Morphometric Evidence for Non-Pressure-Related Arterial Wall Thickening in Hypertension

Jialiu Liu, Sanford P. Bishop, and Henry W. Overbeck

To investigate the relation of pressure and vascular wall thickening in hypertension, we coarcted the abdominal aorta upstream to the renal arteries in 14 rats. Sham-coarcted (n = 16) and two-kidney, one-clip (Goldblatt) hypertensive rats (n = 13) served as controls. Tail, femoral, and carotid arterial pressures rose (p < 0.01) in the two-kidney, one-clip hypertensives; only carotid pressure rose (p < 0.01) in the coarcted rats, tail and femoral pressures remaining normal (p > 0.25). Thus, the hindquarters of the coarcted rats remained normotensive. Four to six weeks after surgery we perfusion-fixed vascular tissues of the hindquarters, including kidneys, with formalin at in vivo levels of pressure. Glycol methacrylate-embedded tissues were sectioned at 1 μm thickness and vessels quantitatively evaluated. The outer medial and lumen perimeters of abdominal aorta, femoral artery, and renal arterioles were measured; from these measurements, vessel outer and lumen diameters, medial thickness, medial area, and medial thickness-to-lumen radius ratios were calculated. Compared with sham-coarcted rats, abdominal aorta, femoral arteries, and renal arterioles <61 μm outer diameter in rats with coarctation and Goldblatt hypertension had significantly increased (up to +100%) medial area, medial thickness, and medial thickness-to-lumen radius ratios. In general, magnitudes of abnormalities were similar in Goldblatt and coarcted rats. Renal arterioles >60 μm outside diameter in Goldblatt hypertensive, but not coarcted, rats also were thickened. These results indicate that vascular wall thickening occurs in conduit arteries and smaller renal arterioles in the normotensive hindquarters of coarcted rats, providing morphometric evidence for non-pressure-related mechanisms involved in vascular growth in this form of hypertension. (Circulation Research 1988; 62:1001-1010)

There is increasing evidence that the abnormal wall thickening of both heart and arteries in hypertension involves non-pressure-related, as well as pressure-related, mechanisms. To identify the roles of pressure- and non-pressure-related influences, many investigators have used antihypertensive drugs systemically in hypertensive animals. However, these pharmacological interventions may impair normal growth and also stimulate release of renin angiotensin, catecholamines, or other cardiovascular-active substances. Furthermore, the pharmacological agents may have specific effects on cellular growth. As an alternative approach we have studied animals with hypertension due to aortic coarctation. In such animals, the vascular bed distal to the aortic constriction remains normotensive, thus allowing study of normotensive and hypertensive beds in the same animal without the need of drugs. In the normotensive hindquarters vascular beds of these animals, we have observed and reported chemical and hemodynamic evidence for wall thickening, in addition to evidence from in vivo study of the microcirculation. Such wall thickening cannot be attributed to pressure-related mechanisms.

The purpose of the present study was to examine quantitatively the vascular morphology of these normotensive beds in animals with coarctation hypertension. The results support the hypothesis that non-pressure-related factors play an important role in arterial wall thickening in hypertension.

