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Wild blueberry (*V. angustifolium*)-enriched diets alter aortic glycosaminoglycan profile in the spontaneously hypertensive rat☆

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Abstract

Glycosaminoglycans (GAGs) are essential polysaccharide components of extracellular matrix and cell surface with key roles on numerous vascular wall functions. Previous studies have documented a role of wild blueberries on the GAG profile of the Sprague–Dawley rat with a functional endothelium as well as in the vascular tone of the spontaneously hypertensive rat (SHR) with endothelial dysfunction. In the present study, the effect of wild blueberries on the composition and structure of aortic GAGs was examined in 20-week-old SHRs after 8 weeks on a control (C) or a wild blueberry-enriched diet (WB). Aortic tissue GAGs were isolated following pronase digestion and anion-exchange chromatography. Treatment of the isolated populations with specific GAG-degrading lyases and subsequent electrophoretic profiling revealed the presence of three GAG species, i.e., hyaluronic acid (HA), heparan sulfate (HS) and galactosaminoglycans (GalAGs). A notable reduction of the total sulfated GAGs and a redistribution of the aortic GAG pattern were recorded in the WB as compared to the C group: a 25% and 10% increase in HA and HS, respectively, and an 11% decrease in GalAGs. Fine biochemical analysis of GalAGs at the level of constituent disaccharides with high-performance capillary electrophoresis revealed a notable increase of nonsulfated (18.0% vs. 10.7%) and a decrease of disulfated disaccharides (2.2% vs. 5.3%) in the WB aorta. This is the first study to report the redistribution of GAGs at the level of composition and their fine structural characteristics with implications for the endothelial dysfunction of the SHR.

Keywords: Wild blueberry; SHR; Aorta; GAGs; Heparan sulfate; Chondroitin sulfate

1. Introduction

Numerous studies suggest a protective role of dietary polyphenols against cardiovascular disease (CVD) [1–3], due to direct antioxidant activity [4,5] and/or signaling effects [6,7]. Wild blueberries, a rich source of anthocyanins and other phenolics [8–10], have demonstrated beneficial properties for various vascular functions [11–13]. Glycosaminoglycans (GAGs) are linear polysaccharides composed of repeating disaccharide units of hexosamine, D-galactosamine or D-glucosamine, and uronic acid (UA), D-glucuronic acid or L-iduronic acid. Four major classes of GAG molecules, determined by the hexosamine and UA type as well as sulfation pattern, are present in the vascular wall: (a) hyaluronic acid (HA); (b) galactosaminoglycans (GalAGs), comprised of chondroitin sulfate (CS) and dermatan sulfate (DS); (c) heparan sulfate (HS) and (d) keratan sulfate [14]. Hyaluronic acid is the only GAG molecule that is not sulfated and not linked to a protein core, whereas HS and GalAGs occur as part of proteoglycan (PG) molecules that may contain more than one GAG type, such as HS- and CS-containing syndecan, glypican or CS- and DS-containing versican, decorin and bigycan [15].

Glycosaminoglycans, as prevalent components of the extracellular matrix (ECM) and cell surface, interact with numerous proteins and ligands, modulating crucial biological processes such as cell growth and development [16]. In particular, the glycocalyx associated with the vascular endothelium is increasingly gaining appreciation as a determinant of signaling, mechanotransduction, vascular permeability and inflammatory response and thereby the development of endothelial dysfunction and CVD [17,18]. Additionally, the initiation of atherosclerotic process by lipid accumulation and retention in the ECM is largely mediated by CS/DS proteoglycans [19].

Abnormalities in GAG synthesis, such as CS elevation and increased sulfation, observed in the vasculature of the spontaneously hypertensive rat (SHR) are among the early events that contribute to the vascular pathology of this animal model of endothelial

Abbreviations: C, control; CVD, cardiovascular disease; CS, chondroitin sulfate; DS, dermatan sulfate; ECM, extracellular matrix; GalAG, galactosaminoglycan; GAG, glycosaminoglycan; HA, hyaluronic acid; HPCE, high-performance capillary electrophoresis; HS, heparan sulfate; PG, proteoglycan; SD, Sprague–Dawley; SHR, spontaneously hypertensive rat; UA, uronic acid; WB, wild blueberry.

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dysfunction [20–24]. In comparison with its normotensive genetic control, the Wistar Kyoto (WK) rat, the SHR aorta is characterized by a thicker subendothelial matrix and a higher CS synthesis leading to a higher concentration of total GAGs and elevated peripheral resistance and blood pressure [22]. The increased sulfate incorporation in PG molecules in the resistance arteries of the SHR [23] is another hallmark of endothelial dysfunction in this animal model [24].

