The effect of gradual or acute arterial occlusion on skeletal muscle blood flow, arteriogenesis, and inflammation in rat hindlimb ischemia

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Background: **Current experimental models of critical limb ischemia are based on acute ischemia rather than on chronic ischemia. Human peripheral vascular disease is largely a result of chromic ischemia. We hypothesized that a model of chronic hindlimb ischemia would develop more collateral arteries, more blood flow, and less necrosis and inflammation than would acute hindlimb ischemia. We therefore developed a rat model of chronic hindlimb ischemia and compared the effects of chronic ischemia with those of acute ischemia on hindlimb skeletal muscle.**

Methods: **Acute or chronic ischemia was induced in 36 male Sprague-Dawley rats. Chronic ischemia caused blood flow, as measured by laser Doppler scanning and confirmed by muscle oxygen tension measurements, to gradually decrease over 1 to 2 weeks after operation.**

Results: **Histologic analysis showed chronic hindlimb ischemia better preserved muscle mass and architecture and stimulated capillary angiogenesis, while lacking the muscle necrosis and inflammatory cell infiltrate seen after acute ischemia. Surprisingly, the chronic ischemia group recovered dermal blood flow more slowly and less completely than did the acute ischemia group, as measured by laser Doppler (0.66 ± 0.02 vs 0.76 ± 0.04, $P < .05$) and tissue oxygen tension (0.61 ± 0.06 vs 0.81 ± 0.05, $P < .05$) at 40 days postoperatively. Consistent with poorer blood flow recovery, chronic ischemia resulted in smaller diameter collateral arteries (average diameter of the five largest collaterals on angiogram was 0.01 ± 0.0003 mm vs 0.013 ± 0.0007 mm for acute, $P < .005$ at 40 days postoperatively). Acute ischemia resulted in decreased tissue concentrations of vascular endothelial growth factor (VEGF) (0.96 ± 0.23 pg/mg of muscle for acute vs 4.4 ± 0.75 and 4.8 ± 0.75 pg/mg of muscle for unoperated and chronic, respectively, $P < .05$ acute vs unoperated), and in increased tissue concentrations of interleukin (IL)-1β (7.3 ± 4.0 pg/mg of muscle for acute vs undetectable and 1.7 ± 1.6pg/mg of muscle for unoperated and chronic, respectively, $P < 0.05$ acute vs unoperated).**

Conclusions: **We describe here the first model of chronic hindlimb ischemia in the rat. Restoration of blood flow after induction of hindlimb ischemia is dependent on the rate of arterial occlusion. This difference in blood flow recovery correlates with distinct patterns of muscle necrosis, inflammatory cell infiltration, and cytokine induction in the ischemic muscle. Differences between models of acute and chronic hindlimb ischemia may have important consequences for future studies of mechanisms regulating arteriogenesis and for therapeutic approaches aimed at promoting arteriogenesis in humans suffering from critical limb ischemia. (J Vasc Surg 2005;41:312-20.)**

Clinical Relevance: **Despite the substantial clinical differences between acute and chronic ischemia, researchers attempting to develop molecular therapies to treat critical limb ischemia have only tested those therapies in experimental models of acute hindlimb ischemia. We present here a novel model of chronic hindlimb ischemia in the rat. We further demonstrate that when hindlimb ischemia is developed chronically, collateral artery development is poorer than when hindlimb ischemia is developed acutely. These findings suggest that further tests of molecular therapies for critical limb ischemia should be performed in chronic hindlimb ischemia models rather than in acute hindlimb ischemia models.**

Critical limb ischemia in humans is untreatable by current catheter-based or surgical strategies in up to 150,000 patients every year in the United States.[1] The progression of atherosclerotic peripheral vascular occlusive disease in these patients often leaves amputation as their only option. Therefore, investigators have concentrated on establishing a molecular therapy to increase the development of new capillaries (angiogenesis) or to promote the enlargement of pre-existing collateral vessels (arteriogenesis). Several promising molecules have been identified that enhance angiogenesis or arteriogenesis (or both) in experimental models of hindlimb ischemia.[2-7] However, such molecules have been less successful in randomized human clinical trials.[8-10] This lack of success in humans is likely to be multifactorial, but one reason may be that current experimental models do not reflect accurately chronic atherosclerotic arterial occlusion.

Current experimental models of critical limb ischemia rely on acute ischemia that is generated by ligating or

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Supported by the National Institutes of Health NRSA Fellowship HL68402 (G.L.T.), William J. von Liebig Foundation (D.S.C), National Institutes of Health Grant HL75353 (L.M.M.), training grant T32GM08258 (D.S.C), and the Pacific Vascular Research Foundation. R.S. is the recipient of a Mentored Clinical Scientist Development Award (HL04435) from the Lifeline Foundation and the National Heart, Lung and Blood Institute (NIH).

Competition of interest: none.

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0741-5214/$30.00

doi:10.1016/j.jvs.2004.11.012

excising the femoral artery.[11-13] All three of the currently identified major mechanisms that promote arteriogenesis—change in shear stress within collateral arteries,[14] inflammation,[15-17] and endothelial progenitor cell recruitment[18]—are likely to be triggered by the acute ischemia.

