Effects of Docosahexaenoic Acid on Vascular Pathology and Reactivity in Hypertension

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Previous studies have shown that docosahexaenoic acid (DHA) has an antihypertensive effect in spontaneously hypertensive rats (SHR). To investigate possible mechanisms for this effect, vascular pathology and reactivity were determined in SHR treated with dietary DHA. SHR (7 weeks) were fed a purified diet with either a combination of corn/soybean oils or a DHA-enriched oil for 6 weeks. Histological evaluation of heart tissue, aorta, coronary, and renal arteries was performed. Vascular responses were determined in isolated aortic rings. Contractile responses to agonists, including norepinephrine (10^{-9} to 10^{-4} M), potassium chloride (5–55 mM), and angiotensin II (5×10^{-7} M) were assessed. Vasorelaxant responses to acetylcholine (10^{-9} to 10^{-4} M), sodium nitroprusside (10^{-9} to 10^{-6} M), papaverine (10^{-5} to 10^{-4} M), and methoxyverapamil (D600, 1–100 μM) were determined. DHA-fed SHR had significantly reduced blood pressure ($P < 0.001$) and vascular wall thicknesses in the coronary, thoracic, and abdominal aorta compared with controls ($P < 0.05$) Contractile responses to agonists mediated by receptor stimulation and potassium depolarization were not altered in DHA-fed SHR. Endothelial-dependent relaxations to acetylcholine were not altered which suggests endothelial-derived nitric oxide production/release is not affected by dietary DHA. Other mechanisms of vascular relaxation, including intracellular cyclic nucleotides, cGMP, and cAMP were not altered by dietary DHA because aortic relaxant responses to sodium nitroprusside and papaverine were similar in control and DHA-fed SHR. No significant differences were seen in relaxant responses to the calcium channel blocker, D600, or contractile responses to norepinephrine in the absence of extracellular calcium. These results suggest that dietary DHA does not affect mechanisms related to extracellular calcium channels or intracellular calcium mobilization. Moreover, the contractile and vasorelaxant responses are not differentially altered with dietary DHA in this *in vivo* SHR model. The findings demonstrate that dietary DHA reduces systolic blood pressure and vascular wall thickness in SHR. This may contribute to decrease arterial stiffness and pulse pressure, in addition to the antihypertensive properties of DHA. The antihypertensive properties of DHA are not related to alterations in vascular responses. Exp Biol Med 228:299–307, 2003

Key words: omega-3 fatty acids; fish oil; endothelium; vascular smooth muscle; artery

It has been established in human and animal studies that dietary fish oil rich in ω-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) has a blood pressure-lowering effect in hypertension (1–6). Increasing evidence suggests that DHA alone has antihypertensive properties (7–9). DHA administered in the diet to spontaneously hypertensive rats (SHR) increases the levels of ω-3 fatty acids, replacing ω-6 fatty acids in vascular tissue and organs (10). Alterations in vascular fatty acid composition in hypertension may affect vascular structure and contractile processes important to blood pressure regulation. Hypertensive vasculature exhibits endothelial dysfunction and increased levels of intracellular free calcium (11–14). In addition, there is a reduction in vascular smooth muscle cell membrane fluidity associated with hypertension (15). These characteristics may contribute to an increase in peripheral resistance and high blood pressure.

Dietary supplementation with DHA may result in the incorporation of ω-3 fatty acids into vascular smooth muscle cells. The subsequent increase in membrane fluidity may affect properties involved in contraction including: ion transport, receptor activities, and electrical potentials. In the hypertensive rat, possible mechanisms for DHA's blood pressure-lowering effects may be related to reduced vascular reactivity to norepinephrine (NE; Refs. 5, 16), blunting of the renin–angiotensin–aldosterone system by decreasing adrenal synthesis of aldosterone (9), changes in renal ara-

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chidonic acid metabolism (9), modulation of calcium release and influx in vascular smooth muscle cells, and activation of vascular ATP-sensitive potassium channels by vasodilatory prostanoids (17).

To our knowledge, the effects of dietary DHA on vascular morphology are not known. A previous study showed dietary fish oil reduced right ventricular hypertrophy and pulmonary arterial muscularization in a hypoxia-induced rat model of pulmonary hypertension (18). It is possible that dietary DHA may affect the structural components of arteries by modifying the fatty acid composition of the arterial wall. A thinner vascular wall may contribute to alter the mechanical properties of large arteries.

The present study was designed to determine the effects of dietary DHA on vascular pathology and reactivity in the hypertensive rat model. We evaluated contractions induced by NE, potassium chloride (KCL), and angiotensin II (ANG II) as well as endothelial-dependent relaxations mediated by acetylcholine in thoracic aortae of SHR, with and without DHA dietary intervention. Relaxations mediated by intracellular cyclic nucleotides and modulation of extracellular and intracellular calcium in SHR vascular responses were also assessed.