Although the role of bioactive dietary ingredients on ECM and GAG metabolism has not been thoroughly examined to date, various plant-derived micronutrients [25], vitamins E and C [26], flavonoids [27], tea polyphenols [28], sorghum phenolics [29] and genistein [30] have been reported to positively affect signaling pathways and enzymatic processes related to ECM and GAG metabolism. Wild blueberries consumed with diet have been documented to elicit reduced sulfation and population redistribution of aortic GAGs in the Sprague–Dawley (SD) rat with a normal endothelium [31]. Hence, in the present study, the effect of a wild blueberry diet on the aortic GAG profile and fine structural characteristics was examined in the SHR model of endothelial dysfunction after 8 weeks of diet consumption.

2. Methods and materials

2.1. Chemicals

Pure NaCl, KCl, $CaCl_2$, $MgSO_4$, KH_2PO_4, $NaHCO_3$ and dextrose for the physiologic salt solution (PSS) as well as heparinase I, II and III, chondroitinase AC and ABC, standards for HA, HS and CS/DS analysis and standard preparations of sulfated D-disaccharides from CS/DS [Δ-di-di(2,6)S, Δ-di-di(2,4)S, Δ-di-mono6S, Δ-di-mono4S and Δ-di-nonS] were purchased from Sigma (St. Louis, MO, USA). Chondroitinase AC I was purchased from Seikagaku America (Ijamsville, MD, USA), the Blyscan assay kit from Accurate Chemical (Westbury, NY, USA) and PRONASE Protease from Calbiochem (San Diego, CA, USA).

2.2. Animal model and diets

The animal welfare and the experimental protocols conformed to the Animal Care and Use Committee of the University of Maine standards (IACUC Protocol A2008-2006-2005). Twenty male SHRs (Charles River Laboratories, Wilmington, MA, USA) at the age of 12 weeks were randomly assigned to one of two diets: control diet (C) (modified AIN-76, $n=10$) and wild blueberry diet (WB) (control diet+8% wt/wt freeze-dried wild blueberry powder substituting for dextrose, $n=10$), for a period of 8 weeks. The animals were housed in the Small Animal Facility at the University of Maine in individual stainless-steel mesh-bottomed cages in a room controlled for temperature (22°C) and light conditions (12:12-h light:dark cycle). Tap water and food were provided *ad libitum*. Food consumption was measured daily; and body weights, weekly. The diets were prepared in our laboratory from purified diet ingredients, stored at 4°C and used within 5 to 7 days. The diet ingredients, dextrose, egg white solids, vitamin mix (A.O.A.C. Special Vitamin Mixture), DL-methionine, biotin, corn oil, were purchased from Harlan Teklad (Madison, WI, USA); custom-made mineral mix was purchased from MP Biomedicals (Solon, OH, USA). Wild blueberries, provided as a composite by Wyman's (Cherryfield, ME, USA), were freeze-dried and powdered with standard procedures by FutureCeuticals (Momence, IL, USA). Twenty-one different anthocyanins were detected in the wild blueberry powder with the main anthocyanins, malvidin 3-galactoside and peonidin 3-glucoside, representing approximately 13% of the total anthocyanin content (1.6±0.2 mg/100 mg) [8].

2.3. Aortic sample collection and preparation

After 8 weeks of dietary treatment, rats were anesthetized with 95%CO_2/ 5%O_2; the thoracic aorta was excised and transferred in PSS, composed of NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 12.5 and dextrose 11.1 mM. Aortas were cleaned of the adherent connective and fat tissue, frozen in liquid N_2 and stored at −80°C. After all samples were collected, frozen specimens were powdered under liquid N_2, defatted twice with methanol/chloroform solution (1:1, vol/vol), incubated for 16 h at 4°C, rinsed with acetone and vacuum dried.

2.4. Tissue digestion and separation of GAGs

Dry defatted aortas were rehydrated overnight at 4°C with 2× distilled H_2O, 30 μl/mg aorta. The rehydrated samples were digested overnight at 37°C with PRONASE (1.6 units/mg aortic tissue) in 50 mM Tris-HCl, pH 8.0. The reaction was stopped with 0.6 M NaCl and transfer of vials in 100°C for 1 min. Aortic digests were centrifuged for 5 min at 12,000*g*, and the collected supernatant was diluted in 4.5 volumes of ethanol solution with 2.5% sodium acetate. After overnight incubation at 4°C, samples were centrifuged for 5 min at 12,000*g*, supernatant was decanted, and the precipitate was dissolved in 2× distilled H_2O, 5 μl/mg aortic tissue.

2.5. Uronic acid and total sulfated GAG determination

Carbazole reaction modified by Bitter and Muir was applied on aliquots from each sample to determine UA content [32]. Individual samples were also analyzed colorimetrically for total sulfated GAGs (sGAGs) with Blyscan.

