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Altered active but not passive properties of mesenteric resistance arteries from the vitamin E-deprived rat

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1 We tested the hypothesis that lowering antioxidant protection through dietary vitamin E deprivation would alter active and passive mechanical properties in resistance arteries of the rat. Specifically, we hypothesized that vascular tone in isolated mesenteric arteries of the vitamin E-deprived rats would be altered due to impaired endothelial influences of nitric oxide and/or prostaglandins.

2 Lumen diameter and wall thickness were measured in pressurized arteries ($\approx$250 μm diameter) from control ($n=9$) and vitamin E deprived ($n=9$) Sprague-Dawley female rats by use of a dimension analysing system.

3 Treatment with a cyclo-oxygenase inhibitor (meclofenamate) did not affect the basal vascular tone in either group. Treatment with a nitric oxide synthase inhibitor (N^G-methyl-L-arginine) caused a significant increase in basal tone only in the vitamin E-deprived rats (% tone: 6.2 ± 1.1 vs 1.2 ± 0.3%; $P<0.05$). When tone was induced to 25% of the initial diameter with phenylephrine, treatment with the nitric oxide synthase inhibitor resulted in a greater potentiated tone in the vitamin E-deprived rats compared to the controls (26.5 ± 2.7 vs 16.4 ± 3.4%; $P<0.05$); suggesting a greater nitric oxide affect in the vessels from the vitamin E-deprived rats. Meclofenamate treatment in the induced tone arteries significantly relaxed (-17.4 ± 4.0%; $P<0.05$) only the arteries from the vitamin E-deprived rats, indicating that a vasoconstrictor was modifying tone. The passive characteristics of distensibility and stress-strain relationship were not different between the two groups of rats.

4 In summary, vitamin E deprivation in the rat enhanced the modulation of vascular tone by both the nitric oxide and cyclo-oxygenase pathways but did not alter passive characteristics of mesenteric arteries.

Keywords: Vitamin E deficiency; mesenteric arteries; endothelium; vascular; nitric oxide; prostaglandins; cyclo-oxygenase; eicosanoids

Introduction

An imbalance between pro-oxidant and anti-oxidant forces (oxidative stress) has been implicated as an aetiological factor in many vascular diseases, including atherosclerosis (Hennig & Chow, 1988), hypertension (Halliwell & Gutteridge, 1985) and preeclampsia, a disorder of pregnancy (Hubel *et al*., 1989b). In addition, vascular remodelling has been shown to occur in systemic hypertension (Cox, 1981), pulmonary hypertension (Langleben *et al*., 1988) and atherosclerosis (Schwartz *et al*., 1991). However, it is not known whether oxidative stress leads to vascular remodelling and altered vascular tone.

One experimental model for studying oxidative stress is vitamin deprivation in the rat. Vitamin E, is a potent antioxidant that scavenges peroxyl radicals in biological lipid phases such as cellular membranes (Tappel, 1980). Further, several epidemiological studies have provided correlative evidence for lower levels of vitamin E and risk for cardiovascular disease (Rimm *et al*., 1993; Stampfer *et al*., 1993). We (Hubel *et al*., 1989a; Davidge *et al*., 1993; 1994) and others (Rubino *et al*., 1993; 1994; Ralevic *et al*., 1995) have shown an impairment of endothelial-mediated vascular function in the vitamin E-deprived rat. In our previous study, using an isometric myograph system, we observed an endothelial-dependent increase in a cyclo-oxygenase-dependent vasoconstrictor and an enhanced release of an endothelial-dependent vasorelaxer (probably nitric oxide) modulating the vascular response in mesenteric arteries of the vitamin E-deprived rat (Davidge *et al*., 1993). This work indicated that lipid peroxidation influenced the vasoactive properties of the vessels. However, vascular tone and the passive properties of the arteries could not be accurately evaluated with this system. In the present study, we evaluated vascular tone and passive characteristics of the arteries using a pressurized system. The pressurized system has the advantage of distending arteries in a natural geometric shape while intraluminal pressure varies. The pressure-diameter curve can be measured directly rather than inferred from a length-tension curve produced with the isometric wire system. Therefore, the ability to study structure-function relationships of the vascular wall may be studied in greater detail.

The pressurized myograph system was used to test the hypothesis that lowering antioxidant protection through dietary vitamin E deprivation would alter active and passive mechanical properties in resistance arteries of the rat. Furthermore, we hypothesized that altered vascular tone in arteries of the vitamin E-deprived rats would be due to impaired endothelial influences of nitric oxide and/or prostaglandins.