Materials and Methods

Models of Hypertension

We randomly divided normotensive Sprague-Dawley rats (Zivic-Miller Laboratories, Allison Park, Pennsylvania) weighing between 150 and 200 g into three groups. In one group, we created coarctation hypertension by placing a partially constricting silver clip (i.d. 0.813 mm) around the abdominal aorta upstream to the origin to both renal arteries under ether anesthesia. In a second group (sham-coarcted normotensive control rats), we similarly placed a clip (i.d. 1.70 mm) too large to constrict the aorta. To create two-kidney, one-clip hypertension in the third group, we placed a constricting clip (i.d. 0.390 mm) on the left renal artery with the right kidney remaining intact. Postoperatively, we maintained the rats on standard rat chow (PROLAB R-M-H 1000, Agway, Syracuse, New York; 0.4% Na, 0.7% K) and tap water ad libitum. We measured systolic arterial blood pressure weekly by the tail plethysmographic method.
Four to six weeks postoperatively, we prepared the rats for hemodynamic and morphometric studies. Rats were anesthetized by injection of pentobarbital (55 mg/kg i.p.). We then cannulated the left carotid and femoral arteries for measurement of blood pressure with Statham P23Gb transducers (Gould, Cleveland, Ohio) and a Hewlett-Packard Model 8805C pressure amplifier and 7702B recorder (Palo Alto, California). Next, blood was sampled for measurement of hematocrit, plasma sodium, and potassium (flame photometry; model 643, Instrumentation Laboratory, Dayton, Ohio) and creatinine (Creatinine Assay, Sigma Chemical, St. Louis, Missouri), and the rat was given heparin (200 units i.v.). The thorax and abdomen were opened, and the heart was excised and weighed. The abdominal aorta was cannulated at a point 1 cm upstream to the origin of the right renal artery (downstream to the level of the clip in the coarcted rats). Through the cannula, we pump-perfused (low output roller pump, model 3500, Sarns, Ann Arbor, Michigan) the hindquarters vascular bed with 0.9% NaCl solution (flush) followed by 10% buffered formalin solution for 15 minutes. For both saline and formalin we set and maintained perfusion pressures at the level of hindquarters mean blood pressure determined to be present in vivo in each type of rat: 90 mm Hg for coarcted and sham-coarcted rats and 155 mm Hg for two-kidney, one-clip rats.

Following perfusion fixation, 3-mm sections of the terminal abdominal aorta, the right femoral artery, and the right kidney were excised and immersed in 10% buffered formalin. Additionally, a standardized 1-cm segment of the remaining abdominal aorta distal to the renal arteries was dissected free of adventitia, blotted dry, and weighed. This segment was then oven-dried at 60°C and the constant dry weight recorded. Fixed tissues were embedded in glycol methacrylate, sectioned at 1 μm, and stained with toluidine blue. Formalin fixation and methacrylate embedding do not alter volume of myocardial cells.

At the end of the procedure, each rat underwent necropsy, and clip type and placement, as well as general health, were verified.

Morphometric Analysis

Morphometric analysis was by an investigator (J. Liu) who did not know the tissue source. Figure 1 is a composite photomicrograph of representative arteries and arterioles studied in the three groups of rats. We analyzed vessel morphology with a Graf/pen sonic digitizer (Science Accessories, Southport, Connecticut; resolution 0.1 mm) interfaced to a computer system programmed to determine area within an enclosed boundary. The system was calibrated with a ruled microslide, and sections of aorta and femoral artery were measured at a magnification of approximately ×80, renal arterioles at approximately ×800. We traced the outer perimeter of the medial smooth muscle and the lumen of each vessel. We used only vessels that were adequately perfusion fixed as evidenced by a straight internal elastic lamina. For the aorta and femoral artery, two to four serial sections from each block were measured and the mean value used for each vessel. Renal arterioles and arteries were measured in a single section of cortical tissue from the right (nonclipped) kidney. We included only those vessels with a long-to-short axis ratio of less than 1.3, thus excluding vessels cut at greater than 40° from true cross section, ensuring a maximum of 30% overestimation of true cross-sectional area. Due to the exponential nature of the increasing percent error with increasing sectioning angle, 50% of vessels have an overestimation of less than 5%. Furthermore, the error is equal in all groups, thus not affecting statistical comparison between groups. Medial area was determined by subtraction of lumen area from outer medial area, and from these values we calculated mean vessel medial thickness, lumen diameter, outer vessel diameter, and medial thickness-to-lumen radius ratio.

