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Dependence of Media Cross-Sectional Area on Circumference of Aortic Rings from Normal and Hypertensive Rats (42787)

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Abstract. The purpose of this study is to determine if cross-sectional area and media thickness depend on circumference in excised vessels. Measurements were obtained from rat aortic rings chemically fixed after excision. Rings were fixed without distention or after mounting on circular rods of different diameters. Media cross-sectional area increased with circumference but thickness decreased. Thickness and area were significantly greater in the SHR than the WKY rat at all circumferences. Results of a theoretical analysis predict that cross-sectional area and thickness will depend on circumference in excised rings. Best-fit curves of the experimental data to the theoretical relationships show that neither thickness nor cross-sectional area of the aorta is consistently better correlated with circumference in SHRs and WKY rats. The following conclusions are made: (i) Media cross-sectional area and thickness depend on circumference in excised-aortic rings, and (ii) for a specific method of preparation, thickness and cross-sectional area are equally accurate when comparing aortic rings from normal and hypertensive rats. If an experimental determination of the relationship between circumference and cross-sectional area (or thickness) cannot be done, we suggest that a reasonable alternative is to compare these measurements at the same circumference.

An increase in vessel wall mass by hypertrophy, hyperplasia, and other mechanisms has been widely studied as a contributing factor in established human hypertension. The general hypothesis is that an increase in wall mass causes tissue to encroach into the vessel lumen and restrict blood flow (1). Experimental indices of wall mass include thickness and cross-sectional area. These indices have been measured in perfused vessels (2–6) and in isolated rings and strips (7–11).

It has been concluded in some studies of perfused vessels that cross-sectional area measurements are more reliable than thickness measurements when comparing normal and hypertensive animals (3, 4, 6). This preference is based on observations of a change in wall thickness with vasoconstriction or passive distention (3, 12) but no change in cross-sectional area (4).

In studies of excised rings, thickness and cross-sectional area are measured as part of mechanical experiments or after fixation at the end of an experiment (13–16, 11). A significant difference between perfused vessels and excised rings is that axial length of perfused vessels does not change *in vivo* with circumferential distention (17) but the width of excised rings are free to change. This raises the possibility that dimensional changes of excised rings are different from the changes of perfused vessels when circumference is altered.

The purpose of this study is to determine, by theoretical analysis and by experimental measurement, if the cross-sectional area and thickness of excised-aortic rings depend on circumference. Because cross-sectional area and thickness were found to change with ring circumference we have investigated the influence of this relationship on comparing the excised aorta from normotensive WKY rats and spontaneously hypertensive rats (SHR). Preliminary results have been presented in abstract form (Physiologist **28**:290, 1985.).

Methods. *Animals.* Eight male spontaneously hypertensive rats (SHR) and eight of their controls (WKY) were obtained from the Charles River Co. The vessels were tested when the rats were 16-weeks old. Systolic pressures were obtained by the tail cuff method (Narco Bio-Systems, Inc.).

Vessel preparation. A segment from the thoracic aorta was placed in a dissection bath containing physiological salt solution (PSS) at 37°C and bubbled with 95% O_2 and 5% CO_2. Adventitia was trimmed from the vessel and three rings were cut from the segment. Ring width (approximately 2.0 mm) is the distance between two successive trans-

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verse cuts in the segment. Two rings were distended by mounting them on plastic cylindrical rods (approximately 1.6 and 2.0 mm diameters). This procedure maintained the circular geometry of the rings. The third ring was chemically fixed without mounting on a rod. The rods had tapered ends and were made by machining a Delron plastic rod.

