Flow-induced arterial remodeling in rat mesenteric vasculature

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Tulis, David A., Joseph L. Unthank, and Russell L. Prewitt. Flow-induced arterial remodeling in rat mesenteric vasculature. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H874–H882, 1998.—This study was designed to characterize in vivo arterial remodeling of male Wistar rat small mesenteric arteries exposed to varying levels of elevated blood flow in the presence of normal arterial pressure. Through a series of arterial ligations, respective ileal artery and second-order branch blood flows acutely increased ~36 and ~170% over basal levels. The respective diameters increased 12 and 38% and their wall area increased 58 and 120% in a time-dependent fashion between 1 and 7 days postligation compared with same-animal control vessels. Medial extracellular connective tissue increased concomitantly with medial wall hypertrophy. Immunostaining for proliferating cell nuclear antigen and nuclear profile analyses suggest that both smooth muscle and endothelial cell hyperplasia contribute to flow-induced vascular remodeling. The initial stimulus in this model is flow-mediated shear stress, with possible augmentation by hoop stress, which is increased ~7% by the resultant vasodilation. Stable wall thickness-to-lumen diameter ratios at 1, 3, and 7 days, however, suggest chronic hoop stress is tightly regulated and remains constant. The model described herein allows analyses of two arteries with different degrees of flow elevation within the same animal and demonstrates that the magnitude of vessel remodeling in vivo is directly dependent on the duration of flow elevation after abrupt arterial occlusion.

shear stress; mesenteric arteries; medial wall hypertrophy; cellular hyperplasia

FLUID SHEAR STRESS, the tractive frictional force exerted by flowing blood on the intimal endothelium, has been shown to elicit a wide range of physiological (27, 28), biochemical, and molecular (6, 10, 16, 19) responses in both cell culture and whole animal experiments. Immediate shear stress-dependent dilation (7, 21, 22) and chronic luminal enlargement from elevated blood flow (24, 26) have been well studied. Results from these studies suggest that acute changes in vascular tone and long-term alterations in structural diameter are regulated by blood flow through endothelium-dependent mechanisms that act to restore wall shear stress toward normal homeostatic levels.

In vivo studies investigating vessel wall remodeling associated with chronic alterations in shear stress are limited and have yielded somewhat conflicting results. Reductions in blood flow have been shown to reverse or inhibit vascular growth in both large and small vessels. Langille et al. (11) showed that 70–80% reductions in blood flow for 1 mo decreased lumen diameters and inhibited developmental proliferation of both cellular and extracellular wall components in carotid arteries of young rabbits. Adult rabbits experienced structural reductions in vessel lumen diameter in the flow-restricted artery with no significant changes in medial wall constituents or vessel mass. Wang and Prewitt (28) examined unilaterally orchidectomized cremaster arterioles in juvenile rats and found mean lumen diameters and medial cross-sectional wall areas significantly reduced after 3 wk of flow reduction. Chronic elevation of flow in vivo has also been shown to stimulate arterial growth and enlargement. After 6 mo of chronically elevated blood flow using an iliac arteriovenous shunt in monkeys, Zarins et al. (30) found a significant twofold increase in lumen diameter and medial cross-sectional area with no changes in medial wall thickness compared with contralateral control arteries. Collateralization of rabbit cerebral basilar arteries (12) and rat mesenteric arteries (24, 26) yielded significant increases in medial cross-sectional areas associated with luminal enlargement, with normal medial thickness-to-radius ratios. Although these in vivo studies with native arteries demonstrate a clear correlation between chronic changes in wall shear stress and arterial growth and development, several investigators have found shear stress inversely correlated with vascular growth using models of in vivo graft implants (9), cultured cells (23), and organ culture (1).

The existing studies have not clearly established the sequence of remodeling events associated with chronic changes in blood flow. Recent studies demonstrated that small resistance arteries in the rat mesentery undergo rapid changes in lumen diameter subsequent to abrupt elevation of wall shear forces (24, 26). The most rapid changes occurred within the first week and involved significant adaptations in both the intima and media. The aim of the present study is to further characterize the early arterial wall remodeling that is associated with rapid, shear-mediated luminal expansion. This study investigated the hypothesis that the degree of luminal expansion and the nature of the associated remodeling would be influenced by the duration of flow elevation. To accomplish this, modification of an existing model (26) made it possible to evaluate in the same animal small arteries exposed to two different degrees of blood flow elevation over a 7-day period without alteration of the distending pressure.