Statistical Analysis

The renal arterioles and arteries ranged in size (vessel diameter) from 14 to 301 μm. To compare vascular morphometry over the range of vessel diameters, a regression analysis was done for each group. The slopes and intercepts of the regression lines for the various measurements were then compared among the groups. To further analyze the data, the vessels from each animal were arbitrarily divided into four size categories; <31 μm, 31–60 μm, 61–100 μm, and >100 μm. Two to four sections of aorta and femoral artery and one to 26 sections (average four) of renal arterioles and arteries at each size range in each rat were examined. The mean value from each category for each animal was then used to calculate group means, and the results analyzed by one-way analysis of variance followed by two preplanned (coarcted versus sham-coarcted; Goldblatt versus sham-coarcted) treatment contrasts. Probability values <0.05 were considered significant.

Results

All rats reported had been gaining weight or were of stable weight and appeared healthy on the day tissue was obtained. No significant lesions were found at necropsy. As previously found, hematocrit and plasma concentrations of sodium, potassium, and creatinine did not differ among the groups of rats. Table 1 presents tail systolic blood pressures in conscious rats measured over the 6 postoperative weeks. There were no statistically significant differences in tail pressure between coarcted and sham-coarcted rats, as we have previously reported. In contrast, tail blood pressures in the two-kidney, one-clip rats were significantly elevated after the 1st week (p < 0.001).

Figure 2 presents final body weights, directly measured carotid and femoral mean blood pressures, heart weight-to-body weight ratios, and wet and dry weights of the standardized segments of abdominal aorta. There were no significant differences in body weight among the three groups. Heart weight expressed in terms of
body weight was elevated in both the coarcted and the Goldblatt hypertensive groups. Carotid mean arterial blood pressures were elevated equally in the two hypertensive groups, but, in contrast, femoral mean pressure was elevated only in the Goldblatt hypertensive rats. Both wet and dry weights of the abdominal aorta, as well as difference between wet and dry weights (Figure 2), were elevated in both hypertensive groups, as we have previously reported.25

Morphometry of the abdominal aorta (Figure 3) revealed no statistically significant differences in vessel outer or lumen diameters among the three groups, although there were trends for increases in outer diameters in the rats with coarctation or Goldblatt hypertension. The intima appeared normal in all groups of rats and was limited to one or two cell layers (Figure 1). In contrast, there were statistically significant increases in aortic medial area in these hypertensive rats. Medial thickness and the medial thickness-to-lumen radius ratio were also elevated in the Goldblatt rats with similar trends in coarctation hypertension. Findings in the femoral artery were generally similar (Figure 4); however, increases in medial thickness and medial thickness-to-lumen radius ratio reached significance in both the Goldblatt and the coarcted rats.

Renal arterioles and arteries ranging in size from 14 to 301 µm o.d. were also studied. Data were grouped as <31 µm, 31–60 µm, 61–100 µm, and >100 µm for analysis, and Figures 5–8 present these data.

<table>
<thead>
<tr>
<th>Group</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
<th>5th week</th>
<th>6th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-coarcted</td>
<td>120.8 ± 2.2 (16)</td>
<td>118.5 ± 2.4 (16)</td>
<td>115.1 ± 2.8 (16)</td>
<td>116.2 ± 1.6 (16)</td>
<td>115.0 ± 2.2 (14)</td>
<td>120.3 ± 1.3 (11)</td>
</tr>
<tr>
<td>Coarcted</td>
<td>118.9 ± 1.9 (14)</td>
<td>119.2 ± 2.1 (14)</td>
<td>116.0 ± 1.6 (14)</td>
<td>117.0 ± 2.3 (14)</td>
<td>119.9 ± 2.6 (11)</td>
<td>123.2 ± 4.6 (4)</td>
</tr>
<tr>
<td>Goldblatt</td>
<td>126.4 ± 3.6 (13)</td>
<td>137.5 ± 5.3* (12)</td>
<td>147.7 ± 5.1† (12)</td>
<td>163.1 ± 4.0‡ (13)</td>
<td>171.8 ± 5.5‡ (12)</td>
<td>163.8 ± 5.7‡ (6)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; numbers of observations in parentheses.

