DOI:10.1021/jf101839u

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

A Wild Blueberry-Enriched Diet (*Vaccinium angustifolium*) Improves Vascular Tone in the Adult Spontaneously Hypertensive Rat

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The effect of a wild blueberry-enriched diet on vasoconstriction and vasorelaxation was examined in the adult, 20-week-old spontaneously hypertensive rat (SHR) after 8 weeks of a control (C) or an 8% wild blueberry (WB) diet. Nitric oxide (NO)- and cyclooxygenase (COX)-mediated aortic responses were examined ex vivo with the agonists L-phenylephrine (Phe) and acetylcholine (Ach), in the absence or presence of the NO synthase (NOS) inhibitor L-N^G-monomethyl arginine (L-NMMA) or the COX inhibitor mefenamic acid (MFA). The vasoconstriction elicited by Phe was reduced in the WB group, attributed to the NO pathway, favoring a lower vascular tone under basal conditions. Acetylcholine-induced vasorelaxation in the WB group was possibly mediated through the COX, but not the NO pathway. These findings document the potential of wild blueberries to modify major pathways of vasomotor control and improve the vascular tone in the adult SHR with endothelial dysfunction.

KEYWORDS: Spontaneously hypertensive rat; wild blueberry; *Vaccinium angustifolium*; vasoconstriction; vasorelaxation

INTRODUCTION

Numerous epidemiological and clinical studies have documented a protective role of bioactive compounds and flavonoids in particular against cardiovascular disease (CVD) (*1*). Dietary polyphenols favorably affect vasomotor tone and the function of the endothelium (*2*−*5*), a crucial determinant of CVD development and progress (*6*). In addition to a direct antioxidant activity (*7*, *8*), these bioactive compounds modify enzymatic systems and signaling mechanisms important for vascular homeostasis and exert cardioprotective effects independently or in concert with their antioxidant properties (*3*, *4*, *9*, *10*).

Wild blueberries (*Vaccinium angustifolium*) are a rich source of anthocyanins and other polyphenolic compounds (*11*−*13*). Dietary animal studies in our laboratory have documented the capacity of wild blueberries to improve endothelial function and structure (*14*−*17*). They reduced vasoconstriction of Sprague−Dawley (SD) rat aortic rings through endothelium-mediated pathways (*14*, *15*). In the young spontaneously hypertensive rat (SHR), a wild blueberry-enriched diet enhanced acetylcholine (Ach)-mediated vasorelaxation with the involvement of the cyclooxygenase (COX) pathway (*16*). Furthermore, structural changes of the aortic glycosaminoglycans (GAGs), conducive to a less atherogenic profile, were observed in SD rats (*17*).

The endothelium plays an indispensable role in the maintenance of vascular tone and homeostasis (*6*). Vascular tone is regulated through the controlled release of endothelium-derived relaxing (EDRF) and endothelium-derived contracting factors (EDCF). Under physiological conditions EDRFs, NO, the endothelium-derived hyperpolarizing factor (EDHF), and the COX-derived prostacyclin (PGI_2) counteract EDCFs, such as endothelin-1 (ET-1), thromboxane A_2 (TXA_2), prostaglandin H_2 (PGH_2), and superoxide (*18*). A hallmark of the SHR endothelial dysfunction is augmented oxidative stress and reduced NO availability (*19*, *20*). Additionally, multiple alterations of COX pathway components and signaling, such as increased release and/or sensitivity of the vascular smooth muscle to PGH_2 and TXA_2, further increase endothelium-dependent contractions (*21*, *22*).

Bioactive compounds have been reported to improve endothelial dysfunction in the SHR through their effects on various systems (*4*, *23*, *24*).

