

The American Journal of Sports Medicine

<http://ajs.sagepub.com/>

Use of Autologous Platelet-rich Plasma to Treat Muscle Strain Injuries

Jason W. Hammond, Richard Y. Hinton, Leigh Ann Curl, Joaquin M. Muriel and Richard M. Lovering
Am J Sports Med 2009 37: 1135 originally published online March 12, 2009
DOI: 10.1177/0363546508330974

The online version of this article can be found at:
<http://ajs.sagepub.com/content/37/6/1135>

Published by:



<http://www.sagepublications.com>

On behalf of:



American Orthopaedic Society for Sports Medicine

Additional services and information for *The American Journal of Sports Medicine* can be found at:

Email Alerts: <http://ajs.sagepub.com/cgi/alerts>

Subscriptions: <http://ajs.sagepub.com/subscriptions>

Reprints: <http://www.sagepub.com/journalsReprints.nav>

Permissions: <http://www.sagepub.com/journalsPermissions.nav>

Use of Autologous Platelet-rich Plasma to Treat Muscle Strain Injuries

Jason W. Hammond,* MD, Richard Y. Hinton,* MD, MPH, Leigh Ann Curl,[†] MD, Joaquin M. Muriel,[‡] PhD, and Richard M. Lovering,^{†§} PhD, PT

From *Union Memorial Hospital, Department of Orthopedic Surgery, Baltimore, Maryland,

[†]Harbor View Hospital, Department of Orthopedic Surgery, Baltimore, Maryland, and [‡]University of Maryland School of Medicine, Department of Physiology, Baltimore, Maryland

Background: Standard nonoperative therapy for acute muscle strains usually involves short-term rest, ice, and nonsteroidal anti-inflammatory medications, but there is no clear consensus on how to accelerate recovery.

Hypothesis: Local delivery of platelet-rich plasma to injured muscles hastens recovery of function.

Study Design: Controlled laboratory study.

Methods: In vivo, the tibialis anterior muscles of anesthetized Sprague-Dawley rats were injured by a single (large strain) lengthening contraction or multiple (small strain) lengthening contractions, both of which resulted in a significant injury. The tibialis anterior either was injected with platelet-rich plasma, was injected with platelet-poor plasma as a sham treatment, or received no treatment.

Results: Both injury protocols yielded a similar loss of force. The platelet-rich plasma only had a beneficial effect at 1 time point after the single contraction injury protocol. However, platelet-rich plasma had a beneficial effect at 2 time points after the multiple contraction injury protocol and resulted in a faster recovery time to full contractile function. The sham injections had no effect compared with no treatment.

Conclusion: Local delivery of platelet-rich plasma can shorten recovery time after a muscle strain injury in a small-animal model. Recovery of muscle from the high-repetition protocol has already been shown to require myogenesis, whereas recovery from a single strain does not. This difference in mechanism of recovery may explain why platelet-rich plasma was more effective in the high-repetition protocol, because platelet-rich plasma is rich in growth factors that can stimulate myogenesis.

Clinical Relevance: Because autologous blood products are safe, platelet-rich plasma may be a useful product in clinical treatment of muscle injuries.

Keywords: skeletal muscle; injury; strain; muscle regeneration

Muscle injury occurs from either acute or repetitive trauma and results in a decreased ability to produce force that does not recover after a short period of rest. When an activated muscle lengthens because the external load exceeds the tension generated by the muscle contraction, this is termed a *lengthening* (or *eccentric*) contraction. Submaximal lengthening contractions are

used in everyday life, but it is well known that high-force lengthening contractions are associated with muscle damage and pain.^{13,18,30} The force generated during a maximal lengthening contraction is approximately 2-fold the force developed during a maximal isometric contraction.^{9,18}

The generation of high force by muscles is a goal of strength training; this is evident in training protocols that use lengthening contractions, or "negatives," to increase strength. Although lengthening contractions are common and often occur without causing damage, high-force lengthening contractions are more likely to produce damage than either isometric or concentric contractions, resulting in pain and damage.^{16,18,23,30} In clinical lexicon, the injury resulting from a high-force lengthening contraction is termed a *muscle strain*; such strains are the most common cause of muscle injuries.^{10,37}

[§]Address correspondence to Richard M. Lovering, PhD, PT, University of Maryland School of Medicine, Department of Physiology, 685 W. Baltimore St, HSF-1, Room 580-E, Baltimore, MD 21201 (e-mail: rlovering@som.umaryland.edu).