The rings were transferred from the dissection bath to a buffered fixative. The fixative consisted of 2% glutaraldehyde in a 0.07 *M* phosphate buffer with a pH of 7.4 (18). The rings were in the fixative solution for approximately 7 hr then rinsed in 0.14 *M* PO_4-buffered solution. Following rinsing, the rings were removed from the cylindrical plastic rods and dehydrated in increasing concentrations of ethanol. Once the rings were rinsed in 95% ethanol they were infiltrated in methyl methacrylate (JB-4) for 4–5 hr. Following infiltration, the rings were oriented in Sorvall plastic embedding molds with catalyzed JB-4. Sorvall block holders were placed in plastic molds and cured for 3–4 hr at room temperature. The blocks were mounted directly into a Sorvall JB-4 microtone. Thick sections (2 μm) were cut and stained in aqueous toluidine blue and basic fuchsin and mounted on slides.

Experimental measurements. Media thickness was measured with an Olympus WF 10× measuring ocular and light microscope. Thickness was measured at four points around the ring at approximately 90° apart. Thickness is reported as the average value for the four measurements. Media is defined as the layer of the arterial wall which contains the internal and external elastic laminae, collagenous fibrils, elastic fibrils, and smooth muscle cells. The media cross-sectional area was measured on a Houston Hi-Pad digitizing tablet and an Apple II computer. Images of the rings were projected onto the Hi-Pad with a Prado microprojector at 64× magnification. Media cross-sectional area (A) was computed as the difference between the area within the external circumference of the media and the area within the internal circumference of the media (A_i). The internal circumference (C_i) of all rings was determined by two methods from the Hi-Pad images. First, it was measured by tracing with an electronic ruler/calculator (Panasonic JE-8210U). Second, the internal circumference was calculated from the internal cross-sectional area or $C_i = 2\ (\pi A_i)^{1/2}$. There was no difference in the two methods. The theoretical analysis requires a midwall circumference (C). This circumference was computed as $C = C_i + \pi h$ where h is the media thickness. The effects of rat strain (or blood pressure) and vessel circumference on media thickness and cross-sectional area was determined by an analysis of variance (ANOVA). P values, variance ratio (F), and freedom (df) are given for each ANOVA test. A second analysis of the results was made with the two-tailed unpaired Student's t test. Comparisons were made between vessel rings from normal and hypertensive animals that were treated with the same amount of distention (i.e., unstretched, mounted on 1.6-mm rods, or mounted on 2.0-mm rods). The data in the figures and tables are given as the mean value ± the standard error of the mean.

Theoretical analysis. A theoretical analysis of changes in cross-sectional area and thickness with circumferential distention of isolated rings is based on the principle of Conservation of Mass and assumes the vessel wall to be incompressible (17) and isotropic (19). The results are given as equations for the cross-sectional area–circumference relationship and the thickness–circumference relationship. Best-fit curves to the experimental data were obtained by adjusting the constant β in the corresponding equation (see Eqs. [5] and [6] in theoretical analysis) to estimate its least squares best-fit value. The best value for the constant is defined as having the lowest sum of squares of residual error values (SSE). A computer program written specifically for these equations was provided by Lundon Software, Inc., P.O. Box 21820, Cleveland, Ohio 44121. The computation of the best value is accurate to 1%. In addition, the constant was computed from the average initial values of cross-sectional area, thickness, and circumference that were measured directly in the unstretched rings.

Results. *Results of theoretical analysis.* The dimensions of a vessel ring segment are width = w, thickness = h, and the midwall

circumference = C. According to the Conservation of Mass, the total amount of tissue mass (m) in the vessel wall must remain constant with increasing circumference (distention) then

$$m = \rho_0 V_0 = \rho V, \quad [1]$$

where ρ_0 and ρ are the densities of the tissue and V_0 and V are the volumes in the unstretched and distended vessel, respectively.

For an incompressible material the density is unchanged with distention (i.e., $\rho = \rho_0$) then $V_0 = V$. This is expressed as $w_0 \cdot h_0 \cdot C_0 = w \cdot h \cdot C$ (19) or

$$(w/w_0)(h/h_0)(c/c_0) = 1. \quad [2]$$

If the *in vivo* segment is restrained in the longitudinal direction such that its length does not change then $w = w_0$ and Eq. [2] becomes

$$h = \alpha C^{-1}, \quad [3]$$

where $\alpha = h_0 C_0$. The cross-sectional area (A) is

$$A = h \cdot C = h_0 \cdot C_0 = A_0 = \text{constant}. \quad [4]$$

Equations [3] and [4] show that wall thickness decreases in direct proportion to the increase in circumference whereas cross-sectional area remains unchanged in a longitudinally restrained vessel.