MATERIALS AND METHODS

Rat mesenteric shear stress model. All experimental protocols used in this research project comply strictly with the guidelines of the institutional Animal Care and Use Committee. Three groups of 10 male Wistar rats (~300 g) were anesthetized with pentobarbital sodium (60 mg/kg; Im; Anpro Pharmaceutical, Arcadia, CA) and given 10,000 U penicillin G
potassium im (Marsam Pharmaceutical, Cherry Hill, NJ). Supplemental injections of pentobarbital sodium (10% of original dose) were given intramuscularly as needed to maintain a surgical level of anesthesia. Abdomens were shaved and cleaned with a topical antiseptic-antimicrobial agent. Using sterile technique, we made a midline laparotomy, and the cecum, adjoining section of the small intestine, and supporting mesenteric membranes and vasculature were exteriorized and placed on a sterile surgical drape. The cecum, intestines, and mesentery were kept moist throughout the procedure with sterile physiological saline. The fourth, fifth, and sixth ileal arteries proximal to the ileocecal junction were isolated, and arterial ligations were made using sterile 10–0 Ethilon monofilament nylon suture (Ethicon, Somerville, NJ) as indicated in Fig. 1. Ileal arteries are defined as nonbranching first-order vessels leading from the superior mesenteric artery to the ileum, where each divides into two second-order branches. A marginal artery is defined as a transverse vessel segment joining two adjacent small arterial arcades (Fig. 1). Marginal and second-order arteries give rise to first-order arterioles that directly feed the bowel wall. If a marginal artery was identified in the arcade of interest, it was ligated to form a complete barrier to blood flow from outside the feeder artery. The length of the flow-dependent region (the section of the intestinal wall subject to blood flow from the unligated feeding ileal artery) was measured, the number of first-order arterioles feeding the intestinal wall in the flow-dependent region was counted, and a brief diagram of the vascular network was made for each animal. The cecum, intestines, and mesentery were gently placed back inside the peritoneal cavity, the abdominal wall was sutured with sterile surgical clips. The animals were allowed to recover completely, placed back in the animal care facility, and given water and standard rat chow ad libitum for 1, 3, or 7 days.

After the appropriate number of days, the rats were anesthetized with pentobarbital sodium (60 mg/kg im). Mean arterial pressure (MAP) was taken through tail-artery cannulation for purposes of obtaining an established pressure for perfusion-fixation. A medial laparotomy was performed followed closely by a pneumothorax as the means for animal death. The left renal artery and both femoral arteries were ligated, the inferior vena cava below the kidneys was severed, and the thoracic aorta above the diaphragm was isolated and cannulated with a 14-gauge catheter. The intestines, mesenteric vasculature, and right kidney were perfusion-fixed at the approximate MAP with a warmed vasodilator mixture [100 µM sodium nitroprusside (Sigma, St. Louis, MO), 100 µM verapamil hydrochloride (Sigma), 100 µM papaverine hydrochloride (Aldrich Chemical, Milwaukee, WI), and 0.9% physiological saline, 37°C] followed by warmed 10% buffered Formalin phosphate (Fisher Scientific, Fair Lawn, NJ). The right kidney was extracted and placed in 10% buffered Formalin phosphate. The mesenteric vasculature was extracted and placed under a dissection microscope (StereoZoom 4, Bausch & Lomb), and the high-flow ileal and second-order arteries from the flow-dependent region were carefully isolated and extracted. In addition, same-animal ileal and second-order control arteries were taken from the ninth ileal artery and second-order branch proximal to the ileocecal junction. All tissues remained in 10% buffered Formalin phosphate for ~4 h before processing.