Methods

Animal model

Ten week old female Sprague-Dawley rats were divided into two groups and housed at Magee-Womens Research Institute (American Association of Laboratory Animal Care accredited). One group ($n=9$) received a diet deficient of vitamin E while the other ($n=9$) received an equivalent diet supplemented with 50 iu ($\pm$)-α-tocopherol acetate (U.S. Biochemical

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Corp, Cleveland, OH) kg^{-1} diet. The animals were maintained on these diets for 10 weeks to deplete adequately plasma and tissue stores of α-tocopherol in the rats receiving the diet deficient of vitamin E (Bieri, 1972).

Assessment of peroxidative state

Rats were killed while under light anaesthesia with methohexitone sodium (50 mg kg^{-1} body weight) and blood was collected by heart puncture. Samples were kept at 4°C until centrifugation and sera were then stored frozen at −70°C until assayed. Serum vitamin E levels were analysed by high pressure liquid chromatography (h.p.l.c.) according to Bieri *et al*. (1979), as modified by Chow and Omaye (1981). In this modified assay the presence of the antioxidant butylated hydroxytoluene (0.024%) is utilized during the extraction with heptane. All samples were analysed in one run of the assay to avoid inter-assay variation. Malondialdehyde, a product of lipid peroxides detectable in sera, was used as an indicator of lipid peroxidation. Malondialdehyde concentrations were determined by h.p.l.c. with the technique of Wong *et al*. (1987). The detection limit of this assay was 0.15 μmol of malondialdehyde 1^{-1} sera. All samples were analysed in one run of the assay to avoid inter-assay variation.

Vessel preparation

A section of the mesentery 5 to 10 cm distal to the pylorus was rapidly removed and placed in ice cold N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulphonic acid] (HEPES) buffered physiological saline solution (HEPES-PSS). One mesenteric artery was dissected free from surrounding adipose tissue and cut into two. Arteries were transferred to a dual chamber arteriograph (Living Systems Instrumentation, Burlington, VT) and each vessel was mounted on two microcannulae. The cannulae rested in a 3 ml glass-jacketed organ baths with HEPES-PSS solution kept at 37°C. Residual blood was flushed from the lumen and the distal cannula was occluded to prevent flow. After the arteries were tied to the microcannulae, the arteriograph was placed on the stage of a compound microscope. The proximal cannula was joined in series with a pressure transducer connected to a servo-controlled peristalic pump. This allows a desired intraluminal pressure to be set. For the active tone studies, intraluminal pressure was set to 75 mmHg. A video camera on the microscope provides an image of the artery on a television monitor and measurements of lumen diameter and wall thickness were made by use of a video dimension analyser and direct observations with a filar. This vessel system has been described in detail elsewhere (Halpern *et al*., 1984).

Tone

Spontaneous or basal tone of the arteries was determined by the % difference of the diameter of the vessel in HEPES-PSS relative to the relaxed (presence of 10^{-4} M papaverine and EGTA) diameter of the artery. To determine the influence of vasoactive agents on tone, nitric oxide synthase and cyclooxygenase were inhibited by N^G-methyl-L-arginine (NMA: 0.1 mM) and meclofenamate (1 μM), respectively. Since there was little basal tone in these arteries *in vitro*, experiments were also conducted when tone was induced to 25% of the initial diameter with phenylephrine. Nitric oxide synthase and cyclooxygenase were then inhibited and changes in vascular tone were measured.

Passive mechanics

In order to compare distensibility of arteries between the vitamin E-deprived rats and the control rats, the active contractile activity must be eliminated. In this study, vascular smooth muscle was maximally deactivated by papaverine (10^{-4} M) and studies were conducted in buffer containing 0.1 mM ethylene glycol-bis(β-aminoethyl ether)- N, N, N′, N′-tetraacetic acid (EGTA) to remove the effect of extracellular calcium. The inactivation of smooth muscle contractile activity was confirmed by the lack of contraction during exposure to potassium (124 mM). Inner and outer diameters were measured at 11 pressures ranging from 0–150 mmHg. Passive pressure-diameter relationships were determined for the arteries and this information was used for subsequent calculations (Mackey *et al*., 1992). Distensibility is defined as the relative change in diameter per unit change in pressure. The stress-strain relationship was also compared to evaluate further the passive mechanical properties of the arteries. These parameters were normalized for wall thickness and therefore characterized the stiffness of the components that comprise the vascular wall. Stress was defined as the force exerted on the vascular wall per unit of tissue and was calculated by the following equation: $\text{stress} = (P \times D)/2T$ where P is pressure in dynes cm^{-2}, D is diameter, and T is wall thickness. Circumferential strain represents the response of an artery to the force or intraluminal pressure it experiences. Strain was calculated as (D2-D1)/D1 where D1 is the initial diameter at a pressure of 3 mmHg and D2 is the initial diameter at the new pressure.

