The Cut Intramuscular Nerve Affects the Recovery in the Lacerated Skeletal Muscle

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ABSTRACT: The recovery of lacerated skeletal muscles are said to be slow and incomplete. Often the intramuscular (IM-) nerve is concomitantly cut, but never repaired. We questioned whether the IM-nerve should also be reanastamosed before repairing the skeletal muscle. Before answering this, it was necessary to know if the cut IM nerve would have an effect on the recovery of the segment of muscle distal to the level of the laceration. This study investigates the recovery of lacerated muscles after repair, and compares a complete muscle laceration where the main IM-nerve was concomitantly cut and an incomplete muscle laceration where the IM-nerve was preserved intact. The medial gastrocnemius (MG) of the adult male New Zealand White rabbit was used, with the contralateral muscle as a sham control. The laceration was at the proximal quarter of the muscle, distal to the entry point of the nerve branch from the tibial nerve into the muscle belly. Twenty-eight weeks post-repair, the lacerated MG with the IM-nerve intact showed improved muscle wet weight, near normal morphology and contractile properties, and return of muscle fiber type mix and size. The repaired lacerated MG with their IM-nerve concomitantly cut demonstrated loss of muscle wet weight, obvious fibrosis, mononuclear proliferation with fatty infiltration, increase in type-1 fibers and muscle fiber atrophy in the distal portion. We postulate that it might be important to repair the intramuscular nerve branch by microanastomosis when repairing a vital skeletal muscle that is lacerated. © 2005 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 24:102–111, 2006

Keywords: animal model; denervation; fibrosis; muscle atrophy; medial gastrocnemius

INTRODUCTION

Lacerations of skeletal muscles are common traumatic injuries, which often result in the impairment of limb function. The general practice has been to repair the muscle by epimysial suturing of the cut ends together.[1–5] Muscle has the potential to regenerate itself after an injury, depending on the extent of the laceration. Most often the recovery and the return of muscle function is slow, incomplete, and sometimes unpredictable.[1,2,4–8] While muscle fibers are regenerating, scar tissue might be deposited, and the interaction between the two processes determines the speed and quality of healing. Scarring or fibrosis at the repair site also has a direct effect on the muscle continuity, subsequently affecting the development of muscle fibers, connective tissue, nerves, and blood vessels across the repair site.[6] The degree of neural activity is known to play an important role in influencing the development of the muscle form and morphology, the adaptation and maturation of the muscle fibers and connective tissue, and the functional recovery of an injured muscle.[9–11] In skeletal muscle lacerations, little is known of the role of the intramuscular nerve branches that may also be severed. This essentially equates to a partial denervation of the muscle segment distal to the level of the

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laceration. At present, there are no directions on what needs to be done in these situations.[12]

This article addresses how, if at all, the main intramuscular nerve branch contributes to the repair process in a lacerated skeletal muscle. The model chosen was the medial gastrocnemius in the adult male rabbit, lacerated at the proximal quarter of the muscle. The aim was to evaluate the importance of the main intramuscular nerve branch, in the recovery after repair of a completely lacerated muscle. The study examines the potential need in the clinical situation to repair the main intramuscular nerve branch by microanastomosis, if it was cut. The study assesses the long term recovery of the portion of the muscle that is distal to the level of the laceration at 28 weeks after repair.

MATERIALS AND METHODS

Animal Model

The study was approved by the Ethics Committee of the Animal Holding Unit at the National University of Singapore. All animal care and surgery were in accordance with the policy governing the use and care of animals in research and teaching at the National University of Singapore (http://www.nus.edu.sg/corporate/research/rsch animalcare.htm). All surgical procedures were performed by the same surgeon, in an aseptic environment. The left medial gastrocnemius (MG) of the adult New Zealand White male rabbit ($n = 14$) was chosen as the lacerated skeletal muscle model. The right limb was used as the control where no surgery was performed. All rabbits were acclimatized under the same conditions at the Animal Holding Unit, where they were housed individually in metal cages (35.6 cm $\times$ 40.6 cm $\times$ 45.7 cm) and kept on a 12:12-h, light–dark cycle at room temperature (28°–30°C). The rabbits were fed with laboratory chow and water, ad libitum. The animals were anesthetized with intraperitoneal injection of 1:1 combination of ketamine and xylazine (0.8 ml/kg), placed in a prone position, and maintained on an oxygen-halothane mixture (1%–2%) through an endotracheal tube. The lower limb was extended at the hip, knee, and ankle to allow the popliteal fossa to be exposed. A skin incision on the posterior aspect of the mid-thigh to about 1 cm proximal to the calcaneum was made. The skin flap was dissected exposing the popliteal fat and the two bellies of the gastrocnemius muscle. The bellies are enclosed in a layer of fascia that formed a raphe in the midline, between the two bellies, joining distally at the common calcaneal tendon. The popliteal vein, artery, and the sciatic nerve and branches were isolated, exposing the nerve branches arising from the tibial nerve, to the bellies of the gastrocnemius and soleus. The nerve to the medial belly of the gastrocnemius was seen passing obliquely to the entry point (motor point) between the proximal quarter and distal three quarters of the belly. This branch measured an average 5.0–6.0 mm in length, and was on average about 0.4–0.6 mm in diameter.