Hemodynamic parameter measurements. In a duplicate series of experiments hemodynamic parameters were measured before and initially after creation of the model by arterial ligation. The left common carotid artery was cannulated to measure MAP. After a medial laparotomy and surgical protocol as described above, a perivascular ultrasonic flow probe (0.5-V probe and model 206 flowmeter, Transonic Systems, Ithaca, NY) was placed on the sixth ileal artery proximal to the ileocecal junction (Fig. 1). The proximal second-order branch off the fourth ileal artery proximal to the ileocecal junction was ligated. In six animals, the proximal branch of the sixth ileal artery was cannulated retrogradely for the purpose of determining if creation of the model altered arterial pressure in the experimental mesenteric arteries. The cannula was constructed from 31-gauge MicroFil tubing (World Precision Instruments, Sarasota, FL) sealed in PE-10 and PE-50 tubing. The fifth ileal artery proximal to the ileocecal junction was ligated. The sites of arterial cannulation and ligations are included in Fig. 1. Measurements of blood flow, arterial pressure, and internal diameters of the sixth ileal artery and its distal second-order branch were made before and after each cannulation or

**Fig. 1.** Rat mesenteric flow model indicating general mesenteric arterial arrangement, sites of ligation, and placement of pressure cannula and flow probe. Diagram depicts model used for both acute hemodynamic measurements and chronic morphological assessments. In chronic studies, proximal branch of high-flow 6th ileal artery was ligated. Results showed significantly increased blood flows in both the ileal and second-order arteries, with significantly elevated calculated shear rate at wall for high-flow branching artery compared with control predilation levels. No significant changes in mean carotid or local mesenteric arterial pressures were detected. This model allows for same-animal control and high-flow vessels. Control vessels were obtained from 9th ileal artery and 2nd-order branch proximal to ileocecal junction and are not included in illustration.
ligation as described above. Ileal artery blood flow measurements were corrected for zero offsets, and measurements of internal diameters were made through videomicroscopy. Before we accepted each pressure measurement, the stopcock connecting the cannula to the pressure transducer was opened to the atmosphere to verify that the cannula was patent as demonstrated by the entrance of blood into the cannula. Finally, vessels were completely relaxed through topical administration of a dilator cocktail (10^{-4} M adenosine, 10^{-3} M sodium nitroprusside), with subsequent measurement of flow, pressure, and diameters. Average shear rate at the wall was calculated from these data using the formula \( WSR = (4Q)/(\pi r^2) \) where \( Q \) is blood flow (ml/s) and \( r \) is the vessel radius (cm).

Tissue and slide preparation. The tissues were processed in graded alcohols, paraffin-embedded, and stored at room temperature. Four-micrometer sections were cut using a rotary microtome (American Optical 820, Buffalo, NY) and placed immediately in ribonuclease-free 30% ethanol. The sections were then preheated to heated deionized H_2O and placed on precleaned Superfrost/Plus microscope slides (Fisher Scientific) and allowed to air dry.

Morphological analysis. For morphological analysis, slides were dewaxed in xylene, rehydrated in graded ethanols, and stained with toluidine blue for 45 s at 37°C. The slides were then dehydrated in graded alcohols and xylene and coverslipped. Microscopic measurement and quantitation of morphological parameters were performed using J A V A Video Image Analysis Software System (Jandel Scientific). Pixel gray-level thresholds were set for optimum contrast, and inside and outside circumferences were automatically traced and digitized. Data transformations provided values for lumen area, wall area, and inside and outside diameters. Between three and six cross sections per vessel were measured for morphometric analysis.

Extracellular connective tissue staining. Tissue cross sections were stained for extracellular connective tissue using a modification of the protocol included in Masson's trichrome stain kit (Sigma). Slides were deparaffinized, rehydrated, and treated with Bouin's histological reagent solution for 15 min at 56°C. Slides were washed and hematoxylin stained (Gill's no. 3) with sodium cobalt nitrate and Cresyl fast blue for 2 min to stain muscle fibers and cytoplasm. Slides were treated with a phosphotungstic-phosphomolybdic acid solution for 2 min to stain muscle fibers and cytoplasm. Slides were treated with Vectastain elite avidin-biotinylated horseradish peroxidase (Vector) and allowed to air dry.