2.6. GAG fractionation and identification

The intra-differences in UA and total sGAGs within each diet group were not significant. Therefore, all GAG samples obtained from each diet group (C, $n=10$ and WB, $n=10$) were pooled due to limited tissue availability from each rat and applied to ion-exchange chromatography on a DEAE-Sephacel column (7×1.6 cm i.d.), eluted with 0.1 M NaCl (3 volumes) and a NaCl linear gradient (0.1 to 0.9 M, 10 volumes), as previously described [31]. Fractions of 0.8 ml were collected and analyzed for UA. Uronic acid recovery from the DEAE column was higher than 90% in both C and WB pooled samples. The UA-positive DEAE fractions containing distinct GAG populations were pooled, precipitated overnight with 4.5 volumes of absolute ethanol at 4°C and centrifuged at 12,000*g* for 15 min. The precipitate was then dissolved in 2× distilled H_2O at the final concentration of 1 μl/μg UA. Glycosaminoglycan populations obtained by DEAE fractions were identified by cellulose acetate membrane electrophoresis using known standards of HA, HS and CS/DS and a running buffer of 0.1 M pyridine–0.1 M formic acid, pH 3.1, at a constant current of 0.5 mA/cm of membrane width for 50 min. Toluidine blue in 15% vol/vol aqueous methanol was applied for a 10-min membrane staining. Membranes were rinsed with water and scanned immediately [31].

2.7. GAG degradation with specific lyases

Digestions of pooled samples with a mixture of chondroitinases AC and ABC (0.01 units/10 μg UA) in 0.1 M sodium acetate–0.1 Tris-HCl buffer, pH 7.3, and a mixture of heparin lyases I, II and III (0.05 units/25 μg UA) in 20 mM acetate buffer, pH 7.0, with 1 μmol calcium acetate were performed overnight at 37°C and stopped by transfer of samples in 100°C for 1 min [31].

2.8. High-performance capillary electrophoresis (HPCE) analysis

Following enzyme digestions, GAG profile analysis was conducted as previously described [31,33] on an $HP^{3D}CE$ instrument (Agilent Technologies, Waldronn, Germany) with a built-in diode array detector set at 232 nm, using uncoated fused-silica capillary tubes (50 μm i.d., 64.5 cm total and 56 cm effective length) at 25°C. Phosphate buffer, 50 mM, pH 3.0, was used as a running buffer. NaOH 0.1 M (1 min), 2× distilled H_2O (1 min) and operating buffer (5 min) were used before each run to wash the capillary tube. Migration of Δ-disaccharides from the cathode to the anode by electrophoretic mobility and against the electroosmotic buffer flow was achieved by applying samples at the cathodic end under 50 mbar and 30 kV for 5 s.

2.9. Statistical analysis

Student's *t* test was used for comparisons of food consumption, body weights, and liver and aorta weight between the diet groups. Two-way analysis of variance was applied in ranked observations of UA and sGAG concentration of individual aortic specimens. Statistical analysis was performed with Sigmastat Statistical Program version 2.0 (SPSS Inc., Chicago, IL, USA). Values are given as mean±standard deviation (S.D.) or mean±standard error of mean (S.E.M.); differences are considered statistically significant at $P\leq.05$.

3. Results

3.1. Food intake and animal growth

The daily food intake was similar between the control and the wild blueberry diet-fed animals: 20±0.4 g in both diet groups. Growth rate, assessed by weekly measurement of body weight, did not differ significantly between the two diet groups (data not shown). As

Table 1
Body weight (BW), liver weight and aortic tissue weight[a] of the two diet groups

Diet group	BW (g)	Liver (g)	Aorta (mg)[b]
C	343.0±2.7	13.2±1.0	10.4±2.4
WB	353.0±5.3	12.8±0.9	10.0±2.2

[a] Mean±S.D., $n=10$ rats per diet group.
[b] Aorta dry weight.

presented in Table 1, no significant difference between diet groups was detected in the final body weights or liver wet weight. Furthermore, thoracic aorta dry weight did not differ between the two diet groups.

3.2. Wild blueberry diet affects total content of aortic sulfated GAGs

Aortic samples were analyzed for UA and total sGAGs content after tissue defatting and proteolytic digestion. No significant differences were found in the UA concentration of the aortas isolated from either diet group: 5.2±0.3 vs. 4.6±0.3 μg/mg aorta in the WB and C group, respectively (Table 2). Notably, the concentration of total sGAGs was significantly lower in the WB group (12.6±0.2) as compared to the control (13.7±0.2 μg/mg aorta, $P<.05$, Table 2).