Experimental Design

For Group A ($n = 7$, mean body mass $= 3.15 \pm 0.32$ kg; Fig. 1A), the whole muscle belly of the MG was divided transversely using a sharp scalpel blade, 2–3 mm distal to the entry point of the nerve branch (the motor point). Distal to the laceration site, the nerve was seen under 10$\times$ magnification, to bifurcate into three branches within the distal segment of the cut muscle belly. The concomitant cut nerve in the proximal segment was observed to have two to three fascicles. For Group B (two animals died prematurely at 5 months, hence $n = 5$, mean body mass $= 2.95 \pm 0.22$ kg; Fig. 1B), the MG was also lacerated in the same way except that, in this group, care was taken to ensure that the main intramuscular (IM) nerve branch and the tissue surrounding it were intact.

In both groups, the cut ends of the MG were surgically repaired using 4-0 Poly-propylene (Prolene, Ethicon, Sommerville, NJ) Kessler core sutures and a continuous 5-O poly-propylene epimysial suture. The myofascia was reattached, and the skin closed with a 4-O silk thread. The wound was dressed with iodine spray, and the animals were put on Cephalexin (10 mg/kg/dose) Virbac, Australia and paracetemol postoperatively for 1 week. The limbs were kept in a cast, with the knee and ankle locked at 90 degrees, and the rabbits were monitored daily. The cast and sutures were removed 7–10 days after the operation, and the animals were permitted cage activity.

Electrical Stimulation

After a postoperative period of ~28 weeks (~7 months), the body mass of the animals were recorded. Under general anesthesia as before, the two bellies of the MG were exposed, down to the Achilles' tendon insertion at the calcaneum. The popliteal vein, artery, and the tibial nerve were isolated, and the nerve branches to the bellies of the gastrocnemius and the soleus identified. With the ankle dorsiflexed at 45 degrees and the knee fully extended, the muscle length was measured from the origin of the MG to the point of insertion of the distal tendon at the calcaneum. This length was used as the isometric length for the electrical stimulation study. The median raphe was then divided distally at the tendon insertion, separating the two bellies. The MG was also freed from the soleus with blunt dissection. The distal tendon was then released from its insertion point, and attached to a force transducer (DFG, Lloyds, Chatillon, Greensboro, NC) with a 4-O nylon suture (4%

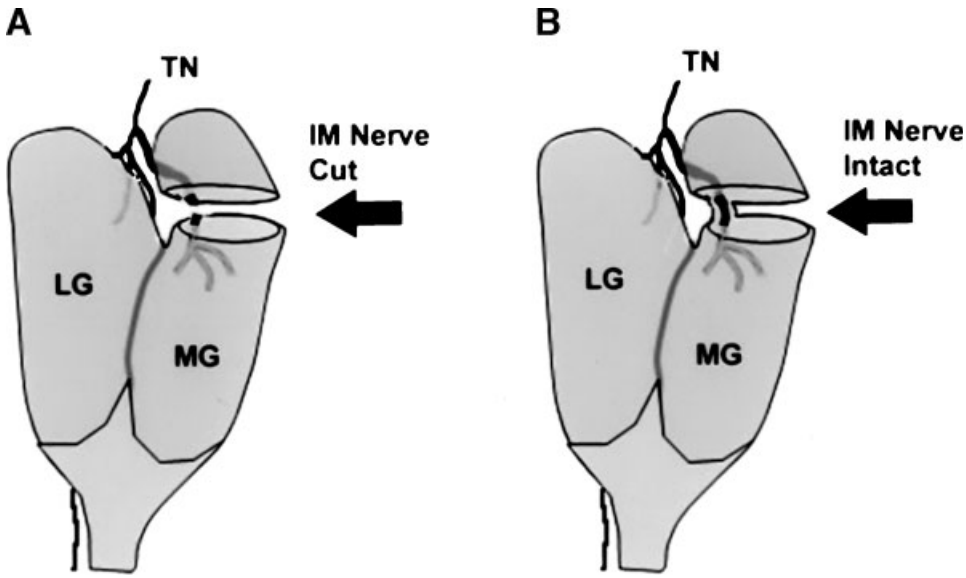

Figure 1. Schematic diagram of the two experimental groups. (A) Group A: The MG was completely lacerated at the proximal quarter of the muscle dividing their belly as well as the intramuscular nerve (IM). (B) Group B: The MG was lacerated at the same level as in Group A, preserving the IM-nerve branch, creating an incomplete (90%) muscle laceration model. Both muscles were later repaired in the same way by epimysial suturing the cut ends together. LG, lateral gastrocnemius; MG, medial gastrocnemius, TN, tibial nerve.

elongation at a break-point of 800-N) via a series of pulleys. The MG was electrically stimulated (Grass S88 Stimulator, Quincy, MA) with a fine wire mono-polar electrode hooked over their nerve branch, just proximal to the motor point. The stimulation pattern was three trains (each 2–3 s with 25-ms pulse width at 20 Hz, 2V using a constant current source of 20 mA, with a 100-ms delay between each train), repeated two times, with 3-min rest intervals between stimulations to avoid fatigue; during this time, the muscle was kept moist with saline solution. The isometric force at the distal tendon ends was recorded at a sampling rate of 100 Hz through an Analog to Digital system (National Instruments, Austin, TX) with the muscle length maintained. This was also carried out on the contralateral MG. Only four rabbits from each of the Groups A and B were used. Unfortunately, the initial three animals in each group were not tested, as the electrical stimulator was not available.

