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Wall Remodeling after Wall Shear Rate Normalization in Rat Mesenteric Arterial Collaterals

Key Words

Collateral
Arterial growth
Arterial remodeling
Wall shear
Intima
Media
Resistance artery

Abstract

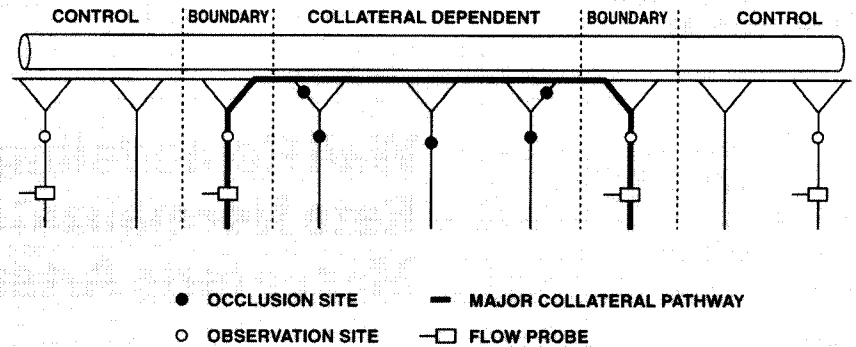
Previous studies have demonstrated endothelial and smooth muscle hyperplasia occur during arterial luminal expansion associated with elevation of arterial wall shear rates. The current study investigated whether remodeling induced by elevated wall shear would ultimately result in a vessel with intimal and medial cell densities and other wall characteristics similar to control arteries. A rat mesenteric model was used in which collateral wall shear is restored to normal 4 weeks after arterial occlusion. Twelve weeks after shear elevation, paired *in vivo* measurements indicated that the maximum collateral inner diameter was increased 27–75%. Morphometric evaluation of collateral cross sections indicated that, relative to control arteries, luminal and medial areas were increased 79 ± 22 and $56 \pm 15\%$. Collateral medial cell density was decreased (1.12 ± 0.044 vs. 1.35 ± 0.005 nuclei/1,000 μm^2) but intimal cell density was similar (2.86 ± 0.166 vs. 2.49 ± 0.102 nuclei/100 μm luminal perimeter). Medial thickness to radius ratio was also similar between control and collateral arteries. Thus, for the wall characteristics evaluated, there are many similarities between enlarged collaterals and control arteries. Comparison of nuclear numbers in arterial cross sections of the current and previous studies suggest that intimal and medial cellular regression is correlated with a decrease in wall shear force toward normal levels.

Introduction

Although collateral development is commonly associated with ischemia [1–3], we have recently demonstrated that collateral enlargement is correlated with increases in arterial blood flow and shear stress and can occur independent of tissue ischemia [4, 5]. Our observations are consistent with other studies which have demonstrated that the structural diameter of arteries is altered by

chronic changes in blood flow or shear stress [6–9]. Our model to study collateral development in the rat mesentery provides a clearly defined collateral pathway and the ability to make paired observations on small resistance arteries which form collaterals. When wall shear rate is abruptly increased by about 175%, we have observed increases of ~30 and 65% in collateral diameters at 1 and 4 weeks, respectively [4, 5]. Very significant alterations must occur within the arterial wall to allow this rapid and

Fig. 1. Drawing illustrates the model used in this study. Sites of arterial ligation and measurements of diameter and flow are shown. The collateral pathway originated from a patent mesenteric artery on each side of the boundary region and continued through the marginal arteries towards the center. Diameter measurements were repeated at exactly the same locations before and 12 weeks after arterial ligation.



substantive enlargement of the collateral lumen. Vascular wall remodeling associated with changes in luminal diameter may involve reorganization, degradation, and/or proliferation of the existing wall constituents [10]. In our earlier study [5], luminal expansion subsequent to abrupt flow elevation was associated with wall hypertrophy and cellular proliferation in both the intima and media [5].

As we contemplated the observations of our collateral growth studies [4, 5], we considered that in the ideal scenario the remodeling process would produce a larger artery with normal wall characteristics. Review of the literature demonstrated a paucity of information on the remodeling of the arterial wall during the progression of collateral enlargement and existing studies were controversial. For example, wall thinning and wall thickening have both been reported for enlarging collateral arteries [1, 11, 12]. Furthermore, existing studies have not considered how shear forces may correlate with specific remodeling events within the arterial wall. Since shear forces have been shown to alter the expression of many growth factor genes in vascular endothelium [for recent reviews, see 12–14], the level of shear forces could have a major influence on the wall remodeling at a particular time point. Four weeks after creation of our model [5], wall shear rate had been restored to normal levels by expansion of the collateral lumen. At this time, medial cell density and the ratio of medial thickness to luminal radius were similar between collateral and control arteries, but intimal cell density remained elevated. As we gave additional consideration to these observations, we wondered if the remodeling process was complete at this time when wall shear rate had been restored to normal. If so, the elevated intimal cell density might have important functional implications for these vessels. Alternatively, intimal remodeling might continue after the normalization of wall

shear forces and ultimately result in normal spacing between cells.