Materials and Methods

Animals and Diet Preparation. Male SHR aged 7 weeks were purchased from Harlan (Indianapolis, IN). They were housed at constant temperature (26°C), humidity (60%), and lighting (12:12-hr light/dark cycle) and randomly assigned to one of two dietary groups. The diets were prepared with a fat-free basal mix (Research Diets, Inc., New Brunswick, NJ) and enriched with either a combination of corn (25 g%) and soybean oils (25 g%; control, CSO) or corn (15 g%), soybean (15 g%), and DHA oil (20 g%; DHASCO®, Martek Biosciences Corp., Columbia, MD). The diets contained similar proportions of saturated, monounsaturated and polyunsaturated fatty acids with DHA (8.8%) provided in the experimental diet. The dietary ingredients and fatty acid composition have been reported previously (10). The diets were provided fresh daily and stored at 0°C. The animals were fed one of the two diets for 6 weeks. All experimental procedures were reviewed and conducted in accordance with the guidelines of the Committee on Animal Research at the University of California, San Francisco.

Blood Pressure Measurement. Systolic blood pressure was determined at room temperature by a photoelectric tail cuff system (Model 179, IITC, Inc., Woodland Hills, CA). Values are presented as the average of three separate measurements.

Vascular Reactivity Studies. After the dietary treatments, anesthesia was administered with a mixture of halothane (5%), oxygen (70%), and nitrous oxide (30%). Thoracic aortae were excised rapidly, trimmed of adhering tissues and cut into rings (3 mm in length). The rings were mounted in tissue baths (Radnoti Glass Technology Inc., Monrovia, CA) and submersed in Krebs-Ringer bicarbonate solution (composition in mM): 118.3 NaCl, 4.7 KCL, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25.0 $NaHCO_3$, and 11.1 glucose. The solution (pH 7.4) was constantly aerated with oxygen (95%) and carbon dioxide (5%) and warmed to 37°C. Force displacement transducers (Grass FT03, Grass Instrument Co., Quincy, MA) coupled to an eight-channel chartwriter (model WR3701, Western Graphtec, Inc., Irvine, CA) were used to record isometric tension. A computerized system (PO-NE-MAH, Gould, Inc., Cleveland, OH) was used for data acquisition. Tension adjustments and bath washes were automated and controlled (STC 400, Buxco Electronic Inc., Troy, NY). Aortic rings were equilibrated for 60–90 min before each experiment and were maintained at an optimal resting tension of 2 g. Tissue viability was assessed with KCL (30 mM) and the integrity of the endothelium was assessed by acetylcholine-induced (1 μM) relaxation.

The first series of experiments included cumulative concentration-response curves to the contractile agonists, NE (10^{-9} to 10^{-4} M) and KCL (5–55 mM). Contraction in response to a single physiological concentration of ANG II (5×10^{-7} M) was also studied. NE and KCL-induced contractions are mediated by different mechanisms, i.e., pharmacomechanical coupling and receptor-operated calcium channels, and electromechanical coupling and voltage-dependent calcium channels, respectively. KCL-induced contractions were generated in the presence of phentolamine (1 μM) to prevent release of endogenous catecholamines. ANG II-induced contractions are mediated by ANG II type I receptor (AT_1 R) stimulation of phospholipase C and production of inositol phosphates.

In the second series of experiments, the vasorelaxant responses to acetylcholine (ACH,10^{-9} to 10^{-4} M), sodium nitroprusside (SNP, 10^{-9} to 10^{-6} M), papaverine (PAP, 10^{-5} to 10^{-4} M) were determined. The relaxation induced by ACH is dependent on intact endothelium; whereas SNP and PAP act directly on vascular smooth muscle to increase intracellular nucleotides, cGMP and cAMP, respectively.

In the last set of experiments, the effects of methoxyverapamil (D600, 1–100 μM) were determined. D600 induces vasorelaxation by acting as an extracellular calcium channel antagonist in smooth muscle. Biphasic contractions (phasic, tonic) to NE (10^{-6} M) in calcium-free medium containing ethylene glycol-bis (β-aminoethylether)-*N,N'*-tetraacetic acid (EGTA, 2 mM), a calcium chelating agent, were also investigated. Aortic rings were equilibrated initially in Krebs solution with $CaCl_2$ (2.5 mM) and were then washed four times at 4-min intervals for a total of 20 min in Ca^{2+}-free EGTA-containing solution. Administration of NE under these conditions induces biphasic contractile responses (phasic, tonic) both due to calcium release from intracellular Ca^{2+} pools, i.e., sarcoplasmic reticulum and plasma membrane Ca^{2+} storage sites, in the rat aorta.

Chemicals. The chemicals used in these studies were obtained from Sigma Chemical Co. (St. Louis, MO).

Histological Studies. After the dietary treatment, anesthesia was administered and the heart, aorta, and renal arteries were excised, trimmed of adhering tissues, placed in buffered formalin (10%), and fixed. The tissues were weighed after the fixation process. Specimens from each animal were paraffin embedded and thick sections (4 μm) were stained with hematoxylin eosin for light microscopy studies. The Masson trichrome stain and the Verhoff's stain were used to evaluate organ collagen content and expression and distribution of elastin fibers, respectively. The histological evaluation of heart tissue was performed in a semiquantitative manner as previously described (19–23), which measures, in particular, the aspect and the thickness of small arteries and arterioles. The hearts were cut according to the "bread loaf procedure," and in representative sections of each organ. All of the coronary arteries with an external diameter ranging from 20–200 micron were photographed so that the mean arterial thickness could be determined by a computerized image analysis. which automatically corrects the vertical and longitudinal arterial diameters when the vascular sections are elliptical. Two pathologists blinded to the experimental protocol reviewed all of the slides and the measurements in the photographs and their scores were averaged to obtain a single score. The same procedure was adopted for evaluations of aortic and renal artery wall thickness and for the count of the elastic fibers (Verhoff's staining) in these vessels.