Acute arterial occlusion leads to a sudden pressure gradient between the arteries within the proximal nonischemic region and the arteries in the distal ischemic region. This pressure gradient leads to increased flow and, thereby, increased shear stress within the collateral arteries bypassing the occlusion. Increased shear stress leads to early and late changes in gene expression through shear stress responsive elements.[19,20]

Inflammatory cells infiltrate into the nonischemic and ischemic muscle in response to acute ischemia, and some areas of ischemic muscle undergo necrosis. Monocytes and macrophages are thought to play an important role in arteriogenesis, possibly through the release of cytokines such as monocyte chemotactic protein-1.[2,15,17,21,22] The infiltrating inflammatory cells or, potentially, endothelial progenitor cells[18] that invade in response to acute ischemia may assist arteriogenesis and blood flow recovery in animal models that rely on ligation or excision. As time passes, the acutely ischemic muscle undergoes fibrosis and regeneration.[23,24]

In humans, critical limb ischemia results mainly from atherosclerosis gradually occluding arteries over a time span of months to years. This gradual onset of ischemia allows the muscle tissue of the leg to accommodate to the gradual decrease in blood flow by adaptations in muscle fiber type and energy metabolism.[25] These adaptations allow the leg muscles to avoid the necrosis, inflammation, and regeneration seen after acute ischemia. In addition, increases in shear stress occur gradually rather than acutely, allowing more time for collateral artery adaptation and development.

A single study reported the use of ameroid constrictors to gradually occlude the femoral artery, creating a state of chronic hindlimb ischemia in rabbits.[26] An ameroid constrictor consists of a stainless steel casing surrounding a hygroscopic casein material that has an internal lumen. The casein gradually absorbs water, thereby swelling and occluding the arterial lumen that it encases.

These constrictors have been used previously on pig and dog coronary arteries to simulate atherosclerotic coronary artery disease.[27,28] However, no models of gradual arterial occlusion leading to chronic hindlimb ischemia in the rat have been reported. In addition, to our knowledge, no prior studies have investigated how the rate of arterial occlusion affects spontaneous arteriogenesis and blood flow recovery in any vascular bed.

We hypothesized that chronic ischemia would induce collateral arteries to develop more fully, blood flow to return more completely, and muscle fibers to show less necrosis and inflammation than would occur in response to acute hindlimb ischemia. To test our hypothesis, we developed the first rat model of gradual arterial occlusion by using ameroid constrictors. We then compared several different outcome variables against those resulting from our previously described acute critical hindlimb ischemia model in the rat.[23]

MATERIALS AND METHODS

Animals. Forty-two male Sprague-Dawley rats (Simonsen, Gilroy, Calif) weighing 280 to 350 g were used. The rats were housed in an environmentally controlled room and were given chow and water ad libitum. The care of the rats complied with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, Washington, DC, National Academy Press, 1996). All protocols were approved in accordance with the Committee on Animal Research at the University of California, San Francisco.

Study design. The rats were divided into two study groups: acute hindlimb ischemia created by artery ligation[23] (n = 18) and chronic hindlimb ischemia created by ameroid constrictors (n = 18). Laser Doppler perfusion imaging was performed serially at intervals of 3 to 4 days throughout the duration of the study. The rats were also studied on days 3, 14, and 40 postoperatively to measure oxygen tension in the gastrocnemius muscle, muscle weights (n = 6 per group), and histology (n = 3 per group). Angiograms were performed on three rats in each group at each time point. Six rats were used for unoperated controls.

Hindlimb ischemia. Hindlimb ischemia was created in the acute ischemia group by ligation of the left common iliac artery and left femoral artery and all their branches as described.[23,29] The chronic ischemia group had a similar operation performed, but instead of ligating the left common iliac artery at the aortic bifurcation and the left femoral artery above the saphenopopliteal bifurcation, a 0.75-mm internal diameter ameroid constrictor was placed around the artery at each location. These custom ameroid constrictors (Research Instruments SW, Escondido, Calif) were sterilized with ethylene gas prior to implantation. No arteriotomies were performed. The right limb served as an internal control for each rat.

Clinical ischemia index. All rats were observed every 3 to 4 days for a clinical assessment of ischemia based on a previously published clinical ischemia index.[23] The rats were scored according to an ischemia grade scale (0 = normal, 1+ = pale foot, 2+ = pressure sores, and 3+ = necrosis) and abnormal gait (limping or no limping). In addition, after sacrifice, muscle mass was measured to quantitate recovery for a surrogate marker of muscle function.

Laser Doppler perfusion imaging. The rats were anesthetized with 1% isoflurane with a constant oxygen flow rate of 1 L/min and placed on a heating pad to maintain a constant temperature. Hair was removed with an electric shaver followed by Surgi-Prep depilatory cream (Sparta Surgical, Roseville, Calif). A laser Doppler perfusion imager (Moor Instruments, Ltd., Devon, UK) was used to estimate dermal blood flow in the calf and foot. Ratios of the operated–nonoperated limb were compared

to minimize differences between ambient temperature and lighting.