No potential conflict of interest declared.

To conduct a well-controlled study, we developed an animal model of muscle injury and a reproducible mechanism for generating muscle strain. We recently reported that recovery of contractile function after injury by a single, large-strain lengthening contraction involves repair of damaged sarcolemma with minimal myogenesis, whereas recovery from multiple, small-strain lengthening contractions requires myogenesis, with minimal sarcolemmal repair.²⁵ Here we used both protocols in our established injury model to test the effects of autologous platelet-rich plasma (PRP) on recovery in the tibialis anterior muscle of rats. Platelet-rich plasma is isolated by a technique involving centrifugation of whole blood, allowing extraction of the specific part of the plasma containing a high concentration of platelets. These platelets are rich in growth factors that can stimulate myogenesis^{7,27,32,36} and mitigate inflammation.^{8,26} We hypothesized that the local delivery of PRP to injured skeletal muscles accelerates recovery, and we present data from experiments that support this hypothesis.

MATERIALS AND METHODS

Injury Model

All protocols were approved by the university Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats ($n = 72$) weighing 341 ± 21 g (approximately 4 months of age) were anesthetized with isoflurane (2% with oxygen flow rate of 0.5 L/min). The Sprague-Dawley rats were inbred, allowing us to consider them syngeneic. The injury model results in a significant and reproducible injury and has been described previously.^{11,23-25,33} In brief, anesthesia was confirmed by lack of response to a normally painful stimulus (pinching the foot); the left hindlimb was stabilized and the foot was secured onto a footplate. The axis of the footplate was attached to a stepper motor (T8904 NMB Technologies, Chatsworth, California), a potentiometer to record angular position, and a torque sensor (QWFK-8M, Sensotec, Columbus, Ohio) to measure torque. The fibular nerve was stimulated via subcutaneous needle electrodes (Harvard Apparatus 723742, Cambridge, Massachusetts), and proper electrode position was determined by a series of isometric twitches. In addition to visual confirmation of isolated dorsiflexion, an increase in twitch torque in response to increasing voltage indicated that opposing plantar flexor muscles were not being simultaneously stimulated.³ A custom program was used (Labview version 8.5, National Instruments, Austin, Texas) to synchronize contractile activation and onset of ankle rotation. Impulses generated by an S48 square pulse stimulator (Grass Instruments, West Warwick, Rhode Island) were 1 millisecond in duration and passed through a PSIU6 stimulator isolation unit (Grass Instruments).

To induce injury in the tibialis anterior muscle (TA), we superimposed a lengthening contraction onto a maximal isometric contraction (Figure 1), using either a single repetition (large strain) or multiple repetitions (small strain). Specifically, a maximal isometric contraction was

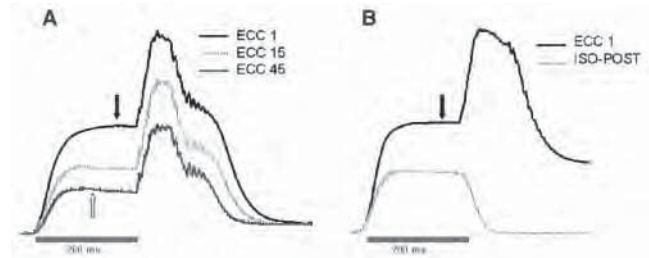


Figure 1. Representative trace recordings of torque from the lengthening contractions. For both single and multiple repetitions, muscles were stimulated for 200 milliseconds to induce a peak isometric contraction before lengthening by the footplate. Maximal isometric torque (without lengthening) was measured before injury (not shown, but equal to the plateau of the isometric portion of the trace recordings, indicated by filled arrows). A, superimposed recordings from the multiple-repetition protocol (a 60° arc of motion) showing the first (ECC 1), middle (ECC 15), and last (ECC 45) eccentric contractions. Note that even with injury, the eccentric torque is still relatively high compared with isometric torque (the peak isometric torque measured after injury is not shown but is similar to the isometric portion of the last trace recording, indicated by the open arrow). B, superimposed recordings of the single-repetition protocol (ECC 1, a 90° arc of motion) and the isometric contraction to measure torque loss after injury (ISO-POST).

