"Tissue Need" and Limb Collateral Arterial Growth

Skeletal Contractile Power and Perfusion During Collateral Development in the Rat

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Among the factors that might influence collateral arterial growth after arterial occlusion, the capacity to deliver blood flow in relation to metabolic need and work performance are obvious candidates. In this study in rats after superficial femoral artery ligation, we assessed collateral arterial growth (by arteriography), basal and peak limb blood flow during acetylcholine-induced vasodilation (by electronic drop counting), pressure-flow relations, and contractile power of the gastrocnemius muscle (force transduction during sciatic nerve stimulation) at intervals over 3 months after superficial femoral artery ligation. Basal and peak blood flow and muscle contractile power were clearly reduced 1 week after ligation but had returned to normal by 3 weeks. Major collateral arterial growth, however, progressed between 3 weeks and 3 months. The limb perfusion pressure-blood flow relation was still altered at 3 weeks, with blunting of the normal autoregulation, and became more normal by 3 months after superficial femoral artery ligation. Collateral arterial growth continues after blood flow adequate to maintain work performance has been restored and may reflect a response to more subtle abnormalities involving distal pressure delivery, evident in altered pressure-flow relations. (Circulation Research 1992;70:546-553)

KEY WORDS: blood flow autoregulation, arteriography, skeletal muscle work, acetylcholine blood flow

John Hunter, the first to recognize collateral arterial growth, suggested over 200 years ago that the force promoting collateral development is "tissue need." The specific term he used, "the stimulus of necessity," was typically vitalist and thus required no further definition. This reasonable concept has often been repeated, need being translated into physiological and biochemical events related to tissue ischemia.

Recent observations have also focused attention on the ischemic zone as a crucial source of information for arterial growth. Hyperplasia, evident in increased tritiated thymidine incorporation into vascular elements, begins within 24 hours, primarily in vessels near the ischemic zone and shows centripetal spread from there over the next several days. This pattern has suggested that a complex communication system exists and that the ischemic zone is a crucial source of information for collateral arterial growth. The hyperplastic process gradually grows quiescent, but there is evidence of continued collateral arterial growth for many weeks. What physiological forces might be responsible? Obvious candidates have included basal blood flow delivery, flow velocity, pressure delivery, blood flow in relation to support of metabolic work, and the products of metabolism. Recent observations, however, have revealed a remarkably rapid return to normal of blood flow and the metabolic machinery to support work in rat skeletal muscle after superficial femoral artery ligation in the rat. Because the time course was not congruent with observations on blood vessel growth, although none had been reported in the rat, we undertook to systematically assess blood vessel growth after superficial femoral artery ligation by arteriography in relation to basal limb blood flow, the maximal capacity of the limb blood supply to deliver flow during acetylcholine-induced vasodilation, and the capacity of the collateral-dependent skeletal muscle to contract. All are responsible surrogates for tissue need. All of these indexes of collateral arterial function had returned to normal within 3 weeks, but collateral arterial growth continued thereafter, suggesting that the forces at work to promote and regulate vascular growth lie beyond some of the intuitively obvious candidates.

Materials and Methods

The studies were performed in 192 Sprague-Dawley male rats weighing between 350 and 400 g. The very large number of rats studied reflected the fact that it was necessary to measure blood flow and contractile power and to obtain arteriograms in different groups of rats for technical reasons. Moreover, four time points...
were assessed. During ligation of the superficial femoral artery to promote the growth of a collateral arterial tree, ether anesthesia was used; the artery to the left hind limb was ligated in a region just underlying the rectus abdominis muscle, about 1.5–2 cm above the superficial epigastric artery. The skin incision was closed with skin clips, and the animals were returned to cages for a follow-up study between 1 and 12 weeks after arterial ligation. In the normal controls (sham-operated group), a similar surgical procedure was used, but the artery was not ligated.

For follow-up studies, the rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and the trachea was cannulated to maintain an airway. For blood flow studies, the right carotid and left femoral arteries were catheterized with PE90 siliconized polyethylene tubing (0.86 mm i.d., 1.27 mm o.d.; Clay Adams, Parsippany, N.J.). Catheter sizes were selected to provide the largest possible diameter and thus minimize resistance to flow. The jugular vein was also catheterized with a PE90 catheter for administration of fluids and supplemental doses of anesthesia as required. For arteriographic studies, the carotid artery catheter was advanced to the abdominal aorta and the femoral artery was not catheterized.