Skeletal muscle oxygen tension. Muscle oxygen tension was measured by using Licox Clark-type oxygen and temperature probes (Integra LifeSciences, Plainsboro, NJ) in the left and right gastrocnemius at 3, 14, and 40 days after operation, as described previously.[23] A ratio of the left limb–right limb was used for analysis.

Angiograms. Angiograms were performed as previously described.[6,7] Briefly, the rats were anesthetized with 2% inhaled isoflurane. The infrarenal abdominal aorta was ligated proximally and cannulated distally with a 20-gauge polyethylene catheter. Warmed heparinized saline (10 U/mL, 4 mL total volume) was injected into the aortic catheter. Barium sulfate (EZpaque) (0.6 g/mL, 2.5 mL total volume) was then injected into the aortic catheter, and the aorta and vena cava were ligated. The skin was removed from the hindlimbs to avoid imaging the dermal vasculature. Images were acquired by using a single enveloped Kodak X-OMAT TL film at 500 mA, 50 kV, and 0.5-second exposure. The rats were sacrificed prior to imaging.

Three blinded observers independently scored the images by counting the number of vessels that crossed a standardized grid overlaying the image. The number of vessels was then divided by the lines of the grid in the area of interest to produce an angioscore. Vessel diameters were measured using Fovea Pro software (Reindeer Graphics, Inc, Asheville, NC) to analyze images digitally acquired by scanning the angiographic films.

Tissue preparation. The rats were sacrificed after tissue oxygen measurements and the gastrocnemius and tibialis anterior from the right and left sides were harvested and weighed for muscle mass. Middle sections of muscle were processed into optimal cutting temperature compound (Tissue-Tek, Fisher Scientific, Fairlawn, NJ) for morphologic analysis to assess the extent of ischemic necrosis. Hematoxylin and eosin stained sections were analyzed by using Fovea Pro software (Reindeer Graphics, Inc) to measure the percent necrotic area of muscle sections. Immunohistochemistry was also performed to determine capillary density.

Immunohistochemistry. Immunohistochemistry was performed on muscle sections of the gastrocnemius and tibialis anterior in three rats per group at each time point. Capillary–muscle fiber ratios were determined by staining for the endothelial-specific antigen CD31 (1:500 dilution, Serotec, Raleigh, NC) as described.[23]

Enzyme-linked immunosorbent assay. Commercially available enzyme-linked immunosorbent assays (ELISA) for VEGF, IL-1β, and IL-6 were performed on homogenized gastrocnemius (calf) and gracilis (thigh) muscle per the manufacturer's instructions (mouse VEGF Quantikine ELISA kit, cross-reacts 70% with rat VEGF; rat IL-1β Quantikine ELISA kit; rat IL-6 Quantikine ELISA kit, R&D Systems, Minneapolis, Minn).

Briefly, leg muscle harvested from sacrificed rats was snap frozen in liquid nitrogen and pulverized by using a prechilled mortar and pestle on dry ice. Approximately 100 mg of each muscle was homogenized in 1.5 mL NP-40 lysis buffer (50 mM HEPES, pH 7.5; 150 mM NaCl, 10% glycerol, 1.5 mM $MgCl_2$, 1 mM EDTA, 100 mM NaF, 1% NP-40) containing protease inhibitors (10 μg/mL aprotinin, 10 μg/mL leupeptin, and 1 mM 4-[2-aminoethyl]-benzenesulfonyl-fluoride, hydrochloride [Pefabloc, Roche Applied Science, Indianapolis, Ind]).

Muscle homogenates were centrifuged at 14,000 rpm. The surface lipid layer was removed, and the supernatant was saved at −80°C for ELISA and protein assay. Protein concentration was measured by using a 1:500 dilution of the supernatant in the microtiter plate micro-BCA protein assay per the manufacturer's instructions (Pierce, Rockford, Ill). Supernatant dilutions of 1:5, 1:3, and 1:2 were used for the VEGF, IL-1β, and IL-6 ELISA assays, respectively.

Statistical analysis. All results are expressed as the mean ± standard error of the mean (SEM). Statistical significance was determined using the unpaired Student *t* test for comparison between two groups or analysis of variance followed by Student-Newman-Keuls post-hoc analysis for comparison among multiple groups. Differences in the clinical ischemia score were determined by the Fisher exact test. A *P* value of <0.05 was considered to be statistically significant.

RESULTS

Induction of ischemia. Critical limb ischemia was induced by acute arterial occlusion but not by gradual arterial occlusion according to the clinical ischemia index. All rats in the acute ischemia group developed clinical signs of critical limb ischemia, including pressure sores, muscle atrophy, pale foot, and limping, with a maximum ischemia index of 2+ at 14 to 17 days and a final 40-day index of 1+. The rats in the chronic ischemia group never developed signs of critical limb ischemia but did develop mild muscle atrophy and pale feet (without limping), with a maximum ischemia index of 1+ at 40 days. A significant difference was noted in the clinical ischemia index at 14 days between the acute and chronic groups ($P < 0.01$ by the Fisher exact test).