Although the SHR is a commonly used experimental model of endothelial dysfunction, this condition is age-dependent (*25*). Our previous study on young (12 weeks old) SHRs fed wild blueberries before or during the development of endothelial dysfunction indicated an effect of wild blueberries on the vessel reactivity to the muscarinic, but not the adrenergic agonist, suggesting that wild blueberries may employ alternative pathways, such as COX, to potentially prevent the development of endothelial dysfunction in this model (*16*). In the present study, we examined the role of wild blueberries in adult (20 weeks old) SHRs, with already established endothelial dysfunction, in relation to phenylephrine (Phe)-mediated vasoconstriction (basal conditions) and Ach-mediated vasorelaxation (stimulated conditions), to determine whether a wild blueberry-enriched diet administered for 8 weeks after the development of endothelial dysfunction can improve vascular tone in the adult SHR.

 Published on Web 10/22/2010

MATERIALS AND METHODS

Animal Model. Forty male SHRs (Charles River Laboratories, Wilmington, MA) at the age of 12 weeks were randomly assigned to one of two diets: control (C) (modified AIN-76) and wild blueberry (WB) diet (C+8% w/w freeze-dried wild blueberry powder substituting for dextrose) for a period of 8 weeks (*14*, *15*). One set of 20 SHRs, assigned to either the C diet ($n = 10$) or the WB diet ($n = 10$), was used for the vasorelaxation and an identical set was used for the vasoconstriction experiments. 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 weight weekly. The animal welfare and the experimental protocols conformed to Institutional Animal Care and Use Committee of the University of Maine standards (IACUC Protocol A2008-2006-2005).

Animal Diets. Diets were prepared in our laboratory from purified ingredients, stored at 4 °C, and used within 5−7 days. Dextrose, egg white solids, vitamin mix (AOAC Special Vitamin Mixture), DL-methionine, biotin, and corn oil were purchased from Harlan Teklad (Madison, WI), and custom-made mineral mix was from MP Biomedicals (Solon, OH). Wild blueberries, provided as a composite by the Wild Blueberry Association of North America (WBANA) were freeze-dried and powdered according to standard procedures by FutureCeuticals (Momence, IL). Twenty-one different anthocyanins were detected in the wild blueberry powder with the main anthocyanins, malvidin 3-galactoside (Mv-3-gal) and peonidin-3-glucoside (Pn-3-glc), representing approximately 13% of the total anthocyanin content (1.6 ± 0.2 mg/100 mg) (*11*).

Drugs and Chemicals. Pure NaCl, KCl, $CaCl_2$, $MgSO_4$, KH_2PO_4, $NaHCO_3$, and dextrose for the Physiologic Salt Solution (PSS), acetylcholine chloride (Ach), L-phenylephrine (Phe), L-N^G-monomethyl arginine (L-NMMA), and mefenamic acid (MFA) were purchased from Sigma-Aldrich (St. Louis, MO).

Aortic Ring Preparation. At 20 weeks of age, rats were briefly anesthetized with 95% CO_2/5% O_2. Thoracic aortic rings were prepared as previously described (*14*). Briefly, each aortic ring was suspended between two stainless steel weightless wire triangles and submerged in a 20 mL Radnoti tissue bath (Radnoti Glass Technology Inc., Monrovia, CA) containing PSS (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) at 37 °C and aerated with 95%O_2/ 5%CO_2 (pH 7.4). The isometric tension (g) developed in the aortic rings was transmitted to a digital analyzer (model 410 Micromed, Louisville, KY) and recorded in a personal computer by DMSI-210 software (version 1.01 Micromed). All isolated rings were transferred in the tissue bath within 60 min from the administration of anesthesia and introduced to a passive tension of 1.5 g preload, set as the baseline for the experiments.

Experimental Design. As previously described (*16*), rings were preconditioned for 10 min with Ach (10^{-8} M) and Phe (10^{-8} M) under baseline and randomly assigned to treatment with either no inhibitor or L-NMMA (10^{-4} M) (NOS I, II, and III inhibitor) or MFA (10^{-5} M) (COX I and II inhibitor). Vasoconstriction experiments were conducted with seven cumulative concentrations of the α_1-adrenergic agonist Phe in 3-fold concentration steps (10^{-8}−10^{-5} M) applied in all rings in the absence or presence of an inhibitor. A single Ach dose (3×10^{-6} M) was applied at the end of the Phe concentration−response curve to confirm endothelial integrity through Ach-induced vasorelaxation. Vasorelaxation experiments were performed in rings precontracted with a maximal Phe dose (10^{-6} M) followed by the application of eight cumulative concentrations of the muscarinic agonist Ach (10^{-9} to 3×10^{-6} M), in the absence or presence of an inhibitor as described above. A 6 min drug−tissue contact time followed the addition of each concentration of the agonist to achieve maximum response.