3.3. Distribution of GAG content is substantially affected in WB vs. control rats

Fractionation, isolation and identification of GAGs were performed as earlier described [31]. In brief, UA-positive populations were separated with ion-exchange chromatography of C and WB aortic samples. The identity of these populations was confirmed with cellulose acetate electrophoresis before and after treatment with specific GAG-degrading lyases (not shown). Glycosaminoglycan concentration (μg/mg aortic tissue) estimated by the amount of recovered UA is presented in Table 3. In both diet groups, HS was quantitatively the major GAG population, followed by GalAGs and HA. Although the total GAG content between the two groups tested was similar, the distribution of GAG populations was notably different between the diet groups. Hyaluronic acid concentration was significantly higher (25% increase) in the WB (154.2 vs. 123.6 μg/mg in control group). Heparan sulfate was also 10% higher in the WB (1370.3 vs. 1242.2 μg/mg), whereas GalAGs content was 11% lower in the WB group (960.8 vs. 1081.4 μg/mg, Table 3, Fig. 1).

3.4. Wild blueberry diet increases nonsulfated GalAGs disaccharide content

Analysis of the isolated aortic GalAG population with HPCE after combined digestion with chondroitinases AC and ABC showed that Δdi-mono4S was the main GalAG disaccharide in both diet groups (52.4% in C and 49.5% in WB). The other monosulfated disaccharide, Δdi-mono6S, was also slightly lower in the WB group (30.3% vs. 31.6%). Notably, as shown in Table 4, the content of GalAGs in nonsulfated disaccharides increased twofold in WB group (18.0% vs. 10.7%), and that in disulfated disaccharides (Δdi-di(2,6)S) decreased by 60% (2.2% vs. 5.3%).

4. Discussion

This is the first study to document that a wild blueberry-enriched diet elicited multiple alterations of aortic GAGs in the SHR model of endothelial dysfunction. A significant reduction of total sulfated GAGs was observed in the SHR thoracic aortas after 8 weeks of wild blueberry consumption (Table 2). Although the total aortic GAG content was similar with only a slight increase (2%) in the WB group, wild blueberry consumption resulted in a redistribution of GAG populations. The levels of HA and HS were 25% and 10% higher and the GalAGs 11% lower in the aortic tissue isolated from the WB group (Table 3, Fig. 1). The GalAG disaccharide analysis revealed that the disulfated and monosulfated GalAG disaccharides were reduced due to the wild blueberry diet, whereas a higher percentage of nonsulfated GalAG disaccharides was observed in the SHR aortas from the WB group (Table 4).

The elevated peripheral resistance and blood pressure of the SHR are related to an increase of vascular PGs [22]. Furthermore, in comparison with the parental WK strain, the SHR aorta is characterized by a thicker subendothelial matrix and a higher CS synthesis leading to a higher concentration of total GAGs [22]. With most CSPGs contained in the media layer of the vascular wall, hypertrophy of this layer in small and large SHR vessels agrees with the elevated CS in the SHR [34]. Hypertrophy of the aortic media observed in young SHRs before the development of hypertension is most likely responsible for the lower compliance and distensibility of the SHR aortic wall [35].

Additionally, GalAGs seem to promote atherogenesis, since CS and DS are highly involved in low-density lipoprotein (LDL) binding [36], and especially biglycan is a critical CS/DS-containing PG for LDL retention [37]. Subendothelial retention of apoB100 lipoprotein, the major apolipoprotein of human LDL, is an early step in atherogenesis, with the atherogenicity of the LDL particle linked to its binding affinity for arterial PGs [19,38]. Another indicator of the role of GalAG in atherosclerosis is the recently documented predictability for the postoperational CS4 levels in patients undergoing coronary artery bypass surgery based on preoperational apoB and apoE levels [39].

In the present study, we observed lower GalAGs concentration after 8 weeks of WB treatment, although it was not determined whether the lower GalAGs reflected a decrease in CS or DS or both types. The decrease of GalAGs due to wild blueberry consumption was accompanied by higher concentrations of both HA and HS in our adult

Table 2
Uronic acid[a] and total sGAGs[b] concentration in aortic tissue of the two diet groups

Diet group	UA (μg/mg)[a]	sGAGs (μg/mg)[b]
C	4.6±0.3	13.7±0.2
WB	5.2±0.3	12.6±0.2*

Individual aortas were analyzed for uronic acid concentration with carbazole reaction and sGAG content with Blyscan. Values are given in μg/mg of dry defatted aortic tissue.
[a] Mean±S.E.M., $n=6$.
[b] Mean±S.E.M., $n=10$.
* Statistically significant at $P\leq.05$.