Statistics. Statistical significance was determined by the Student's *t* test for unpaired observations. One-way analysis of variance (ANOVA) was performed for multiple comparisons followed by the Scheffe procedure for statistically significant F values. The Kruskal-Wallis test for group comparisons was used when the assumption of equal variances required for the ANOVA was not satisfied. For comparisons of concentration–response curves, repeated-measures ANOVA with Greenhouse-Geisser adjustment for multi-sample asphericity was used to avoid excessive Type I error (24). Significance criteria was set at 0.5 and when multiple comparisons were performed at each concentration, the test of simple main effects with Bonferroni correction was used, e.g., 0.05/5 = 0.01. All results are expressed as means ± SEM with n = the number of rats. Two to three aortic rings per rat were used on average for each permutation. Relaxations are expressed as a percentage of the maximum tension produced by the contractile agonists. EC_{50} values represent the negative logarithm of the molar concentration of NE causing 50% of the maximal contraction. For the histological studies, statistical evaluation of all data was performed using the *t* test for independent samples according to a program formulated by Statistica® L Statistica for windows (volume 1–5, Stat Soft Inc., 1995, Tulsa, OK).

Results

Blood Pressure. Systolic blood pressure was significantly lower in DHA-fed SHR compared with CSO-fed SHR at 3–6 weeks (Table I).

Table I. Systolic Blood Pressure in SHRs Fed a Control (CSO) or DHA-enriched Diet

Time	Systolic blood pressure (mm Hg)	
	CSO	DHA
Baseline	119 ± 1.8	118 ± 1.8
Week 1	126 ± 2.2	122 ± 2.3
Week 2	150 ± 4.3	138 ± 3.8
Week 3	165 ± 2.3	146 ± 3.5**
Week 4	185 ± 3.9	173 ± 4.4*
Week 5	201 ± 1.9	170 ± 2.9**
Week 6	201 ± 4.1	165 ± 2.9**

Values represent mean ± SEM; n = 10 per group; *P < 0.05; **P < 0.001.

Vascular Reactivity Studies. *Contractile Response to NE, KCL, and ANG II.* As seen in Figure 1, NE (10^{-9} to 10^{-4} M)-induced contractions were not significantly (NS) different in aortic rings from the control (37 ± 7 to 1413 ± 122 mg, n = 9) and DHA group (44 ± 20 to 1354 ± 79 mg, n = 9). Comparable EC_{50} values were seen in both groups (control, −6.580 ± 0.12, n = 9; DHA, −6.645 ± 0.18, n = 9, NS). Cumulative concentration–response curves to KCL (5–55 mM) also reflected no significant differences between the 2 groups (control, 32 ± 10 to 745 ± 78 mg, n = 9; DHA, 10 ± 5 to 646 ± 48 mg, n = 9) as seen in Figure 2. ANG II (5×10^{-7} M)-induced contractile responses (Fig. 3) were 475 ± 45 mg in the control (n = 10) and 481 ± 47 mg in the DHA (n = 10) group (NS).

Vasorelaxant Responses to ACH, SNP, PAP, and D600. Comparable concentration-response curves to ACH, SNP, PAP, and D600 were seen in both groups (NS). ACH (10^{-9} to 10^{-4} M)-induced relaxant responses (Fig. 4) in KCL (30 mM)-contracted rings were −8 ± 8 to −611 ± 31 mg, n = 8 in the control group and −15 ± 9 to −706 ± 43 mg, n = 8 in the DHA group (NS). Sodium nitroprusside (10^{-9} to 10^{-6} M)-induced responses (Fig. 5) to NE (10^{-6} M)-contracted rings were −243 ± 65 to −1752 ± 110 mg, n = 10 in the control and −242 ± 16 to −1728 ± 157 mg, n = 10 in the DHA group (NS). Papaverine (10^{-5} to 10^{-4} M)-

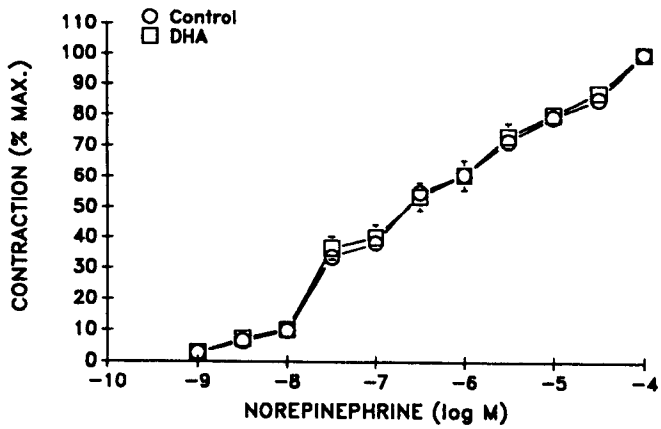

Figure 1. Contractile response to NE in SHR aorta. Data are expressed as the percentage of the maximum contraction induced by NE. Values represent mean %, n = 5–9 rats.