obtained in the dorsiflexors and after 200 milliseconds they were lengthened through an arc of motion at 90 deg/s. The majority of torque produced by the dorsiflexors is from the TA,¹⁵ and we have shown previously that this model results in injury to this muscle.^{11,23-25} The TA remained stimulated throughout lengthening and was injured using 1 of 2 protocols: a single lengthening contraction through a 90° arc or 45 lengthening contractions through a 60° arc. For multiple repetitions, the lengthening contractions were spaced 2 minutes apart.

Outcome Measures

For both protocols, a maximal isometric contraction (200-millisecond duration) of the dorsiflexors was used to measure maximal torque before injury. For each animal, maximal isometric torque was also measured 4 minutes after injury (to measure force lost because of injury). Animals were returned to their cages after recovery from anesthesia, and maximal isometric torque was retested under anesthesia at selected time points (3, 5, 7, 14, and 21 days after injury, see Table 1). All isometric contractions were performed at the same point in the range of motion (with the foot orthogonal to the tibia, considered 0°).

After functional data were collected, tibialis anterior muscles were harvested from the anesthetized rat, snap frozen in liquid nitrogen, and stored at -80°C . The animal was then sacrificed by carbon dioxide inhalation or with pentobarbital sodium (200 mg/kg) administered intraperitoneally.

TABLE 1
Timeline for an Individual Animal^a

Day	
0	Measure maximal isometric torque Induce injury Wait 5 minutes and retest torque Inject PRP or PPP
3	Measure maximal isometric torque Inject PRP or PPP
5	Measure maximal isometric torque Inject PRP or PPP
7	Measure maximal isometric torque Inject PRP or PPP
14	Measure maximal isometric torque
21	Measure maximal isometric torque

^aPRP, platelet-rich plasma; PPP, platelet-poor plasma. Each mouse was anesthetized, and maximal isometric torque was measured *in vivo*. An injury was induced, and the loss of force was determined by another measure of maximal isometric torque. Subsequent measures of torque and injections of PRP/PPP were as shown.

Platelet-rich Plasma

Twenty milliliters of whole blood was collected from 5 adult male Sprague-Dawley rats (withdrawn from the femoral vein, renal vein, or cardiac puncture). The syngeneic nature of the Sprague-Dawley inbred rat allowed us to consider the blood of one animal autologous to blood from another animal.³⁶ Autologous PRP was then separated from the blood using the Symphony II Platelet Concentration System (Depuy, a Johnson & Johnson Company, Warsaw, Indiana). The centrifugation results in the formation of 2 layers within the plasma: a platelet-poor plasma (PPP) component and a PRP component. The PPP supernatant was carefully removed and used as a control vehicle. The remaining PRP was conditioned using 10 seconds of high-frequency ultrasound to lyse platelets and release growth factors, thereby enriching the PRP before injection.³⁶ One hundred microliters of PRP was injected into the TA of the injured hindlimb in each rat of the treated group at days 0 (day of injury), 3, 5, and 7 (Table 1). Both the conditioned PRP and the PPP were refrigerated and used within a few days.

