Sustained Peripheral Arterial Insufficiency Durably Impairs Normal and Regenerating Skeletal Muscle Function

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Abstract: Peripheral vascular occlusive diseases are frequently observed in humans, and studies with animal models have been largely used. However, the effects of sustained lower limb ischemia on normal and regenerating hindlimb skeletal muscles are not well known in the mouse model. Therefore, prolonged unilateral hindlimb ligation was generated by femoral artery ligation. Normal (myotoxic-untreated) and regenerating (myotoxic-treated) ischemic muscles were studied by analyses of the in situ contractile properties and histological parameters. Concerning normal mouse muscles, we found that femoral artery ligation reduced hindlimb perfusion and altered muscle structure and function. Thus, 7 days after ligation, maximal tetanic force was reduced by about 70% (p < 0.05). By 56 days after ligation, muscle weights and cross-section areas of muscle fibers were still reduced (p < 0.05). Concerning myotoxic treated muscles, we report that ligation reduced the recovery of muscle weight and maximal tetanic force and increased fatigue resistance at 56 days (p < 0.05). In conclusion, our results demonstrate that sustained peripheral arterial insufficiency in mice induces long-term as well as acute detrimental effects in both normal and regenerating muscles.

Key words: injury, regeneration, hindlimb ischemia, arterial insufficiency, contractile properties, mouse, laser doppler perfusion imaging.

Peripheral arterial occlusive disease is known to lead to disability and possibly to limb loss (reviewed in [1, 2]). Mouse models of peripheral vascular diseases, i.e., peripheral artery ligation or excision, are widely used to study arteriogenesis and mechanisms of angiogenesis, as well as cellular and gene therapies [3–5]. Since mouse hindlimb perfusion is not fully restored rapidly after femoral artery ligation or excision [3, 6], ischemia is sustained in this kind of animal model. Therefore, it is quite likely that these experimental procedures of peripheral artery occlusion do not lead to ischemia-reperfusion injury in mice (reviewed in [7]). Only a very few investigations have been concerned with the effect of peripheral artery ligation or excision in mice on hindlimb skeletal muscle functions [8]. Thus, very little information exists on the rate and on the ultimate extent of muscle contractile recovery following prolonged peripheral arterial occlusive events in this animal model. An examination of muscle contractile properties appears to us as the most pertinent way to appreciate the functional outcome of peripheral ischemia.

Moreover, blood circulation is thought to play an important role in muscle repair [9–11]. However, very little research has been realized to directly investigate this role in relation to peripheral vascular disease. In favor of such a role is the impairment of muscle regeneration by the administration of a hemorrhagic agent [12]. Revascularization, i.e., muscle blood supply, might also be considered critical, since the centripetal gradient of muscle regeneration stages (from the periphery toward the center of the muscle) is observed in terminal devascularized muscle graft and is reversed when graft is wrapped around vessels [13]. To our knowledge, no study has analyzed the effect of femoral artery ligation on the functional recovery of hindlimb muscles that are treated by myotoxic agents. These agents are widely used in studies of muscle regeneration, since they destroy muscle fibers, but they leave intact the precursors of muscle cells without disrupting nerve branches, blood vessels, and muscle tendons that are elements known to play a major role in muscle repair (reviewed in [11]).

We report here the results of experiments in mice aimed at assessing the outcome of prolonged peripheral arterial insufficiency in both normal and regenerating hindlimb muscles. First, for a wider insight on the potential effects of sustained reduced blood flow in normal hindlimb muscles, we performed a femoral artery ligation on the mouse. More specifically, we wanted to determine whether a complete recovery of normal muscles could be reached in the mouse model of peripheral vascular occlusion.
sive diseases. Second, to analyze the effect of hindlimb ischemia on regenerating muscles, we created extensive muscle necrosis by injecting myotoxic agents into the muscles [14–16]. Myotoxic-treated muscles were then made ischemic by femoral artery ligation. We tested the hypothesis that peripheral ischemia would reduce hindlimb muscle repair. The muscle status in normal (myotoxic untreated) and regenerating (myotoxic treated) ischemic muscles was evaluated here by examining muscle function. Our findings indicate that sustained experimental peripheral arterial insufficiency, i.e., femoral artery ligation, influences normal and regenerating muscles in a detrimental way. These results could provide helpful knowledge in relation to vascular and muscular diseases.