Blood flow to the hind limb was measured by means of a drop counting system that we have described in detail. In brief, a disposable intravenous solution administration set (Travenol Laboratories, Inc., Deerfield, Ill.) was used, and the chamber was filled with liquid silicone of low viscosity (Dow Corning 200 Series; viscosity, 2.0 centistokes at 25°C). Heparin was administered intravenously (400 units). Arterial blood entered the system via the cannulated carotid artery and then traversed the chamber as a series of constant-sized drops, about three drops per milliliter. The outflow from the chamber entered the iliac artery at a level just above the origin of the collateral arteries to supply the vascular bed of the limb only via the collateral route. The active dead space of the system was 3.0 ml, which was filled at the beginning of the experiment with an artificial rat plasma. The artificial rat plasma was prepared from donor animals, which were bled. The red blood cells were separated, and the heparinized plasma was dialyzed for 8 hours against a Krebs solution to remove low molecular weight vasoactive factors. The protein and salts were stored dry after lyophilization and reconstituted with sterile distilled water just before the experiment.

A photocell (Grass Instrument Co., Quincy, Mass.) encompassed the drop chamber to register drop rate, representing blood flow to the limb. The system was calibrated with heparinized rat blood. Mean systemic arterial blood pressure and arterial perfusion pressure in the chamber were measured by means of Statham P23DC pressure transducers. Photoelectric and pressure transducer outputs were recorded continuously on a Grass polygraph. Drop rate was registered directly and also translated into a flow rate by means of an ordinate recorder, as previously described.

After the surgical preparation was complete and the drop counting system was active, the rats, with rectal thermometers in place, were set on a heating pad to maintain a body temperature of 36–37°C. Perfusion pressure to the limb could be reduced by graded external compression on the inflow side of the drop counting system. The relation between perfusion pressure and blood flow was assessed by reducing perfusion pressure as a series of 10 mm Hg steps (see Figure 5) and assessing blood flow at each perfusion pressure between 1 and 3 minutes after the new pressure level had been reached, when blood flow achieved a new steady state. For purposes of presentation, complete autoregulation has been defined as a flow rate that is independent of perfusion pressure (slope not significantly different from 0) over a range of perfusion pressures of 30 mm Hg or greater.

Blood flow was assessed in 47 rats at rest and during acetylcholine-induced vasodilation obtained by injecting graded doses of 1.0–1,000 μg in 0.1 ml of saline as a series of boluses into a side arm of the catheter in the iliac artery. Acetylcholine induced the same peak blood flow as did maximal skeletal muscle work in both the normal and collateral-dependent limbs. Perfusion pressure was measured via the same catheter. At higher acetylcholine doses a depressor response occurred, but the maximal blood flow increase occurred before blood pressure fell.

The ability of the skeletal muscles of the normal and collateral-dependent hind limbs to contract was examined in 37 animals. The sciatic nerve was exposed, and two electrodes were placed in contact with the nerve. A 0.5-cm-wide parafilm strip was placed loosely around the nerve, and both ends were sealed together to maintain separation of the electrodes. The wound was sutured, and the knee joint was fixed in position on a plexiglass board by means of a brass screw. The distal end of the muscle mass was attached to a Grass force transducer. Maximal force was generally obtained within 1–2 minutes and maintained throughout the balance of the 5 minutes of stimulation. The maximum force measured was the value used to describe work capacity of the limb. The muscle was adjusted to the optimal length for maximal isometric contraction. The muscles of the hind limb were stimulated supramaximally via the sciatic nerve with a Grass Model SD9c stimulator (1 msec, 3 V) at 2–3 Hz for 5 minutes. The muscles were allowed to recover for 15 minutes and restimulated to verify the result.

For arteriographic studies, meglumine diatrizoate or micropaque was infused into the abdominal aorta of 23 rats by pump injection of 0.8 ml over 15 seconds. Arteriograms were obtained with a Machlett microfocal spot tube with nominal size of 0.1 mm and a Gigantor generator (Siemens Medical Systems Inc., Erlangen, FRG). Films were obtained with 3M Alpha-3 screens and with 3M XUD film. The conditions were 75 KVP, 80 MA, and 25 MS. The collateral arteries were too small for measurement by objective, computer-assisted methods. To ascertain whether there was continued growth of collateral arteries between the third week, when blood flow and work had returned to normal, and 3 months after superficial femoral artery ligation, we made a coded assessment of the arteriograms. One observer ranked, on a coded basis, each film set obtained at 3 weeks and 3 months and after acute occlusion.