Enzyme-linked immunosorbent assays (ELISAs) (R&D Systems, Minneapolis, Minnesota) were performed following the manufacturer's instructions, to determine whether PRP is enriched in myogenic growth factors. Both PRP and PPP were separated and subjected to ELISAs to detect and quantify the presence of platelet-derived growth factor (PDGF) and insulin growth factor-1 (IGF-1). We also assayed conditioned plasma, as described previously.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Two micrograms of total RNA was isolated from frozen rat tibialis anterior muscle with TRIzol (Invitrogen, Carlsbad, California) and was reversed-transcribed with Superscript II

First strand Synthesis System (Invitrogen), per the manufacturer's instructions. The resulting complementary DNA was used as a template for PCR amplification. The primers for rat MyoD were (forward) 5'-CACTCCTCCAATTGTCC-3' and (reverse) 5'-CTTATTTCCAACACCTGAGC-3'. The primers for rat myogenin were 5'-CACCTTCCCAGATGAAACC-3' and 5'-AAGAAGTCACCCCAAGAGC-3'. The primers for rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were 5'-ACGACCCCTTCATTGACC-3' and 5'-ATCACGCCACAGCTTTCCC-3'. The NCBI reference numbers are M24393, M017008, and M84176 for MyoD, myogenin, and GAPDH, respectively. The PCR products were run on 1% agarose gels, stained with ethidium bromide, and scanned. The intensity of bands was quantified using NIH Image J software, and relative expression was quantified based on the total GAPDH expressed in a particular muscle sample.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Immunoblotting

Western blot analysis was used to assess semiquantitative changes in the levels of MyoD and myogenin proteins. Extracts of unfixed TA muscles were snap frozen in liquid nitrogen, pulverized, and homogenized with a PowerGen 125 homogenizer (Fisher Scientific, Waltham, Massachusetts) at a wt/vol ratio of 0.05 in homogenate buffer (10 mmol/L NaPO₄, 2 mmol/L EDTA, 140 mmol/L NaCl, 1% NP40, pH 7.4) with protease inhibitors (Complete Protease Inhibitor Tablets, Roche Diagnostics, Indianapolis, Indiana). Samples were boiled and centrifuged, and the protein concentration of the supernatant was determined using a Bradford assay. Samples were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 4% to 12% gradient gel and transferred onto nitrocellulose electrophoretically. The nitrocellulose was blocked in 3% milk-PTA, washed, and then incubated with anti-myoD (sc 760) or anti-myogenin (sc 576) polyclonal (rabbit) antibodies (Santa Cruz Biotechnology, Santa Cruz, California) for 6 hours. Excess antibodies were washed off and the nitrocellulose was incubated with donkey anti-rabbit secondary antibodies conjugated to alkaline phosphatase (Jackson Laboratories, Bar Harbor, Maine). The excess secondary antibodies were then washed off and the bands were visualized by a chemiluminescent assay method (Tropix, Bedford, Massachusetts).

Hematoxylin and Eosin Staining

Tissue was frozen in isopentane-cooled liquid nitrogen, and transverse sections were cut on a cryostat (10- μ m thickness). Sections were collected onto glass slides (Superfrost Plus; VWR, West Chester, Pennsylvania) and stained with hematoxylin and eosin for counting centrally nucleated fibers. Sections were randomized and viewed at $\times 100$ magnification in a Zeiss Axioskop light microscope, and pictures were taken with a digital camera (AxioCam HR using AxioVision 3.0 AXIOVISION 3.0, Carl Zeiss Inc, Germany).

Each optical field contained an average of 38 ± 7 fibers, and more than 45 fields were counted per muscle.

Statistical Analysis

Contractile data from each experiment were analyzed using a single-factor analysis of variance (ANOVA, Sigma-Stat, San Rafael, California). When a significant ratio was found, a Tukey post hoc analysis was performed to determine where significant differences had occurred ($P < .05$). Each ELISA contained 3 replicates, and the results were analyzed with a 1-way ANOVA. For statistical analysis of RT-PCR, densitometry was performed for messenger RNA (mRNA) of myoD or myogenin and GAPDH for each blot. The mRNA/GAPDH ratios were calculated and analyzed using the Holm-Sidak pairwise multiple comparison test.