Histology

The MG and the soleus from both limbs in all the animals were harvested under anesthesia, and their wet weights measured. The soleus muscles from both limbs were used as a control to assess the amount of compensation by the contralateral limb, since the rabbits were likely to function on the nonoperated limb during the initial recovery period. The freed MG from both limbs was divided into three transverse portions and labeled—the proximal portion, proximal to the laceration site; a mid-portion, which included the site of the laceration; and the distal portion, which was distal to the laceration site. For this investigation, only the distal portion was studied. The biopsies were kept in cryovials, snap frozen by liquid nitrogen, and later transferred to a -80° storage freezer. Serial transverse 10-μm thick sections were obtained from each distal segment, using a cryostat (Leica, CM 3050 S, Leica Microsystems, Nussloch, Germany) at -20°C, and mounted on ploy-L-lysine coated slides. The slides were stained for hematoxylin and eosin (H&E) with adjacent sections stained for modified Masson's trichrome, reduced nicotine adenine dinucleotide, tetrazolium reductase (NADH-TR), a marker for oxidative enzyme activity; and for myofibrillar actomyosin adenosine triphosphatase (mATPase), after alkaline (pH 9.4) and acidic ($pH = 4.3$, 4.6) pre-incubations using standard techniques described previously.[13] The classification of muscle fiber types was based on the mATPase-stained sections.[13,14] Acetyl cholinesterase (AchE) staining for motor endplate was also employed to assess the neuromuscular innervation. For this, 30-μm thick sections were used.

Morphologic Evaluation

The degree of nonspecific morphological abnormalities assessed were based on six parameters: 1) muscle fiber atrophy, 2) fiber size variation, 3) mononuclear

proliferation, 4) fatty infiltration, 5) fibrosis, and 6) muscle fiber regeneration.[28] These were assessed from blinded H&E-stained sections from each specimen. This evaluation was done by one pathologist. According to the summation of all abnormalities, each section was then classified into one of four categories by a routine pathological score (0 = normal, 1 = slight, 2 = moderate, and 3 = severe pathology). The mean score was used to compare the differences between the operated side of Groups A and B. In addition, to verify the score, modified Masson's trichrome stain was used to confirm the extent of fibrosis, while a quantitative morphometric evaluation of the myofiber caliber, their distribution and grouping of fiber types, as well the interstitial connective and adipose tissue was done, by two other persons.

Morphometric Evaluation

Images of the histological stained sections were obtained using the Olympus BX-51 with an attached Sony CCD camera (Olympus America, Inc., Melville, NY; Sony Corp., Tokyo, Japan) and the Olympus Microimage 4.5.1 analytical software (Image Pro Plus, Media Cybernetics, Silver Spring, Maryland). From the H&E-stained sections, the entire cross-section of the biopsy was digitized at low-power (10×, magnification) using five to seven contiguous fields. The cross-sections, stained with mATPase at pH 4.3 and 4.6 pre-incubations, were digitized at 40× original magnification and used to determine the fiber type distribution, and the area of the muscle fibers, interstitial area, as a ratio of the entire cross-section of the section. The percentage of each fiber type was also calculated from the number of fibers of a given fiber type divided by the total number of fibers, from a sample of 800–2,000 fibers. The mean diameter, shape factor, and cross-sectional area of each myofibril type in each sample were also estimated to investigate the occurrence of muscle fiber atrophy and fiber size variation.

Data Analysis

Group A represents the completely lacerated MG muscle that was repaired, leaving the intramuscular (IM) nerve cut. Group B represents the so-called incomplete lacerated MG muscle that was repaired, where the IM nerve was left intact. The results are based on stained sections taken from the portion distal to the laceration site, and the corresponding section from the contralateral muscle. Values are presented as means and standard error of means (SEM). Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL). The mean body mass of the animals from both groups were compared before the operation and at the 28-week assessment time point. The wet mass of the MG and soleus muscles of the operated limb was compared to that of the contralateral limb to obtain a value representing a percentage of the control. The paired Student's t-test was used to compare the left and right sides, to determine any significance between their means. In the isometric contractile force data, the ratio between the left and right side for Group A and Group B, were also compared and tested. Analysis of variance (ANOVA) was used to compare any differences between the groups for muscle fiber diameters of types 1, 2A, and 2B fibers, and their fiber type ratio. Significant differences were accepted at a $\alpha = 0.05$ level of significance. The Bonferonni's post-hoc test was used to determine differences between the sub-groups.