Immunocytochemistry protocol for proliferating cell nuclear antigen. Slides were dewaxed in xylene, rehydrated in xylene and graded alcohols. Endogenous peroxidase activity was blocked with 0.4% hydrogen peroxide in methanol, followed by treatment with 5% normal goat serum (Vector). Mouse monoclonal anti-proliferating cell nuclear antigen (anti-PCNA) clone PC-10 (Boehringer Mannheim, Indianapolis, IN) was used in a 1:400 dilution for 30 min. Slides were treated with Vectastain elite avidin-biotinylated complex immunoperoxidase system (Vector) for 30 min, followed by 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma) solution (0.1 M imidazole, 0.2% DAB, hydrogen peroxide, and phosphate-buffered saline) chromagen treatment for 5 min. Slides were counterstained with hematoxylin (Gill's formulation no. 1, Fisher), dehydrated in graded alcohols and xylene, and coverslipped. Positive and negative nuclei were counted in the medial smooth muscle layer, and between three and five cross sections per vessel were analyzed. The right kidney served as a positive control for DNA replication, and the negative control slides received only the secondary antibody.

Statistical analyses. Data were stored and analyzed on personal computers using Excel (Microsoft version 5.0), Instat 2 (Graphpad Software, version 2.02), and SigmaPlot (Jandel Scientific version 2.0). Flow, pressure, and WSR data were analyzed using a repeated-measures analysis of variance (ANOVA) with post hoc Student-Newman-Keuls analysis. Morphological and endothelial count data were analyzed using a two-way ANOVA with post hoc Bonferroni-corrected paired and unpaired t-tests. All other data were analyzed using a two-tailed paired Student's t-test. The null hypothesis was rejected at an alpha of 0.05. Unless otherwise specified, data are presented as means ± SE.

RESULTS

Rat mesenteric shear stress model. Thirty rats were successfully ligated for this part of the experiment. During establishment of the arterial ligations in the shear stress model protocol, several parameters were measured for purposes of maintaining consistency throughout the experiment. The mean length of the flow-dependent region, defined as the section of the intestinal wall subject to blood flow from the unligated ileal artery, was 41.83 ± 0.88 mm. The number of first-order arteries that feed the intestinal wall in the flow-dependent region was 19.3 ± 0.67, whereas the MAP (measured for purposes of setting a pressure for perfusion-fixation) was 89.3 ± 1.52 mmHg.

Hemodynamic parameter measurements. Same-animal control and postligation hemodynamic parameter data are summarized in Table 1. Eight additional rats were successfully evaluated for this part of the study. Neither mean arterial (carotid) nor local mesenteric pressures changed significantly after ligation. Before any ligations, ileal artery blood flow averaged 0.65 ± 0.08 ml/min. After ligation of only the proximal branch of the ileal artery, ileal artery blood flow decreased 35 ± 5% (data not shown). For control conditions before any ligations, it was assumed that flow through the second-order branch artery was equal to one-half of the flow through the parent ileal artery. This assumption seems reasonable considering that the in vivo inner diameters of the two branches were similar under control conditions (220 ± 18 µm distal branch vs. 213 ± 13 µm proximal branch). After ligation of the proximal branch, the flow through the ileal artery and the only remaining branch would be equal. Given this assumption, after ligation of the proximal second-order branch of the sixth ileal artery and ligation of the adjacent fifth ileal artery, blood flows through the
supplying ileal artery and distal branch were increased 36 ± 13% (P < 0.01) and 170 ± 25% (P < 0.001), respectively, relative to preligation control conditions. Significant flow-induced dilation was demonstrated in the ileal artery (~7%; P < 0.001), whereas the second-order artery did not experience a significant increase (~5%) in internal diameter after ligation. The ileal artery exhibited a nonsignificant (~10%) increase in calculated average WSR, whereas average WSR in the second-order branch was elevated 118 ± 27% (P < 0.001). Measurements of completely dilated vessels indicate that control ileal arteries possess ~6% tone, whereas control second-order arteries have ~9% tone under resting conditions. Morphological analysis. Figure 2 graphically illustrates shear stress-induced alterations in inside lumen diameter (Fig. 2A) and medial wall area (Fig. 2B) for ileal and second-order arteries after 1, 3, and 7 days of increased blood flow. Significant increases in lumen diameters were observed in both same-animal control (P < 0.002) and high-flow (P < 0.005) ileal arteries with time, with no differences between them in the amount of change over the 7-day period (P = 0.11). The second-order arteries, however, demonstrated a significant (P = 0.004) interaction between flow and the duration of flow exposure for changes in lumen diameter. Post hoc independent comparisons revealed +14 and +38% increases in lumen diameter for high-flow second-order vessels at 3 and 7 days, respectively, whereas same-animal controls did not change. The two-way ANOVA (Fig. 2B) suggests significantly greater increases with time in the medial wall area of the high-flow ileal arteries (P = 0.014) and second-order arteries (P = 0.005). Post hoc analysis indicates that after 7 days of increased flow, ileal artery wall area was significantly (+58%) elevated compared with its same-animal control. Similarly, post hoc analyses show that high-flow second-order arteries exhibit significant medial wall area enlargement compared with corresponding same-animal control vessels after 3 (+53%) and 7 (+120%) days of elevated flow. Medial wall areas for control ileal and second-order arteries did not change significantly with time.