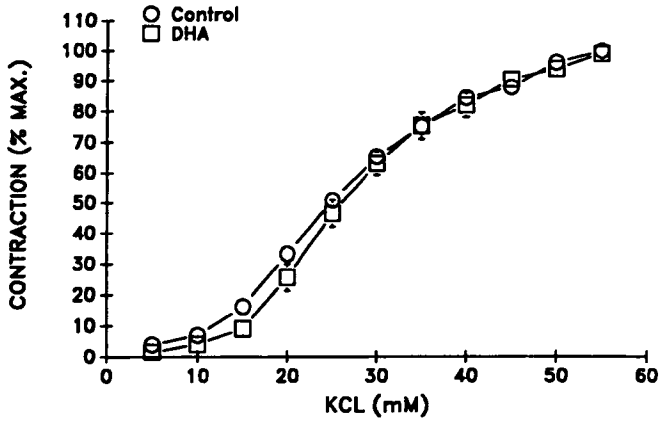

Figure 2. Contractile response to KCL in SHR aorta. Data are expressed as the percentage of the maximum contraction induced by KCL. KCL-contracted rings were pretreated with phentolamine (1 μM, 20 min) to prevent endogenous release of catecholamines. Values represent the mean %, n = 5–9 rats.

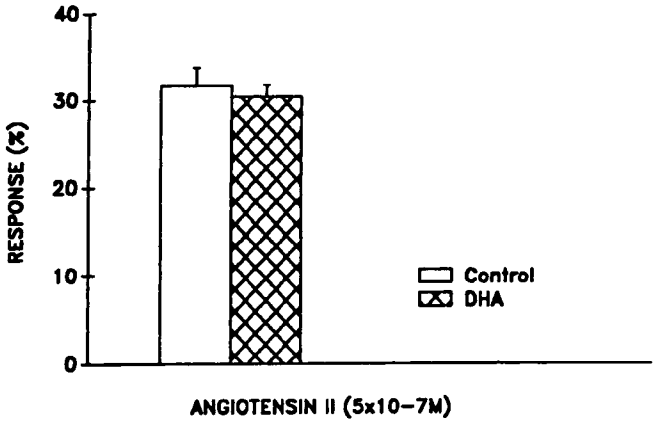

Figure 3. Contractile response to ANG II in SHR aorta. Data are expressed as percentage of the NE-induced (10^{-6} M) contraction. Values represent the mean %, n = 10 rats per group.

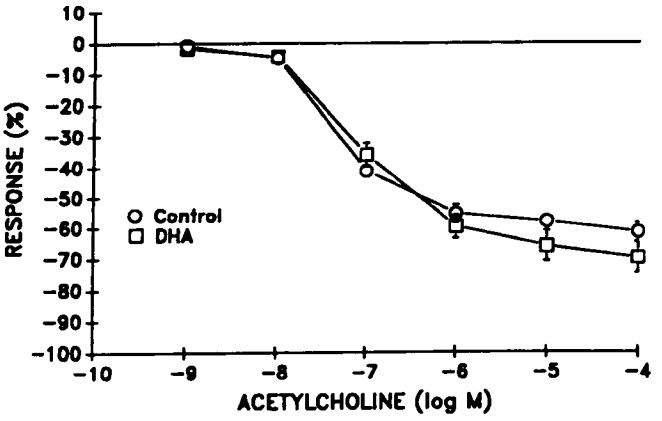

Figure 4. Relaxant response to ACH in KCL-contracted SHR aorta. Data are expressed as the percentage of KCL-induced (30 mM) contractions. Values are the mean %, n = 8 rats for each group.

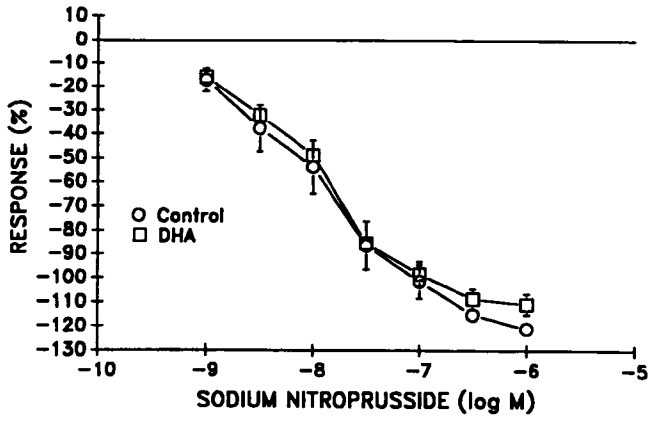

Figure 5. Relaxant response to sodium nitroprusside in NE-contracted SHR aorta. Data are expressed as the percentage of NE-induced (10^{-6} M) contractions. Values are the mean %, n = 4 rats for each group.

