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Direct Correlation between Calcium Content and Blood Pressure: A Study of Aortic Coarctation in Rats (42799)

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Abstract. The calcium content of aorta was measured by atomic absorption after coarctation in the rat. At 7 and 14 days, the calcium content was elevated on the proximal side of the coarctation, where pressure was increased significantly. On the distal, low pressure side of the aortic coarctation, calcium was reduced significantly. There is a direct correlation between the blood pressure and the content of calcium ($r = 0.69$, $P < 0.001$). The width of the aortic media on the high pressure side was increased significantly at 7 and 14 days after coarctation, whereas no significant changes in width were present on the low pressure side of the constriction. We conclude that pressure regulates the aortic calcium content, likely acting through a local effect.

Alterations in handling of calcium have been implicated in the pathogenesis of hypertension (1, 2). The precise mechanism by which calcium exerts its effects however is not clear although it is likely that calcium acts upon smooth muscle (3). Nickerson and Yang (4) reported an elevated calcium content in the aorta of rats made hypertensive with deoxycorticosterone (DOC). In that study, it was proposed that pressure exerts a direct effect on the content of aortic calcium. To test this hypothesis further, coarctation of the aorta was studied because an elevated blood pressure is established on the proximal side of the coarctation, whereas a low pressure is present on the distal side of the coarctation in the same animal (5).

Materials and Methods. Four-month-old female Holtzman rats weighing 275–300 g were obtained from the Holtzman Breeding Co. (Madison, WI). Under ether anesthesia, the abdomen was opened with a middle-ventral incision and a 0.40-mm-diameter probe was placed in contact with the wall of the abdominal aorta between the renal arteries. The aorta was ligated around the probe with surgical silk and the probe was removed immediately, leaving a constriction to the vascular lumen equal to the diameter of the probe (5). In sham-operated control animals, the same operative procedure was employed but the ligature was not tightened.

Blood pressures in conscious rats were measured with a photoelectric tail-cuff pulse detector (Narco Instruments, Houston, TX) 1 day before surgery and 1 day before sacrifice. Direct blood pressure measurements were recorded with a p-1000B pressure transducer (Narco Instrument, Houston, TX) in animals anesthetized with intraperitoneal injection (80 mg/kg body wt) of Inactin (BYK Gulden Konstanz, West Germany); cannulas (PE-50) were inserted in the left carotid artery and in the right femoral artery.

Animals were killed at 7 and 14 days after induction of the coarctation. The aorta was immediately removed, gently cleaned of adherent fat and connective tissue while briefly immersed in 140 m*M* NaCl, 5 m*M* KCl, and 10 m*M* La_2O_3, pH 7.0, at 4°C, blotted with ash-free filter paper, and weighed. The aorta was divided into a proximal portion (between the coarctation and the bifurcation of the aorta). The area of the aorta including several millimeters above and below the coarctation was excluded from the sample.

The total calcium in aorta from individual animals from the high (proximal) and low (distal) pressure sides of the coarctation was measured with a Perkin–Elmer atomic absorption spectrometer. Aorta was dried at 100°C to constant weight and ashed in an evacuated plasmod chamber (TEGAL Co., Novato, CA) and the ash was dissolved in concentrated HCl and diluted with 10 m*M* La_2O_3. Heart and kidneys were fixed in 10% neutral phosphate-buffered formalin before being weighed. A 3-mm cross section of thoracic and abdominal aorta was removed rapidly from four animals in each group and

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fixed in 2% glutaraldehyde, 2% paraformaldehyde in 0.1 *M* potassium phosphate, pH 7.6, containing 0.1% tannic acid (Malinkrodt, St. Louis, MO) and 2% potassium pyroantimonate (6). While in fixative, 1-mm slices were cut through the media, allowing the aorta to be embedded so that sections could be cut perpendicular to the lumen. Tissue was fixed for 3 hr, washed in several changes of 0.1 *M* potassium phosphate with 2% potassium pyroantimonate (pH 7.6), postfixed in 1% osmium tetroxide in phosphate-buffered pyroantimonate (pH 7.6), and processed by conventional technique for electron microscope (6). One-micrometer sections were cut from four blocks for each of four animals in each group. The thickness of the media was measured directly on the 1-μm sections with a filar ocular micrometer (American Optical, Buffalo, NY) at 440×. Thickness was measured from the subendothelial space to the border of the adventitia.