Table 3
Glycosaminoglycan-derived UA concentration[a] in each population isolated from pooled aortic samples with DEAE-Sephacel anion-exchange chromatography in the two diet groups

	C	WB
HA	123.6 (5.1%)	154.2 (6.2%)
HS	1242.2 (50.8%)	1370.3 (55.1%)
GalAGs	1081.4 (44.2%)	960.8 (38.7%)
Total GAGs	2447.3	2485.4

[a] μg UA/mg of dry defatted aortic tissue. Percentages of each type of GAG are provided in parentheses.

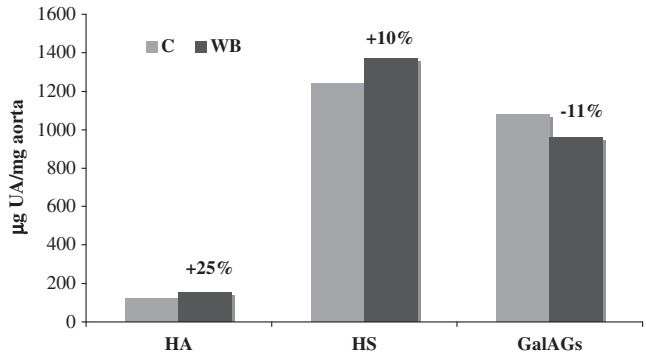

Fig. 1. Glycosaminoglycan-derived UA concentration (μg UA/mg of dry defatted aortic tissue) in each population isolated by DEAE-Sephacel anion-exchange chromatography on pooled aortic samples from the two diet groups.

Table 4
Galactosaminoglycan disaccharide composition as determined by HPCE analysis of pooled aortic samples after combined digestion with chondroitinases AC and ABC[a] in the two diet groups

Disaccharide	C	WB
Δdi-di(2,6)S	5.3	2.2
Δdi-di(2,4)S	–	–
Δdi-mono6S	31.6	30.3
Δdi-mono4S	52.4	49.5
Δdi-nonS	10.7	18.0

Dashes indicate nondetectable disaccharides.
[a] Percentage of total recovered Δ-disaccharides.

SHRs. In young SHRs, an increase of HA aortic content after diuretic treatment was positively correlated with total systemic compliance [40]. Hyaluronic acid of the arterial glycocalyx is necessary for activating flow-induced nitric oxide (NO) release from the endothelial cells by sensing and transferring the shear stress to the endothelium [41]. Furthermore, the hydrophilicity of HA is crucial for the structural stability of the glycocalyx [17]. Heparan sulfate localized on the luminal surface is another mechanosensor that mediates NO synthesis in response to shear stress [42]. In addition to the key role of HS on vascular tone, several antiatherogenic characteristics, such as inhibition of oxidized LDL and monocyte binding to the ECM and inhibition of smooth muscle cell proliferation, have been attributed to HSPGs and especially perlecan [43]. As opposed to GalAGs, HS is not involved in LDL binding [36], and perlecan, the main HS-containing subendothelial PG, does not co-localize with human apoB and therefore appears reduced in human atherosclerotic lesions [37]. The greater amount of HA and HS in the SHR aorta observed in the WB-fed rats further supports the beneficial role of wild blueberries on CVD.

Higher HA and HS levels might be related to our recent findings that documented an NO pathway-mediated attenuation of the vascular tone in response to adrenergic stimuli in the adult SHRs treated with wild blueberries [44]. The effect of wild blueberries on vascular tone, acting through the endothelium and more specifically on the NO pathway, has been also documented in the SD rat [45,46].

Besides the beneficial redistribution of GAG populations, the wild blueberry diet caused a significant decrease in GAG sulfation in the SHR aorta. Reynertson et al. have reported a higher sulfate incorporation into aortic PG in the SHR compared to the WK rat [23], as well as in other experimental models of hypertension [47]. The higher sulfation of PG molecules from vascular smooth muscle cells of mesenteric resistance arteries observed in the young prehypertensive SHRs indicates that alterations of PG synthesis occur before the establishment of vascular dysfunction and hypertension in this model [24]. The degree of sulfation is also positively correlated with LDL binding affinity to PGs [37]. A lower LDL retention induced by statin treatment was attributed to the decreased sulfation incorporation into smooth muscle cell PGs [48].

The attenuation of sulfation in the SHR aorta after 8 weeks of wild blueberry consumption in the present study suggests that dietary wild blueberries play an important role in aortic GAG remodeling and may improve structural characteristics of the vascular wall related to the development and progress of CVD. This is an important finding, especially in the light of the emerging role of GAGs as vascular agents for prevention of atherosclerosis and CVD, with special attention given to their sulfation pattern [49].