RESULTS

Assessment of Growth Factors

We quantified growth factors such as PDGF and IGF-1, both known to stimulate myogenesis, in the PRP and PPP groups. We examined conditioned (sonicated) and unconditioned PRP and PPP using ELISA kits. Figure 2 shows that the concentrations of PDGF and IGF-1 ($20\,745 \pm 520$ pg/mL and $65\,550 \pm 500$ pg/mL, respectively) in PRP were significantly higher than in PPP ($P < .001$), and concentrations were further increased (a 5-fold increase in PDGF and a 27% increase in IGF-1) by mechanical perturbation of the platelets (see Methods). Because of the significant increase of growth factors with conditioning, we tested the effects of conditioned PRP on muscle injury, as stated in the methods.

Functional Recovery

We induced injury and studied recovery of function in the whole-ankle dorsiflexor group. We harvested and then examined TA muscles, which account for most of the torque generated by this muscle group.¹⁵ The peak isometric torque generated by each animal was measured before injury and was considered 100% for that animal (the mean \pm SD torque for all animals was 44 ± 5 N·m). For each animal, peak isometric torque was also measured after injury. The torque measurements immediately after injury and during recovery were expressed as the percentage of maximal isometric torque (out of 100%) for a given animal. The mean percentage of recovery at each time point is presented in Figure 3. Both injury protocols resulted in a significant loss of muscle function ($P < .001$) followed by gradual recovery. The multiple-repetition protocol results in a larger force deficit and takes longer to recover.²⁵ The PRP treatment had little effect on the single-repetition protocol but did significantly ameliorate the force loss ($P = .003$) at 1 time point (day 3, Figure 3A), even though the animals had received only 1 injection by this time point (injections were immediately after injury and on days 3, 5, and 7 after injury). In the multiple-repetition protocol, PRP treatment significantly improved contractile function at 2 time points (days 7 and 14, $P < .001$),

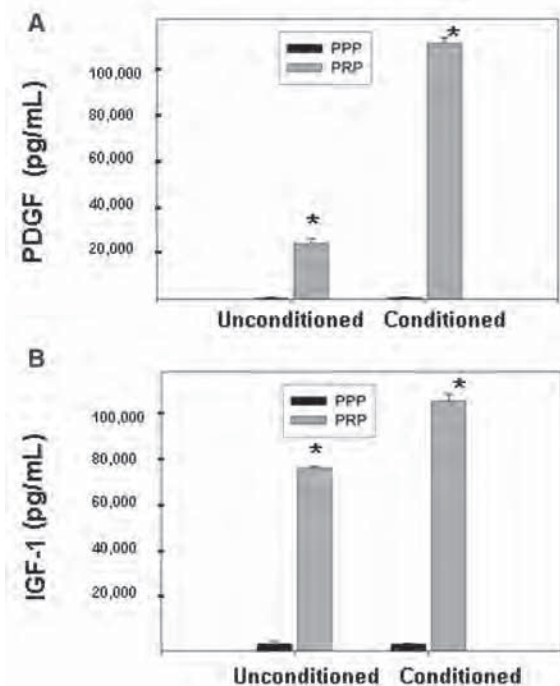


Figure 2. Results of enzyme-linked immunosorbent assays (ELISAs) to confirm that platelet-rich plasma (PRP) is enriched in myogenic growth factors platelet-derived growth factor (PDGF) and insulin growth factor-1 (IGF-1). Both PRP and platelet-poor plasma (PPP) were separated and subjected to ELISAs to detect and to quantify the presence of growth factors, such as PDGF (gray bar to left in A) and IGF-1 (gray bar to left in B), both known to stimulate myogenesis. We also assayed conditioned plasma (gray bars to right), as described in the methods. The PRP was clearly rich in the 2 tested growth factors and was further enriched by conditioning. Results are shown compared with ELISAs of PPP. * $P < .05$.

effectively shortening the time to full recovery from 21 days to 14 days (Figure 3B).