*p<0.05, †p<0.01, ‡p<0.005 compared with sham-coarcted rats.
COARCTATION HYPERTENSION (n=14) • NORMOTENSIVE CONTROL (n=16) • 2 KIC HYPERTENSION (n=13)

FIGURE 2. Terminal body weights, directly measured carotid and femoral mean blood pressures (pentobarbital anesthesia), heart weight-to-body weight ratios, and wet and dry weights of a standardized 1 cm segment of the excised abdominal aorta. Mean ± SEM. Probability values indicated for comparison of hypertensive groups with control group by analysis of variance.

classified renal vessels > 100 µm o.d. as "arteries" and smaller vessels as "arterioles." Again, by light microscopy we observed no gross abnormalities in the intima of these vessels from any group. In contrast, in groups of vessels <101 µm from the rats with two-kidney, one-clip hypertension there were significant increases in medial area, thickness, and medial thickness-to-lumen radius ratio with similar trends in arteries greater than 100 µm. In the rats with coarctation hypertension, on the other hand, there were increases in these variables only in arterioles less than 60 µm diameter. In magnitude, these increases were similar to those seen in the small arterioles of the Goldblatt hypertensive rats. There were no significant differences in vessel diameters among the three groups, indicating valid sampling procedures. The highly significant (p<0.0001) relation between medial area and vessel diameter in each group of rats studied is plotted in Figures 9 and 10. These figures again indicate that in arterioles less than 61 µm diameter, equivalent wall thickening occurs in both hypertensive groups. In contrast, in arterioles and arteries greater than 60 µm diameter, only thickening in the Goldblatt group persists.

Thus, to summarize, wall thickening occurs in conduit arteries and small renal arterioles in the normotensive hindquarters of coarcted rats. The magnitude of thickening is no less than that in rats with Goldblatt

ABDOMINAL AORTA

COARCTATION HYPERTENSION (n=12) • NORMOTENSIVE CONTROL (n=16) • 2 KIC HYPERTENSION (n=11)

FIGURE 3. Morphometry of the abdominal aorta. Mean ± SEM. Legend indicates numbers of animals in each group. Probability values indicated for comparison of hypertensive groups with control group.
hypothesis. In contrast, such thickening does not appear to occur in the larger renal arteries of coarcted rats, at least during the first 6 weeks of the hypertensive process.

Discussion

Renal tissue downstream to a partial occlusion of the renal artery regulates downstream renal artery pressure and, hence, perfusion pressure of the kidney at near normal levels. Murphy et al. suggested that, in part, the mechanism of this regulation involves increased renal resistance and atrophy of the downstream kidney; the resulting decreased renal flow reduces the pressure gradient across the occlusion. Coarctation of the abdominal aorta upstream to the origin of the renal arteries appears to evoke similar mechanisms. The presence of renal tissue downstream to the coarctation serves to normalize perfusion pressure of the kidney and, hence, of the entire contiguous hindquarters vascular bed of the animal.

These normal hindquarters pressures in coarctation hypertension have been documented repeatedly by us and by several other investigators. This documentation includes 24-hour monitoring of abdominal aortic pressures in instrumented conscious rats at several stages in the development of the hypertension.

Because systolic, diastolic, mean, and pulse pressures in the hindquarters, including kidneys, of coarcted rats do not exceed normal levels, the coarctation model of hypertension provides an excellent test for the
role of pressure in the production of vascular abnormalities accompanying hypertension. Any abnormalities in conduit arteries that might be observed in these normotensive hindquarters beds could not be attributed to mechanisms related to increased pressure or wall stress. Although pressures in smaller arteries and arterioles of the hindquarters have not been measured in coarctation hypertension, it is probable that they too remain normotensive and that pathology of these smaller arteries also is non-pressure-related.