Blood flow recovery. Chronic ischemia caused slower and less complete blood flow recovery as measured by laser Doppler perfusion imaging. Laser Doppler perfusion imaging was used to measure blood flow in the foot and leg in both treatment groups for up to the 40-day time point (Fig 1, *A*). For the rats in the acute ischemia group, blood flow was lowest immediately postoperatively and recovered over 2 weeks to 60% of that measured in the unoperated leg. During the next 2-week period, blood flow recovered more slowly until the time of sacrifice on postoperative day 40, achieving a final ratio of 66%. A different pattern of blood flow recovery occurred in the rats in the chronic ischemia group; blood flow was lowest, varying between 7 and 17 days postoperatively. Forty days after the induction of ischemia, rats in the chronic ischemia group had a lower foot dermal blood flow than those in the acute ischemia group (0.66 ± 0.02 vs 0.76 ± 0.04, n = 6 rats per group, $P < .04$). Blood flow recovery in the calf in both groups paralleled that in the foot (Fig 1, *B*).

Oxygen tension. Rats that underwent gradual arterial occlusion had lower gastrocnemius tissue oxygen tensions than rats treated by acute arterial occlusion. In addition to multiple determinations of dermal blood flow by laser Doppler perfusion imaging, we directly measured tissue oxygen tension in the gastrocnemius muscle on postoperative days 3, 14, and 40 (Fig 1, *C*). These results were consistent with those from laser Doppler blood flow measurements. By postoperative day 40, rats in the acute ischemia group showed recovery of the gastrocnemius tissue oxygen tension, as expressed as a left–right ratio, to 0.81 ± 0.05; significantly higher than the 0.61 ± 0.06 seen in the chronic ischemia group (n = 6 rats per group, $P < 0.033$).

Muscle mass and architecture. The rats that were treated by gradual arterial occlusion showed preserved muscle mass and architecture without early muscle necrosis or late fibrosis. The ischemic limb of the rats in the acute ischemia group initially had greater calf muscle mass than the contralateral side, secondary to edema (Fig 2, *A*). Subsequently, muscle mass decreased secondary to muscle necrosis and fibrosis (Fig 2, *B*). The gastrocnemius muscle showed less fibrosis and more regeneration than the tibialis anterior muscle, returning to normal muscle mass by postoperative day 40 (Fig 2, *B* and *C*). The rats in the chronic ischemia group had better preserved muscle mass (ratio of left–right tibialis anterior muscle mass of 0.90 ± 0.015 vs 0.51 ± 0.077 at postoperative day 40, n = 6 rats per group, $P < .005$) and no evidence of the muscle necrosis, regeneration, or inflammatory cell infiltration seen after acute ligation (Fig 2, *A* shows representative histology; Fig 2, *C* shows muscle weights in the tibialis anterior muscle left panel and gastrocnemius muscle right panel).

Angioscores and vessel diameter. Angioscores were equivalent after acute and chronic ischemia, but vessel diameter was greater in acute ischemia. Angioscores based on the number of vessels were equivalent on postoperative day 40, (Fig 3, *A* and *B*, left panel, 4.11 ± 0.35 vs 4.57 ± 0.56, $P = .52$). However, the diameter of the largest five vessels seen on each angiogram was greater in the acute ischemia group than in the chronic ischemia group (Fig 3, *B*, right panel, 0.013 ± 0.0007 vs 0.010 ± 0.0003, $P < .005$). There was complete arterial occlusion at the site of each ameroid constrictor in all rats studied by angiogram at postoperative day 40.

Capillary counts. Capillary counts were increased more by acute ischemia than by chronic ischemia. Although capillary counts increased significantly in the rats in the acute ischemia group (Fig 3, *C* left panel), the muscle fiber number increased approximately the same amount, leaving the capillary–muscle fiber ratio unchanged (Fig 3, *C* right panel). The rats in the chronic ischemia group had increased capillary–muscle fiber ratios when they were most ischemic, as determined by tissue oxygen measurements and laser Doppler perfusion ratios, on postoperative day 14. This ratio returned to normal by day 40, despite persistent ischemia in the chronic ischemia group.