Muscle Regeneration

We harvested TA muscles from at least 2 animals at each time point for each protocol. We used 2 different markers to assay muscle regeneration. The first was to assay levels of myoD and myogenin. These are major muscle regulatory factors (MRFs) and are only expressed when satellite cells are activated to proliferate.⁶ The GAPDH was used as an internal control. The RT-PCR data (Figure 4A) show that mRNA transcripts for both of these muscle-specific transcription factors were present 7 days after injury but clearly elevated in the muscles treated with PRP compared with sham-treated muscles (PPP) or controls (not shown). Muscle tissue from other time points was also analyzed (data not shown), but the most obvious differences in mRNA levels occurred on day 7 ($P < .001$). The GAPDH transcript was tested and did not show altered transcription after muscle injury. Densitometry was performed and

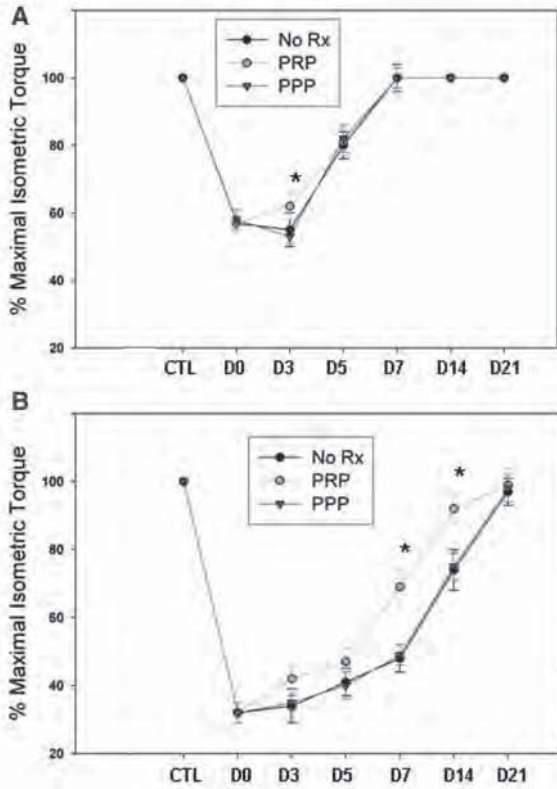


Figure 3. Maximal torque was measured in each animal before injury (CTL) and immediately after injury (D0), as well as at selected time points after injury (days 3, 5, 7, 14, and 21). One hundred percent represents peak torque before injury. The percentage of recovery for each animal was calculated and the mean is expressed as the percentage of maximal isometric torque (out of 100%) at each time point. A, after a single repetition through a 90° arc of motion, there is a significant decrease in torque and gradual recovery to full contractile function by day 7. The PRP had a significant effect only on day 3 ($P = .003$; $n = 8$ animals each group). B, after multiple repetitions through a 60° arc of motion, there is a significant loss of torque followed by a gradual recovery by day 21. The PRP had a significant effect on days 7 and 14 ($P < .001$), by which time the injured muscle had returned to the pre-injury level of strength ($n = 8$ animals each group). No Rx, injury only (no injections). * $P < .05$.

normalized to GAPDH to compare relative expression of myoD and myogenin (Figure 4B). To confirm that changes were not just in the expression of mRNA, we performed Western blotting and probed for myoD and myogenin. The results confirm that these 2 markers of muscle regeneration were increased in muscle samples injected with PRP (Figure 4C).

The second assay we used to detect myogenesis was quantification of centrally nucleated fibers (CNFs). Muscle fibers are multinucleated with the nuclei located at the periphery of the fibers, but within weeks after injury, nuclei are observed in the cytoplasm (Figure 5A) and these CNFs are widely accepted as a marker of muscle regeneration.^{6,12,16}