Data were expressed as the mean ± SE and analyzed statistically by the Student *t* test. A correlation coefficient was calculated for blood pressure and aortic calcium content.

Results. *Blood pressure and body weight.* There were no significant differences in body weight between groups at the start of the experiment. After 7 days, the body weight of rats in the coarctation group was reduced significantly as compared to that of controls; by Day 14, the body weight of the coarctation group, although reduced, was similar to that of the control group (Table I).

Seven days after induction of the coarctation, the blood pressure was undetectable by the conventional tail-cuff method. By direct cannulation, the blood pressure proximal to the coarctation (carotid artery) was elevated and the blood pressure distal to the coarctation (femoral artery) decreased significantly at Days 7 and 14 (Table I). The blood pressure of control animals as measured by either carotid artery or femoral artery cannulation remained at the normotensive level (Table I) compared with that measured by the tail-cuff method (control at 7 days: 118 ± 2 mm Hg; control at 14 days: 122 ± 2 mm Hg).

Aortic calcium content. Total calcium content of the coarctation group at Days 7 and 14 was significantly increased on the high pressure side and decreased on the low pressure side of the circulation (Table II). No significant difference in calcium content on the two sides of the aorta (proximal side: aortic arch to aorta between origins of renal arteries; distal: aorta between origins of renal arteries to aorta bifurcation) was found in control animals. There is a positive correlation between direct blood pressure readings and aortic calcium content ($r = 0.69$ for combined proximal and distal values) which is statistically significant ($P < 0.001$). The same positive correlation was also shown for each side of the coarctation ($r = 0.55$ on the proximal side, blood pressure measured in carotid artery, $P < 0.001$ and $r = 0.42$ on the distal side, blood pressure measured in femoral artery, $P < 0.02$).

Organ weights and aortic width. In the coarctation group, the right kidney situated proximal to the coarctation was significantly larger than the left kidney located distal to the coarctation (Table III). The weight of the heart (located proximal to the coarctation)

TABLE I. EFFECT OF AORTIC COARCTATION ON BODY WEIGHT AND BLOOD PRESSURE

Group	Time (days)	Body weight (g)			Blood pressure (mm Hg)		
		Initial	Final	*P* value[b]	Carotid artery	Femoral artery	*P* value[c]
Control	7	295 ± 3[a]	294 ± 5	NS	107 ± 11	96 ± 10	NS
Coarctation	7	296 ± 6[NS]	259 ± 8[d]	<0.001	140 ± 7[d]	34 ± 6[e]	<0.001
Control	14	301 ± 8	310 ± 9	NS	117 ± 6	112 ± 7[NS]	NS
Coarctation	14	304 ± 5[NS]	290 ± 8[NS]	NS	153 ± 5[e]	57 ± 7[e]	<0.001

[a] Mean ± SE; NS = not significant.
[b] Comparison of initial and final weight.
[c] Comparison of pressures in carotid and femoral arteries.
[d] $P < 0.05$; [e] $P < 0.001$ are comparisons and coarctation groups.

TABLE II. EFFECT OF COARCTATION ON TOTAL AORTIC CALCIUM CONTENT AND AORTIC MEDIA WIDTH

Group	Time (days)	Aortic calcium content (mM/kg aortic dry wt) Proximal side (high pressure)	Distal side (low pressure)	P value[b]	Aortic media width (μm) Proximal side (high pressure)	Distal side (low pressure)	P value[b]
Control	7	9.82 ± 0.25[a]	8.96 ± 0.46	NS	88.6 ± 3.4	84.3 ± 1.4	NS
Coarctation	7	11.21 ± 0.18[d]	6.94 ± 1.03[NS]	<0.001	97.5 ± 1.9[c]	81.9 ± 1.4[NS]	<0.001
Control	14	9.55 ± 0.38	8.95 ± 0.40	NS	85.8 ± 2.0	80.7 ± 1.8	NS
Coarctation	14	13.35 ± 1.01[c]	5.14 ± 0.79[d]	<0.001	110.2 ± 5.7[d]	83.7 ± 2.0[NS]	<0.001

[a] Mean ± SE; NS = not significant.
[b] Comparison of proximal and distal sides within a group.
[c] $P < 0.01$.
[d] $P < 0.001$.

was similar to that of the control at 7 days; by 14 days, the heart was larger in the coarctation group, but the difference was not significant statistically (Table III).