The current study was undertaken as an extension of our previous studies [4, 5] to determine, in small resistance arteries subjected to abrupt elevation of wall shear force, what if any additional changes in intimal and medial cell densities occur within the arterial wall after the normalization of the shear forces.

Methods

All procedures performed in this study were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee. A collateral dependent region of small intestine was created as previously described [4] and a technique for repeated observation of the intestinal vasculature was utilized [15]. Male Wistar rats (~200 g, Harlan Sprague-Dawley, Indianapolis, Ind.) were initially anesthetized with sodium pentobarbital (50 mg/kg, i.m.) and administered atropine (0.4 mg/kg, i.m.) to prevent airway congestion. When mobility was lost, the rat's abdominal region was shaved. The rat was then placed on a heating pad to maintain rectal temperature at 37°C. Supplemental anesthesia (pentobarbital, 10 mg/kg) was administered as needed. The abdominal region was wiped with an alcohol pad, a liquid adhesive (Mastisol, Ferndale Laboratories, Ferndale, Mich.) applied, and an intestinal support chamber [15] attached with modified surgical adhesive drape. A midline abdominal incision was made through the skin and linea alba. The terminal ileum was exteriorized into the support chamber for the creation of a collateral-dependent intestinal segment as previously described. A standardized length of small intestine located near the appendix was selected so that the intended collateral-dependent region would contain 45–55 first-order arterioles after the ligation of 3–4 sequential mesenteric arteries. The bowel was covered with prewarmed (~37°C) phosphate-buffered saline (PBS) or plastic wrap at all times during the procedure. The bowel segment was maximally dilated (10^{-4} M adenosine and 10^{-5} M sodium nitroprusside in PBS applied topically to the bowel) and video images of the mesenteric arteries at the control and boundary regions (fig. 1) were recorded for later diameter measurement (Olympus SZH Dissecting Microscope,

Olympus, Lake Success, N.Y.; Hamamatsu Model C2400-50 CCD video camera; Hamamatsu Photonics KK, Japan; Sony SVO-9500MD SVHS VCR and Sony Trinitron Monitor Model PVM-1343MD, Sony Medical Systems, Montvale, N.J.; total magnification $\sim 50\times$). At locations shown in fig. 1, mesenteric arteries were carefully isolated from adjacent tissues and ligated with 8-0 nylon suture. The isolation and ligation were performed using the dissecting microscope with extreme care taken to avoid the adjacent vein, lymphatics and nerve. After completing the arterial ligations, the bowel was carefully placed back into the peritoneal cavity, and the incision was closed with a running suture (3-0 Dexon[®], Davis & Geck, Mauati, PR). The rats were administered antibiotics for 3 days postoperatively (tetracycline, 1.1% in drinking water) and allowed free access to food and water.

At the final time of observation (12 weeks later), rats were again anesthetized, then shaved in the abdominal region. A tracheostomy was performed to ensure a patent airway, and the femoral artery was cannulated to verify that mean arterial pressure was greater than 90 mm Hg when the arterial diameter measurements were made. The intestinal support chamber was attached as above, and the collateral-dependent bowel segment located. Videography was repeated as above for the measurement of inner arterial diameters at exactly the same location on the control and collateral arteries as when the model was created. Diameter and flow measurements were made during both control and maximally dilated conditions. However, because of the amount of manipulation required to place flow probes and position the arteries to record diameter, the tone of the arteries prior to dilation may not represent the normal resting tone under *in vivo* conditions. Arterial blood flow was measured (0.5 V perivascular ultrasonic flow probes and Model 206 Flowmeter, Transonics Systems, Ithaca, N.Y.) in collateral arteries at the boundary region and in adjacent control arteries as shown on figure 1. Zero offsets were determined for each artery and subtracted from each flow measurement. Arterial diameters were obtained simultaneously with the flow measurements. The average wall shear rate (WSR) at the arterial wall was calculated using the formula: $WSR = (4Q)/(\pi r^3)$ where Q is blood flow (ml/s) and r is the vessel radius (cm). To eliminate the effect of possible changes in flow probe calibration from one day to the next, collateral flows and shear rates are expressed as percent of control artery flow and shear rate within the same animal.