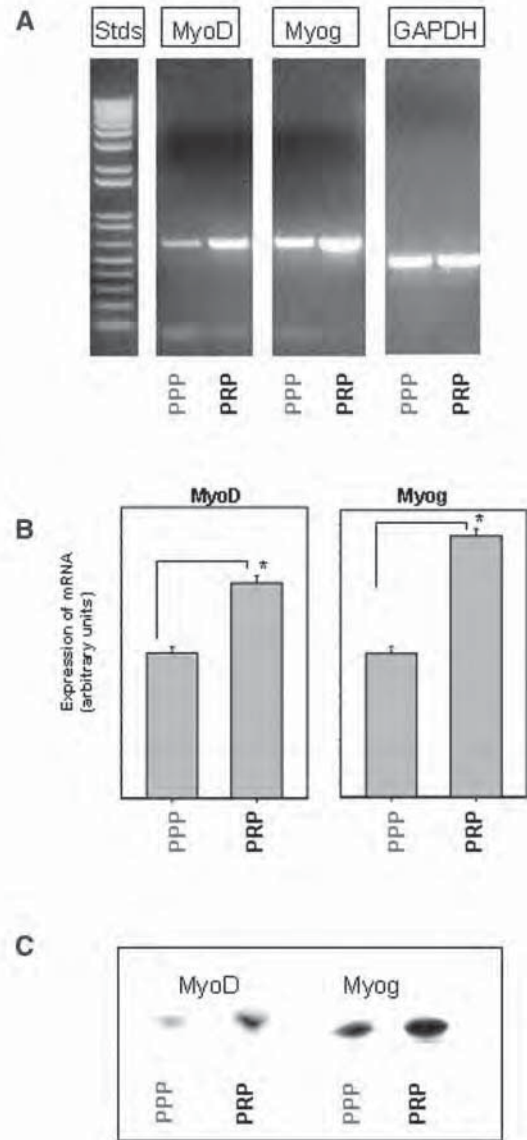


Figure 4. Myogenesis after the multiple-repetition injury. A, 2 μ g of total RNA was isolated from frozen rat tibialis anterior muscle, and reverse transcriptase polymerase chain reaction (RT-PCR) was performed at various time points after injury using primers for 2 different genes involved in muscle regeneration (myoD and myogenin) as well as a gene used as an internal control (glyceraldehyde 3-phosphate dehydrogenase, or GAPDH). The gel shows representative PCR products from muscles injected with PPP or PRP 7 days after injury. B, densitometry of the bands was performed and the results quantified relative to expression of the total GAPDH expressed in a particular muscle sample, as described in the methods. Thus, the histogram shows the mRNA transcript levels of myoD and myogenin from muscles injected with PPP or PRP ($n = 3$). C, muscle samples from the same time point (day 3) were homogenized and proteins separated by electrophoresis. Immunoblots confirmed the increase protein expression of myoD (38 kD) and myogenin (36 kD). * $P < .05$.

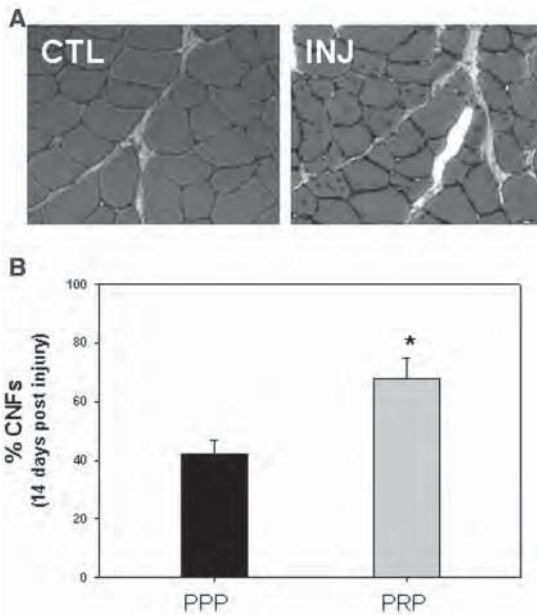


Figure 5. Tibialis anterior muscles were harvested at various time points after injury. The micrograph (A) shows representative cross-sections of noninjured control muscle (CTL) and of injured muscle (INJ) 14 days after the multiple-repetition injury, when some of the normally peripherally located nuclei (CTL) are seen in the middle of the fiber (INJ). These centrally nucleated fibers (CNFs) are a marker of muscle regeneration and the peak in CNFs occurred on day 14. The histogram (B) shows the percentage of fibers that had CNFs at this time point in the injured TA muscles injected with platelet-poor plasma (black bar) and platelet-rich plasma (gray bar). * $P < .05$

As shown previously,²⁵ the number of fibers with CNFs was significantly higher ($P < .001$) in the multiple-repetition protocol, peaking at about 2 weeks after injury (the single-repetition protocol does not result in a significant increase in the number of CNFs²⁵—data not shown). The PRP-treated TAs had an even higher number of CNFs than injured TAs with a sham treatment (PPP) or no treatment (not shown), indicating that the PRP was effective in stimulating further myogenesis (Figure 5B).