In contrast to the normal hindquarters pressures in coarcted rats, there may be derangements in blood flow through the hindquarters beds. In a recently reported investigation involving microsphere measurement of regional flows in conscious rats 4 weeks after coarctation of the abdominal aorta, Stanek et al. found evidence for an increase averaging 16% (p<0.05) in flow through all tissues distal to the coarctation. Of importance for interpretation of data in the present investigation, this included a trend for increase (about 20%) in flow through the hind limb and a trend for increase (about 5%) in renal blood flow; however, neither of these latter increases reached statistical significance. In contrast, others have reported significant reductions in hindquarters flow in conscious coarcted dogs and in anesthetized coarcted rats. In summary, as for pressure, it appears unlikely that the morphological changes we observed in the arteries, especially in renal arterioles, of the coarcted rats are attributable to flow-related mechanisms.
Abnormalities in vascular resistance have also been observed in the hindquarters of coarcted animals. Stanek et al. report a decrease in resistance of the hindquarters averaging 22% (p < 0.05) with trends for lesser falls in hind limb and renal resistances not reaching statistical significance. In contrast, we and others have reported increases in vascular resistance in the normotensive hindquarters vascular beds of animals with coarctation hypertension, similar to the increases in renal resistance in Goldblatt hypertension reported by Murphy et al. In the hindquarters, these increases involve all components of resistance: neural, humoral-myogenic, and structural. It is possible that the increased structural component (elevated resistance at maximal vasodilation) alone accounts for the increases in the other resistance components; Folkow has discussed the hemodynamic implications of increases in the structural component of resistance (wall-to-lumen ratio) in hypertension.

Abnormalities in vascular composition and structure have also been reported. Water, sodium, and potassium contents, as well as the dry weight, of the abdominal aorta are increased. Similar observations were made in the present investigation. In a more recent study of the microcirculation of the hindquarters, Plunkett and Overbeck observed 12-33% increases in wall thickness and the wall-to-lumen ratio in third- to fifth-order cremaster arterioles in coarcted compared with sham-coarcted rats. These structural abnormalities measured in vivo persisted after maximal arteriolar relaxation with topical nitroprusside.
In the present investigation, we sought quantitative morphometric evidence that structural changes occur in arteries and arterioles of the normotensive hindquarters of rats with coarctation hypertension. The thickening of the wall of the abdominal aorta and femoral artery that we observed provide additional evidence for non-pressure-related wall thickening of conduit arteries. In renal arterioles less than 60 μm in diameter in the hypertensive rats, there was also clear-cut evidence for increases in wall medial area, thickness, and wall-to-lumen ratio. The magnitude of this wall thickening in these small arterioles in rats with coarctation hypertension was indistinguishable from that in rats with Goldblatt hypertension. The wall thickening in general cannot be explained by differences in diameter of the selected vessels. The increases in wall area specifically cannot be attributed to artifacts in vessel distention during fixation nor to differences in contractile state of the medial vascular smooth muscle between hypertensive and normotensive rats. The tight linear relation between medial area and lumen diameter (Figures 9 and 10) provides further evidence for validity of our measurements.

In contrast to smaller arterioles, in arterioles 61–100 μm diameter thickening was greater in Goldblatt hypertensive rats than in coarcted rats. In renal arteries greater than 100 μm in the Goldblatt hypertensive rats, trends suggested that the abnormal wall thickening persisted; there was no similar evidence for thickening of these larger renal arteries in the coarcted rats.

In summary, small renal arterioles appear to participate in the non-pressure-related arterial wall growth that occurs in coarctation hypertension in rats, in addition to the conduit arteries and arterioles of the cremenster and hind limb circulations. Structural changes in renal arterioles may help explain the increased renal resistance downstream to a partial renal arterial occlusion, representing one mechanism by which the perfusion pressure of downstream renal tissue is regulated.