Next, the vessels were prepared for histological evaluation. To preserve the arteries, a cotton suture was placed around a bowel segment containing the collateral-dependent region and adjacent arteries supplying control tissue. The suffusion solution was replaced with prewarmed 10% neutral buffered formalin-containing vasodilator cocktail (10^{-4} M adenosine and 10^{-5} M sodium nitroprusside). In this manner, the vessels were fixed at their maximum diameter for the prevailing *in vivo* arterial pressure. The bowel segment was tied off with the suture and the rats were euthanized with an overdose of anesthesia (~ 150 mg/kg). The bowel segment was then excised and placed in 10% formalin overnight. After overnight fixation, bowel segments were transferred to PBS. In our experience, this method of fixation provides excellent histological preservation at the light microscopy level and the arterial cross sections are consistently more circular than when obtained with perfusion fixation. Segments of the mesenteric vascular bundle containing the artery, vein and lymphatic were removed from one boundary and control region (fig. 1) within each rat. Each segment was dehydrated in ethanol, embedded in plastic (JB4, Polysciences, Warrington, Pa.), and sectioned (3 μ m thick) so that at least 10 μ m intervened between each section. Extreme care

was taken to orient the longitudinal axis of the embedded arterial segment perpendicular to the microtome knife using the adjustments and adapters on the JB4 microtome. Three sections from each segment were stained with methylene blue-basic fuchsin for morphologic assessment. All cross sections were characterized by media of uniform thickness with vascular smooth muscle cells oriented circumferentially.

Video images of the processed arterial cross sections were acquired and stored using an image analysis system (Olympus BHMJ Microscope with Image-1/AT, Universal Imaging, West Chester, Pa.; total magnification $\sim 400\times$). Measurements of the *in vivo* inner diameters of arteries were made with the image analysis system before and 12 weeks after creation of model. After contrast enhancement, the luminal area (A_L), luminal + intimal areas (A_{L+I}), and luminal + intimal + medial areas (A_{L+I+M}) of the arterial cross sections were determined by image analysis with gray level thresholding to select only the region to be measured. Preliminary measurements confirmed that this method gave results similar to manual tracing. The luminal perimeters were determined with the image analysis software. The medial area (A_M) was then calculated: $A_M = A_{L+I+M} - A_{L+I}$. The average medial thickness (T_M) was also calculated by assuming circularity of the arterial cross sections: $T_M = (A_T/\pi)^{0.5} - (A_{L+I}/\pi)^{0.5}$. The total number of nuclei in the endothelial layer of the intima and in the media of each section were manually counted via the microscope at $200\times$. From the arterial cross sections, the circumferential and radial dimensions of medial nuclei and area of intimal nuclei were also determined with image analysis of the video images.

Statistical Analysis

All data were entered into a spreadsheet (Microsoft Excel v5.0). The histological data from the 3 cross sections of each artery were averaged and individual animal means were determined for each parameter for control and collateral arteries. The data were then imported into a statistical software package (Sigma Stat for Windows v1.0, Jandel Scientific, San Rafael, Calif.) for comparisons. All data are reported as animal averages \pm standard error of the mean. Statistical comparisons were made using repeated measures analysis of variance (RM ANOVA) or paired t test as indicated in table and figure legends.

Results

Nine male Wistar rats (Harlan Sprague-Dawley, Indianapolis, Ind.) were used for this study. The average animal weights were 213 ± 11 g at the time of model creation, and 499 ± 10 g 12 weeks later. *In vivo* measurements of maximum diameters and arterial blood flows were successfully made in all 9 animals. As depicted in figure 2, the maximally dilated *in vivo* diameters were initially similar between the control and collateral arteries (247 ± 6 vs. 253 ± 11 μ m, respectively). Twelve weeks after the model was created, the maximal *in vivo* diameters of the control arteries were not significantly increased (271 ± 11 μ m) but the maximum diameter of the collateral arteries had increased ($45 \pm 6\%$, $p < 0.001$) from the

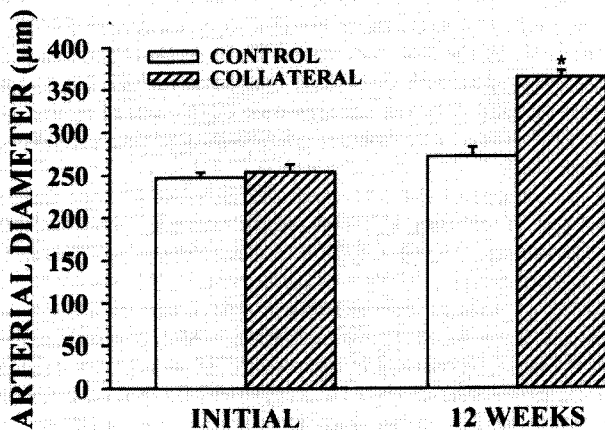


Fig. 2. The averages of in vivo diameters before and 12 weeks after ligation are reported for the collateral and control arteries ($n = 9$ for paired comparisons). Two-way RM ANOVA indicated significant differences between collateral and control vessels ($p = 0.004$) and differences at the 2 times of observation ($p < 0.0001$). There was significant interaction between vessel type and time ($p = 0.002$). Specific differences ($p < 0.05$) between groups were identified by pairwise multiple comparisons using the Student-Newman-Keuls method.