Figure 3 demonstrates that medial wall thickness-to-lumen diameter ratios for high-flow ileal and second-order arteries were not significantly different from same-animal control vessels at any time. Figure 4 illustrates that the percentage of connective tissue in the medial wall remains constant in both ileal and second-order branch vessels after exposure to elevated flow. This indicates that medial extracellular connective tissue increases concomitant with medial wall hypertrophy.

Immunocytochemistry results for PCNA/smooth muscle cell nuclear profile analysis. To determine the ratio of medial smooth muscle cells (SMCs) undergoing mitotic cell cycling, we performed immunocytochemical analysis for PCNA, and medial SMC nuclei were counted. Figure 5 shows the percentage of PCNA-positive medial SMC nuclei for high-flow vessels divided by those for control vessels from the same animal. Data were best represented in this fashion to account for elevated basal PCNA counts, which averaged between 7 and 21% in both ileal and branch arteries. These data indicate that ileal artery medial SMCs undergo significant cell cycling after 3 and 7 days of elevated flow (+189 and +179% compared with same-animal controls, respectively), whereas significantly less cell cycling occurs in the second-order arterial branch after 7 days (+407% compared with control).

In the case of polyploidy, whereby cells undergo DNA replication without cell division, cells can stain positively for PCNA without a change in cell number. Figure 6A shows absolute medial nuclear counts with significant SMC hyperplasia indicated in both high-flow ileal (+30%; P < 0.04) and second-order arteries (+73%; P < 0.05) after 7 days. When data were normalized to square millimeter of medial wall area (Fig. 6B), SMC nuclear density remained constant in the presence of enlarged wall areas for almost all time points in both the ileal and second-order arteries. However, the data indicate that the second-order artery experiences significant SMC hypertrophy after exposure to elevated flow for 7 days, as shown by a decreased (~22%; P < 0.02) medial nuclear density concomitant with an increased wall area. These results imply that flow-mediated vessel wall growth in vivo occurs through a combination of SMC hyperplasia and cellular hypertrophy in small branching mesenteric arteries. Additionally, the second-order branch may be experiencing nuclear polyploidy after exposure to flow for 7 days, as suggested by results showing increased PCNA staining in the presence of a decreased nuclear (and cellular) density.

Endothelial cell nuclear profile analysis. Figure 7 illustrates absolute (Fig. 7A) and normalized (Fig. 7B) intimal endothelial cell (EC) nuclear counts. Absolute EC nuclear counts were significantly elevated in both the ileal (+17, +50, and +65%, respectively) and second-order (+33, +89, and +81%, respectively) arteries compared with same-animal control vessels at 1, 3,
and 7 days, respectively. When data were normalized to 100-µm luminal perimeter, significant interactions between flow and EC density were detected in both the ileal and branch vessels. Post hoc independent comparisons of control vs. treatment for each time point are indicated. Data were evaluated with 2-way ANOVA using post hoc Bonferroni-corrected t-tests. Values represent means ± SE; n = 10 for each group. Between 3 and 6 cross sections per vessel were analyzed. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control; †P < 0.05, ††P < 0.01 over time.