MATERIALS AND METHODS

Animals. All procedures were performed in accordance with national and European legislations, using young adult rodents. The study was approved by the ethical committee for experimentation at Université Paris 12. After at least 7 days of acclimatization, surgery was performed on animal legs to induce hindlimb ischemia and/or to inject a myotoxic agent into the muscles. The animals were anesthetized with pentobarbital (60 mg/kg). A few days after surgery, they generally became normal with respect to mobility.

Femoral artery ligation. Unilateral hindlimb ischemia was generated. The right hindlimbs of the mice were made ischemic. The femoral artery was ligated immediately upstream of the bifurcation from the superficial epigastric artery. No evidence of toe, foot, or leg necrosis, nor of autoamputation, was found. Muscles from the ligated legs were normal (L group) or regenerating (LM, see below). The contralateral (left) legs were left intact and were used to produce control (nonischemic) muscles.

Myotoxic treatment. Extensive muscle necrosis was created by injecting myotoxic agents into the muscles [14–16] immediately before femoral artery ligation. Cardiotoxin (C-3987, Sigma-Aldrich, 10 μM) was injected into the right medial gastrocnemius muscles (M and LM groups) with 70 μl of normal saline. A needle connected to the microsyringe was inserted near the distal tendon, pushed up to the proximal tendon, and the myotoxic solution was injected into the muscle, the needle being pulled up in order to deliver the solution all along the muscle. The contralateral (left) legs were left intact and used to produce control (uninjured) mouse muscles.

Hindlimb blood perfusion assessment. Repeated mouse hindlimb flow measurements over the region of interest (leg and foot) were taken immediately after surgery and 5, 8, and 12 days after artery ligation through the use of laser Doppler perfusion imaging (Moor Instruments) as previously described [3]. The animals were anesthetized with pentobarbital (60 mg/kg, immediately after surgery) or ketamine (100 mg/kg) and xylazine (15 mg/kg) (5, 8, and 12 days after surgery). The right (ischemic) hindlimb perfusion is expressed as a ratio of that of the left (normal or control) hindlimb.

Contractile measurements. The isometric contractile properties of the left and right mouse medial gastrocnemius muscles were studied in situ (7, 14, 28, and 56 days after surgery). The measurements were taken according to methods previously detailed [15–17]. The animals were anesthetized with pentobarbital (60 mg/kg). Supplemental doses of anesthetic were given as required to maintain deep anesthesia during experiments. All isometric contraction measurements were made at an initial muscle length of L0 (length at which maximal tension was obtained during the twitch). Briefly, the knee and foot were fixed with clamps. The distal tendons of plantaris, lateral gastrocnemius, and soleus muscles were cut. The distal tendons of medial gastrocnemius muscles (Achilles tendon) were cut and attached to an isometric transducer (Harvard Bioscience, Les Ulis, France). Medial gastrocnemius muscles were prepared free from the surrounding tissue, thus minimizing the effects of the other muscle contractions. Great care was taken to ensure that the blood and nerve supply remained intact during surgery. All data provided by the isometric transducer were recorded and analyzed on a microcomputer, using a PowerLab system (4SP, ADInstruments) and software (Chart 4, ADInstruments). Body temperature was maintained at 37°C using radiant heat. The sciatic nerves (proximally crushed) were stimulated by a bipolar silver electrode using a supramaximal square wave pulse of 0.1 ms duration. Muscle force responses were successively recorded following single or repetitive electric stimulation (at pulse frequency from 6.25, 12.5, 25, 50, 100, and 143 Hz; train duration of 500 ms). At least 1 min was allowed between stimulations. The following parameters were studied: maximum twitch force (Pt), time to peak tension (CT), half relaxation time (1/2RT), and maximal tetanic force (P0). Specific forces (P0/m) were also calculated (P0/m = P0 [g]/muscle mass [g]). Fatigue resistance (FR) was then determined after a 5 min rest period. The muscles were stimulated at 50 Hz during 45 s, and the time (s) corresponding to a tension decrease by 20% was noted.

After contractile measurements, the animals were killed with an overdose of pentobarbital. The medial gastrocnemius muscles were then weighed, frozen in isopentane precooled by liquid nitrogen, 80°C until histology.

Histological analysis. Transverse serial sections of medial gastrocnemius muscles (10 μm) obtained by using a cryostat were stained with hematoxilin solution. The images of muscle sections were acquired with a video camera mounted on a bright-field microscope and attached to a personal microcomputer. A cross-sectional area of muscle fibers (100 fibers) of a different region within a section of muscles was measured with a computer mouse to trace
the outline of muscle fibers using an image software application (Photoshop 4, Adobe Systems Incorporated; San Jose, California). The area unit of a cross-sectional area (CSA) was arbitrary.