Table 1. Collateral artery blood flow, diameter and estimated wall shear rate under conditions of rest and maximal dilation

	Flow	Diameter	Wall shear rate
Rest	$195 \pm 20^*$	$124 \pm 8^*$	120 ± 19
Dilated	$156 \pm 8^*$	$132 \pm 8^*$	79 ± 13

Expressed as percent of control artery within the same rat ($n = 8$ animals). * $p \leq 0.05$ vs. control (100%).

preligation value. Relative to the diameter of control arteries within the same animals, collateral diameters were $103 \pm 9\%$ ($p = 0.56$) of control initially and $138 \pm 7\%$ ($p < 0.005$) after 12 weeks. Because these diameter measurements were made under maximally dilated conditions, the increase in diameter represents structural changes to expand the lumen rather than dilation due to smooth muscle relaxation.

Within each animal, average collateral diameter, flow and calculated wall shear rate were expressed as percent of the control artery and are reported in table 1. One rat was eliminated from the comparisons because its estimated wall shear rate for the control artery was 2.8 standard

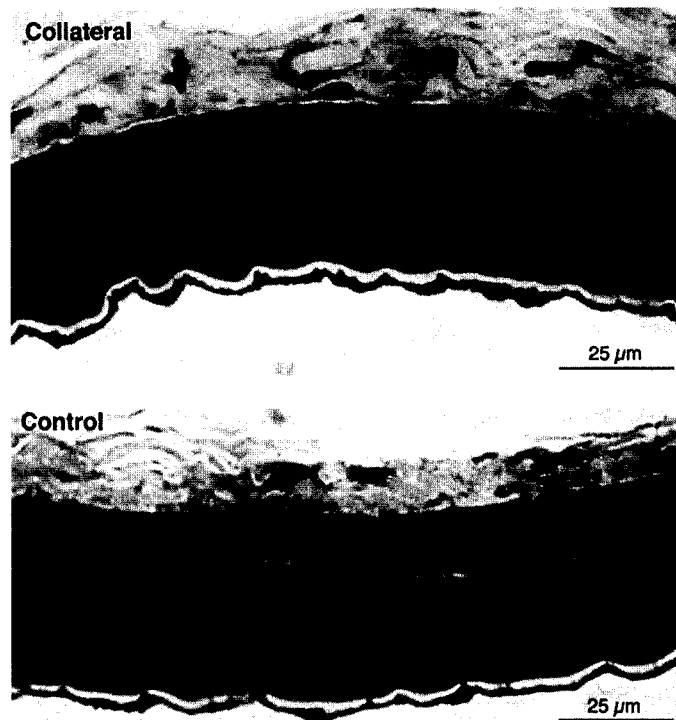


Fig. 3. Micrographs of cross sections of the wall of representative control and collateral arteries from the same rat, 12 weeks after ligation. The contrast between the nuclei and tissue was increased by both analog and digital methods using the camera control box and the image analysis software. The vessel cross sections were stained with a methylene blue-basic fuchsin dye.

deviations above the average for control arteries in all animals. Diameters and flows were elevated in collaterals but the estimated wall shear rates were similar between collateral and control arteries.

Representative cross sections of a control and collateral artery from the same animal are shown in the micrographs of figure 3. The intima, media, and internal elastic membrane were easily identified in all cross sections. The internal elastic membrane was distinct and intact. In all vessels, the appearance of the intima and media was perfectly normal – no atypical characteristics were apparent such as the intimal hyperplasia that occurs in injured vessels. Longitudinal sections indicated that the intimal cell nuclei were uniformly oriented parallel to the direction of flow (longitudinal vessel axis). Paired histological comparisons were possible for 6 animals; 1 animal died before the vessels could be fixed and 1 control and 1 collateral artery from 2 different animals were excluded because properly oriented cross sections could not be obtained from the plastic sections. Histological and morphometric

Table 2. Morphometric and histological comparisons between control and collateral arteries