VEGF and inflammatory cytokines. VEGF decreased and inflammatory cytokines increased after acute ischemia but not after chronic ischemia. VEGF levels, as detected by ELISA performed on muscle homogenates from either the gastrocnemius (calf) or gracilis (thigh) muscles, demonstrated a surprising decrease in the levels of gastrocnemius muscle VEGF at postoperative days 3 and 14 in the acute ischemia group compared with unoperated controls (Fig 4, *A*). However, levels of muscle IL-1β were significantly higher in the acute ischemia group in both the gracilis and gastrocnemius muscles at postoperative day 14 (Fig 4, *B*). IL-6 levels trended higher at postoperative days

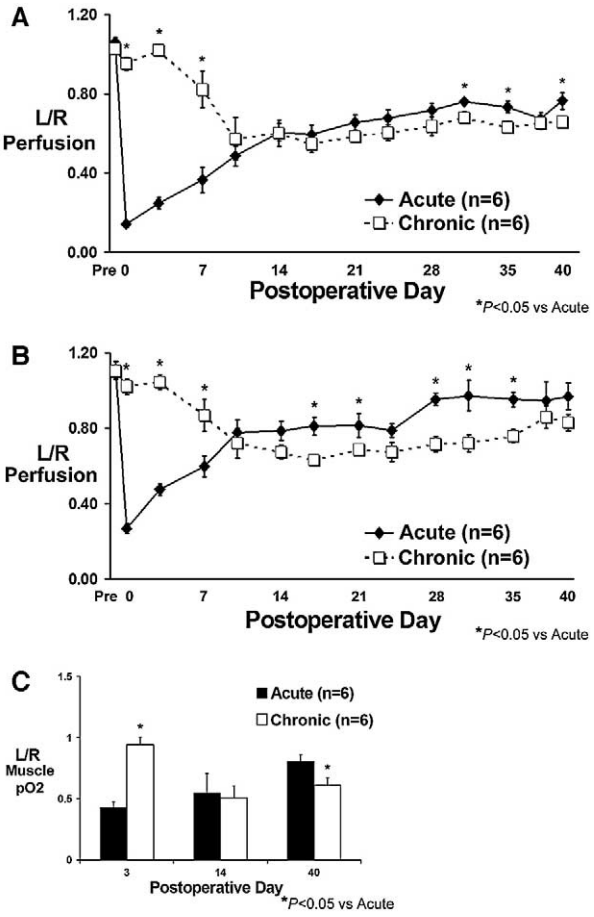

Fig 1. Hindlimb ischemia induced gradually leads to delayed foot and calf blood flow recovery as determined by laser Doppler perfusion imaging and persistent deficits in gastrocnemius tissue oxygen tension. Blood flow is expressed as the ratio of the averaged laser Doppler perfusion flux values of the left (ischemic) over the right (nonischemic) foot (**A**) or calf (**B**) region. Laser Doppler perfusion imaging was performed pre-operatively, postoperatively, and every 3-4 days afterward out to 40-days postoperatively. **C,** Tissue oxygen tension (pO_2) was measured in the right and left gastrocnemius muscles of rats anesthetized by pentobarbital at 3, 14, and 40 days postoperatively. The left–right ratio of gastrocnemius muscle oxygen tension correlated with the laser Doppler perfusion ratio and was lower in the rats that underwent gradual arterial occlusion than in the rats that underwent acutely arterial occlusion (0.61 ± 0.06 vs 0.81 ± 0.05 respectively, $*P < .05$ vs acutely induced ischemia group, n = 6 rats per group.)