Data Analysis. The maximum force of vasoconstriction, F_{max} (g), was selected as the highest value of each Phe concentration−response curve among the highest responses to each agonist concentration. The highest response to each Ach concentration was used to calculate the percent relaxation to the initial precontraction and the maximal vasorelaxation (%). The EC_{50} values were obtained by the semilog transformation of the concentration response curves. Receptor agonist interaction or vessel reactivity, pD_2, was calculated as the negative $\log_{10}$ EC_{50} (*14*).

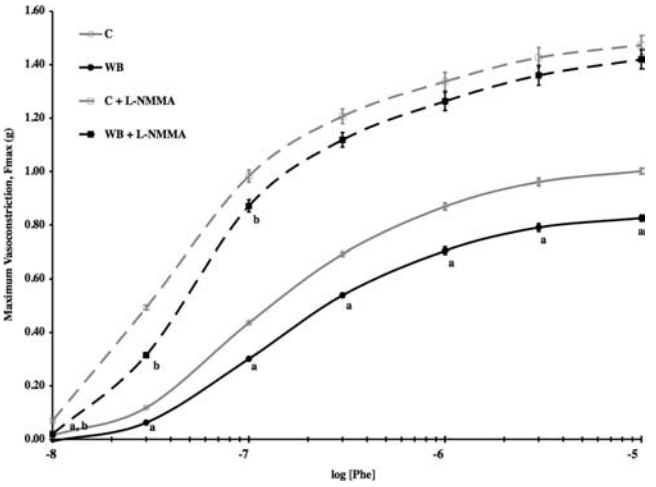

Figure 1. Phenylephrine concentration−response curve in SHRs fed a control (C, gray) and wild blueberry-enriched diet (WB, black), ($n = 10$), in the absence (continuous line) or the presence (dashed line) of L-NMMA (10^{-4} M). a, significant difference at $p \leq 0.05$ in comparison with controls in the absence of inhibitors; b, significant difference at $p \leq 0.05$ in comparison with controls in the presence of L-NMMA.

Statistical Analysis. A Student *t* test was used to determine the effect of diet on rat body weights and food consumption. Two-way analysis of variance (ANOVA) with Student−Newman−Keuls comparisons was applied on equal numbers of rank-ordered observations of the response to Phe and Ach concentrations and their pD_2 values, in the absence or presence of inhibitors, to determine possible differences between treatment groups. Statistical analysis was performed with Sigmastat Statistical Program version 2.0 (SPSS Inc., Chicago, IL). All values were given as mean ± SEM (standard error of mean); differences were considered to be statistically significant at $p \leq 0.05$.

RESULTS

Rat Growth and Weight. Growth rate, assessed by weekly measurement of body weight during the 8 week dietary treatment, was similar between the two diet groups (data not shown). Additionally, there was no difference in the final body weights, WB, 353 ± 5.34 g, and C, 343 ± 2.70 g, or in the daily food intake, 20 ± 0.36 g in both diet groups.