Mean values have been presented with the standard error of the mean as the index of dispersion. Linear regression was used to assess the pressure–flow relation over selected pressure ranges. The coded assessment of arteriograms was analyzed by the Fisher Exact Test.
One-way analysis of variance (ANOVA) was used to assess the time course of flow changes after superficial femoral artery ligation. The null hypothesis was rejected when the probability was less than 0.05.

Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those of the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

**Results**

One week after femoral artery ligation, basal limb blood flow was reduced from the normal 3.5±0.17 ml/min to 2.3±0.47 ml/min (p<0.025; Figure 1 [top panel]). Perfusion pressure was 85.1±3.2 and 86.0±7.3 mm Hg, respectively. By 3 weeks after superficial femoral artery ligation, basal limb blood flow had returned to 3.5±0.5 ml/min. Peak limb blood flow during acetylcholine infusion showed a similar pattern, falling from a normal 7.3±0.4 ml/min to 5.6±0.8 ml/min (p<0.05) at 1 week and returning to 8.8±2.0 ml/min at 3 weeks (Figure 1 [top panel]).

An essentially identical time course was seen in the contractile response of the gastrocnemius muscle to sciotic nerve stimulation after superficial femoral artery ligation (Figure 1 [bottom panel]). At 1 week, the contractile response had fallen from a normal 21.0±2.7 g to 12.9±2.0 g (p<0.01). By 3 weeks, the contractile response had returned to a normal value of 21.3±6.6 g, which exceeded the value at 1 week significantly (p<0.025).

The evaluation of the arteriograms for collateral arteries revealed a different time course. No collateral arteries were visible in the first 30–60 minutes after superficial femoral artery ligation (Figure 2). At 1 week, collateral arteries were visible but were very small and poorly defined (not shown). By 3 weeks, definite collateral arteries were routinely visible, but they remained small (Figure 3). In 10 rats studied at 3 months (Figure 4), all but two had better collateral arterial development than did the best of those studied at 3 weeks in a coded assessment, and none of the other nine studied at 3 weeks had a collateral arterial tree that was as well developed (p<0.005).

The anticipated blood pressure–flow relation was observed in the hind limb of normal rats (Figure 5 [top panel]). Autoregulation of blood flow was “complete” over a wide range of perfusion pressures, from 60 to 120 mm Hg. Blood flow showed no change with increases in perfusion pressure over that range. Conversely, below that pressure range, flow increased linearly from 0.4±0.2 ml/min at a perfusion pressure of 10 mm Hg to 4.3±0.3 ml/min at 60 mm Hg. Over the plateau pressure range, from 60 to 120 mm Hg, flow equaled 4.4±0.18 ml/min (slope=0.4=0.015, F=0.010).

On acute occlusion of the femoral artery, blood flow fell to minimal levels and remained there for 60 minutes.

In animals studied 3 weeks after occlusion of the femoral artery, there was a clear change in the pressure–flow relation (Figure 5 [middle panel]). There was no evidence of an autoregulatory break point. Blood flow apparently rose linearly with increasing perfusion pressure over the entire range of pressures studied, from 50 to 130 mm Hg (slope=0.0378±0.0069 (SD), r=0.90, F=30.2, p<0.01). Blood flow was identical to that in the normal animal at a perfusion pressure of about 100 mm Hg and rose to supranormal levels with higher pressures. Even over the more limited pressure range, from 70 to 130 mm Hg, a positive relation was identified

![Figure 1](http://circres.ahajournals.org/)

**Figure 1.** Top panel: Basal limb blood flow and peak blood flow during acetylcholine-induced vasodilation in normal rats and rats studied 7 or 21 days after superficial femoral artery (SFA) ligation. Bottom panel: Peak force of contraction of the gastrocnemius muscle in response to sciatic nerve stimulation at the same time intervals. Note that all measures were reduced significantly at 7 days after SFA ligation but had returned to normal by 3 weeks.
Figure 2. Arteriograms of the lower aorta and iliac arterial system in two rats. In each rat the left femoral artery was ligated (arrow labeled a) 30 minutes before the arteriogram. Small amounts of contrast have reached the distal arterial tree, but distinct collateral arteries are not visible.
between pressure and blood flow (slope=0.023±0.007 (SD), r=0.82, F=10.2, p<0.001).

In animals studied 3 months after femoral artery occlusion, the results were somewhat ambiguous, but autoregulation of femoral blood flow was evident (Figure 5 [bottom panel]) over the pressure range of 60–110 mm Hg (slope=0, F=0.13). Blood flow over this pressure range averaged 4.03±0.3 ml/min (compare the top and bottom panels of Figure 5).