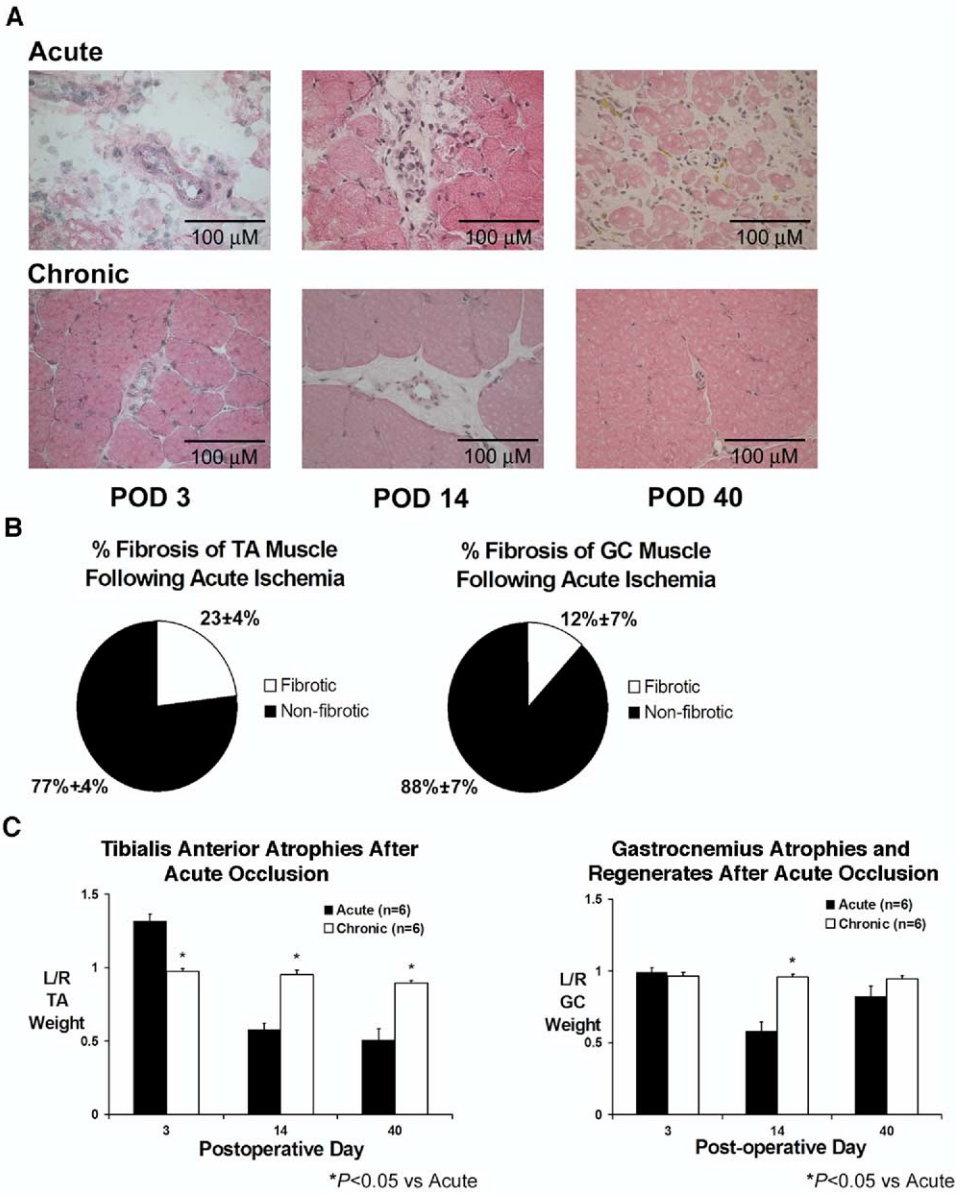

Fig 2. Chronic ischemia does not alter muscle architecture or induce an inflammatory cell infiltrate. **A,** Hematoxylin and eosin stained frozen sections of tibialis anterior muscle harvested from rats that underwent arterial occlusion acutely or gradually. Other than mild perivascular edema at postoperative day 14, no changes in muscular architecture were seen in the chronic ischemia group. **B,** Pie graph demonstrating the percentage of tissue fibrosis seen in tibialis anterior (TA) and gastrocnemius (GA) muscle sections harvested at postoperative day 40 after acute ischemia. No fibrosis was seen in any muscle sections from the chronic ischemia group (n = 3 rats per group). **C,** Tibialis anterior (TA) and Gastrocnemius (GA) muscle weights after sacrifice (n = 6 rats per group).

3 and 14 in the acute ischemia group, but this result did not achieve statistically significance (Fig 4, *C*).

DISCUSSION

We created a novel model of chronic hindlimb ischemia in the rat by using ameroid constrictors and used this model to demonstrate significant differences in blood flow recovery, arteriogenesis, and morphologic outcomes of skeletal muscle subjected to ischemia created acutely or gradually. We demonstrated that rats implanted with ameroid constrictors reliably developed blood-flow deficits over the 1- to 2-week period after operation and blood flow nadirs 7 to 17 days after the implantation of the constrictors.

Tissue oxygen content was decreased, and capillary angiogenesis was stimulated transiently in response to this ischemia. Moreover, chronic ischemia did not result in