If a ring segment is isolated from the vessel, it is not restrained in the longitudinal direction, and w is not equal to w_0. However, if the wall is isotropic then $w/w_0 = h/h_0$.

From Eq. [s] $(h/h_0)^2 = (C/C_0)^{-1}$ or

$$h = \beta \cdot C^{-1/2} \quad [5]$$

$$A = \beta \cdot C^{1/2}, \quad [6]$$

where $\beta = h_0 C_0^{1/2} = A_0/C_0^{1/2}$. Equations [5] and [6] show that wall thickness as well as cross-sectional area depend on circumference in the isolated ring. Further, the same constant, β, relates changes in thickness and cross-sectional area to changes in circumference. All relationships are expressed independently of mechanical forces in the wall and the constant β may be calculated from the initial values of cross-sectional area (A_0), circumference (C_0), and thickness (h_0). Cross-sectional area must increase with circumference because ring width decreases and tissue mass remains constant.

Effect of circumference on experimental measurement of thickness and cross-sectional area in excised rings. The systolic blood pressure was 126 ± 4 mm Hg in WKY rats and 186 ± 5 mm Hg in SHR rats (n = 8).

Media thickness measurements from SHRs and WKY rats are shown in Fig. 1 for the three groups of ring circumferences (unstretched, mounted on 1.6-mm rod, and mounted on a 2.0 mm-rod). The mean thickness values are placed at the corresponding mean values of inside circumference in each group of rings. The results in Fig. 1 show that media thickness in isolated rings from the aorta of SHRs and WKY rats decreases with increasing ring circumference. The media thickness of SHR rings is significantly greater ($P < 0.001$) than those of WKY rings when compared between groups that are treated with the same amount of distention (i.e., unstretched, mounted on 1.6-mm rods, or on 2.0-mm rods). The results of ANOVA analysis (27) shows that $P < 0.01$ for the effect of circumference (F = 73; df = 2, 42) and for the effect of rat strain, WKY versus SHR (F = 306; f = 1, 42).

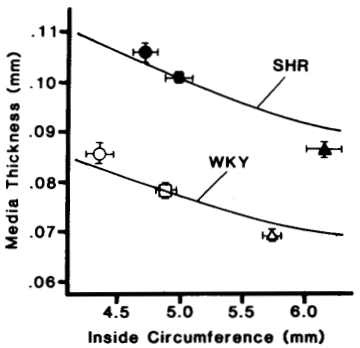

FIG. 1. Media thickness for three treatments of circumferential distention: unstretched, mounted on 1.6-mm rod, and mounted on 2.0-mm rod. The means and standard errors of media thickness are shown at the means and standard errors of inside circumference that were measured in each ring from SHR and WKY rats. The symbols for unstretched rings are circles, for rings on 1.6-mm rods are squares, and for rings on 2.0-mm rods are triangles. Filled symbols are SHRs and open symbols are WKY rats. Best-fit curves for thickness–circumference data from SHR and WKY rats according to Eq. [5] are shown.

Media cross-sectional area measurements from SHRs and WKY rats are shown in Fig. 2. The results in Fig. 2 show that cross-sectional area in isolated rings increases with increasing circumference. The cross-sectional area of SHR rings is significantly greater ($P < 0.001$) than WKY rings when compared between groups that are treated with the same amount of distention. The results of ANOVA analysis shows that $P < 0.01$ for the effect of circumference ($F = 33$; $df = 2, 42$) and for the effect of rat strain ($F = 470$; $df = 1, 42$).