Phenylephrine-Induced Vasoconstriction. Concentration−response curves in the absence of any inhibitor or in the presence of L-NMMA (10^{-4} M), a NOS inhibitor, or MFA (10^{-5} M), a COX inhibitor, were generated by cumulative concentrations of Phe (10^{-8}−10^{-5} M). In the absence of the inhibitors, the vasoconstriction force was lower in the WB group at all doses of Phe; the maximum contraction (F_{max}) was 0.83 ± 0.01 vs 1.00 ± 0.01 g, $p \leq 0.05$, for the WB and C groups, respectively (**Figure 1**; **Table 1**). The addition of L-NMMA in the tissue bath caused a significant shift in the concentration−response curve of both dietary groups with a higher contractile force generated at all Phe concentrations, but no difference on F_{max} was observed between the two groups (**Figure 1**; **Table 1**). When MFA was applied, the vasoconstriction response in the WB was lower in all but two doses of Phe, 3×10^{-8} and 10^{-7} M. The maximal response was significantly lower in the WB, 0.68 ± 0.01, versus 0.79 ± 0.01 g in the C rats, $p \leq 0.05$ (**Figure 2**; **Table 1**). Vessel sensitivity (pD_2) to Phe was similar between the diet groups in the absence of an inhibitor. The addition of L-NMMA reduced vessel sensitivity to Phe in both diet groups, whereas MFA decreased the vessel sensitivity to Phe only in the C group, $p \leq 0.05$ (**Table 3**).

The presence of L-NMMA reduced the vessel sensitivity to Phe in the SHR fed wild blueberries, 7.15 ± 0.02, versus control,

Table 1. Maximum Vasoconstriction Force[a], F_{max}, in Response to Phe in SHRs Fed a Control (C) or Wild Blueberry-Enriched Diet (WB), ($n = 10$), in the Absence or the Presence of either L-NMMA (10^{-4} M) or MFA (10^{-5} M)

	F_{max} (g)		
diet group	Phe	Phe + L-NMMA	Phe + MFA
C	1.00 ± 0.01	1.47 ± 0.04	0.79 ± 0.01
WB	0.83 ± 0.01 *	1.42 ± 0.04	0.68 ± 0.01*

[a] Mean ± SEM. *, significantly different from the C group at $p \leq 0.05$.

Table 2. Maximum Vasorelaxation[a] in Response to Ach after Initial Precontraction with Phe (10^{-6} M) in SHRs Fed a Control (C) or Wild Blueberry-Enriched Diet (WB) ($n = 10$), in the Absence or the Presence of either L-NMMA (10^{-4} M) or MFA (10^{-5} M)

	maximum vasorelaxation (%)		
diet group	Ach	Ach + L-NMMA	Ach + MFA
C	94.63 ± 0.55	45.39 ± 0.50	97.76 ± 0.55
WB	91.93 ± 0.55*	46.41 ± 0.50	102.48 ± 0.55*

[a] Mean ± SEM, *, significantly different from the C group at $p \leq 0.05$.

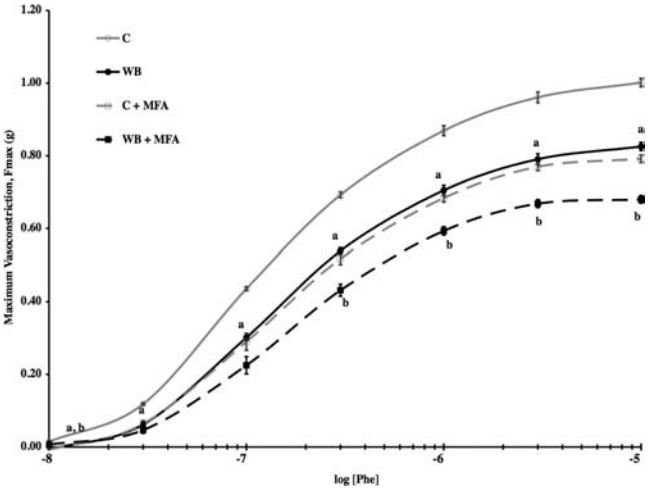

Figure 2. Phenylephrine concentration−response curve in SHRs fed a control (C, gray) and wild blueberry-enriched diet (WB, black), ($n = 10$), in the absence (continuous line) or the presence (dashed line) of MFA (10^{-5} M). a, significant difference at $p \leq 0.05$ in comparison with controls in the absence of inhibitors; b, significant difference at $p \leq 0.05$ in comparison with controls in the presence of MFA.

7.28 ± 0.02, $p \leq 0.05$, whereas COX inhibition with MFA caused a significant increase of pD_2, 7.74 ± 0.06, versus 7.41 ± 0.06, $p \leq 0.05$ (**Table 3**).