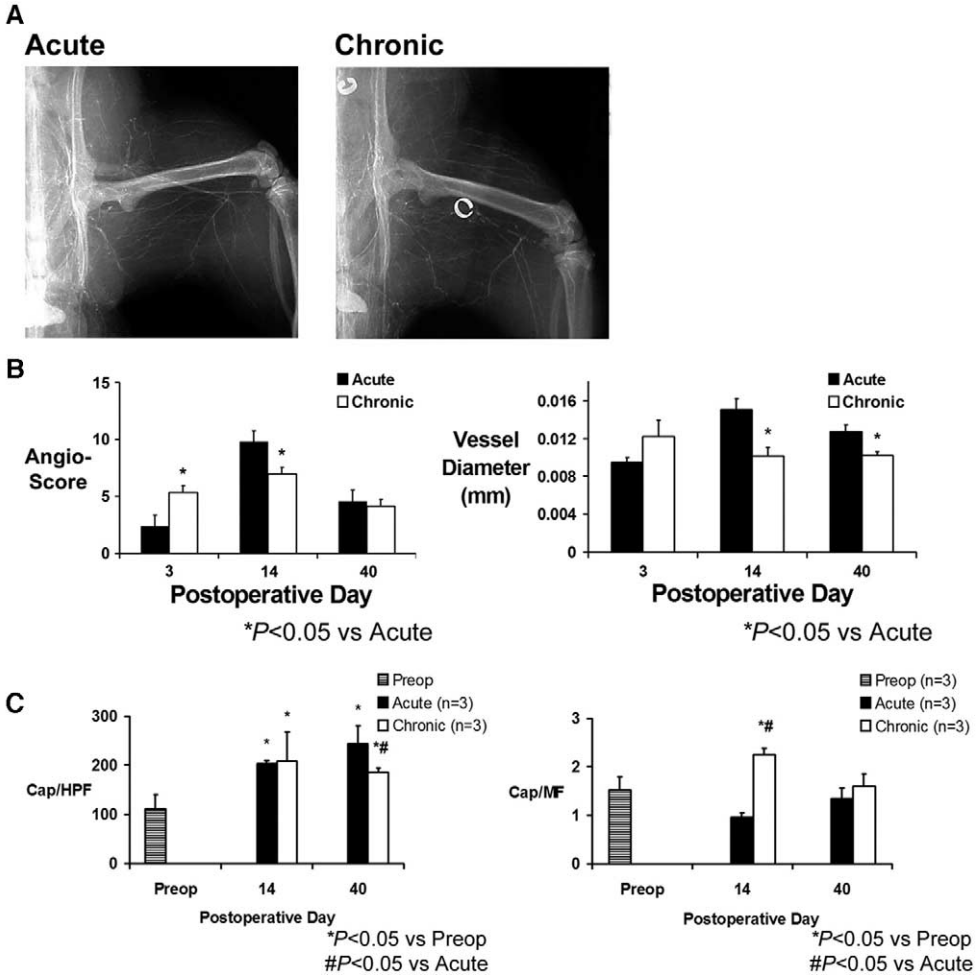

Fig 3. Chronic ischemia leads to similar collateral artery angioscore but smaller diameter collateral arteries than does acute ischemia. **A,** Representative angiograms on postoperative day 40 of rats that underwent arterial occlusion acutely or gradually. Vessel diameters are larger for rats that underwent acute arterial occlusion. **B,** Angioscores at postoperative days 3, 14, and 40 (n = 3 rats per group per time point). **C,** Gastrocnemius capillary (CAP) counts (left panel) and capillary-to-muscle fiber (MF) ratios (right panel) at postoperative days 3, 14, and 40 for rats that underwent arterial occlusion acutely or gradually. Unoperated gastrocnemius capillary count or capillary–muscle fiber ratio is shown for comparison (*$P < .05$ vs unoperated, #$P < .05$ vs acute, n = 3 rats per group per time point).

increases in inflammation, necrosis, or fibrosis, as opposed to acute ischemia. The more complete and rapid recovery of blood flow seen after acute arterial occlusion may be due to the inflammation that develops in response to the extensive skeletal muscle necrosis, which was absent in chronic ischemia induced by gradual arterial occlusion using ameroid constrictors.

A widely held clinical view is that the chronic onset of ischemia due to peripheral vascular occlusive disease induces greater collateral blood flow and less tissue injury than acute ischemia due to trauma or thrombosis. Our results suggest that gradual occlusion induces less tissue injury but not greater collateral blood flow. The magnitude of the differences in recovery of blood flow measured by laser Doppler, while not large was significant. Direct measurement of skeletal muscle oxygen tension showed an even greater difference. We view the tissue oxygen data as more precise than the laser Doppler-measured blood flow because it is a direct measurement of the level of oxygen delivery to the ischemic *muscle* as opposed to a proportional measurement of *dermal* blood flow.

We speculate that the lack of inflammation may account for the slower recovery of blood flow in rats whose hindlimb ischemia was created chronically rather than acutely. In contrast, acute ischemia resulted in increased concentrations of IL-1β and inflammatory cells seen by histology in the gastrocnemius muscle. Profound muscle necrosis and atrophy of the tibialis and gastrocnemius may have contributed to the initial rapid phase of blood flow recovery after acute ischemia. Muscle necrosis may also explain the low