Comparison of experimental measurements to theoretical curves. Best-fit curves to the thickness–circumference data were obtained according to Eq. [5]. Similar curves for the cross-sectional area–circumference data were obtained according to Eq. [6]. These curves are shown in Figs. 1 and 2. The significance level is less than 1% for the four curves. The correlation coefficient, R, from thickness measurements is the same as from cross-sectional area measurements.

A second method of determining the constant β is by direct calculation from the measurements of thickness (h_0), cross sectional area (A_0), and circumference (C_0) in unstretched rings (see Theoretical Analysis).

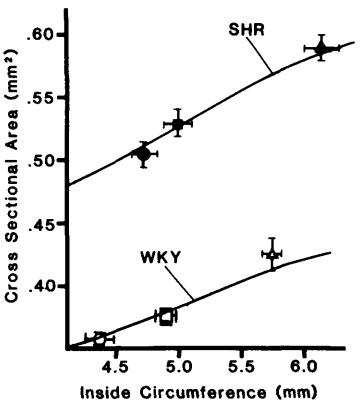

FIG. 2. Media cross-sectional area at different amounts of circumferential distention: unstretched, mounted on 1.6-mm rod, and mounted on 2.0-mm rod. The means and standard errors of cross-sectional area are shown at the means and standard errors of the inside circumference that was measured in each ring from WKY and SHR rats. Symbols are the same as in Fig. 1. Best-fit curves for cross-sectional area–circumference data from SHR and WKY rats according to Eq. [6] are shown.

The R values from a calculated β were lower than the R values of the best-fit curves. However, the significance level of the R values for the calculated β is less than 1%.

Computing cross-sectional area from thickness and circumference measurements. Cross-sectional area may be computed as $A = h \cdot C$ where h is thickness and C is midwall circumference. The computed area for SHR rings is significantly greater than WKY rings for each treatment of distention. The constant β in Eq. 6 for computed cross-sectional area is the same as β from experimental measurements of cross-sectional area for both WKY rats and SHRs. The correlation of computed area with circumference is significant ($P < 0.05$). The results of ANOVA analysis shows that $P < 0.01$ for the effect of circumference ($F = 13$; $df = 2, 42$) and for the effect of rat strain ($F = 462$; $df = 1, 42$).

Discussion. The hypothesis in this paper is that cross-sectional area of an excised-vessel ring as well as its thickness depends on the amount of circumferential distention. Our hypothesis is supported by theoretical analysis based on the principle of Conservation of Mass. The analysis predicts that cross-sectional area of excised-vessel rings will increase with circumference but thickness will decrease. The change in cross-sectional area must be opposite to the change in thickness and ring width in order for the tissue mass to remain constant. In a previous study (14) we have found that ring width decreases with increasing circumference. Our theoretical predictions for excised rings were confirmed experimentally by measurements from the aorta of SHR and WKY rats. Cross-sectional area was highly correlated with circumference in the WKY rat and in the SHR. Correlation coefficients for thickness were similar to the coefficients for cross-sectional area. These results show that cross-sectional area and thickness measurements of excised rings are affected equally by circumference.

Although *in vivo* circumferences of blood vessels are usually less in hypertensive animals than in normal animals, comparisons of excised-vessel segments are not done at these circumferences or at their equivalent muscle lengths. Early studies of the intrinsic contractility and sensitivity of smooth muscle in arterial strips were compared at the

same preload. However, it is now well established that the effects of length, or circumference, must be determined in order to compare the intrinsic properties of vessels in hypertension. Wall thickness and cross-sectional area are also intrinsic characteristics of each vessel. Therefore, distinguishing the effect of vessel circumference from the effect of hypertension on cross-sectional area and thickness measurements is similar to studies in hypertension that measure length-tension relationships and length-dependent sensitivity. If an experimental determination of the relationship between circumference and cross-sectional area (or thickness) cannot be done, we suggest that a reasonable alternative is to compare these measurements at the same circumference.