RESULTS

Twenty-eight weeks after repairing the lacerated left MG, the mean body mass measured was 3.72 ± 0.46 kg and 3.31 ± 0.76 kg for Group A and Group B, respectively, with no significant difference between the two groups ($p = 0.26$, t-test). The mean muscle mass of the MG and soleus muscles are summarized in Table 1. The muscle mass of the soleus on the left and right sides, used to assess the degree of compensation, was found not to be significantly different (ANOVA, $p = 0.89$, $F = 0.02$). Between the left and right MG, there was a significant decrease in the mass of the operated muscle in Group A by 33% (paired t-test, $p = 0.01$), with no significant difference noted between the paired data in Group B (paired t-test, $p = 0.37$). The L/R-muscle mass ratio of the MG, between Groups A and B, was also found to be significantly different (ANOVA, $p = 0.021$, $F = 7.498$).

Electrical Stimulation Study

The isometric contractile force developed by the MG in the two groups is outlined in Table 2. For Group A, the mean isometric force contraction of the operated MG was about 63% of the contralateral MG, yet not shown to be statistically significant (paired t-test, $p = 0.07$). For Group B, the isometric force between the left and right MG were not significantly different (paired t-test, $p = 0.395$). This would suggest that there was insufficient evidence to show a return to normal contractile function, although a likely trend would be inferred. No significant differences in the percentage isometric force over the controls, between Group A and Group B were noted ($p = 0.2$). There was no significant difference ($p = 0.121$) between the percentage paired ratio of left to right between Group A (63%) and Group

Table 1. The Mean (±SEM) Muscle Wet Mass (g) of the Soleus and Medial Gastrocnemius of Both Hind Limbs, in Group A ($n = 7$) and Group B ($n = 5$), 28 Weeks After the Repair of the Lacerated Left Medial Gastrocnemius (MG), with a Percentage Comparison of the Left (L) Operated to the Right (R) Control Side

	Muscle Mass (g)					
	Soleus			Medial Gastrocnemius		
Group[a]	Operated Side (L)	Contralateral Control (R)	(L/R%)	Operated Side (L)	Contralateral Control (R)	(L/R%)
A	6.36 ± 0.95	6.17 ± 0.74	(104%)	5.11 ± 0.90	7.69 ± 1.26*	(67%)**
B	5.76 ± 0.95	5.61 ± 1.00	(103%)	5.14 ± 0.68	6.11 ± 1.16	(86%)

[a]Group A, IM nerve cut; Group B, IM nerve intact.
*Group A (MG): L versus R, paired t-test, $p < 0.01$.
**L/R: Group A (MG) versus Group B (MG), ANOVA, $p = 0.02$, $F = 7.498$.

B (90%). This was attributed to the small sample size. Also, one sample in Group A recorded an 87% recovery of contractile force. So, it might be inferred that there was a trend for the isometric contractile properties to recover better in Group B, than Group A.

Morphological Evaluation

In Group A (IM nerve cut), the portion distal to the site of the laceration of the left MG exhibited extensive morphological abnormalities compared to the contralateral limb (Fig. 2), including grouped fiber atrophy with greater variation of muscle fiber sizes. There was also more fatty infiltration, monocyte proliferation, and interstitial infiltration with few regenerating muscle fibers (i.e., centronucleated fibers) compared to the same portion of the contralateral muscle. (Fig. 2c, e) This group also demonstrated poorly organized AchE-positive end-plate spots with several multiple and polyneural reinervation observed. In contrast, the left MG of Group B (IM nerve intact, Fig. 2d, f), demonstrated obvious improved muscle morphology, with numerous centronucleated regenerating myofibers. The general morphology closely represented the contralateral muscle (Fig. 2b). The degree of abnormalities from the sum of the pathology scores, for Group A and Group B (Fig. 2g) was also significantly different ($p < 0.01$). The distribution of the AchE-positive end-plate spots in this group was similar to the contralateral controls, albeit being less dense.

Morphometric Evaluation

The morphometric evaluation of muscle fiber type distribution and sizes were made in the portion distal to the site of the laceration (Fig. 3). For Group A (IM Nerve cut), the repaired MG compared to their contralateral had a higher proportion of type 1 (slow, oxidative) muscle fibers which appeared to have more than a twofold increase between their means in some cases with a concomitant reduced number of type 2 (fast)

Table 2. Summary of the Mean ± SEM Peak Isometric Force (N) over Three Stimulation Trains of 2–3 s, each

		Mean Isometric Force (N)		
Group[a]	n	Operated Left MG	Control Right MG	% (L/R) paired ratio
A	4	3.44 ± 0.41*	5.66 ± 0.50	63 ± 11
B	4	4.61 ± 0.59**	5.10 ± 0.38	90 ± 10***

MG, medial gastrocnemius; SEM, standard error of mean.
[a]Group A, IM nerve cut; Group B, IM nerve intact.
*Group A, L versus R: paired t-test, $p = 0.07$, not statistically significant.
**Group B, L versus R: paired t-test, $p = 0.39$, not significantly different.
***Comparison of the percentage (L/R) paired ratio: Group A versus Group B: t-test, $p = 0.12$.