	Control	Collateral
Luminal area, μm^2	54,200 \pm 6,220	91,900 \pm 8,440*
Medial area, μm^2	25,700 \pm 3,050	37,900 \pm 712*
Medial thickness, μm	28 \pm 2.6	32 \pm 1.0
Medial thickness/radius	0.214 \pm 0.022	0.192 \pm 0.014
Number of medial nuclei	34 \pm 3.6	44 \pm 1.2
Medial nuclei/area, n/1,000 μm^2	1.36 \pm 0.05	1.17 \pm 0.04*
Number of intimal nuclei	22 \pm 0.6	32 \pm 1.7*
Intimal nuclei/perimeter, n/100 μm	2.49 \pm 0.10	2.86 \pm 0.17
Medial nuclear width, μm	3.26 \pm 0.075	3.20 \pm 0.184
Medial nuclear length, μm	14.4 \pm 0.53	15.9 \pm 1.24
Intimal nuclear area, μm^2	17.1 \pm 0.47	19.6 \pm 1.91

Paired t test (n = 6 animals). * p \leq 0.05 vs. control.

parameters are presented in table 2. Paired comparisons of control and collateral arteries in these 6 animals indicated that, 12 weeks after creation of the model, the luminal and medial cross-sectional areas of the collateral arteries were greater than control arteries by $79 \pm 22\%$ (p = 0.019) and $56 \pm 15\%$ (p = 0.008), respectively. Intimal cell density (number of intimal nuclei per 100 μm of luminal perimeter) and the ratio of medial thickness to luminal radius were similar between collateral and control arteries (table 2). Medial thickness and number of medial nuclei tended to be higher (p < 0.10) in collaterals by 20 ± 7.5 and $35 \pm 15\%$, respectively. Medial cell density averaged $14 \pm 3\%$ lower in collaterals than control arteries and the number of intimal cell nuclei was increased by $46 \pm 9\%$ in collaterals relative to control arteries. For control and collateral arteries, both intimal and medial nuclei in the cross sections were of similar size as indicated by the dimensional measurements reported in table 2.

Discussion

It should be noted that the intimal and medial cell densities from the current and previous [5] study with this model were estimated from nuclear counts in vessel cross sections. As recently reviewed [16], such an index of cell density is accurate only to the extent that nuclear size and orientation are similar. Although the use of recently developed assumption-free methods [16] would have offered a number of advantages and provided additional information, we consider the technique used in the current study to be adequate for the comparisons made. Examination of our arterial cross sections confirm that

nuclear orientation of smooth muscle cells was similar in collateral and control vessels as shown in figure 3. Nuclear dimensions are similar in the collateral and control vessels (table 2).

This study addressed the hypothesis that shear-mediated arterial growth would produce a larger vessel with normal wall characteristics. In many respects, the collaterals of this study, observed 12 weeks after abrupt elevation of wall shear, have properties similar to control arteries. Although the collaterals are significantly enlarged (fig. 2, 3, tables 1, 2) and have elevated blood flows (table 1), as also shown in our previous studies [4, 5], wall shear rates (table 1), the ratio of medial thickness to luminal radius, and intimal cell densities (table 2) are similar to control arteries within the same animals. Medial cell density (table 2) is only slightly decreased in the collateral relative to control arteries. These observations in healthy animals demonstrate that, at least for the wall parameters evaluated, arterial growth associated with elevated shear rates ultimately produces a vessel with major properties close or similar to control arteries. However, since flow-mediated growth and remodeling are dependent upon the endothelium [6, 17], it is important to note that this process might be impaired in diseased animals characterized by endothelial dysfunction, such as occurs in diabetes, hypertension, and hyperlipidemia – primary risk factors for vascular disease. In fact, preliminary studies in our laboratory indicate that shear-mediated growth and remodeling are altered in animal models of essential hypertension and insulin-dependent diabetes [18, 19].

It is interesting to note that although body mass is increased more than 100% during the 12-week interval between observations, the maximum diameter of the con-

trol arteries is not increased (fig. 2). It would seem to be a logical assumption that maximum vessel diameter would increase with body mass. However, our previous studies indicate that bowel growth is proportional to increases in body mass only very early during juvenile life [15, 20, 21]. While bowel growth in rats is proportional to body growth from ages 5–10 weeks [15, 20], only minimal intestinal growth occurs in normal rats after 10 weeks of age [21]. Our available data indicate that while adaptations occur in the terminal microvasculature (small arterioles and capillaries) [15, 20, 21], significant diameter enlargement of control small arteries (fig. 2) [5] and large arterioles [20, 21] does not occur in the intestine or mesentery of the age of rats used in this study.