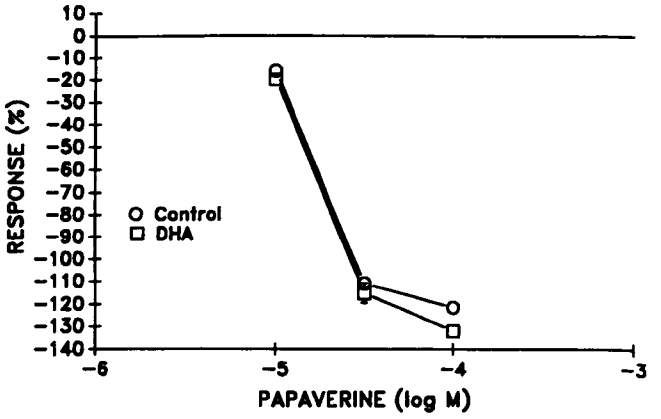

Figure 6. Relaxant response to PNP in NE-contracted SHR aorta. Data are expressed as the percentage of NE-induced (10^{-6} M) contractions. Values are the mean %, n = 3 rats for each group.

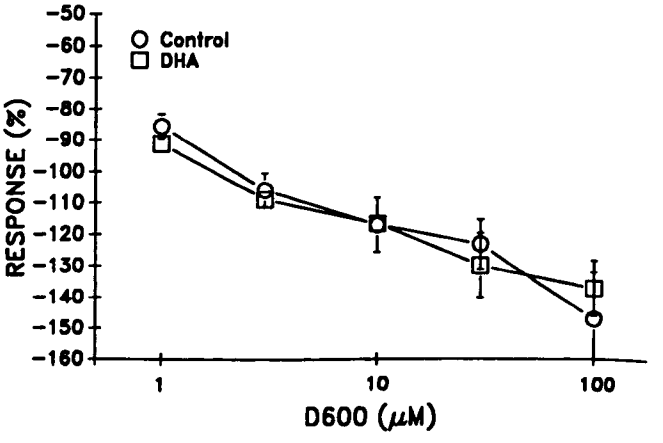

Figure 7. Relaxant response to D600 in KCL-contracted SHR aorta. Data are expressed as the percentage of KCL-induced (55mM) contractions. Values are the mean %, n = 9 rats for each group.

induced responses (Fig. 6) in NE (10^{-6} M)-contracted rings were −280 ± 13 to −1753 ± 84 mg, n = 3 in the control group and −277 ± 49 to −1822 ± 163 mg, n = 3 in the DHA group (NS). D600 (1–100 μM)-induced relaxant responses (Fig. 7) in KCL (55mM)-contracted rings were −621 ± 48 to −1023 ± 55 mg, n = 9 in the control group and −587±47 to −871 ± 69 mg, n = 9 in the DHA group (NS).

Contractile Responses to NE in the Absence of Extracellular Calcium. Phasic and tonic contractile responses (Fig. 8) to NE (10^{-6} M)-induced contractions were

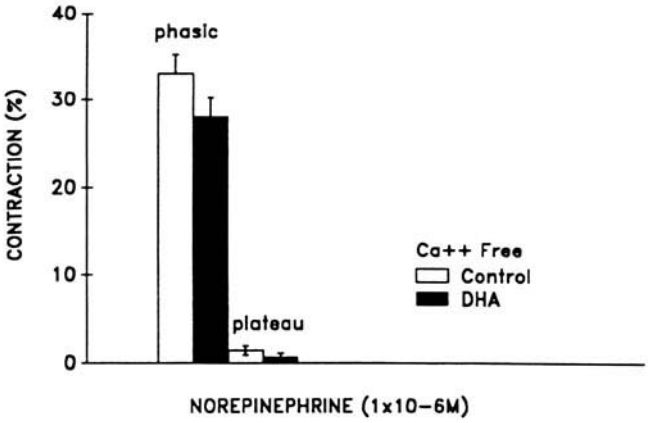

Figure 8. Contractile response to NE in the absence of extracellular calcium. Data are expressed as percentage of NE-induced (10^{-6} M) in Krebs (Ca^{2+}-containing) solution. Values are the mean%, $n = 8$–10 rats for each group.

not significantly different among the two groups. The contractile responses to NE in the control group were 493 ± 55 mg (phasic), 19.7 ± 6 mg (tonic), $n = 8$ and 436 ±3 2 mg (phasic), 11 ± 6 mg (tonic), $n = 10$ in the DHA group (NS).

Histological Studies. *Heart.* No significant changes were observed in the myocardial structure of the control SHR. However, several branches of the coronary arteries independently from their location but especially the descending ones showed moderate to marked hypertrophy of the media and, occasionally, minimal periadvential edema. The vascular thickness resulted in the reduction of their lumens. This thickening was not observed in the arteries of SHR treated with DHA, which had a rather thin media, well patent lumen, and some more evident periadvential edema. The decreased thickness of the media of these vessels resulted in a statistically significant difference between the two groups (Table II). Representative arteries from SHR in both dietary groups are shown in Figure 9. Arterioles of the papillary muscles were also thicker in the DHA-fed SHR.

Aorta. The aortic wall of the DHA-fed SHR was thinner than that of their control counterparts and it contained a smaller number of elastin bands (Fig. 10 A and B). This was also true for both aortic sections of the thoracic region as well as that of the abdominal region.

Renal Arteries. No difference was observed in the thickness of the wall of the renal arteries when the DHA-fed SHR were compared with the control-fed SHR.

Trichrome Staining. No differences were observed in the two groups for the expression of connective tissue either in the hearts or in the large vessels.