The reduced sulfation in the SHR due to the wild blueberry diet is consistent with past observations in aortic tissue from SD rats [31]. Wild blueberries, other than being a rich source of bioactive polyphenols and anthocyanins, are a good source of manganese [50], a trace element documented to decrease sulfation of HS in SD rat aorta [51].

Whereas further studies are required to elucidate the mechanisms of actions of wild blueberries on the aortic GAG profile, the possibility cannot be excluded that consumption of wild blueberries alters GAG synthesis and turnover through an effect on enzymatic processes and related signaling pathways. Phenolics from sorghum bran were reported to inhibit hyaluronidase activity, with implications in the balance between HA synthesis and degradation in the ECM and hence connective tissue remodeling and homeostasis [29]. Flavonoid compounds can inhibit the activity of HA-splitting enzymes based on the number of their hydroxyl groups [27]. Plant-derived compounds (ascorbic acid and quercetin) and tea extract induced alterations of the ECM composition leading to reduction of monocyte adhesion in the endothelium [25]. High concentrations of genistein can inhibit GAG synthesis through an effect on epidermal growth factor-dependent pathway in patients with mucopolysaccharidoses, a condition of excessive GAG accumulation in lysosomes [30]. Signaling processes initiated by vitamins E and C [26] or tea catechins [28] that modulate ECM environment with effects on vascular remodeling and maintenance of vascular wall function also indicate a positive role of dietary and bioactive compounds on vascular integrity.

Current biomedical research has been targeting the endothelium in an attempt to uncover vascular-directed agents for the prevention of atherosclerosis and CVD [49]. Glycosaminoglycans are rendered as such molecules, with special attention given to their chain elongation and sulfation patterns [48]. In the present study, wild blueberries were involved in primary aspects of GAG metabolism such as population redistribution and sulfation in the SHR aorta. We are the first to document the role of a wild blueberry diet on arterial GAG remodeling in the SHR. Wild blueberries modulate structural characteristics that potentiate cardiovascular risk, but most importantly enhance the protective features of GAG molecules associated with endothelial dysfunction and vascular pathology in the SHR. Therefore, wild blueberries may be employed as a component of endothelium-targeted nutrition to prevent endothelial dysfunction and CVD development.

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References

[1] Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Coleman R, et al. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am J Clin Nutr 2000;71:1062–76.
[2] Bertelli AA, Das DK. Grapes, wines, resveratrol and heart health. J Cardiovasc Pharmacol 2009;54:468–76.
[3] Engler MB, Engler MM, Chen CY, Malloy MJ, Browne A, Chiu EY, et al. Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. J Am Coll Nutr 2004;23:197–204.
[4] Aldini G, Carini M, Piccoli A, Rossoni G. Procyanidins from grape seeds protect endothelial cells from peroxynitrite damage and enhance endothelium-dependent relaxation in human artery: new evidences for cardio-protection. Life Sci 2003;73:2883–98.
[5] Ungvari Z, Labinskyy N, Mukhopadhyay P, Pinto JT, Bagi Z, Ballabh P, et al. Resveratrol attenuates mitochondrial oxidative stress in coronary arterial endothelial cells. Am J Physiol Heart Circ Physiol 2009;297:H1876–81.
[6] Oak MH, Bedoui JE, Madeira SV, Chalupsky K, Schini-Kerth VB. Delphinidin and cyanidin inhibit PDGF(AB)-induced VEGF release in vascular smooth muscle cells by preventing activation of p38 MAPK and JNK. Br J Pharmacol 2006;149:283–90.