As identified in the Introduction, although shear-mediated arterial growth [10] and collateral luminal expansion [1, 4, 5, 11, 22–25] are well documented, very little information is available concerning how the intima and media are altered as growth progresses. Masuda et al. [26] found that endothelial hypertrophy and hyperplasia occurred before medial changes in the canine carotid artery after elevation of arterial flow by construction of an arteriovenous fistula. Schaper's group [27] has observed endothelial proliferation to precede smooth muscle mitosis in canine coronary collaterals. At least in some instances, endothelial proliferation does not occur with flow elevation as indicated by the study of Walpole et al. [28] in carotid arteries of adult rabbits. Medial hyperplasia has also been observed in collateral vessels. A study of cerebral collaterals by Lehman et al. [11] demonstrated that 4 weeks after arterial ligation, the medial cross-sectional area was increased in collaterals by smooth muscle hyperplasia rather than hypertrophy. However, specifically lacking are studies which correlate the rate of luminal expansion and the specific nature of the remodeling process with changes in wall forces associated with arterial flow and pressure.

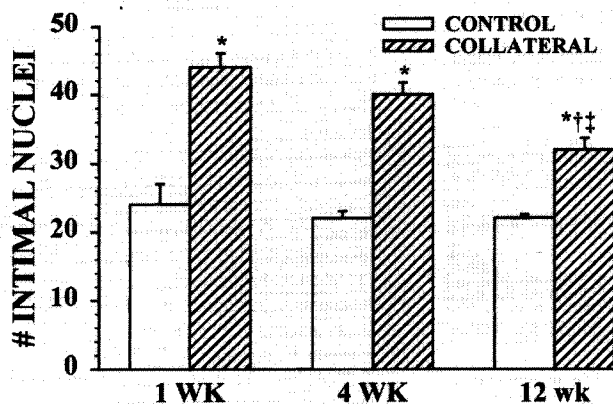
We have observed that intimal hyperplasia and medial hypertrophy occur within the 1st week when wall shear forces remain elevated [5]. After 4 weeks, when wall shear forces have decreased toward normal, medial hyperplasia has occurred, smooth muscle cell density is similar but endothelial cell spacing remains elevated in collaterals relative to controls. The current study demonstrates that, in the exact same model at 12 weeks after the abrupt elevation of flow, endothelial cell spacing is normal and smooth muscle cell density is slightly reduced in the collaterals (table 2). While a more precise technique such as the dissector method might reveal that differences still exist at 12 weeks, our data clearly show that, in terms of cell den-

sity, the collaterals are evolving to become more like control arteries within the same animals.

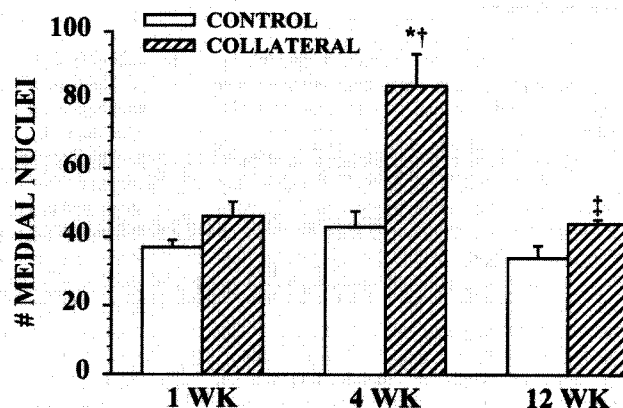
Although our studies have been correlative and do not establish changes in shear forces as the cause of the time-dependent alteration in the nature of the remodeling process, it seems likely that the changes in shear forces would be involved to at least some extent. Our findings imply that the remodeling process, associated originally with elevation of wall shear forces, may not have been completed by the time the wall shear rate is normalized. It is important to note that the relationship between medial thickness and luminal radius is not altered during the course of collateral development in this model (table 2) [5]. This observation suggests that as luminal expansion occurs to normalize shear forces, circumferential wall stress is also maintained.

Data from our current and previous [5] studies suggest that the initial proliferative response to abruptly elevated shear forces produces an overabundance of cells in both the intima and media which is followed by cellular regression/rarefaction. To illustrate this, the data from these two studies are summarized together in figure 4. [Although caution should be taken in comparing data between studies, we believe it is legitimate to make these comparisons. The studies were performed in the same laboratory by the same investigators within a 1-year period. The techniques, methods, and equipment utilized were identical. The tissues were processed for histological and morphometric analysis by the same histotechnologist. At all time points studied (1, 4, and 12 weeks) the number of intimal and medial nuclei observed in the cross sections of control arteries were similar.] At 1 week, the number of intimal nuclei in the cross sections of collaterals was ~90% greater than in control arteries. At 12 weeks, there were significantly fewer intimal nuclei in the collateral cross section than observed at either 1 or 4 weeks. The results is a normal intimal cell density at 12 weeks (table 2). The number of medial nuclei observed in collaterals is increased at 4 weeks. By 12 weeks the number of medial cells in arterial cross section has decreased to be similar to the number of medial cells observed in both control arteries and 1-week collaterals.