**Statistical analysis.** The data were analyzed with the use of Statistica 5.5 software (StatSoft, Paris, France). The groups were statistically compared using three-way variance analysis (times × myotoxic treatment × ischemia). If necessary, a subsequent contrast analysis was also performed. To simplify data presentation, the effect of time was not shown in the tables or figures. The values are means ± SE. The values of ischemic muscles, myotoxic-treated muscles, and myotoxic-treated ischemic muscles were also expressed in the percentage of control (normal) muscles, i.e., contralateral muscles.

**RESULTS**

**Effect of femoral artery ligation on normal skeletal muscles**

The femoral arteries of the right legs were ligatured. Hindlimb perfusion in the ligated and the contralateral control legs was compared. Immediately after surgery, artery ligation induced an important reduction of hindlimb perfusion (Fig. 1, *p* < 0.05). Some hindlimb perfusion recovery was already visible 8 days after artery ligation (*p* < 0.05). A full restoration of hindlimb perfusion was noted 12 days after surgery when no difference was found between ligated and control legs (Fig. 1, *p* < 0.05).

The medial gastrocnemius muscles from ligated legs (L group, Table 1) were compared to contralateral control muscles (control group, Table 1) at different times after surgery. In situ muscle contraction in response to electric nerve stimulation shows notable changes after artery ligation. By 7 days, absolute and specific maximal tetanic (P0 and P0/m) forces were markedly and significantly reduced in L in comparison to control muscles (Table 1, *p* < 0.05). Significant recovery of P0 and P0/m was observed at days 28 and 56, since they significantly increased as compared to previous days (Table 1, *p* < 0.05). By 56 days, no significant difference in P0 or P0/m was observed between the L and control muscles (*p* > 0.05). However, it is noteworthy that mean P0 in L muscles was only 73.5% of that in control muscles (Table 1). A full recovery of fatigue resistance (FR) was observed in L muscles by day 56 (Fig. 2, A and B, *p* > 0.05). On the other hand, the kinetics of the twitches (CT and 1/2RT, data not shown) of L and control muscles showed no significant difference at any time of observation (*p* > 0.05).

Histologically, contrary to control muscles, L muscles exhibited a few centronucleated muscle fibers (Fig. 3, A and B). These regenerating muscle fibers indicate that artery ligation had previously led to focal muscle fiber death. They fill about 10% of the muscle cross-sectional area. By 56 days, muscle fiber cross-section areas were significantly lower in L muscles in comparison to control muscles (Fig. 3B, *p* < 0.05). Thus the muscle weights were not fully recovered 56 days after surgery and were significantly decreased by artery ligation, (Fig. 4, *p* < 0.05).

**Effect of femoral artery ligation on regenerating skeletal muscles**

To determine the effects of hindlimb ischemia on mouse regenerating muscles, femoral artery ligation was performed (right legs) and medial gastrocnemius muscles from ligated legs were injected with a myotoxic agent (cardiotoxin) (LM group, Table 1). These muscles were compared to contralateral control muscles (left legs) and to myotoxic treated muscles from nonligated legs (M group, Table 1) at different times after surgery.

The analyses of contractile properties show the marked effects of myotoxic treatment on muscles and also of artery ligation in myotoxic-treated muscles. By day 7, similar to M muscles, P0 and P0/m were significantly decreased in LM muscles in comparison to control muscles (Table 1, *p* < 0.05). Contrary to M muscles, the recoveries of P0 and P0/m were not observed until day 56 in LM muscles (*p* < 0.05). Moreover, contrary to M muscles, the LM muscles still exhibited reduced P0 in comparison to control muscles 56 days after injury (*p* < 0.05). P0 was significantly lower in LM muscles in comparison to M muscles at all times of observation (Table 1, *p* < 0.05).

We found no significant effect of myotoxic treatment on muscles from nonligated legs and no effect of artery ligation on myotoxic-treated muscles concerning CT and ½ RT (*p* > 0.05; data not shown). However, contrary to M muscles, FR was significantly reduced in LM muscles as compared to control muscles 14 and 28 days after injury (Fig. 2, A and B, *p* < 0.05). By day 56, FR had recovered in the LM muscles (*p* < 0.05). Similar to M muscles, FR in LM muscles was significantly increased in comparison to control muscles by day 56 (Fig. 2, A and B, *p* < 0.05). Moreover, FR was significantly higher in LM-treated
muscles in comparison to M muscles 56 days postinjury (Fig. 2A, p < 0.05).