[7] Loke W, Proudfoot J, Stewart S, Mckinley A, Needs P, Kroon P, et al. Metabolic transformation has a profound effect on anti-inflammatory activity of flavonoids such as quercetin: lack of association between antioxidant and lipoxygenase inhibitory activity. Biochem Pharmacol 2008;75:1045–53.
[8] Del Bò C, Ciappellano S, Klimis-Zacas D, Martini D, Gardana C, Riso P, et al. Anthocyanin absorption, metabolism, and distribution from a wild blueberry-enriched diet (*Vaccinium angustifolium*) is affected by diet duration in the Sprague–Dawley rat. J Agric Food Chem 2010;58:2491–7.
[9] Kalt W, Ryan DA, Duy JC, Prior RL, Ehlenfeldt MK, Vander Kloet SP. Interspecific variation in anthocyanins, phenolics, and antioxidant capacity among genotypes of highbush and lowbush blueberries (*Vaccinium section cyanococcus* spp.). J Agric Food Chem 2001;49:4761–7.
[10] Rimando AM, Kalt W, Magee JB, Dewey J, Ballington JR. Resveratrol, pterostilbene, and piceatannol in vaccinium berries. J Agric Food Chem 2004;52:4713–9.
[11] Kalea AZ, Clark K, Schuschke DA, Kristo AS, Klimis-Zacas DJ. Dietary enrichment with wild blueberries (*Vaccinium angustifolium*) affects the vascular reactivity in the aorta of young spontaneously hypertensive rats. J Nutr Biochem 2010;21:14–22.
[12] Shaughnessy KS, Boswall IA, Scanlan AP, Gottschall-Pass KT, Sweeney MI. Diets containing blueberry extract lower blood pressure in spontaneously hypertensive stroke-prone rats. Nutr Res 2009;29:130–8.
[13] Youdim KA, McDonald J, Kalt W, Joseph JA. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. J Nutr Biochem 2002;13:282–8.
[14] Vynios DH, Karamanos NK, Tsiganos CP. Advances in analysis of glycosaminoglycans: its application for the assessment of physiological and pathological states of connective tissues. J Chromatogr B Analyt Technol Biomed Life Sci 2002;781:21–38.
[15] Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. Pflugers Arch 2007;454:345–59.
[16] Sasisekharan R, Raman R, Prabhakar V. Glycomics approach to structure–function relationships of glycosaminoglycans. Annu Rev Biomed Eng 2006;8:181–231.
[17] Broekhuizen LN, Mooij HL, Kastelein JJ, Stroes ES, Vink H, Nieuwdorp M. Endothelial glycocalyx as potential diagnostic and therapeutic target in cardiovascular disease. Curr Opin Lipidol 2009;20:57–62.
[18] Weinbaum S, Tarbell JM, Damiano ER. The structure and function of the endothelial glycocalyx layer. Annu Rev Biomed Eng 2007;9:121–67.
[19] Karangelis DE, Kanakis I, Asimakopoulou AP, Karousou E, Passi A, Theocharis AD, et al. Glycosaminoglycans as key molecules in atherosclerosis: the role of versican and hyaluronan. Curr Med Chem 2010;17:4018–26.
[20] Castro CM, Cruzado MC, Miatello RM, Risler NR. Proteoglycan production by vascular smooth muscle cells from resistance arteries of hypertensive rats. Hypertension 1999;34:893–6.
[21] Walker-Caprioglio HM, Koob TJ, McGuffee LJ. Proteoglycan synthesis in normotensive and spontaneously hypertensive rat arteries in vitro. Matrix 1992;12:308–20.
[22] Reynertson RH, Parmley RT, Rodén L, Oparil S. Proteoglycans and hypertension. I. A biochemical and ultrastructural study of aorta glycosaminoglycans in spontaneously hypertensive rats. Coll Relat Res 1986;6:77–101.
[23] Reynertson RH, Rodén L. Proteoglycans and hypertension. II. [35S]sulfate incorporation into aorta proteoglycans of spontaneously hypertensive rats. Coll Relat Res 1986;6:103–20.
[24] Risler N, Castro C, Cruzado M, González S, Miatello R. Early changes in proteoglycans production by resistance arteries smooth muscle cells of hypertensive rats. Am J Hypertens 2002;15:416–21.
[25] Ivanov V, Ivanova S, Kalinovsky T, Niedzwiecki A, Rath M. Plant-derived micronutrients suppress monocyte adhesion to cultured human aortic endothelial cell layer by modulating its extracellular matrix composition. J Cardiovasc Pharmacol 2008;52:55–65.
[26] Villacorta L, Azzi A, Zingg JM. Regulatory role of vitamins E and C on extracellular matrix components of the vascular system. Mol Aspects Med 2007;28:507–37.
[27] Hertel W, Peschel G, Ozegowski J-H, Müller P-J. Inhibitory effects of triterpenes and flavonoids on the enzymatic activity of hyaluronic acid-splitting enzymes. Arch Pharm (Weinheim) 2006;339:313–8.
[28] Lo HM, Hung CF, Huang YY, Wu WB. Tea polyphenols inhibit rat vascular smooth muscle cell adhesion and migration on collagen and laminin via interference with cell–ECM interaction. J Biomed Sci 2007;14:637–45.