Recent reviews have considered the mechanisms by which vascular growth in general [14] and more specifically collateral development [12] may be regulated. Mechanical forces such as wall shear and circumferential wall stress can alter the production of growth factors as reviewed by Skalak and Price [14]. Pries et al. [29] have proposed that shear stress and pressure-dependent forces act together to influence vascular diameter and wall thick-



*, †, ‡: $p < 0.05$ vs CONTROL, 1 WK, 4 WK



*, †, ‡: $p < 0.05$ vs CONTROL, 1 WK, 4 WK

Fig. 4. Intimal (a) and medial (b) cell numbers from the current (12 weeks) and our previous study (1 and 4 weeks) [5] are shown. All counts in control and collateral arteries were made at identical branching locations (fig. 1). The results demonstrate a decrease in both intimal and medial cell nuclei in collaterals from 4 to 12 weeks after abrupt elevation of shear, during a time when shear rates were similar in control and collateral arteries. Two-way RM ANOVA was used for the comparisons and indicated significant differences between time points (1, 4, and 12 weeks) and vessel type (control and collateral) for both medial and intimal cell counts. Specific differences ($p < 0.05$) between groups were identified by pairwise multiple comparisons using the Student-Newman-Keuls method and are indicated.

ness. Although monocytes/macrophages may have an important role in some instances of collateral development and remodeling [12] we consider it unlikely that they have a significant role in our model for reasons previously stated [5]. In our model, a correlation exists between the elevation of wall shear rate and the magnitude of luminal expansion [4, 5]. Since shear stress is involved in the regulation of arterial growth [6, 8, 9, 30–32], it is reasonable to expect that changes in shear forces within collateral vessels would be involved in the observed enlargement of the lumen and the associated wall remodeling. This is supported by previous studies which have demonstrated that shear-mediated luminal expansion or reduction is endothelial-dependent [6, 17] and that increases in shear stress induce the expression of endothelial genes for growth factors [12–14]. In these resistance vessels, changes in shear stress may influence vascular tone and alter circumferential wall stress even when pressure remains unchanged. Consequently it seems likely that alterations in shear and circumferential wall stress would modulate the balance between growth inhibitors and promoters and influence the luminal enlargement and wall remodeling of resistance vessels. Thus the balance between paracrine/autocrine growth modulators produced within the local envi-

ronment of the arterial wall may continuously be adjusted as wall forces associated with pressure and flow change during progressive luminal expansion. Such a shift in the balance between growth factors might explain specific cellular events within the collateral wall, including proliferation and apoptosis [12].

In summary, the current study provides to our knowledge the first evidence that, in arteries exposed to abruptly elevated blood flow, cellular regression or rarefaction occurs in both the media and intima after an initial hyperplastic response. Much additional work is needed to clarify the specific stimuli, elucidate the transduction mechanisms, and identify the specific growth modulators involved in this late as well as earlier phases of normal collateral development. Such studies should not only extend our basic understanding of vessel growth but also provide insight for potential new therapies for vascular pathologies.