For many years, abnormal cardiovascular wall thickening in hypertension was attributed to mechanisms related to elevated pressure and wall stress. However, increasing evidence suggests a major role for non-pressure-related processes. In the case of the heart, this is true in genetic and in renal hypertension. Non-pressure-related vascular wall thickening has also been reported in genetic and in experimentally induced forms of hypertension, including classic renovascular hypertension. Nongenetic, non-pressure-related factors that may be involved in vascular wall thickening in hypertension include sympathoadrenergic influences, humoral influences, and growth factors in blood or vascular wall tissues. Our previous investigations suggest that sympathoadrenergic influences may be of minor importance in the non-pressure-related arterial wall thickening we see in coarctation hypertension. In coarcted rats with the sympathoadrenergic system nearly ablated, we observed hemodynamic evidence for increases in wall-to-lumen ratio of hindquarters resistance vessels. More recently, in in vivo studies, we have also directly observed wall thickening in the cremenster arterioles in sympathectomized animals.

It is possible that growth factors released by circulating cells may be responsible for the vascular growth we observed in the hindquarters of coarcted rats. Platelets activated by turbulence associated with the aortic constriction may release platelet-derived growth factor with downstream effects on arterial growth. Other humoral substances, such as angiotensin II or the digitalis-like factor described by Schreiber et al., may be involved. If resistance of renal arterioles, and hence renal perfusion pressure, is being regulated by the arterial thickening that we observed, it would be likely that the kidney in some way controls the release of the factors that produce the arterial wall thickening.

![Morphometry of renal arterioles and arteries with vessel diameter >60 μm. As in Figure 9. Correlation coefficients for the regressions are 0.971, 0.972, and 0.979 for coarctation, Goldblatt, and sham-coarctation groups, respectively. Analysis of variance indicates significant differences among the groups. Slopes of Goldblatt hypertensives (0.517) and sham-coarcted (0.514) groups are not significantly different (p>0.8), but each differs from the slope of the coarcted hypertensive group (0.477; p<0.05). Intercepts of Goldblatt and coarctation hypertensives do not differ (p>0.7), but each differs from the intercept of the sham-coarcted group (p<0.01).](http://circres.ahajournals.org/doi/abs/10.1161/01.RES.62.5.1008)
Regarding conduit arteries, Owens and Schwartz have provided evidence that growth of smooth muscle cells in hypertension occurs by a hypertrophic process characterized by polyplody. They suggest that the polyplody and excess DNA are evoked by early transient rises in arterial pressure. In their view, the arterial smooth muscle cells are thereby programmed to abnormal growth, which in turn promotes the hypertensive process. In contrast, Mulvany et al have more recently reported that in smaller resistance arteries, smooth muscle cells undergo hyperplasia. Thus, the mechanisms of abnormal wall thickening may differ in arterioles and conduit arteries. That different processes may be involved in the thickening of conduit and resistance vessels in our coarcted rats is suggested by the lack of wall thickening in the larger renal arteries. We have no other explanation for the discontinuity of the thickening we observed, which is particularly apparent in Figures 9 and 10.

It is possible that the hyperplasia of resistance arteries in hypertension such as those observed by Mulvany et al may not be pressure-related. Meininger et al have reported normal intravascular pressures in arterioles less than 30 \(\mu\)m in diameter in Goldblatt hypertension, and we observed thickening of the walls of these small arterioles in the Goldblatt hypertensives as well as in the coarcted rats.

In the present investigation, we found evidence for wall thickening of the abdominal aorta and femoral artery, both conduit vessels. The wall thickening involves “waterlogging” as well as increases in dry weight. It remains unclear whether the non-pressure-related increases in dry weight of these conduit arteries involves hypertrophy, hyperplasia, fibrosis, or a combination. The presence of hypertrophy with polyplody would argue that pressure-related mechanisms may not be solely responsible for such abnormal growth, as has been suggested by Owens. Our ongoing investigations are designed to determine the nature of the non-pressure-related wall thickening. These investigations include electron microscopy to detect subtle intimal abnormalities, as well as quantitation of matrix elements and differentiation between smooth muscle hypertrophic and hyperplastic processes.

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