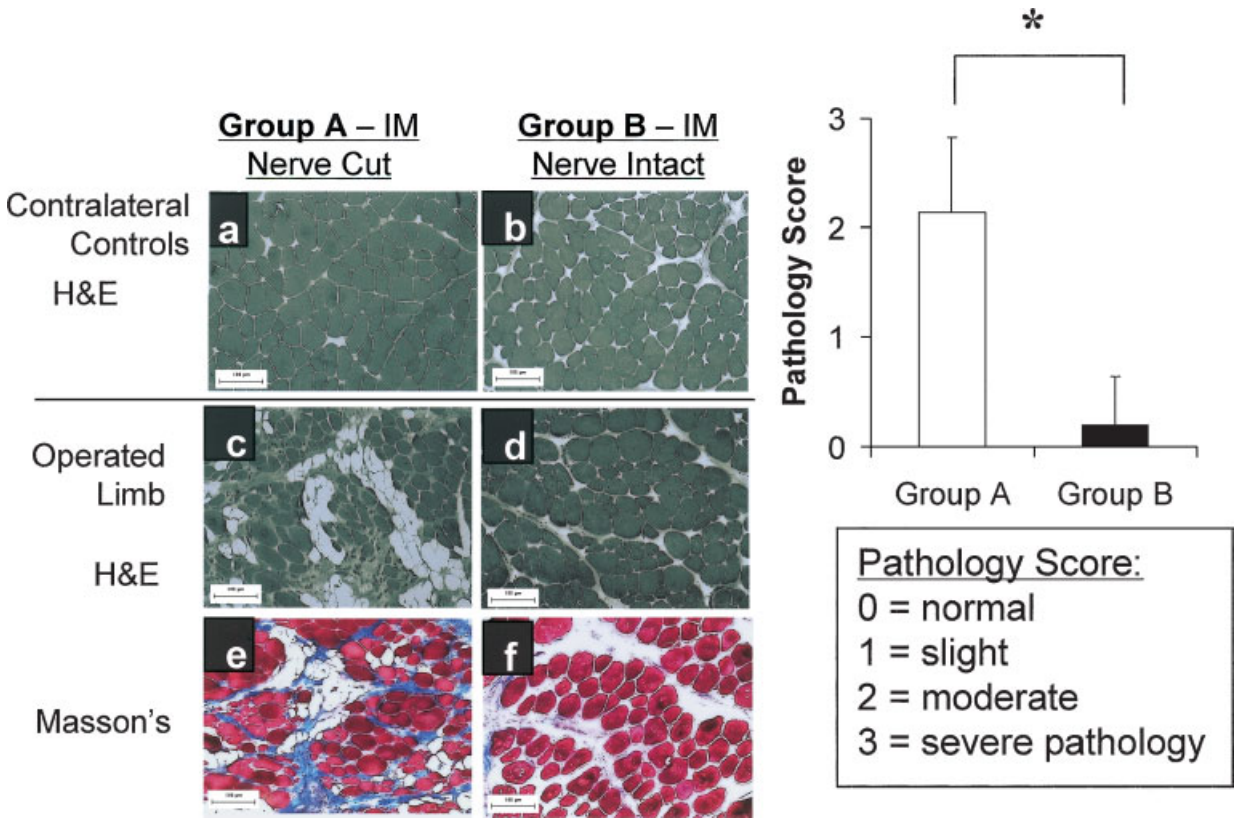

Figure 2. Histopathologic evaluation for damage of the muscle belly distal to the laceration site, 28 weeks after the repair. Top panel (H&E): (a) Group A (control); (b) Group B (control); Middle panel (H&E): (c) Group A (operated side); (d) Group B (operated side); Bottom panel (Masson's trichrome): (e) Group A (operated side); (f) Group B (operated side). (Original magnification, ×50; bar = 100 μm). (g) The morphometric pathology scores for Groups A and B; Statistic: $*p < 0.01$.

fibers, more obvious for the type 2B (fast, glycolytic) fibers (Fig. 3a, c, e); albeit no statistical significance (paired t-test, $p = 0.08$). In one case in Group A, the muscle fiber type on the operated side was found to be 100% type 1 fibers, but overall the mean fiber type 1/type 2 ratio was noted to be 47:53. The mean cross-sectional area of the type 1, 2A, and 2B fibers on the operated limb was found to be 2,234 (SEM, 222), 1,637 (SEM, 304), and 2,458 (SEM, 406) μm^2, respectively; Types 2A ($p = 0.008$) and 2B fibers ($p = 0.002$) were noted to be significantly smaller than the fibers of the contralateral normal MG, while type 1 fibers seem to have recovered their shape and size ($p = 0.21$). (Fig. 3f) In Group B (IM nerve intact), the repaired MG demonstrated a better recovery. The fiber type distribution maintained a type 1/type 2 ratio of about 18:82 (paired t-test, $p = 0.67$, Fig. 3b, d, e). Significant differences were noted between the operated and contralateral controls for type 1 (t-test, $p = 0.01$) and type 2B fibers (t-test, $p = 0.03$), but not the type 2A fibers. The mean fiber diameter for type 1, 2A, and 2B muscle fibers in this group were 2,690 (SEM, 314), 4,059 (SEM, 661), and 6,163 (SEM 654) μm^2, respectively (Fig. 3f).