The width of the aortic media on the high pressure side of the coarctation was increased significantly at 7 and 14 days (Table II), whereas no significant change in width was observed on the low pressure side of the constriction.

Discussion. The present study confirms our hypothesis that blood pressure correlates directly with the aortic calcium content. The coarctation model provides *in situ* two distinct aortic pressures, so that the same animal serves as its own control. The elevation in pressure proximal to the coarctation causes an increase in aortic calcium. Similar elevations in aortic calcium content have been reported for the spontaneously hypertensive rat (7), deoxycorticosterone (DOC)-induced hypertensive rat (8, 9), and rats made hypertensive in the one-kidney, one-clip model (10). These investigations did not report a correlation between the height of the blood pressure attained and the aortic calcium content. Reduced pressure on the distal side of the coarctation however decreases the calcium content and the present paper is the first report of this observation insofar as we can determine; the mechanism for the decrease is not clear.

The question can be asked as to the location of the increased calcium in the aorta inasmuch as atomic absorption spectroscopy measures total calcium. It is likely that the increased total calcium reflects at least in part a greater total and ionic content of the cation within aortic medial smooth muscle cells. Aguas and Nickerson (11) and Nickerson and Yang (4), using the pyroantimonate technique which detects ionic calcium and stereological methods at the electron microscopic level to quantify the reaction product, reported an increased ionic calcium in aortic smooth muscle cells of rats made hyperten-

TABLE III. EFFECT OF AORTIC COARCTATION ON WEIGHT (mg) OF HEART AND KIDNEY

Group	Time (days)	Heart	Right kidney (high pressure)	Left kidney (low pressure)	P value[b]
Control	7	1223 ± 34[a]	1034 ± 33	1044 ± 41	NS
Coarctation	7	1223 ± 21[NS]	1238 ± 47[d]	749 ± 86[c]	<0.001
Control	14	1109 ± 45	1032 ± 44	1028 ± 65	NS
Coarctation	14	1204 ± 40[NS]	1418 ± 37[d]	577 ± 39[d]	<0.001

[a] Mean ± SE; NS = not significant.
[b] Comparison of kidneys on high and low pressure sides of the coarctation.
[c] $P < 0.01$.
[d] $P < 0.001$.

sive with DOC. Ionic calcium is of importance in controlling physiological processes such as contraction of smooth muscle cells (3) which at the level of arterioles is important in regulating blood pressure (12).

Although the mechanism for the increase in calcium within the aorta is unknown, pressure is likely involved. The pathophysiological mechanism in aortic coarctation has not been resolved completely. Evidence for the activation of the renin–angiotensin system has been found in several studies (13, 14), whereas in other studies the results do not support the activation of the system in coarctation (15, 16). Assuming activation of the renin–angiotensin system, there would be an increased generation of angiotensin II which stimulates vascular smooth muscle cells, increasing the entry of calcium (17). It is unlikely, however, that humoral factors play a major role in increasing calcium because both high and low pressure sides of the arterial circulation would be exposed to the elevated levels of the hormone resulting in increased calcium on both sides of the coarctation. The different levels of calcium on the two sides of the coarctation are better consistent with the hypothesis that pressure regulates the aortic calcium content by acting at the local level of the vessel.

An increased thickness of the aortic media is consistent with a pressure-induced stimulation of the smooth muscle cells. Owens and Reidy (18) have reported that hypertrophy and hyperplasia play a role in the increased thickness of the aortic media proximal to the coarctation. The cellular signal for hyperplasia and hypertrophy is not known, although an interaction between the endothelium and smooth muscle cells may well be important (19). Diminished endothelial-dependent relaxation activity has been reported on the high pressure side of an aortic coarctation in rabbits (20) which was not present on the distal low pressure side. These observations, supporting a local response to an elevated blood pressure, are consistent with our conclusion that pressure regulates the aortic calcium content by acting at the local level of the vessel.

It should be noted that on the low pressure side that the aorta media width was not altered, whereas the calcium content was reduced. Thus at reduced pressure, there was a dissociation between medial width and calcium content which was not evident on the high pressure side.

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