Contrary to control muscles (Fig. 3, A), the M and LM muscles (Fig. 3, D) histologically exhibited numerous centronucleated muscle fibers. They fill 70%-90% of muscle cross section in both types of regenerating muscles. By 56 days, the muscle fiber cross-sectional areas were significantly lower in the M and LM muscles in comparison to the control muscles (Fig. 3B, p < 0.05).

The weights of the M and LM muscles were significantly reduced as compared to control muscles by days 7, 14, and 28 (Fig. 4, p < 0.05). Contrary to M-treated muscles, the weights of LM muscles did not significantly recover by day 56 (p < 0.05). Moreover, contrary to M muscles, LM muscles were significantly lighter than control muscles by day 56 (Fig. 4, A and B, p < 0.05). Therefore the weights of LM muscles were significantly lower as compared to those of M muscles by day 56 (p < 0.05).

DISCUSSION

The first aim of this study was to examine the influence of prolonged hindlimb ischemia on the function of murine normal (myotoxic untreated) hindlimb muscles. Contrary to hindlimb ischemia reperfusion, little information is available on the rate and on the ultimate extent of mouse muscle contractile recovery, following sustained femoral artery occlusion. Second, the present study is the first to analyze the effect of prolonged reduced blood flow on the recovery of regenerating (myotoxic-treated) hindlimb muscles. Our analyses of the in situ contractile properties and histological parameters indicate that sustained peripheral artery insufficiency influences the normal and regenerating muscles in a globally long-lasting detrimental way.

Effect of hindlimb ischemia on normal muscles

Laser Doppler imaging has been widely used for the evaluation of perfusion after hindlimb ischemia in mice [3–5, 18–20]. It concerns mainly hindlimb superficial blood flow, which could be quite different from that of buried muscles (such as medial gastrocnemius muscles studied here) [21]. In the present study, hindlimb perfusion deficit, analyzed immediately after artery ligation, appears less important than that observed in the study by Sholtz et al. [19]. This discrepancy might be explained be-

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<th>Ligation, myotoxic treatment</th>
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<tr>
<td>L</td>
<td>Control</td>
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<td>7 days</td>
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<td>P0 (g)</td>
<td>2.1 ± 2.6 a,b,c</td>
<td>78.7 ± 11.2</td>
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<td>(% control)</td>
<td>2.8 ± 1.5 a,b,c</td>
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<td>P0/m (g/g)</td>
<td>34.1 ± 21.1 a</td>
<td>835.4 ± 52.9</td>
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<td>(% control)</td>
<td>4.4 ± 2.8 a,b,c</td>
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<td>14 days</td>
<td>(n = 5)</td>
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<td>P0 (g)</td>
<td>9.6 ± 1.2 a,b,c</td>
<td>72.3 ± 1.0</td>
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<td>(% control)</td>
<td>13.5 ± 5.8 a,b,c</td>
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<td>P0/m (g/g)</td>
<td>169.5 ± 147.9 a,b,c</td>
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<td>(% control)</td>
<td>22.2 ± 11.6 a,b,c</td>
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<td>28 days</td>
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<tr>
<td>P0 (g)</td>
<td>8.0 ± 3.3 a,b,c</td>
<td>64.8 ± 2.5</td>
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<td>12.2 ± 4.7 a,b,c</td>
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<td>P0/m (g/g)</td>
<td>141.3 ± 55.7 a,b,c</td>
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<td>(% control)</td>
<td>19.2 ± 6.8 a,b,c</td>
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<td>56 days</td>
<td>(n = 10)</td>
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<tr>
<td>P0 (g)</td>
<td>50.5 ± 5.7 ab</td>
<td>82.6 ± 3.1</td>
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<tr>
<td>(% control)</td>
<td>60.2 ± 5.8 ab</td>
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<tr>
<td>P0/m (g/g)</td>
<td>617.6 ± 53.0</td>
<td>718.0 ± 53.3</td>
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<tr>
<td>(% control)</td>
<td>86.7 ± 8.3</td>
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Values are means ± SE. LM: myotoxic-treated muscles from ligated legs. M: myotoxic-treated muscles from nonligated legs. L: muscles without myotoxic treatment from ligated legs. P0: maximal tetanic force. P0/m: specific tension. a: significantly different from control (p < 0.05). b: significantly different from M (p < 0.05). c: significantly different from L (p < 0.05).
Skeletal Muscle and Hindlimb Ischemia

The level of hindlimb ischemia varies with mouse strains [19] and also by differences in surgical procedures used to generate peripheral arterial occlusion [18]. It is quite likely that hindlimb-perfusion deficit would be greater for contracting muscles, since blood flow increases several times during muscular exercise in normal muscles [2]. It must be emphasized that we found that femoral artery ligation strongly exerts both short- and long-term effects on normal muscle parameters (see below), despite apparently modest lethal cellular damages. Therefore the absence of major muscle necrosis in our mouse model of peripheral vascular occlusive disease does not prove that residual blood flow was sufficient.