Statistics

Data are presented as the mean ± s.e. Student's two-tailed *t* test was used to determine the statistical difference of the parameters between the control and vitamin E-deprived rats shown in Table 1. Analysis of variance with repeated measures was applied for analysis of tone data and passive mechanics. Where appropriate, *post-hoc* analysis used Fisher's protected least significant difference. Differences with $P < 0.05$ were considered significant.

Results

Animal model

There was no difference in body weights between the vitamin E-deprived and control rats, indicating that growth was not impaired by vitamin E deprivation. As expected, serum vitamin E was significantly lower in the vitamin E-deprived group than in the controls. Sera malondialdehyde concentrations were elevated in the vitamin E-deprived rats indicating an enhanced peroxidative state (Table 1).

Table 1 Animal model: control and vitamin E-deprived rats

Group	n	*Weight* (g)	*Sera Vitamin-E* (μg^{-1})	*Malondialdehyde* (μmol^{-1})
Control	9	319.6 ± 6.9	14.57 ± 1.7	3.39 ± 0.21
Vitamin E-deprived	9	318.2 ± 7.1	2.48 ± 0.32*	4.02 ± 0.17*

Values are mean ± s.e.mean of *n* rats; *$P < 0.05$.

Tone

Spontaneous (basal) tone was not present in the arteries from either control or vitamin E-deprived rats ($1.0 \pm 0.99\%$ vs $1.2 \pm 0.3\%$). Treatment with the nitric oxide synthase inhibitor, NMA, caused a significant increase in basal tone only in the vitamin E-deprived rats (% tone: 6.2 ± 1.1 vs $1.2 \pm 0.3\%$; $P < 0.05$) resulting in a significant difference in tone between the arteries from the control and vitamin E-deprived rats (Figure 1). Treatment with a cyclo-oxygenase inhibitor, meclofenamate, did not affect the basal vascular tone in either group (Figure 1).

Arteries were induced to have 25% tone with phenylephrine (control $= 25.3 \pm 1.9\%$ vs vitamin E-deprived $25.6 \pm 1.3\%$). The dose of phenylephrine needed to produce tone was not different between control ($2.7 \pm 0.75 \times 10^{-7}$ M) and vitamin E-deprived ($4.4 \pm 0.74 \times 10^{-7}$ M) groups. NMA potentiated tone in both groups. However, the increase in tone was significantly greater in the vitamin E-deprived rats compared to the controls ((26.5 ± 2.7 vs $16.4 \pm 3.4\%$; $P < 0.05$); (Figure 2)). These data suggest that vessels from the vitamin E-deprived rats had a greater modification of basal and induced tone by nitric oxide. Treatment of the arteries with induced tone with a cyclo-oxygenase inhibitor significantly relaxed ($-17.4 \pm 4.0\%$; $P < 0.05$) only the arteries from the vitamin E-deprived rats, resulting in a significant difference in tone of the arteries from the control and vitamin E-deprived rats (Figure 2). These data suggest that only the arteries of the vitamin E-deprived rats demonstrated a cyclo-oxygenase-dependent vasoconstriction that was modifying tone.

Passive mechanics

Data from these experiments include the pressure-diameter relationships obtained for arteries from control and vitamin E-deprived rats. This information was used to characterize distensibility and stress-strain relationships for the two groups.

The calculated passive components of the arteries were not different in arteries from control and vitamin E-deprived rats (Figure 3). Distensibility which reflects the relative change in diameter per unit change in pressure was not different for the arteries of the vitamin E-deprived rats compared to the control animals (Figure 3a). Furthermore, the mean diameter response to a particular stress or force experienced per unit of tissue in the vascular wall (stress-strain curves) was similar for the two groups (Figure 3b).

Discussion

The purpose of this study was to characterize the effect of vitamin E-deprivation in the rat on vascular wall mechanics in resistance-sized mesenteric arteries. We hypothesized that lowering antioxidant protection through dietary vitamin E deprivation would alter passive mechanical properties and vascular tone in isolated mesenteric arteries. This study demonstrated that vitamin E deprivation in the rat did not alter passive characteristics of mesenteric arteries, but enhanced the modulation of vascular tone by both nitric oxide and a cyclo-oxygenase-dependent vasoconstrictor.