Acetylcholine-Induced Vasorelaxation. Cumulative concentrations of Ach (10^{-9}−3×10^{-6} M) were applied to generate concentration−response curves in the absence of any inhibitor or in the presence of L-NMMA (10^{-4} M) or MFA (10^{-5} M), NOS and COX inhibitors, respectively. In comparison with controls, vasorelaxation in the WB was significantly increased in response to lower Ach doses (3×10^{-9} and 10^{-8} M), but significantly decreased at higher Ach doses (3×10^{-8}, 10^{-7}, and 3×10^{-6} M), $p \leq 0.05$, when no inhibitor was applied. The maximal vasorelaxation was 91.93 ± 0.55, versus 94.63 ± 0.55, $p \leq 0.05$, in the WB and C rats, respectively (**Figure 3**; **Table 2**). Maximum vasorelaxation was not altered by the addition of L-NMMA in the tissue bath (WB, 46.41 ± 0.50; and C, 45.39 ± 0.50; **Table 2**), although a significantly greater vasorelaxation was observed in the WB group in response to three Ach doses (10^{-8}, 3×10^{-8}, and 10^{-6} M), $p \leq 0.05$, in the presence of L-NMMA (**Figure 3**). Incubation of vascular rings with MFA resulted in a significantly greater vasorelaxation in the wild blueberry-fed SHRs in response to all Ach doses, $p \leq 0.05$ (**Figure 4**).

Table 3. Effect of Diet and Drug Treatment on the Vessel Sensitivity[a], Expressed as pD_2, to the Agonist L-Phe in the Absence or Presence of either L-NMMA (10^{-4} M) or MFA (10^{-5} M)

	pD_2		
diet group	Phe	Phe + L-NMMA	Phe + MFA
C	7.65 ± 0.05	7.28 ± 0.02#	7.41 ± 0.06#
WB	7.78 ± 0.05	7.15 ± 0.02*,#	7.74 ± 0.06*

[a] Mean ± SEM. C, control diet; WB, wild blueberry diet, $n = 10$. *, statistically significant at $p \leq 0.05$ compared to C group; #, statistically significant at $p \leq 0.05$ compared to the absence of inhibitors within the same diet group.

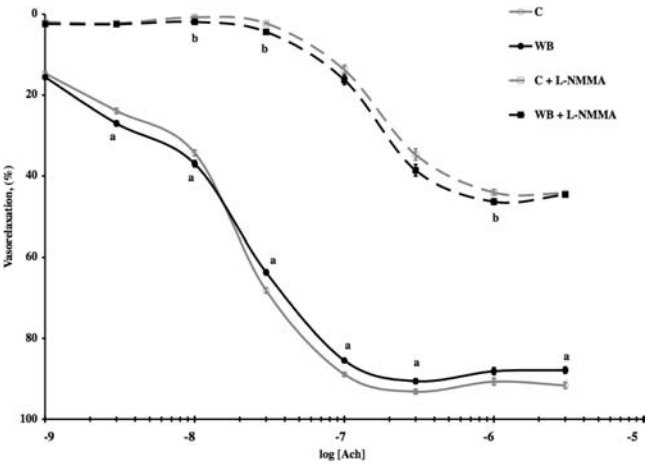

Figure 3. Acetylcholine concentration−response curve in SHRs fed a control (C, gray) and wild blueberry-enriched diet (WB, black), ($n = 10$), after initial precontraction with L-phenylephrine (Phe 10^{-6} M), in the absence (continuous line) or presence (dashed line) of L-NMMA (10^{-4} M). a, significant difference at $p \leq 0.05$ in comparison with controls in the absence of inhibitors; b, significant difference at $p \leq 0.05$ in comparison with controls in the presence of L-NMMA.