DISCUSSION

This study assessed the long term (7-month) recovery of the portion of the muscle distal to the level of the laceration, in a lacerated skeletal muscle that was repaired by epimyseal suturing of the cut ends. The study was motivated by a clinical question on the need to repair the main intramuscular nerve branch by micro-anastomosis, if it was to be cut in the lacerated muscle.[12] Garrett et al. (1984) observed that the distal segment of the lacerated muscle after repair underwent denervation atrophy and that there was a significant functional loss by the 6th and

8th week even after a muscle repair.[2] They concluded that functional return can never go beyond 60% to 80% depending on the level of the laceration. In their study, the laceration was performed at a point 60% of the length of the muscle from their origin. We postulate that the impairment of function is likely to be more significant the more proximal the laceration, as in our study. Most studies on recovery in lacerated muscles suggest that complete muscle regeneration is hindered by the development of fibrosis at the level of the laceration.[4,6,8,15,16] In this study, after 28 weeks, the completely lacerated muscle that had epimysial repair (Group A) was found not to have any statistical significance in the isometric contractile force data, due to the small sample size (Table 2). However, significant loss in muscle mass, obvious fibrosis, mononuclear proliferation, and fatty infiltration in the segment distal to the laceration were observed (Fig. 2c, e) suggesting a trend towards a reduced isometric force contraction. There was also an increase in the proportion of type 1 (slow) fibers, and a corresponding decrease in the proportion of type 2B fibers, with a significant muscle fiber atrophy of types 2A and 2B fibers (Figs. 3f). More importantly, the greater variability in the muscle type mix in Group A compared to Group B suggests that if the nerve is not preserved, the result can be quite unpredictable and indicative of varying degrees of muscle denervation and muscle fiber type changes, which can remain permanent.[2,8,15,17]

In Group B, where the intramuscular nerve was left intact before repairing the lacerated muscle, the improved muscle mass (Table 1), the lack of a difference in the isometric contractile properties between the operated and the contralateral control