Acknowledgement

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References

- 1 White FC, Carroll SM, Magnet A, Bloor CM: Coronary collateral development in swine after coronary artery occlusion. *Circ Res* 1992;71:1490-1500.
- 2 DeFily DV, Chilian WM: Methods for assessing coronary collateral growth: Insights into mechanisms responsible for collateral development; in Schaper W, Schaper J (eds): *Collateral Circulation: Heart, Brain, Kidney, Limbs*. Boston, Kluwer Academic Publishers, 1993, pp 29-40.
- 3 Schaper W: New paradigms for collateral vessel growth. *Basic Res Cardiol* 1993;88:193-198.
- 4 Unthank JL, Nixon JC, Burkhardt HM, Fath SW, Dalsing MC: Early collateral and microvascular adaptations to intestinal artery occlusion in the rat. *Am J Physiol* 1996;271:H914-H923.
- 5 Unthank JL, Fath SW, Burkhardt HM, Miller S, Dalsing MC: Wall remodeling during luminal expansion of mesenteric arterial collaterals in the rat. *Circ Res* 1996;79:1015-1023.
- 6 Langille BL, O'Donnell F: Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science* 1986;231:405-407.
- 7 Longland CJ: The collateral circulation of the limb. *Ann R Coll Surg* 1953;13:161-176.
- 8 Kamiya A, Togawa T: Adaptive regulation of wall shear stress to flow change in the canine carotid artery. *Am J Physiol* 1980;239:H14-H21.
- 9 Zarins CK, Zatina MA, Giddens DP, Ku DN, Glagov S: Shear stress regulation of artery lumen diameter in experimental atherogenesis. *J Vasc Surg* 1987;5:413-420.
- 10 Langille BL: Blood flow-induced remodeling of the artery wall; in Bevan JA, Kaley G, Rubanyi GM (eds): *Flow-Dependent Regulation of Vascular Function*. New York, Oxford University press, 1995, pp 277-299.
- 11 Lehman RM, Owens GK, Kassell NF, Hongo K: Mechanism of enlargement of major cerebral collateral arteries in rabbits. *Stroke* 1991;22:499-504.
- 12 Schaper W, Ito WD: Molecular mechanisms of coronary collateral vessel growth. *Circ Res* 1996;79:911-919.
- 13 Resnick N, Gimbrone MA Jr: Hemodynamic forces are complex regulators of endothelial gene expression. *FASEB J* 1995;9:874-882.
- 14 Skalak TC, Price RJ: The role of mechanical stresses in microvascular remodeling. *Microcirculation* 1996;3:143-165.
- 15 Unthank JL, Bohlen HG: Quantification of intestinal microvascular growth during maturation: Techniques and observations. *Circ Res* 1987;61:616-624.
- 16 Mayhew TM, Gundersen HJ: If you assume, you can make an ass out of u and me: A decade of the dissector for stereological counting of particles in 3D space. *J Anat* 1996;188:1-15.
- 17 Tohda K, Masuda H, Kawamura K, Shozawa T: Difference in dilatation between endothelium-preserved and -desquamated segments in the flow-loaded rat common carotid artery. *Arterioscler Thromb* 1992;12:519-528.
- 18 Fath SW, Burkhardt HM, Dalsing MC, Unthank JL: Shear-mediated growth and remodeling in resistance arteries of hypertensive rats (abstract). *Microcirculation* 1997;4:140.
- 19 Burkhardt HM, Fath SW, Dalsing MC, Unthank JL: Arterial growth and remodeling in diabetic rats (abstract). *Microcirculation* 1997;4:141.
- 20 Unthank JL, Bohlen HG: Intestinal microvascular growth during maturation in diabetic juvenile rats. *Circ Res* 1988;63:429-436.
- 21 Unthank JL, Lash JM, Bohlen HG: Maturation of the rat intestinal microvasculature from juvenile to early adult life. *Am J Physiol* 1990;259:G282-G289.
- 22 Coyle P: Diameter and length changes in cerebral collaterals after middle cerebral artery occlusion in the young rat. *Anat Rec* 1984;210:357-364.
- 23 Conrad MC, Anderson JL, Garrett JB: Chronic collateral growth after femoral artery occlusion in the dog. *J Appl Physiol* 1971;31:550-555.
- 24 Schaper W, Gorge G, Winkle B, Schaper J: The collateral circulation of the heart. *Prog Cardiovasc Dis* 1988;31:57-77.
- 25 Young MA, Vatner SF: Regulation of large coronary arteries. *Circ Res* 1986;59:579-596.
- 26 Masuda H, Kawamura K, Tohda K, Shozawa T, Sageshima M, Kamiya A: Increase in endothelial cell density before artery enlargement in flow-loaded canine carotid artery. *Atherosclerosis* 1989;9:812-823.
- 27 Pasyk S, Schaper W, Schaper J, Pasyk K, Misiewicz G, Steinseifer B: DNA synthesis in coronary collaterals after coronary artery occlusion in conscious dog. *Am J Physiol* 1982;242:H1031-H1037.
- 28 Walpole PL, Gotlieb AI, Langille BL: Monocyte adhesion and changes in endothelial cell number, morphology, and F-actin distribution elicited by low shear stress in vivo. *Am J Pathol* 1993;142:1392-1400.
- 29 Pries AR, Secomb TW, Gaehtgens P: Design principles of vascular beds. *Circ Res* 1995;77:1017-1023.
- 30 Langille BL: Remodeling of developing and mature arteries: Endothelium, smooth muscle, and matrix. *J Cardiovasc Pharmacol* 1993;21(suppl 1):S11-S17.
- 31 Langille BL, Bendeck MP, Keeley FW: Adaptations of carotid arteries of young and mature rabbits to reduced carotid blood flow. *Am J Physiol* 1989;256:H931-H939.
- 32 Wang DH, Prewitt RL: Alterations of mature arterioles associated with chronically reduced blood flow. *Am J Physiol* 1993;264:H40-H44.