We have found that a cross-sectional area computed from the thickness and circumference of excised rings also depends on circumference. A recent study (20) of perfused vessels *in vivo* has found that a cross-sectional area calculated from diameter and wall thickness measurements is a useful indicator of arteriolar hypertrophy and remains the same during vasomotion. Because a computed cross-sectional area depends only on thickness and midwall circumference, a comparison of vessels from normal and hypertensive animals at the same circumference will depend only on thickness. Therefore, a comparison of thickness at the same circumference is equivalent to comparing cross-sectional area.

The SHR aorta rings in this study are thicker and their cross-sectional area is greater than in rings from the WKY rats when compared at the same circumference. Thus, the mass of tissue in the aortic wall of the SHR is significantly greater than in the WKY rat. This shows that thickness and cross-sectional area measurements have the same degree of reliability and support the conclusion of a greater mass of media tissue in excised-SHR aorta rings.

The effect of comparing cross-sectional area or thickness at the same circumference would reduce the difference in excised vessels from normal and hypertensive animals by previous investigators. For example, Arner and Uvelius (8) found significant differences in media thickness and ring circumference of excised-aortic rings from SHR and WKY rats. A sufficient increase in circumference of the SHR aorta in their study would make it equal to the WKY circumference. As circumference increases the difference in thickness between the SHR and WKY aorta will decrease. Our results indicate that measurements of cross-sectional area rather than thickness will not eliminate the effect of circumference.

Because of the dependence of both thickness and wall tension on vessel circumference, a comparison of mechanical stress from excised-SHR vessels and WKY vessels at the same circumference would produce different results than in previous studies. For example, several studies (15, 21) of resistance vessels were made at 80% of the circumference that relaxed vessels would have at a calculated pressure of 100 mm Hg (defined as L1). Brayden *et al.* (21) reported a significantly smaller L1 and a lower active stress in cerebral vessels of the SHR than in the WKY rat. An increase in circumference of their SHR vessels would cause a corresponding increase in active wall tension and decrease in media thickness. This could result in the same active stress from SHR and WKY vessels. In an earlier paper Mulvany and Halpern (22) found no difference in L1 or in the active stress of mesenteric resistance vessels from SHRs and WKY rats.

Although a comparison of cross-sectional area or thickness at the same circumference may resolve some conflicts in previous work, it is possible that an increased wall mass in hypertension exists in some vessels but not in others. Mulvany and Halpern (22) found no increase in wall thickness of second-branch resistance vessels but an increased thickness of first-branch vessels. This may be explained by Joshua *et al.* (23) who concluded that the larger arterioles in renovascular hypertension constrict to protect downstream vessels from the increased pressure. They suggest that this constriction is a structural alteration and as hypertension develops the constriction progresses to smaller vessels in an attempt to maintain normal pressure. Meininger *et al.* (24) also concluded that increases in intraluminal pressure are responsible for initiating and propagating the reduction in arterial diameter in hypertension.

An *in vivo* study of perfused vessels by Friedman *et al.* (3) found increased cross-sectional area and thickness during DOCA hypertension but vessel radius was the same. After cessation of treatment with DOCA pellets and saline water, cross-sectional area was the same but vessel radius was lower and thickness was greater in post DOCA hypertensive rats than in normal rats. Thus, when vessels are compared at the same radius (in this case perfused vessels in DOCA hypertension) the conclusion of increased wall mass is supported by thickness measurements and by cross-sectional area measurements. A similarity of results for cross-sectional area measurements and thickness measurements can be found in other *in vivo* studies of perfused vessels in hypertension (25, 26, 27, 10).

The results from this paper on excised vessels and the results from *in vivo* studies on vessel wall mass may be summarized as follows. The studies on perfused vessels have shown that measurement of thickness is affected by vessel circumference whereas cross-sectional area measurement is not. However, those studies indicate that when vessel thickness is compared at the same circumference the conclusions in hypertension agree with the comparisons of cross-sectional area. When the vessel is excised for *in vitro* experiments both cross-sectional area and thickness are altered by ring circumference. However, when thickness and cross-sectional area in hypertensive animals are compared to those in normal animals at the same ring circumference the conclusions on their respective differences are not different.

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