Concerning the acute effects of ischemia on muscle function, our results indicate that absolute maximal tetanic force (P0) and fatigue resistance (FR) in the mouse were reduced 7 days after artery ligation, in agreement with previous observations in rabbits after iliac artery ligation [22]. This is in contrast with a previous study reporting that contractility returned essentially to a normal 7 days after femoral artery ligation in mice [8]. The basis for

Fig. 2. Fatigue resistance (FR) during continuous tetanus of mouse medial gastrocnemius muscles after femoral artery ligation and myotoxic injury. A: Data expressed as % of control. B and C: Respectively, absolute values of treated and control groups. LM: myotoxic-treated muscles from ligated legs. M: myotoxic-treated muscles from nonligated legs. L: muscles without myotoxic treatment from ligated legs. FR was markedly increased by myotoxic treatment combined with femoral artery ligation by day 56. Values are means ± SE. a: significantly different from control (p < 0.05). b: significantly different from M (p < 0.05). c: significantly different from L (p < 0.05). The number of data per group is 3–10.

Fig. 3. Cross section of mouse muscles 56 days after femoral artery ligation and myotoxic injury. A: Microscopic pictures. (A): control muscles. (B): muscles without myotoxic treatment from ligated legs. (C): myotoxic-treated muscles from nonligated legs. (D): myotoxic-treated muscles from ligated legs. (B), (C), and (D) show centronucleated muscle cells, i.e., regenerating muscle fibers. Femoral artery ligation had induced respectively local damages, whereas extensive muscle damages were observed after myotoxic treatment. B: Estimates of cross-section area (CSA) of mouse muscle fibers (% of control muscle) 56 days after femoral artery ligation and myotoxic injury. LM: myotoxic-treated muscles from ligated legs. M: myotoxic-treated muscles from nonligated legs. L: muscles without myotoxic treatment from ligated legs. All treatments reduced CSA. Values are means ± SE. a: significantly different from control muscles (p < 0.05). The number of data per group is 6–10.
Day 14
Day 56
Control
Day 56
Day 14
Day 28

crease in muscle fiber sizes. Therefore it is possible that in
legs as compared to control muscles, though statistical
28 and 56. However, by day 56 the absolute maximal te-
ables), does not significantly enhance blood flow [1, 19,
response of vasculature to ischemia, i.e., angiogenesis
preexisting collateral arterioles [1, 9, 25]. The second re-
likely resulted from arteriogenesis, the enlargement of
AB C
oral artery ligation and myotoxic injury.

Fig. 4. Mouse muscle weights (% of control muscle) after fem-
Values are means ± SE. a: significantly different from control
p < 0.05). b: significantly different from M (p < 0.05). c: signifi-
the presence of an arterial insufficiency more severe than
that induced in the present study, a complete recovery of
mouse hindlimb skeletal muscles might never be reached.
should be noted that hindlimb muscle parameters are not
altered when femoral artery interruption does not gen-
erate hindlimb ischemia, as in the rat model [26]. Of inter-
est also is the observation that laser Doppler perfusion im-
aging and lethal tissue damage estimates cannot be used
individually as such to easily predict muscle functioning
after sustained hindlimb ischemia. Therefore we strongly
recommend muscle contractile properties to be analyzed
along with other parameters.

Effect of hindlimb ischemia on regenerating
muscles

Since blood circulation is thought to play an important
role in muscle repair [9–13] (see introduction), we there-
fore hypothesized that peripheral arterial insufficiency
would markedly reduce muscle recovery after myotoxic
injury. The present study demonstrates for the first time
that artery ligation has a strong detrimental effect on mus-
cle recovery after myotoxic injury in mice.