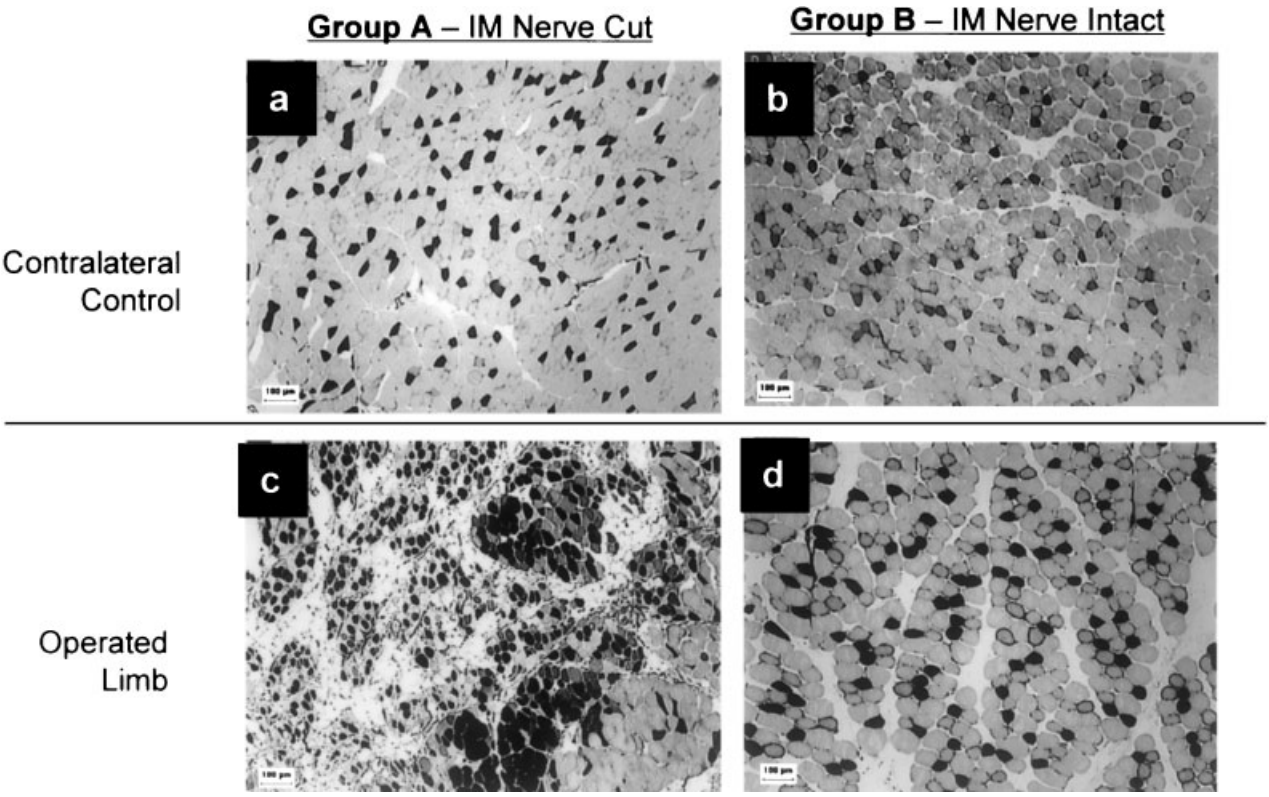

Figure 3. ATPase-stained transverse sections, acidic pre-incubation. (Original magnification, ×20; bar = 100 μm.). Comparison of the muscle fiber ratio (type 1, type 2A, and 2B) and the cross-sectional area of the muscle fibers of the MG between operated left side and contralateral right side. Top panel (Contralateral Control): (a) Group A; (b) Group B; Bottom panel (Operated Limb): (c) Group A; (d) Group B. (e) Percentage distribution of types 1, 2A, and 2B muscle fibers in the two groups. (f) Mean cross-sectional areas of muscle fibers in the two Groups. (White columns represent the contralateral control side; shaded columns represent the operated side.) *, One case recorded 100% type I fibers.

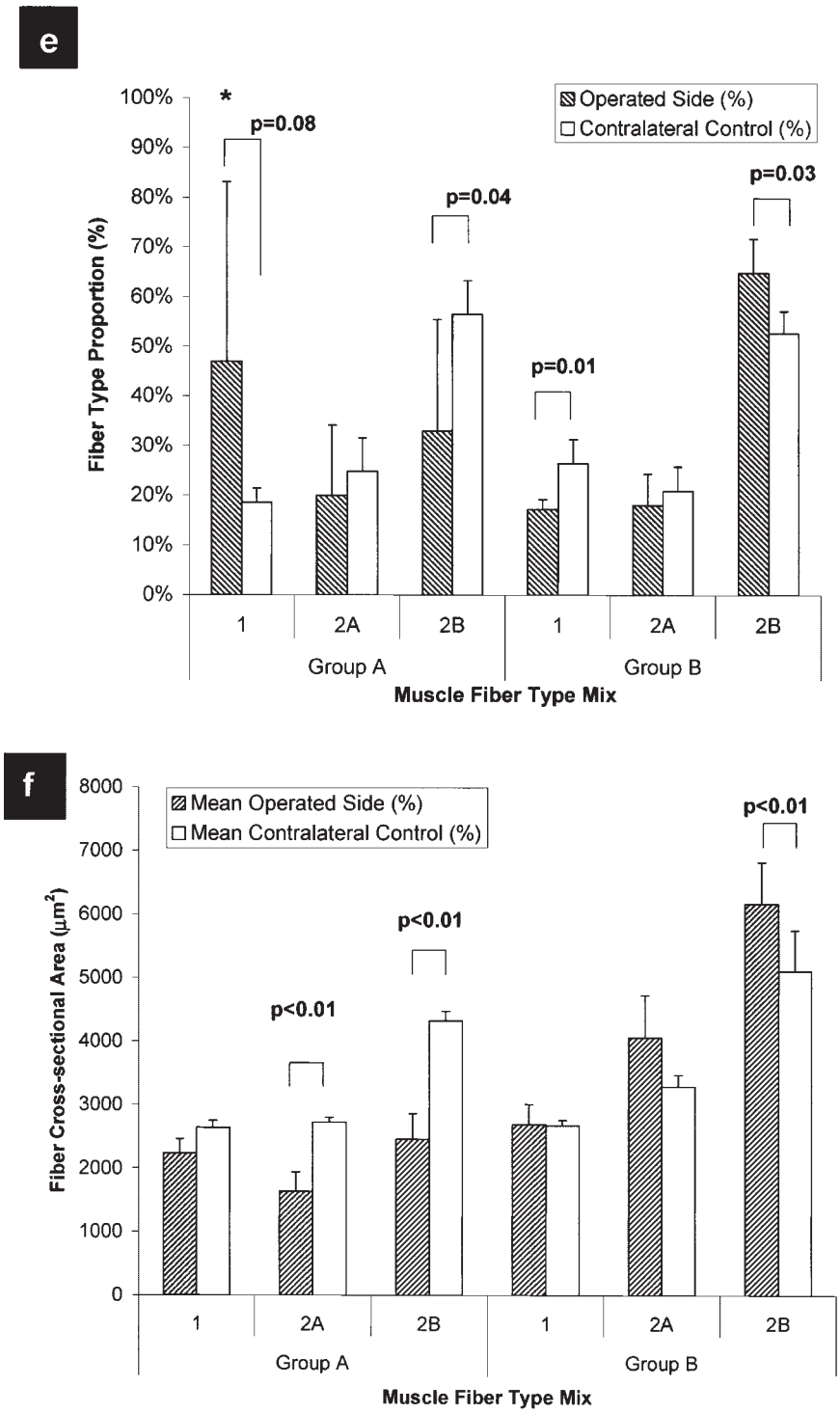

Figure 3. (*Continued*)