DISCUSSION

Previous studies have demonstrated that the structural diameter of large conduit arteries and small resistance arteries is modulated by chronic changes in blood flow and associated wall shear forces. In mesen-

Fig. 2. Shear stress-induced changes in lumen diameter (A) and medial wall (B) area for ileal and 2nd-order vessels. No interaction was detected between flow and time for ileal artery luminal enlargement, with same-animal control and high-flow vessels both significantly increasing with time. Significant interactions between flow and time were detected for 2nd-order artery lumen diameter, as well as for changes in ileal and 2nd-order artery wall area. Post hoc independent comparisons of control vs. treatment for each time point are indicated. Data were evaluated with 2-way ANOVA using post hoc Bonferroni-corrected t-tests. Values represent means ± SE; n = 10 for each group. Between 3 and 6 cross sections per vessel were analyzed. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control; †P < 0.05, ††P < 0.01 over time.

Fig. 3. Medial wall thickness-to-lumen diameter ratios for ileal and 2nd-order arteries show no significant differences between same-animal high-flow and control vessels. Data indicate that chronic hoop stress is tightly regulated and stabilized in presence of normal arterial pressure. Between 3 and 6 cross sections per vessel were analyzed. Values represent means ± SE; n = 10 for each group.

replication leading to increased EC densities. This exaggerated EC hyperplasia is dependent on the duration of flow exposure through 7 days.
teric small resistance arteries of rats, inner diameter can increase ~30% after just 1 wk of elevated flow (26). Major proliferative events in the intima and media are associated with this rapid luminal expansion. The current study utilizes a new experimental model to provide significant insight regarding how the arterial wall accommodates rapid luminal expansion and medial wall remodeling from exposure to elevated blood flow over a 7-day period. Another advantage of this model is the ability to study two arteries (ileal and second-order branch) with different degrees of flow elevation within the same animal without altering local or mean carotid arterial pressures (Table 1). Results demonstrate that flow-mediated vessel wall structural remodeling is manifested as luminal expansion and medial wall hypertrophy, and the magnitude of change of these parameters is dependent on the duration of flow elevation after abrupt arterial occlusion.

In the experimental arteries, blood flow was initially increased 36 and 170%, respectively, in the ileal artery and second-order branch compared with preligation resting levels. Although blood flow was not measured at 1, 3, and 7 days, previous studies by Unthank et al. (26) using a similar ligation protocol that increased blood flow acutely showed that it remained elevated 4 wk later (24). In the present experiments, inner lumen diameters increased by ~13% at day 7 in the ileal artery, whereas the second-order branch, which experienced greater increases in flow, was significantly enlarged by ~14% by day 3 and ~40% by day 7. This heightened response in the second-order branch may indicate a dependency on the degree of flow elevation for flow-induced luminal enlargement. In addition, the luminal expansion experienced by the second-order branch was determined to be dependent on the duration of flow exposure between 1 and 7 days. In contrast, the lumen enlarged in both control and high-flow ileal arteries with time. This implies that significant developmental growth in terms of luminal enlargement may be occurring in normal ~10-wk-old rat mesenteric ileal arteries over a 7-day period. Considering the significant interactions between day and treatment for all other morphological data, however, we believe that age was not a major factor influencing the results of this study. This corroborates the findings of Unthank et al. (25), who showed that minimal developmental growth occurs in normal rat mesenteric microvasculature between 10 and 20 wk of age.

In the current study, the luminal enlargement demonstrated in the high-flow vessels is associated with significant endothelial proliferation. Intimal ECs were rapidly stimulated to replicate from exposure to elevated blood flow, as shown by significant EC hyperplasia after 24 h (Fig. 7A). In addition, EC density increased significantly in a time-dependent fashion on exposure to increased flow (Fig. 7B). This may be part of an adaptive process aimed at maintaining a constant intimal EC density coincident with luminal enlargement. These results are consistent with the recent findings of Unthank et al. (24), who showed the number of EC nuclei in high-flow mesenteric collateral vessels was ~90% greater than those found in control vessels after both 1 and 4 wk. Masuda et al. (14) found EC
density significantly elevated in high-flow canine carotid arteries after 4 wk; however, a corresponding increase in lumen diameter was not detected. Walpola et al. (27) reported a 33% decrease in EC number in ligated rabbit carotid arteries after 5 days exposure to an 80% decrease in blood flow. The results from these combined studies illustrate the significant direct influence of blood flow on intimal EC replication.

Several investigators (3, 13, 17, 31) have found an inverse relation between flow and EC division in cultured aortic ECs. In cell culture, however, flow and shear stress stimulate a phenotypic change from a proliferative cell to a differentiated cell. In addition, cultured cells are usually not preconditioned to flow, as is the case with an in vivo preparation. These variables must be considered when results between in vivo and cell culture experiments are compared.