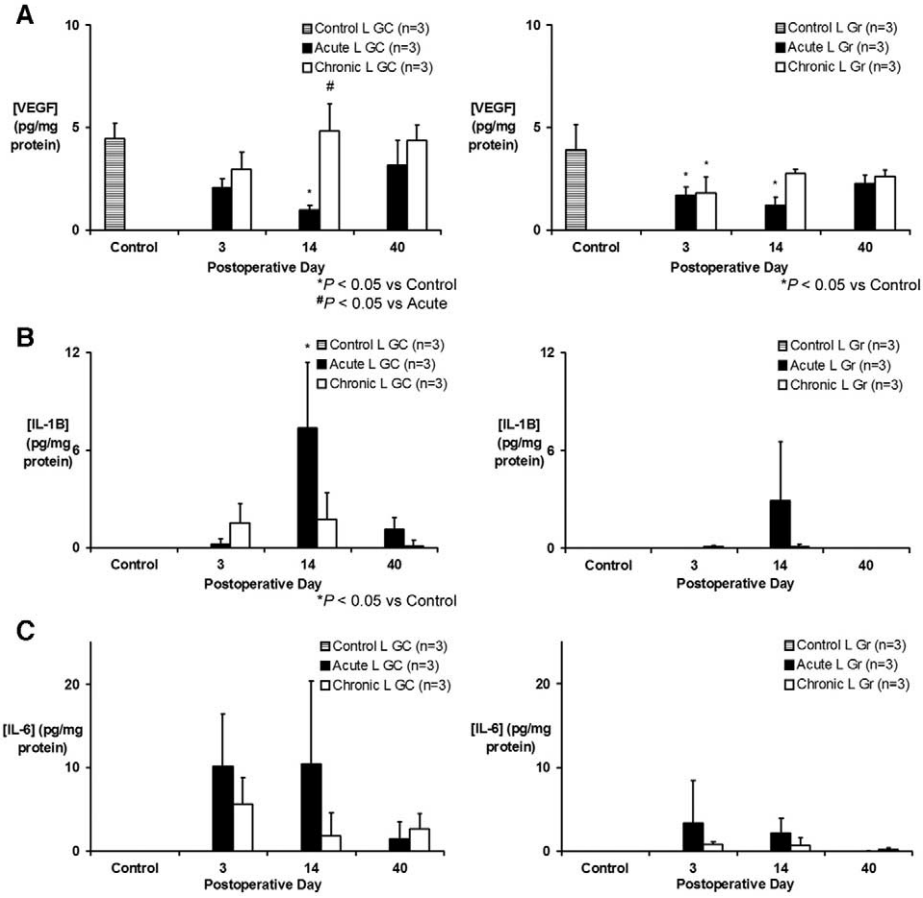

Fig 4. Levels of vascular endothelial growth factor (VEGF), interleukin (IL)-1β, and IL-6 in the gastrocnemius and gracilis muscles differ after the induction of acute or chronic ischemia. Results are presented for the left calf gastrocnemius (GC) muscle (left panel), and the left thigh gracilis (Gr) muscle (right panel) in **A, B,** and **C**. **A,**VEGF levels decreased after the induction of acute ischemia (*$P < .05$ vs control muscle from unoperated rats, #$P < .05$ vs acute ischemia group, n = 3 rats per group per time point). **B,** IL-1β levels are higher after the induction of acute ischemia than after chronic ischemia (*$P < 0.05$ vs control muscle from unoperated rats, n = 3 rats per group per time point). **C,** IL-6 level trended higher after the induction of acute ischemia but this was not statistically significant.

VEGF levels we observed after acute ischemia, as necrotic muscle fibers contribute to muscle mass but not to VEGF production.

Although not addressed in this study, an additional possible mechanism explaining the slower recovery is that much smaller pressure gradients developed[19,20,30] between the collateral arteries and the arteries in the ischemic limb in the chronic ischemia group than occurred in the acute ischemia group. These smaller pressure gradients might contribute to decreased shear stress-responsive arterial remodeling affecting collateral artery development. In support of this were the significant differences between the nadirs of blood flow, a mean of 0.14 ± 0.02 in the acutely induced group and 0.55 ± 0.11 in the gradually induced group, despite occlusion of the same set of arteries in both groups.

It has previously been proposed that studying the later phases of recovery from experimental acute ischemia is a substitute for chronic ischemia.[31,32] Results from our study demonstrate significant differences at the histologic and cytokine level between ischemia induced chronically or acutely. Histologically, after acute ischemia, necrotic muscle fibers, the inflammatory cell infiltrate, and regenerating myocytes can still be seen at postoperative day 14, and fibrosis is still present at postoperative day 40. As this type of tissue damage seen after acute ischemia can increase gene transfer efficiency,[33,34] models that use chronic ischemia are more likely to reflect gene transfer efficiencies and outcomes achievable in humans.

A shortcoming of our model of chronic ischemia is the relatively short time between implantation and occlusion of the ameroid constrictors. The period of 7 to 17 days prior to blood flow nadir might be described as subacute rather than chronic development of ischemia and may not be sufficiently gradual enough to simulate accurately atherosclerotic occlusive disease that develops over years. None-

theless, the lack of significant muscle atrophy or necrosis seen in our model demonstrates that even a relatively short period of time allows skeletal muscle to adapt to gradually increasing ischemia and is sufficient to mitigate the harmful effects of arterial occlusion.

A second shortcoming is that our model lacks the inflammation in the arterial wall that is well known to be associated with atherosclerotic plaques. This weakness might be overcome by inducing chronic ischemia in a mouse model of atherosclerosis, assuming that our findings are not species or model specific and that our methods of gradual arterial occlusion could be successfully adapted to the mouse.

We report here the first rat chronic ischemia model based on gradual arterial occlusion. We speculate that the less complete recovery of limb blood flow and muscle oxygen tension, but prevention of necrosis and preservation of muscle mass after chronic ischemia, may be due to the absence of severe inflammation, necrosis, and high shear rates in the collateral arteries in the hindlimb.

We further speculate that the severe inflammation, necrosis, and high rates of shear stress occurring in experimental models of acute hindlimb ischemia create a proarteriogenic environment. The lack of this proarteriogenic environment in human peripheral vascular disease may explain the failure of molecular therapies to adequately reverse human critical limb ischemia.[8-10]

Finally, we suggest that animal models where ischemia is generated chronically rather than acutely may be more appropriate to study both molecular therapies for peripheral vascular disease and clinically relevant mechanisms of collateral artery development.

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Submitted Apr 20, 2004; accepted Nov 8, 2004.