sides (Table 2), the near normal morphology (Fig. 2d, f), and the return of near normal muscle fiber ratio and size (Fig. 3b, d) clearly suggest an improved recovery over Group A and highlights the importance of the preservation or repair (if cut) of the intramuscular nerve. The data on the soleus muscles which showed no difference in the wet weight between the operated and control side demonstrated that the contralateral limb was not compensating for the impaired function of the

operated limb during the recovery phase. The difference in the wet weight between the left and right MG was therefore not attributable to disuse and atrophy of the operated limb in Group A.[18] It also demonstrates that the nerve branch to the soleus was not damaged in the lacerated model, as the branch from the tibial nerve to the soleus is adjacent and distal to the branch to the MG.

The contractile properties of the MG in Group A were only marginally improved (63%) when compared to the control side. Garrett (1984) found that useful function can be returned after repairing a lacerated muscle, however, the recovery of the muscle form and muscle fiber type mix will always remain incomplete.[2] It may be argued that the clean-cut laceration of the muscle in this experimental set-up and the subsequent epimyseal repair may allow spontaneous reinnervation of the divided intramuscular nerve in the muscle in Group A and contribute to the partial recovery observed. Such clean-cut lacerations are unlikely in the clinical situation, and spontaneous reanastomosis of the intramuscular nerve may not always be expected in injuries to vital limb skeletal muscle. Furthermore, even if reinnervation of a muscle does occur, it might result in a permanent change in the muscle fiber mix.[19] We note this in our study, where there was a higher proportion of type 1 fibers in the MG of Group A, and this was 7-months after the repair, suggesting that a permanent change in the fiber type mix did occur. This need to alter their metabolic state is perhaps an adaptive function to optimizing its energy source,[17] as denervation initially induces progressive atrophy of type 2 fibers and a hypertrophy of type 1 fibers, in trying to optimize the muscle's own resources (i.e., mitochondrial function), and this might remain if reinervation is slow, or if cross-innervation from an inappropriate motorneuron occurs.[8,15,20] Fu and Gordon (1995) have postulated that a change in the fiber mix can compromise the force capacity of the muscle.[20]

The preservation or repair (if cut) of the intramuscular nerve appears to be a very good way of preserving muscle function. The significance of such an exercise will perhaps be more important if the muscle laceration was over the proximal portion of the muscle where the extent of dennervated muscle is likely to be much larger. We have postulated that it might be important to repair the intramuscular nerve branch by micro-anastomosis when repairing a completely lacerated skeletal muscle.[12] Spindle-shaped muscles that have the motor points at the proximal third have been observed to have their intramuscular nerve branch running along the length of the muscle belly. In humans, upper limb muscles like the biceps brachii, triceps brachii, and brachioradialis have this type of muscle form and innervation. These muscles are also superficial and have a higher risk of laceration type injuries. If a complete recovery cannot be established after repairing the muscle as postulated by others, then it appears obvious that preserving or repairing the intramuscular nerve might become a good surgical option. However, identifying the nerve and its location and attempting to reanastomose it remains a challenge to most general surgeons. It has also been suggested that locating the intramuscular nerve in a lacerated muscle may result in more morbidity to the patient and may have an effect on the potential recovery. However, with new emerging developments in operating microscopes and micro-instruments, and with the availability of skilled microsurgeons in most general hospital, these challenges might be better met.

A modified technique to demonstrate the intramuscular nerves has allowed us to come up with some preliminary templates for locating the intramuscular nerve branching patterns.[12] The intramuscular innervation of several upper muscles has recently been demonstrated and this gives a basic road map for surgeons to locate them within the muscle belly.[12,21–25] Although our interest is in the upper limb, we chose the medial gastrocnemius model as there are current available data on the response to denervation of this muscle in the rabbit model.[17] The reason for these findings in Group B is not yet clear. It is certain that innervation, including intramuscular innervation, has some pivotal role in the regeneration and recovery of an injured skeletal muscle, and this would be dependent on other factors like aging, differences in metabolism, types of muscle involved, site of the laceration, activity levels after injury or combinations of these.[4,6,8–11,15,16,26,27] These factors were, however, not addressed in this study but are to be considerations for future work. Yet before attempting to repair the intramuscular nerve branch in a muscle in the clinical setting, which might be technically challenging given the small size of the nerve branch, it was necessary to first demonstrate that the intact intramuscular nerve branch in the lacerated muscle contributes to an improved recovery rate of the repaired lacerated skeletal muscle. This study has demonstrated this concept in the rabbit muscle model at 28 weeks after the repair.

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