A vitamin E-deprived rat model was utilized to study the effects of a nutritionally-mediated oxidative stress on the vascular system. This model was chosen since vitamin E is a potent cellular membrane antioxidant and its major recognized functions to interrupt the free radical chain process of lipid peroxidation (Tappel, 1980). Furthermore,

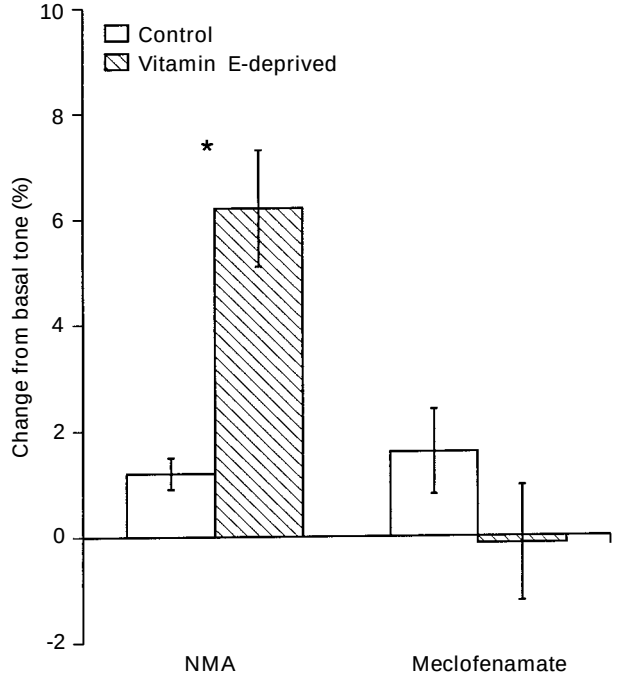

Figure 1 The % change from basal (spontaneous) tone in the absence or presence of 0.1 mM N^G-methyl-L-arginine (NMA; an inhibitor of nitric oxide synthase) or 1 μM meclofenamate (an inhibitor of cyclo-oxygenase) in mesenteric arteries from control ($n = 5$) and vitamin E-deprived ($n = 5$) rats. Columns represent mean $\pm$ s.e.mean. *$P < 0.05$ control vs vitamin E deficient.

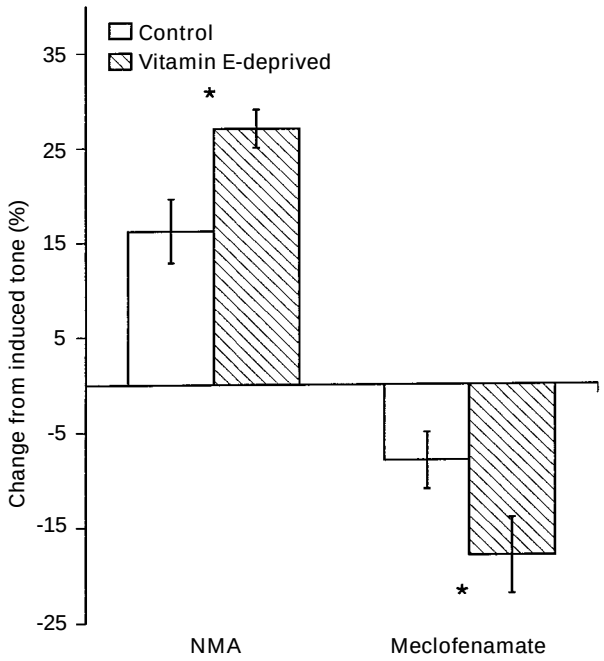

Figure 2 The % change from induced tone in the absence or presence of 0.1 mM N^G-methyl-L-arginine (NMA; an inhibitor of nitric oxide synthase) or 1 μM meclofenamate (an inhibitor of cyclo-oxygenase) in mesenteric arteries from control ($n = 5$) and vitamin E-deprived ($n = 5$) rats. Tone was induced to twenty-five % with phenylephrine (control $= 25.3 \pm 1.9\%$ vs vitamin E-deprived $25.6 \pm 1.3\%$). The dose of phenylephrine needed to produce tone was not different between control ($2.7 \pm 0.75 \times 10^{-7}$ M) and vitamin E-deprived ($4.4 \pm 0.74 \times 10^{-7}$ M) groups. Columns represent mean $\pm$ s.e.mean. *$P < 0.05$ control vs vitamin E deficient.

vitamin E may have a protective effect against cardiovascular disease (Rimm *et al*., 1993; Stampfer *et al*., 1993; Rapola *et al*., 1996), although the mechanism for the effect has not been documented. In the present study, deprivation of the antioxidant vitamin E resulted in elevated sera lipid peroxide levels, as measured by malondialdehyde, suggesting that oxidative stress was achieved in this model. The resistance-sized arteries from the splanchnic circulation were used in these studies, since this vascular bed is a major component in the regulation of peripheral vascular resistance.