Body and Organ Weights: Vascular Wall Thickness. The body weights as well as the weights of the heart, aorta and renal arteries are summarized in Table II. DHA treatment resulted in a small nonsignificant increase in body weight whereas the weight of the heart, aorta, and renal arteries were slightly decreased compared to control SHR.

There was a statistically significant reduction in the wall thickness of the coronary arteries and aorta. The measurements for the aortae were taken at two different sections of the organ: thoracic and abdominal (Table II). The DHA-fed SHR also had a lower number of aortic elastin bands than the control SHR. The wall thickness of the renal artery was slightly lower in DHA-fed SHR, but the difference was not statistically significant.

Table II. Weights and Histological Parameters of Control (CSO) and DHA-fed SHR

Parameters	Control (CSO) diet	DHA diet
Body weight (g)	311 ± 7	321 ± 4
Heart weight/mg 100 g body weight	415 ± 21	386 ± 36
Aorta weight/mg 100 g body weight	24 ± 2	22 ± 2
Renal artery weight/100 g body weight	4.90 ± 1.00	5.20 ± 1.00
Coronary artery wall thickness (μm)	2.43 ± 0.24	1.92 ± 0.09*
Thoracic aortic wall thickness (μm)	9.30 ± 0.33	6.94 ± 0.36**
Abdominal aortic wall thickness (μm)	9.00 ± 0.30	7.00 ± 0.50**
Aortic elastin bands (number)	12.40 ± 0.80	9.50 ± 0.30**
Renal artery wall thickness (μm)	1.75 ± 0.95	1.25 ± 0.60

Values represent mean ± SEM; $n = 7$–9 per group except for renal artery, $n = 3$–4 per group; $^*P < 0.05$; $^{**}P < 0.01$. CSO, corn and soybean oils.

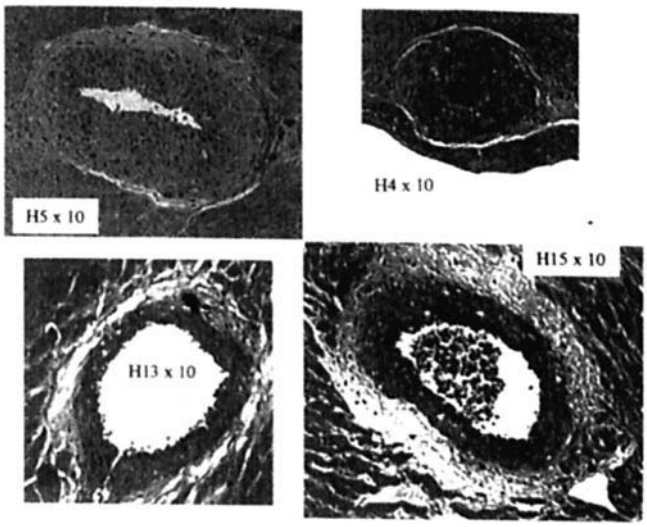

Figure 9. Representative sections of the coronary arteries of controls (H 5 and H 4) and DHA-fed SHR (H 13 and H 15). The wall of the arteries of the controls is thick and the lumen is reduced. The thickness mostly affects the intima of the vessels. Minimal periadventitial edema is evident. The coronary artery wall is thinner and the lumen is wider in DHA-fed SHR. Some periadventitial edema is also present. Staining: hematoxylin & eosin. Magnification: 10x.

Discussion

In the present study, young SHR fed a DHA-enriched diet for 6 weeks had lower systolic blood pressures com-