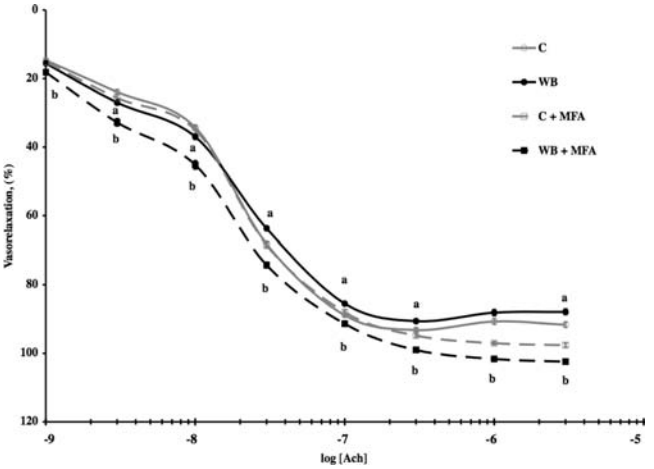

Figure 4. Acetylcholine concentration−response curve in SHRs fed a control (C, gray) and wild blueberry-enriched diet (WB, black), ($n = 10$), after initial precontraction with L-phenylephrine (Phe, 10^{-6} M), in the absence (continuous line) or the presence (dashed line) of MFA (10^{-5} M). a, significant difference at $p \leq 0.05$ in comparison with controls in the absence of inhibitors; b, significant difference at $p \leq 0.05$ in comparison with controls in the presence of MFA.

Table 4. Effect of Diet and Drug Treatment on the Vessel Sensitivity[a], Expressed as pD_2, to the Agonist Ach, in the Absence or Presence of either L-NMMA (10^{-4} M) or MFA (10^{-5} M)

	pD_2		
diet group	Ach	Ach + L-NMMA	Ach + MFA
C	7.59 ± 0.02	7.04 ± 0.02#	7.63 ± 0.02
WB	7.54 ± 0.02	7.17 ± 0.02*,#	7.72 ± 0.02*,#

[a] Mean ± SEM. C, control diet; WB, wild blueberry diet, $n = 10$. *, statistically significant at $p \leq 0.05$ compared to C group; #, statistically significant at $p \leq 0.05$ compared to the absence of inhibitor within the same diet group.

Maximal response was 102.48 ± 0.55 and 97.76 ± 0.55, $p \leq 0.05$, in WB and C groups, respectively (**Figure 4**; **Table 2**). The wild blueberry diet did not alter vessel sensitivity to Ach in the absence of the inhibitors, whereas L-NMMA reduced vessel sensitivity to Ach in both diet groups and MFA increased the vessel sensitivity to Ach in only the WB group, $p \leq 0.05$ (**Table 4**). In the presence of L-NMMA, pD_2 increased significantly, 7.17 ± 0.02 in the WB group and 7.04 ± 0.02 in the C group. Similarly, MFA caused a significant increase in vessel sensitivity in the WB group, 7.72 ± 0.02, versus 7.63 ± 0.02 in the C group (**Table 4**).

DISCUSSION

The ex vivo effect of wild blueberry consumption was examined in thoracic aortas of adult SHRs with a dysfunctional endothelium. Our vasoconstriction studies revealed that aortas from 20-week-old SHRs fed a WB diet for 8 weeks developed a lower force of contraction in response to an adrenergic agonist (**Figure 1**; **Table 1**). The decreased vasoconstriction demonstrated by the WB rat aortas was abolished when a nonspecific NOS inhibitor was present (**Figure 1**; **Table 1**), suggesting that the observed effect of wild blueberries on vasoconstriction under basal conditions was mediated through the NO pathway in the adult SHR. Compared with the absence of the inhibitor, COX inhibition caused a similar downward shift of the Phe curve in both diet groups, which reflects a significantly lower vascular tone for both C and WB groups. Thus, COX participation in the Phe-stimulated contraction can be ruled out, because the degree of inhibition of vasoconstriction due to MFA application is not different between the two groups (**Figure 2**; **Table 1**). Therefore, basal levels of prostanoids do not seem to play a significant role in the effect of wild blueberries to lower vasoconstriction in response to Phe in the adult SHR.