Discussion

The concept of tissue need (never fully defined) as the pivotal determinant of collateral arterial growth is long standing.1 Our hypothesis in this study was that basal blood flow, maximal blood flow that a collateral tree can carry, and the capacity of the perfused tissue to perform work would provide an index for assessing whether a collateral arterial tree had satisfied tissue need. By all of these indexes, the collateral arteries were inadequate at 1 week. At that time they would not support muscle work, and basal and peak blood flow were reduced. Skeletal muscle contractile responses and blood flow, however, clearly had returned to normal by 3 weeks after superficial femoral artery ligation. These observations are in accord with earlier measurements of blood flow and muscle metabolism.12,13 Collateral arterial growth assessed by angiography, on the other hand, showed a different temporal pattern. The collateral arteries, too small to be visualized by arteriography an hour after occlusion, clearly grew between the initial insult and assessment at 7 days but were still too small to be visualized clearly. By 3 weeks collateral arteries were routinely visible and well defined, but collateral vessels at 3 months were substantially larger than they had been at 3 weeks. The force responsible for continued collateral arterial growth, based on blood flow or capacity to perform mechanical work, is unlikely to be dependent on a signal emanating from residual tissue ischemia.

Of the physiological processes assessed in this study, only autoregulation of limb blood flow was still abnormal 3 weeks after superficial femoral artery ligation. Over the autoregulatory range of pressures in the normal animal, arteriolar dilation must compensate for a fall in perfusion pressure to maintain a constant blood flow. Complete autoregulation of blood flow in the normal limb and in normal skeletal muscle has been amply demonstrated in many species.16–19 Although the precise factor responsible for shifts in arteriolar resistance with shifts in perfusion pressure has not been identified, most theses agree that the changes occur in vascular smooth muscle at the arteriolar level.17 Complete autoregulation of blood flow over the pressure range of 60–120 mm Hg has been confirmed in this study. Indeed, the slope of the line relating flow to pressure was precisely zero. Three weeks after arterial ligation, when basal blood flow at normal perfusion pressure and peak blood flow in response to acetylcho-
line had returned to normal and when skeletal muscle metabolism was normal, there was a clear alteration in the relation between blood pressure and blood flow. In this case there are two resistances in series, one provided by the collateral arteries and the other by the downstream arteriolar bed. In the presence of a significant resistance to blood flow provided by the collateral arteries, an alteration in the autoregulatory process would be no surprise. Indeed, one would anticipate that the reduced pressure delivery to the arteriolar level would have resulted in arteriolar dilation, thus limiting dilation as perfusion pressure fell further. Although it is tempting to relate the continued growth of collateral arteries beyond 3 weeks to the disturbance in perfusion pressure–flow relations, there is no direct evidence in this study to support that mechanistic connection, and the relation must be considered speculative. The alternative possibility exists that the alteration in pressure–flow relation reflected vascular injury consequent to the ischemic process, with a time course of recovery that is slower than that of the skeletal muscle. Although possible, this seemed unlikely.

How can one account for a normal peak blood flow during acetylcholine infusion in this model? The only apparent explanation is that acetylcholine was effective in dilating the collateral arteries along with the distal arterioles. Because there has been substantial interest in enhanced responses of collateral arteries to serotonin, with the suggestion that the enhanced response reflects loss of endothelium-dependent relaxation, the fact that acetylcholine was effective in dilating the collateral arteries would be of substantial interest. Clearly, this observation merits further investigation. Whatever the explanation, it is reasonable to conclude that the capacity of the collateral arterial bed to maintain a peak blood flow could account for the return of work performance to normal.

Blood flow in the vascular area subtended by the inflow system after total occlusion of the superficial femoral artery at the level used in this study fell to zero or near zero within seconds of occlusion and remained subnormal for 7 days. How much of the blood flow reduction at 7 days reflects the inadequacy of collateral arterial growth and how much reflects atrophy of disuse related to the injury is not clear. Sham-operated controls showed a similar blood flow level but may well have used their limbs more than did rats with femoral artery occlusion. Thus, the blood flow measured at 2–3 weeks after arterial occlusion reflects some combination of dilation and growth of the available collateral arterial tree. The results in the rat contrast sharply with our earlier findings in the dog, in which at least a partial reconstitution of blood flow and angiographically evident collateral arteries were routinely observed within minutes of arterial occlusion. Presumably, the rat and the dog differ in the size of the available collateral arterial tree to the limb. Whatever the explanation, it is clear that the state of the collateral arterial blood supply 2–3 weeks after vascular occlusion is intermediate. The arteriograms revealed a far more extensive collateral

FIGURE 4. Arteriogram of a rat’s hindquarters 3 months after femoral artery ligation (at arrow labeled a). Note the presence of identifiable collateral arteries (small arrows) and the contrast-filled distal arterial tree (arrow labeled b). Note the better developed collateral arterial tree, which was evident in eight of 10 rats studied at 3 months in a coded assessment.
arterial tree at 3 months, and blood pressure–flow relations had returned to normal by 3 months.