We report that artery ligation has induced a strong re-
duction (about 40%) in absolute maximal force recovery
56 days after myotoxic treatment. The force deficits ob-
served in mouse regenerating muscles from ligated legs
were very likely not due to an increase in cellular death
following myotoxic treatment combined with artery liga-
tion, since we found a similar percentage of regenerating
fibers in ligated and nonligated legs. The functional defi-
cit is mainly explained by muscle atrophy. Artery ligation
reduced the recovery of regenerating muscle weights
without affecting muscle fiber size in cross-section areas.
It is interesting that these observations suggest that the
number of regenerating muscle fibers was decreased in in-
jured muscles from ligated legs. It is possible that part of
the satellite cell reservoir, i.e., the main source of regener-
ating muscle fibers, was lethally affected by ischemia. In
favor of this explanation are data suggesting that muscle

It is important that we find a deficit in muscle contrac-
tility together with a reduced muscle weight and a de-
crease in muscle fiber sizes. Therefore it is possible that in
these divergent results remains to be established. Since
there is a very low amount of regenerating muscle fibers
with central nuclei in ligated legs, it is not possible to at-
tribute force deficit to apparent cellular death in ischemic
muscles. This effect of artery ligation is very likely a re-
sult of unidentified alterations in the complex excitation-
contraction-relaxation molecular processes. Since ATP is
indispensable to muscle contraction, in particular muscle
ATPases (myosin ATPase, sarcoplasmic ATPase, N⁺,K⁺-ATPase, and others) it is very likely that a deficit in
ATP production is the cause of the observed contractile
impairment [6]. Another possibility is that muscle fibers
were sublethally damaged, with alterations of mem-
branes, contractile proteins (such as myosin), or proteins
involved in calcium homeostasis (calcium pump and
channel). Another explanation of the absolute force deficit
is the decreased muscle mass after ischemia, resulting in a
reduced number of myosin-actin cross bridges. Neither
can impairment in neuromuscular transmission be exclud-
It remains to be determined whether some inflamma-
atory response components (such as reactive oxygen sub-
stances, cytokines, and complements) are involved in
these effects induced by sustained ischemia, as with the
model of ischemia reperfusion (reviewed in [7, 23, 24]).

By day 12, a full restoration of hindlimb perfusion was
noted in mouse-ligated legs by laser imaging. This most
likely resulted from arteriogenesis, the enlargement of
preexisting collateral arterioles [1, 9, 25]. The second re-
sponse of vasculature to ischemia, i.e., angiogenesis
(which refers to increase capillaries within ischemic mus-
cles), does not significantly enhance blood flow [1, 19,
25]. Thus muscle contractile recovery occurred by days
28 and 56. However, by day 56 the absolute maximal te-
nanic forces appeared still reduced in muscles from ligated
legs as compared to control muscles, though statistical
significance was not reached.

It is important that we find a deficit in muscle contrac-
tility together with a reduced muscle weight and a de-
crease in muscle fiber sizes. Therefore it is possible that in
recovery increases with the number of satellite cells that escape killing [27]. Also it should not be forgotten that other muscle precursor cells can participate in muscle fiber formation, such as bone-marrow-derived stem cells [28], and their supply could be impaired by artery ligation. It should be underlined that the marked deficit in maximal force production together with the large muscle atrophy observed by day 56 strongly suggest that a complete recovery of some contractile properties of myotonic muscles might never be reached after peripheral arterial insufficiency.

Surprisingly, by day 56 fatigue resistance in myotonic-treated mouse muscles was markedly increased by artery ligation. Higher capillarization in response to ischemia (angiogenesis) might explain the increase in fatigue resistance. It would be interesting to determine whether this increase was associated with a transition from fast to slow muscle fibers in ligated leg, since slow muscle fibers are known to be more fatigue-resistant. However, this was unlikely because we found that the kinetics of the contraction (CT and ½ RT) were not modified by hindlimb ischemia (data not shown).

**Conclusion**

From this study, we can conclude that sustained peripheral arterial insufficiency in mice impairs the structure and function of normal skeletal muscles. Prolonged muscle weakness and atrophy are prominent characteristics of normal muscles that were previously rendered ischemic despite modest lethal cellular damage. We also provide, for the first time, strong evidence that prolonged peripheral arterial insufficiency markedly impairs long-term muscle recovery after myotoxic injury in mice. Therefore a complete recovery of regenerating muscles has not been and might never be achieved after sustained peripheral arterial insufficiency. Recent studies showing that improved revascularization by gene therapy leads to increased muscle regeneration [29, 30], taken together with our own results reported here, should encourage the research of some contractile properties of myotonic muscles might never be reached after peripheral arterial insufficiency.

**REFERENCES**