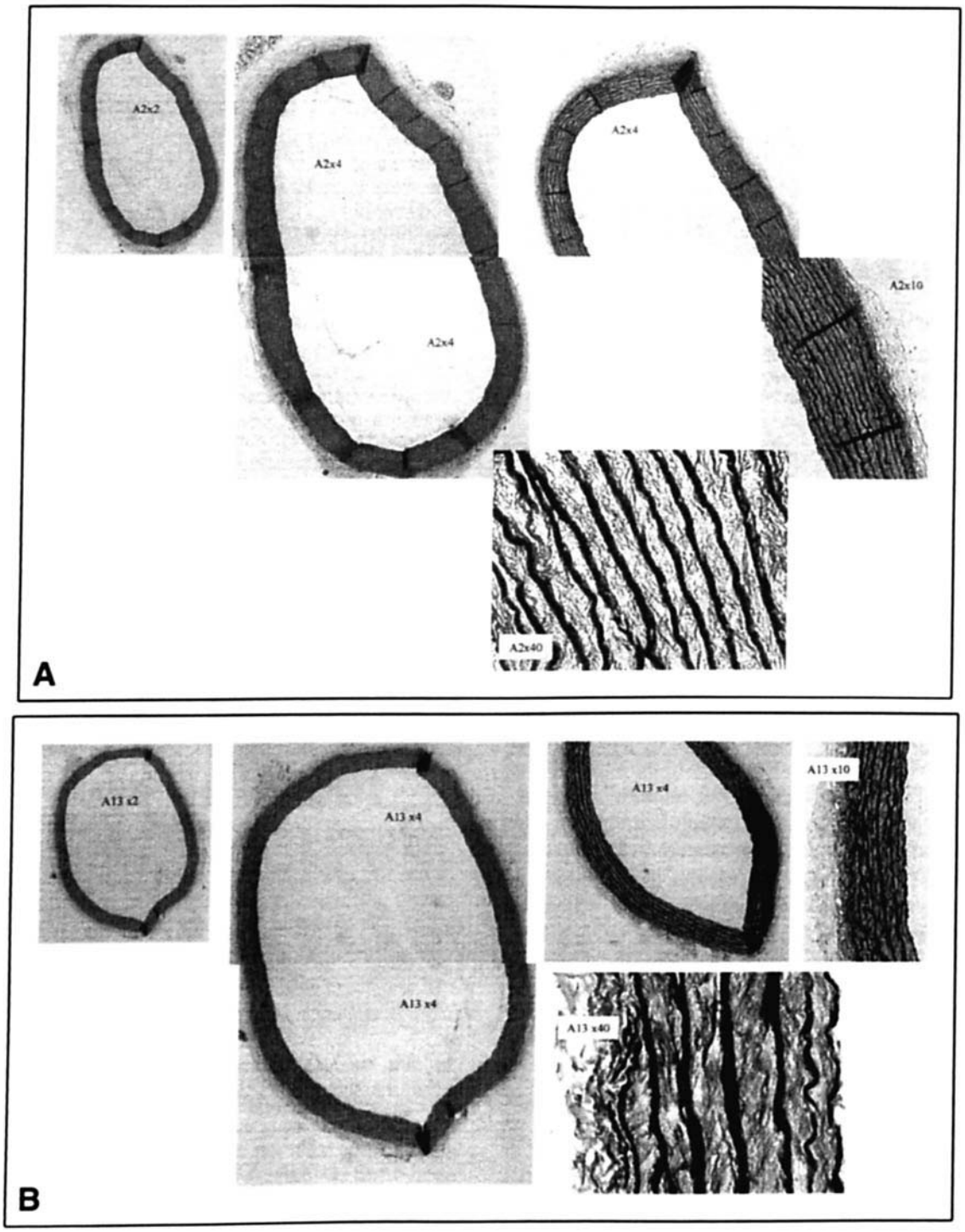

Figure 10. Representative section of the control aorta in (A) and DHA aorta in (B). The vessel wall in (B) is thinner and it has fewer elastin bands than the control section in (A). Staining: hematoxylin & eosin and Verhoff's, Magnification: 10× and 40×.

pared with control animals from weeks 3–6. This result is consistent with our previous work and suggests that DHA attenuates the development of hypertension in prehypertensive rats (9). Other investigators have demonstrated similar reductions in blood pressure in SHR or SHRSP rats fed diets enriched with 4.5–5% DHA for 12–14 weeks (7, 8).

A second important finding is that dietary DHA decreased vascular wall thickness in the coronary artery and aorta of SHR. Increased wall thickness is a common structural feature of hypertensive resistance vessels (25) and conduit arteries such as, the aorta (26). Hypertension is associated with abnormal growth and hypertrophy of vascular smooth muscle cells, which increases arterial wall thickness. The hypertensive structural alteration of the aortic wall

may also affect arterial mechanics. A recent study demonstrated that KCL-induced aortic contractile responses in SHRs are proportional to the development of vascular smooth muscle cell hypertrophy (26). A similar finding in resistance vessels would contribute to an increase in arterial blood pressure. The DHA-induced alterations in the aorta may also decrease arterial stiffness and pulse pressure. Recent evidence suggests that supplementation with DHA and EPA in dyslipidemic subjects improves systemic arterial compliance, reflective of a reduction in pulse pressure (27).

Interestingly, it has been reported that DHA promotes apoptosis in vascular smooth muscle cells (28) and this may account for the observed decrease in vascular wall thickness in the aorta and coronary artery in the present study. Modulation of apoptotic mechanisms by ANG II, ANG II-converting enzyme inhibitors, and ANG II receptor antagonists has been reported in the heart, lungs and vascular smooth muscle (29–31). It has been suggested that this modulatory activity contributes to vascular fibrosis and wall thickening as well as the development of hypertension (29–31). Previous investigators have shown that pharmacologic treatment in SHR also attenuates the hypertensive structural changes in large arteries and this effect is associated with a reduction in blood pressure (32–34).

As an alternative explanation, the diminished thickness of the aorta wall and its lower number of elastin fibers could be a consequence of the blood pressure reduction. According to Laplace's law, vessel wall thickness is related to pressure. As blood pressure increases in hypertension, arterial walls hypertrophy and become thicker. In contrast, a reduction in blood pressure may decrease arterial wall thickness.

Aldosterone also has a direct action on vascular smooth muscle cells by stimulating hypertrophy and proliferation (35). Aldosterone infusion in hypertensive rats is associated with an elevation in blood pressure and increased vascular hypertrophy in aorta and mesenteric arteries. (36). Plasma aldosterone levels are decreased in DHA-fed SHR compared with controls (9). Furthermore, adrenal glomerulosa cells from DHA-fed SHR produce less aldosterone *in vitro* than control SHR in response to ANG II, ACTH, and potassium (9). It is possible that DHA may inhibit aldosterone-induced vascular hypertrophy associated with hypertension by affecting the biosynthesis of aldosterone or interfering with signal transduction pathways in vascular cell membranes. Mineralocorticoid receptors are reportedly predominant in aortic endothelial and vascular smooth muscle cells (37). We have shown that SHR fed a DHA-enriched diet have 5- and 15-fold increases in the levels of DHA in the aorta and renal artery, respectively (10). Another study demonstrated that DHA increases plasma membrane fluidity in aortic endothelial cells (38). Changes in the lipid environment and fluidity may alter membrane-bound receptors and affect receptor-hormone interactions.