We used a drop counting technique to assess blood flow to the rat limb. This method has a number of advantages and limitations. The advantages include great accuracy at very low flow rates, moment to moment measurement, and easy access to the control of perfusion pressure to the limb. The disadvantages include the need for anticoagulation, the need to isolate and open the femoral artery, and the possible local hemodynamic effects of placing a catheter into the very small femoral artery. The catheter used could have added a significant resistance to the flow through the limb. This seems unlikely in view of the close comparability of the pressure–flow relations for the limb identified in this study and described in many earlier reports.16–19

There have been a number of reports that suggest variation in the rate at which recovery of blood flow to the limb occurs after vascular occlusion. In the dog, a number of studies have documented a residual reduction in blood flow in the several weeks after femoral artery occlusion.11,21–23 In the rat, a recent study claimed full recovery of blood flow, not only at rest but also during vasodilatation induced by muscle work.12 None of the studies have reported pressure–flow relations, and indeed few describe measuring arterial blood pressure at the time of individual studies. It is conceivable that earlier, occasional reports of a normal blood flow in

![Figure 5](http://circres.ahajournals.org/doi/abs/10.1161/01.RES.70.3.552?journalCode=circ)

**Figure 5.** Top panel: Pressure–flow relation in the femoral artery of the normal rat. Note complete autoregulation of blood flow from 60–120 mm Hg pressure in the steady state. Mean blood flow=4.4±0.18 ml/min; slope=0. Middle panel: At 2–3 weeks of collateral growth after femoral arterial occlusion, blood flow is a linear function of perfusion pressure over the pressure range of 50–130 mm Hg (line A). Mean blood flow=3.7±0.3833 ml/min; slope=0.0378±0.0069 (SD), r=0.90, F=30.2, p<0.01. Over the more limited pressure range of 70–130 mm Hg (line B), mean blood flow=4.2±0.23 ml/min; slope=0.023±0.007 (SD), r=0.82, F=10.2, p<0.001. The slope in each case differs significantly from 0. Bottom panel: The pressure–flow relation of collaterals at 3 months of development approaches that of normals. Autoregulation is reestablished at pressures greater than 60 mm Hg as the neovascularature matures. Over a pressure range of 60–110 mm Hg, the flat pressure–flow relation has been restored. Mean blood flow=4.03±0.3 ml/min; slope=0, r=0.177, F=0.13.
the early weeks after femoral artery occlusion reflect experiments in which blood pressure was increased.

The recruitment of a collateral arterial supply appears to differ from tissue to tissue. 21, 23 Although there appear to have been no reports on pressure–flow relations for the limb, there have been a substantial number of studies of pressure–flow relations during the growth of coronary collateral arteries. 24–27 Although the experimental protocols are not easy to compare, it appears that coronary collateral flow is more pressure dependent than perfusion of the limb, at least 2 or 3 weeks after femoral artery occlusion. Whether coronary collaterals reach a “mature” state in which pressure–flow relations are normal, as was documented 3 months after femoral artery occlusion in this study, has not been shown in any model.

Earlier reports have contrasted the characteristics of the collateral arterial supply to various vascular beds, and emphasis was given to differences between the collateral arterial supply to the heart and the limb. 3, 21 The latter is thought to have a more immediately available collateral supply that effectively replaces the lost blood supply very quickly. The results of this study suggest that the differences may be more quantitative than qualitative. In the hour after acute occlusion, neither angiography nor direct blood flow measurement revealed an abundant available collateral arterial supply. Three weeks after femoral artery ligation in the rat, a substantial collateral arterial blood supply was evident, both by blood flow measurement and on the basis of anatomic examination by way of arteriography. On the other hand, studies at 3 months make it clear that substantial vascular growth occurs during the interval when followed, when the requirements of tissue need had been satisfied. The assessment of factors that modify vascular growth during that interval might provide insight into what is perhaps the most interesting question: What are the factors promoting vascular growth, and what is the communication system that mediates that process?

References

"Tissue need" and limb collateral arterial growth. Skeletal contractile power and perfusion during collateral development in the rat.

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