Although wild blueberries seem to potentiate the NO pathway under basal conditions in the adult SHR, when aortic rings were stimulated with Ach, NO was not the primary pathway employed by the WB diet to influence vasorelaxation. We observed a higher relaxation in aortic rings of the wild blueberry treated SHRs only at lower Ach concentrations ($< 10^{-8}$ M) (**Figure 3**). The higher vasorelaxation in the WB group in response to lower Ach concentrations seems to be NO-dependent because it was abolished when a NOS inhibitor was present. The alteration noted at higher Ach concentrations could not be attributed to wild blueberry involvement with the NO pathway, suggesting that even though the WB diet up-regulated this pathway under basal conditions, the maximal capacity to respond to Ach-stimulated NO signaling was not affected. However, our data indicate that the COX pathway is involved in the WB effect under stimulated conditions, as evidenced by the higher Ach-mediated relaxation of WB aortic rings due to COX inhibition with MFA (**Figure 4**; **Table 2**). In our study, inhibition of prostanoids did not have an effect on Ach-mediated relaxation in the control group, but improved the maximal response in WB-fed SHRs, which followed an increase in the vessel sensitivity to the agonist (Ach).

Vessel sensitivity in response to Phe was not altered by a wild blueberry-enriched diet. Application of L-NMMA reduced vessel sensitivity to Phe in both groups, whereas the addition of MFA reduced the vessel sensitivity to Phe in the control group, but not in the WB group. This leads us to conclude that a WB diet restores the reduced vessel sensitivity to Phe caused by the inhibition of prostanoids in the C group under basal conditions (**Table 3**). The dietary treatment did not alter vessel sensitivity to Ach unless an inhibitor of the NOS or COX pathway was present. In the presence of either L-NMMA or MFA we observed an increased sensitivity to the muscarinic agonist in the WB group as compared to C (**Table 4**). When NOS was inhibited, the vessel sensitivity to Ach was reduced in both groups, with the reduction being less pronounced in the WB group. On the contrary, inhibition of the COX pathway did not have an effect on vessel sensitivity to Ach in control aortas, but significantly increased the vessel sensitivity to Ach in the WB animals.

Our findings are relevant to the compromised endothelial integrity and function of the SHR associated with augmented superoxide generation and inadequate NO availability (*19*, *20*). The major finding of our study was the reduced adrenergic contractile response in the adult SHRs treated with wild blueberries. The augmented α_1-adrenoreceptor signal transduction and peripheral resistance in the SHR (*26*, *27*) are closely linked with the endothelial dysfunction of this animal (*25*). We demonstrate the ability of wild blueberries to reduce the contractility of the SHR aorta by a NO-mediated effect under basal (non-NO-stimulated) conditions. The outcome of our dietary treatment on the vascular tone was equivalent to the direct pharmacological effect of flavonoids quercetin or flavone (10 μM/L) incubated with aortic rings from 20−21 week adult SHRs during stimulation with cumulative Phe concentrations (*9*, *23*). The results of the Phe application in WB rings are also relevant to the relaxant effect of cumulative concentrations of bioactive compounds in rings precontracted with Phe previously demonstrated (*2*, *5*). Thus, under basal conditions, wild blueberries seem to oppose SHR adrenergic response by targeting the NO pathway, with implications on endothelial dysfunction in this experimental model.

The imbalance between vasorelaxant and vasoconstrictor factors, which is a hallmark of the SHR pathology (*18*), may limit in our experimental setup the potential of wild blueberries to benefit the contractile machinery of the adult SHR aorta beyond basal conditions. Our data show that only lower Ach concentrations resulted in higher vasorelaxation, whereas higher Ach concentrations induced less relaxation in the WB group. This may be in agreement with the biphasic nature of the Ach concentration−response curve documented in the SHR, whereby Ach concentrations higher than 10^{-7} M result in reduced vasorelaxation (*21*). Similarly with the adult SHR, the WB diet-elicited vasorelaxation in the young SHR was observed only in lower ($< 10^{-7}$ M), but not in higher ($> 10^{-7}$ M) Ach concentrations (*15*). Bioactive compounds of wild blueberries may act as partial agonists or antagonists of Ach receptors, as suggested by recent in vitro studies demonstrating that flavonoid compounds interact with (*28*) or modulate muscarinic receptors via allosteric binding (*29*). Additionally, a concentration-dependent vasorelaxant effect by flavonoid compounds (*2*, *5*) also supports the possibility of a pharmacological activity of the wild blueberry vasoactive compounds that may interfere with the Ach-induced NO signaling.