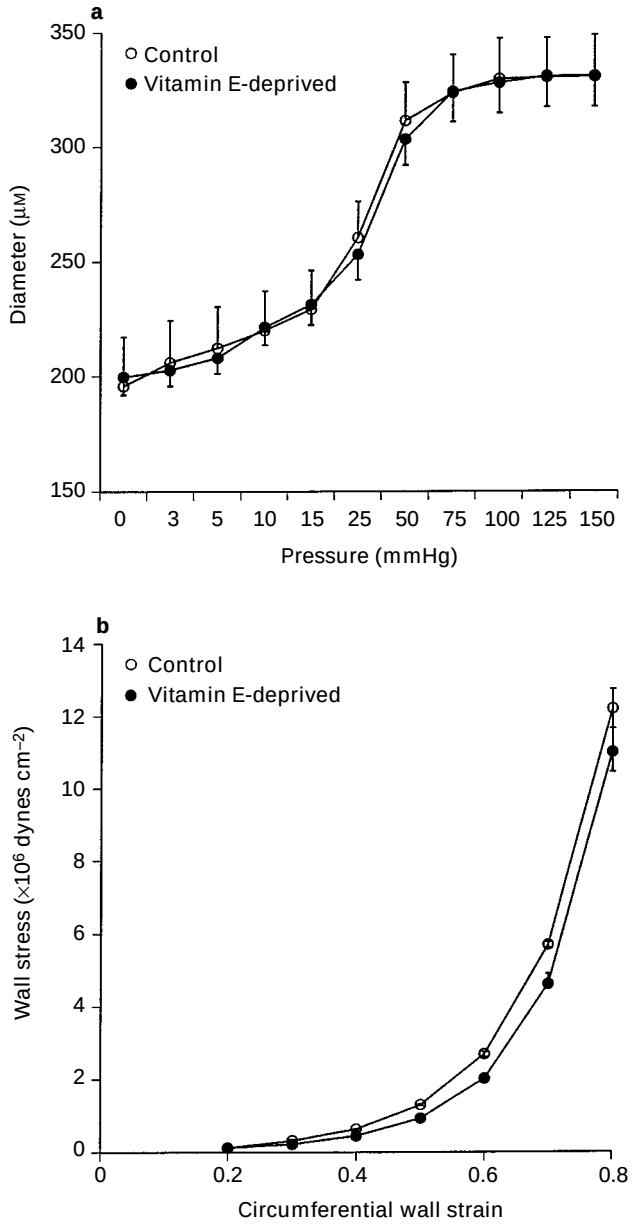

Figure 3 (a) Distensibility or the relative change in diameter per unit change in pressure of mesenteric arteries isolated from control ($n=9$) and vitamin E-deprived rats ($n=9$). These studies were conducted in the presence of papaverine (10^{-4} M) and calcium-free buffer containing of 0.1 mM EGTA in order to remove active contractile activity. Inner and outer diameters were measured at 11 pressures ranging from 0–150 mmHg. (b) Stress-strain relationships were compared for mesenteric arteries isolated from control ($n=9$) and vitamin E-deprived ($n=9$) rats. These parameters were normalized for wall thickness and therefore characterized the stiffness of the components that comprise the vascular wall. Stress was defined as the force exerted on the vascular wall per unit of tissue. Circumferential strain represents the response of an artery to the force or intraluminal pressure it experiences. In (a) and (b), data represent mean and vertical lines show s.e.mean.