[29] Bralley E, Greenspan P, Hargrove J, Hartle D. Inhibition of hyaluronidase activity by select sorghum brans. J Med Food 2008;11:307–12.
[30] Jakóbkiewicz-Banecka J, Piotrowska E, Narajczyk M, Barańska S, Wegrzyn G. Genistein-mediated inhibition of glycosaminoglycan synthesis, which corrects storage in cells of patients suffering from mucopolysaccharidoses, acts by influencing an epidermal growth factor-dependent pathway. J Biomed Sci 2009;16:26.
[31] Kalea A, Lamari F, Theocharis A, Cordopatis P, Schuschke D, Karamanos N, et al. Wild blueberry (*Vaccinium angustifolium*) consumption affects the composition and structure of glycosaminoglycans in Sprague–Dawley rat aorta. J Nutr Biochem 2006;17:109–16.
[32] Bitter T, Muir HM. A modified uronic acid carbazole reaction. Anal Biochem 1962;4:330–4.
[33] Karamanos NK, Vanky P, Tzanakakis GN, Hjerpe A. High performance capillary electrophoresis method to characterize heparin and heparan sulfate disaccharides. Electrophoresis 1996;17:391–5.
[34] Lee RM, Garfield RE, Forrest JB, Daniel EE. Morphometric study of structural changes in the mesenteric blood vessels of spontaneously hypertensive rats. Blood Vessels 1983;20:57–71.
[35] van Gorp AW, Schenau DS, Hoeks AP, Boudier HA, de Mey JG, Reneman RS. In spontaneously hypertensive rats alterations in aortic wall properties precede development of hypertension. Am J Physiol Heart Circ Physiol 2000;278:H1241–7.
[36] Olsson U, Ostergren-Lundén G, Moses J. Glycosaminoglycan–lipoprotein interaction. Glycoconj J 2001;18:789–97.
[37] Tannock L, King V. Proteoglycan mediated lipoprotein retention: a mechanism of diabetic atherosclerosis. Rev Endocr Metab Disord 2008;9:289–300.
[38] Skålén K, Gustafsson M, Rydberg EK, Hultén LM, Wiklund O, Innerarity TL, et al. Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. Nature 2002;417:750–4.
[39] Karangelis D, Asimakopoulou A, Kanakis I, Tagarakis GI, Koufakis T, Triposkiadis F, et al. Monitoring serum chondroitin sulfate levels in patients submitted to coronary artery bypass surgery. Biomed Chromatogr 2010 [Epub ahead of print].
[40] Et-Taouil K, Schiavi P, Levy B, Plante G. Sodium intake, large artery stiffness, and proteoglycans in the spontaneously hypertensive rat. Hypertension 2001;38:1172–6.
[41] Mochizuki S, Vink H, Hiramatsu O, Kajita T, Shigeto F, Spaan JA, et al. Role of hyaluronic acid glycosaminoglycans in shear-induced endothelium-derived nitric oxide release. Am J Physiol Heart Circ Physiol 2003;285:H722–6.
[42] Florian JA, Kosky JR, Ainslie K, Pang Z, Dull RO, Tarbell JM. Heparan sulfate proteoglycan is a mechanosensor on endothelial cells. Circ Res 2003;93:e136–42.
[43] Pillarisetti S. Lipoprotein modulation of subendothelial heparan sulfate proteoglycans (perlecan) and atherogenicity. Trends Cardiovasc Med 2000;10:60–5.
[44] Kristo AS, Kalea AZ, Schuschke DA, Klimis-Zacas DJ. A wild blueberry-enriched diet (*Vaccinium angustifolium*) improves vascular tone in the adult Spontaneously Hypertensive rat. J Agric Food Chem 2010;58:11600–5.
[45] Kalea A, Clark K, Schuschke D, Klimis-Zacas D. Vascular reactivity is affected by dietary consumption of wild blueberries in the Sprague–Dawley rat. J Med Food 2009;12:21–8.
[46] Norton C, Kalea A, Harris PD, Klimis-Zacas D. Wild blueberry-rich diets affect the contractile machinery of the vascular smooth muscle in the Sprague–Dawley rat. J Med Food 2005;8:8–13.
[47] Reynertson RH, Oparil S, Rodén L. Proteoglycans and hypertension: III: aorta proteoglycans in Dahl salt-sensitive hypertensive rats. Am J Med Sci 1987;293:171–6.
[48] Meyers CD, Tannock L, Wight TN, Chait A. Statin-exposed vascular smooth muscle cells secrete proteoglycans with decreased binding affinity for LDL. J Lipid Res 2003;44:2152–60.
[49] Little PJ, Ballinger ML, Osman N. Vascular wall proteoglycan synthesis and structure as a target for the prevention of atherosclerosis. Vasc Health Risk Manag 2007;3:117–24.
[50] Bushway RJ, McGann DF, Cook WP. Mineral and vitamin content of lowbush blueberries (*Vaccinium angustifolium Ait.*). J Food Sci 1983;48:1878–80.
[51] Kalea AZ, Lamari FN, Theocharis AD, Schuschke DA, Karamanos NK, Klimis-Zacas DJ. Dietary manganese affects the concentration, composition and sulfation pattern of heparan sulfate glycosaminoglycans in Sprague–Dawley rat aorta. Biometals 2006;19:535–46.