Our study differs from previous studies in SHRs documenting an increased Ach-induced vasorelaxant response mediated by bioactive compounds, such as quercetin and chlorogenic acid, in that the isolated compounds have been usually administered or applied in vitro directly in the tissue bath at pharmacological concentrations (*4*, *24*, *30*).

Polyphenolic compounds undergo multiple metabolic conversions after ingestion (*31*). Studies on the direct vascular effects of metabolized forms of polyphenols detected in the bloodstream indicate that the metabolism of a bioactive compound can alter its vasoactive properties (*30*, *32*). The Ach-induced contractions in the SHR were reduced by isorhamnetin, the main quercetin metabolite, but not by quercetin per se (*30*), whereas only the conjugated forms of quercetin exhibited vasorelaxant properties in the Wistar Kyoto rat (*32*).

Our study revealed the role of a wild blueberry diet to modify agonist−receptor interactions and the contractile response of the vascular smooth muscle cell by enhancing NO vasorelaxing effects under basal conditions. The SHR endothelium is dysfunctional due to a combination of factors such as deregulation of antioxidant enzymatic systems (*19*, *20*) and an augmented COX vasoconstrictor profile (*21*, *22*). Therefore, the capacity of wild blueberries to improve NO is observed under basal conditions or at low agonist (Ach) concentrations. On the other hand, under Ach stimulation and when prostanoids are eliminated, wild blueberries seem to enhance vasorelaxation, at least partially, due to the increased vessel sensitivity to the agonist.

Previous studies in this laboratory have documented the endothelium-dependent role of wild blueberries in reducing the adrenergic response in the SD rat with normal endothelial function (*14*). Furthermore, involvement of wild blueberries in the NO pathway under basal and stimulated conditions was observed in SD rats (*15*). Most recently, we reported a COX-mediated effect of the WB diet on vasorelaxation in the young SHR, supporting a beneficial role of wild blueberries in the prevention of endothelial dysfunction (*16*). In the present study, wild blueberries incorporated in the SHR diet after the development of endothelial dysfunction resulted in a reduced adrenergic contractile response with the involvement of the NO pathway. Hence, wild blueberries may reverse, at least partially, endothelial dysfunction in the adult SHR.

The unique goal and approach of our study was to examine the dietary effect of wild blueberries, and not isolated bioactive compounds, on vascular tone of the adult SHR. Our data provide clear evidence that the 8 week dietary treatment with 8% wild blueberry in the adult SHR with established endothelial dysfunction results in a significant moderation of the increased aortic vascular tone through an effect on the NO pathway under basal conditions and possibly the COX pathway under stimulated conditions, indicating the potential of the wild blueberry diet to ameliorate endothelial dysfunction and abnormalities of the SHR vascular environment.

ABBREVIATIONS USED

SHR, spontaneously hypertensive rat; C, control; WB, wild blueberry; NO, nitric oxide; NOS, nitric oxide synthase; COX, cyclooxygenase; Ach, acetylcholine; Phe, L-phenylephrine; L-NMMA, L-N^G-monomethyl arginine; MFA, mefenamic acid.

ACKNOWLEDGMENT

We thank the Wild Blueberry Association of North America (WBANA) for contributing the wild blueberries and FutureCeuticals (Momence, IL) for processing them. This is a Maine Agriculture and Forestry Station Scientific Contribution #3151.

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Received for review May 12, 2010. Revised manuscript received August 30, 2010. Accepted September 13, 2010. This work was supported by a WBANA grant to D.K.-Z. and a fellowship by the Greek State Scholarship Foundation (IKY) to A.S.K. This is Maine Agriculture and Forestry Experiment Station scientific contribution 3119.