether cranberry products could directly improve cardiovascular functions.

In summary, using an established animal model of estrogen deficiency, the present study provides novel molecular mechanisms underlying the cardiovascular benefits of chronic CJ consumption. Based on the critical role of RAS and associated oxidative stress in triggering endothelial dysfunction, cranberry products are very effective inhibitors, acting just like AT_1R blockers such as valsartan, of endothelial dysfunction. Considering the well-established effect of cranberry consumption in preventing urinary tract infection, cranberry products, lacking obvious unwanted effects, can be recommended to postmenopausal women as one of dietary supplements to ameliorate endothelial dysfunction and cardiovascular complications. The present results may be useful in developing cranberry products into an effective and safe functional food supplement for postmenopausal women.

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