Since vascular remodelling has been described in diseases associated with oxidative stress (Cox, 1981; Langleben *et al*., 1988; Schwartz *et al*., 1990), we hypothesized that oxidative stress due to vitamin E deprivation would lead to vascular remodelling. Contrary to our hypothesis, arterial distensibility and wall stiffness were not different in the arteries of the vitamin E-deprived rats compared to arteries from control rats. These data indicate that short-term (10 weeks) vitamin E deprivation did not affect passive characteristics of arteries, although the antioxidant protection by vitamin E was reduced in these animals, as evidenced by elevated malondialdehyde levels. We chose to have these animals on a vitamin E deficient diet for 10 weeks, since this allows for depletion of the antioxidant properties of vitamin E without the confounding effects of chronic vitamin E deficiency or age-related vascular changes. Indeed with this model of short-term vitamin E deficiency, we had previously observed altered vasoactive properties of the arteries (Davidge *et al*., 1993; 1994). However, from the present study it does not appear that passive vascular remodelling occurs.

We also studied whether vascular tone was altered by vitamin E deficiency. Although initially there was no basal (spontaneous) tone in either group of animals, nitric oxide inhibition enhanced tone which was significantly greater in the vitamin E-deprived rats. These data indicate a greater modulation of vascular tone by nitric oxide in the vitamin E-deprived group. Treatment with a cyclo-oxygenase inhibitor did not have an observable effect on basal vascular tone in either group of animals, suggesting that a cyclo-oxygenase-dependent vasorelaxant was not modifying tone. However, since little spontaneous tone was observed with these arteries, we could not have been able to detect a modulation of vascular tone by a vasoconstrictor. When arteries are removed from the animal and placed in organ baths, they are removed from factors, such as circulating hormones and nerves, that contribute to tone *in vivo*. Therefore, tone was induced in the arteries with phenylephrine, in order to evaluate modulation that could occur by relaxation of the artery. Treatment with a nitric oxide synthase inhibitor potentiated phenylephrine-induced tone in both groups. However, the increase in tone was significantly greater in the vitamin E-deprived rats compared to the controls, indicating a greater influence of nitric oxide in the arteries of the vitamin E-deprived rats. Treatment, of the arteries with induced tone, with a cyclo-oxygenase inhibitor significantly relaxed only the arteries from the vitamin E-deprived rats, indicating that only the arteries of the vitamin E-deprived rats demonstrated a cyclo-oxygenase-dependent vasoconstriction that was modifying tone.

It is not clear how changes in the oxidative environment affect nitric oxide synthase activity or nitric oxide release. Previous work from our laboratory observed a potentiated response to the endothelium-dependent agent, methacholine in arteries from the vitamin E-deprived rats in the presence of a cyclo-oxygenase inhibitor (Davidge *et al*., 1993). It appears that a cyclo-oxygenase-dependent vasoconstrictor was masking an increased endothelial-dependent vasorelaxant response (likely nitric oxide) in the arteries of the vitamin E-deprived rats. One earlier study by Rubanyi and Vanhoutte (1986a) demonstrated relaxation responses to the oxidative product, hydrogen peroxide in canine coronary arterial rings, that was proposed to be due to an increase production/release of an endothelial-dependent relaxing

factor (nitric oxide). However, in contrast, other studies have demonstrated that a variety of reactive oxygen species can inhibit nitric oxide responses (Gryglewski *et al*., 1986; Rubanyi & Vanhoutte, 1986b; Marczin *et al*., 1992; Todoki *et al*., 1992).

Along with a greater nitric oxide influence on vascular tone, arteries from the vitamin E-deprived rats had a greater modulation of induced-tone by a cyclo-oxygenase-dependent vasoconstrictor. There is some precedence in the literature for a cyclo-oxygenease-dependent vasoconstrictor modifying endothelial-dependent relaxation responses in rat models of hypertension (Diederich *et al*., 1990; Iwama *et al*., 1992) and vitamin E deprivation (Davidge *et al*., 1993). Our present study confirms and extends these observations with evidence of a cyclo-oxygenase vasoconstrictor modifying tone in the arteries of the vitamin E-deprived animal.

Our observations of enhanced modulation of vascular tone by both nitric oxide and a cyclo-oxygenase-dependent vasoconstrictor in the vitamin E-deprived rat remains intriguing, especially with the information regarding nitric oxide-mediated activation of cyclo-oxygenase (Davidge *et al*., 1995) along with the evidence of peroxynitrite activation of the cyclo-oxygenase enzyme (Landino *et al*., 1996). Perhaps in this model of vitamin E deprivation, we are observing an increase in nitric oxide in the presence of oxidative stress leading to a cyclo-oxygenase-dependent vasoconstrictor. We speculate that the increase in the cyclo-oxygenase activation is through peroxynitrite and provides a mechanism that needs to be examined in further detail in conditions that involve oxidative stress and pathologies of the vasculature.

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