Current results show major adaptations also occurred within the medial wall concomitant with rapid luminal expansion. Immunocytochemical results for PCNA (Fig. 5), a cell cycle-dependent cyclin (15), suggest that ileal and second-order branch medial SMCs undergo significant DNA replication after 3 and 7 days of in vivo exposure to increased blood flow. Polyploid cells with DNA replication in the absence of cytokinesis, however, can erroneously be counted as positive according to PCNA data (2). Absolute medial SMC nuclear counts (Fig. 6A) suggest cellular hyperplasia after 7 days of elevated flow in both the ileal and second-order arteries. These data together imply that elevated flow stimulates DNA replication and cellular division in medial SMCs under in vivo conditions. Normal SMC nuclear densities for both ileal and second-order arteries (Fig. 6B) support a role for medial SMC hyperplasia contributing to medial wall hypertrophy in response to elevated flow. After exposure to increased flow for 7 days, however, the second-order branch shows a slightly significant (P = 0.046) decrease in SMC density. Medial wall hypertrophy in this vessel at this time point may involve a combination of cellular hypertrophy and hyperplasia. Nuclear polyploidy may also be involved with numerous SMCs actively engaged in the cell cycle before cytokinesis, as supported by the PCNA data. Once the remodeling process becomes complete, cellular hyperplasia and diploidy would subsequently result. Owens (18) found that large-vessel SMC hypertrophy is often accompanied by development of nuclear polyploidy in hypertensive animals and suggests that polyploidy may act as a gene-amplification adaptive mechanism commensurate with an increased transcriptional requirement of an enlarged cell.
Additionally, as the medial wall hypertrophied in response to flow elevation, synthesis of medial extracellular connective tissue was stimulated, resulting in a constant percentage of connective tissue at all times (Fig. 4). The increase in connective tissue may help maintain normal cell-cell contact concomitant with an enlarged wall area. Alterations in extracellular matrix constituents after exposure to flow have been previously demonstrated by several investigators (1, 29).

An interesting observation in the current study was that even during rapid expansion of the arterial lumen, the wall-to-lumen relationship in the flow-loaded arteries did not deviate significantly from that observed in the same-animal control arteries at each time point (Fig. 3). This would suggest that the parameters controlling luminal enlargement and medial hypertrophy are tightly regulated in these vessels. The exact mechanisms responsible for this are not clear. As supported by other studies (8, 9, 24), we consider the major initial stimulus for vessel remodeling in these arteries to be elevation of the stresses associated with increased blood flow. Acute flow-induced dilation in the ileal artery (~7%) neutralized significant increases in calculated average WSR and may indicate an influence from stretch-induced hoop stress, which alone can stimulate an array of biochemical and molecular signals (4, 16, 20). Using the pressure and radius data from Table 1 combined with the control artery wall area from Fig. 2B allows the calculation of hoop stress before and after ligation. Ileal artery hoop stress increased from $1.81 \times 10^6$ to $1.95 \times 10^6$ dyn/cm², an increase of only 7.7%. The second-order artery, however, did not significantly vasoconstrict on exposure to increased flow, resulting in a highly significant doubling of its calculated WSR. Hoop stress for the second-order artery increased acutely from $1.93 \times 10^6$ to $2.06 \times 10^6$ dyn/cm², a change of 6.7%.

We believe that the forces related to blood flow elevations in this study in both ileal and branch vessels are primarily shearing forces with possible augmentation by cell stretch-induced hoop stress in the ileal artery.

In conclusion, this report examines the influence of blood flow and its associated stresses on arterial remodeling using a unique in vivo preparation. The primary stimulus in this model is believed to be flow-mediated shear stress with possible contribution from cell stretch-mediated hoop stress. Significant luminal expansion and medial wall hypertrophy occurred in a time-dependent fashion after flow elevation for 7 days. These adaptations involved significant SMC and EC hyperplasia and extracellular matrix restructuring.

We thank Suzanne S. Wade for excellent technical assistance, Mary Beth Thompson for expert secretarial skills, and Paul Kolm for expert statistical assistance.
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