The majority of dietary studies in hypertensive rats documenting the vasorelaxant effect of ω-3 fatty acids have used fish oil, which contains both EPA and DHA (5, 7, 39–44). Our previous study demonstrated that acute administration of DHA alone as a free fatty acid, induces a direct vasorelaxant response in SHR aorta by modulating intracellular calcium in vascular smooth muscle cells (17). The lack of effect of dietary DHA on vascular responses in the present study may be attributed to the differences in these experimental conditions. As a free fatty acid, DHA may be incorporated and metabolized quickly in the vascular membrane phospholipids *in vitro* compared with the *in vivo* model where DHA uptake in vascular cells occurs slowly from the diet.

We investigated the effects of dietary DHA on vascular reactivity by using a systematic approach to determine both contractile and relaxant responses. We found that dietary DHA had no effect on NE-, KCL-, or ANG II-induced contractile responses or relaxation responses involving cGMP, protein kinase activation, and calcium membrane flux mechanisms in SHR aorta. NE- and ANG II-induced contractions are mediated by α-adrenoceptor and AT_1R activation, respectively, and subsequent phosphoinositide turnover. Resultant changes in myofilament calcium (Ca^{2+}) sensitivity, increased Ca^{2+} influx through receptor-operated Ca^{2+} channels, and nonselective cation channels, as well as release of Ca2+ from internal stores, increase vascular intracellular calcium $[Ca^{2+}]_i$. In contrast, depolarization by KCL causes Ca^{2+} influx through voltage-gated or L-type Ca^{2+} channels. Myofilament Ca^{2+} sensitivity and phosphoinositide turnover are not affected by KCL-induced contractions (45–49). These findings suggest that dietary DHA is not affecting neurohormonal receptors nor the electromechanical coupling mechanisms that modulate vascular tone.

Endothelial dysfunction is also associated with the pathogenesis of hypertension. We have previously reported on the endothelial-independent vasorelaxant properties of DHA in isolated SHR aortae (39). In the current study, we similarly found no significant effect of the DHA diet on ACH-induced relaxations, which are endothelial-dependent. This suggests that endothelial-derived nitric oxide production/release is not significantly altered by the DHA diet in SHR. It is interesting to note that overweight, mildly hyperlipidemic men supplemented with DHA (4 g/day) for 6 weeks had enhanced vasodilator (ACH, SNP) mechanisms and attenuation of constrictor (NE) response (50). Recently, we have shown that DHA supplementation (1.2 g/day) for 6 weeks improves endothelial function in hyperlipidemic children (51). Species differences may account for the discrepancy in results; therefore, careful extrapolation of results from animal studies to human studies is warranted.

The comparable relaxant responses in both groups to the Ca^{2+} channel antagonist, D600, suggest that L-type Ca^{2+} channels are not a major mechanistic site of action by DHA *in vivo*. The contractile responses to NE in the absence of extracellular calcium were not affected by the DHA diet. Thus, interference by DHA in the release of Ca^{2+} from intracellular storage sites is not likely to occur in this *in vivo*

SHR model. It was also demonstrated that dietary DHA had no significant effect on PNP and SNP induced relaxations which are reflective of increases in cyclic nucleotides which in turn, decrease intracellular calcium and myofilament Ca^{2+} sensitivity (52).

These findings correlate with a recent investigation in which DHA pretreatment had no effect on Ca^{2+} release from internal stores in cultured rat aortic smooth muscle cells (stimulated with 5-hydroxytryptamine-5-HT; Ref. 53). Although, inhibition of Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels (54) and voltage-independent Ca^{2+} channels (53) has previously been reported in cultured vascular smooth muscle cells pretreated with DHA. Recent evidence also suggests that DHA has no effect on endothelial cell $[Ca^{2+}]_i$ levels, nitric oxide production, nor endothelium-dependent relaxation of bovine coronary arteries precontracted with the thromboxane mimic, U46619 (55).

Others have found that dietary fish oil reduces blood pressure in hypertensive rat models that is caused, in large part, by changes in eicosanoid metabolism (5, 40, 56). We recently demonstrated DHA-induced (>30 μM) relaxations in isolated SHR aortae may also be attributed to vasodilatory prostanoids which activate K_{ATP} channels (17). It is possible that vasodilatory prostanoids may be involved in the blood pressure lowering effect seen in this SHR model following dietary DHA intervention.

In summary, the study confirms previous observations that dietary DHA has a blood pressure lowering effect in hypertension (9). Our results also demonstrate that definitive structural alterations in SHR vasculature (coronaries, aorta), characteristic of hypertensive arteries, are ameliorated by dietary DHA. The antihypertensive properties of dietary DHA are not related to alterations in vascular responses.

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