DISCUSSION

Muscle strains are among the most common complaints treated by physicians^{10,17} and account for the majority of all sport-related injuries.^{5,37} Except for complete ruptures of muscles, displaced avulsions, and recalcitrant symptoms from myositis ossificans, almost all muscle injuries are uniformly treated with nonoperative therapy. Standard nonoperative therapy for acute muscle injuries usually involves rest, ice, compression, and elevation (RICE). Beyond the principle of short-term rest and ice, there is no clear consensus on treatment of muscle injuries.⁵

In the laboratory setting, investigators have used toxins, lacerations, freeze damage, and contusions to study muscle damage, but by far the majority of muscle injuries during sports are attributable to excessive strain of an activated muscle (ie, forceful lengthening, or eccentric, contractions).^{5,10,17} Because muscle strains are so common, we used an animal model of contraction-induced injury. Some models that remove part or all of the muscle to perform load to failure or other such tests have certainly yielded useful information, but this is less representative of normal physiology. We used an in vivo model where the neurovascular supply and anatomical attachments are undisturbed, inducing a muscle strain injury under conditions that are as similar to the clinical scenario as possible, while preserving control over the biomechanical parameters to yield a consistent injury. One of the reasons muscle injury is difficult to study is that there are so many models of injury. Even with the in vivo model, the response to injury can vary widely based on the timing of activation, the amount of strain, the number of repetitions, and level of activation.^{13,20,24,25}

We compared the effects of conditioned PRP on 2 in vivo protocols of contraction-induced injury. The 90° arc of plantar flexion uses a large part of the available range of motion, but this magnitude of stretch is required to induce a detectable and reliable injury after just a single contraction, which we used to mimic an acute strain.²⁴ The 60° arc of plantar flexion yields a significant and reliable injury, but only with multiple repetitions. Despite the different protocols, they yield a similar force loss,²⁵ presumably because the amount of active strain (arc of motion in this study) is a key determinant of muscle injury.²⁰

Previously, we found that recovery of muscle contractile function after injury by a single, large-strain lengthening contraction involves sarcolemmal repair, whereas recovery from multiple, small-strain lengthening contractions requires muscle regeneration, with minimal sarcolemmal repair.²⁵ Here we used both protocols to test the effects of autologous PRP on recovery. In the single-repetition protocol, use of PRP did somewhat improve the ability of the muscle to generate force, but only at the day 3 time point. Otherwise, the overall recovery—and time to full return of function—was not altered. Alternatively, in the multiple-repetition protocol, use of PRP resulted in significant improvement at several time points as well as a quicker return to full function. This is most likely attributable to the enhancement of myogenesis, a process required to recover from this protocol.

Myogenesis is not restricted to prenatal development but also occurs in regenerating muscle after some injuries. A large body of evidence suggests that individual growth factors play a role during muscle regeneration/myogenesis. Insulin growth factor-1, fibroblast growth factor-2 (FGF-2), hepatocyte growth factor (HGF), and transforming growth factor- β 1 (TGF- β 1) are thought to be key regulators for myogenesis. For example, IGF-1 is able to stimulate the proliferation and differentiation of myoblasts (precursors of muscle cells) and improves muscle regeneration in mouse skeletal muscles.²⁷ In vivo, FGF-2 enhances the

diameter and number of regenerating fibers.¹⁹ In vitro, HGF is able to activate quiescent satellite cells,¹ the stem cells of skeletal muscle committed to a myogenic lineage. Transforming growth factor- β 1 supports other growth factors, specifically PDGF, which stimulates satellite cell activation.^{14,28,31} Satellite cells are dormant in healthy skeletal muscle but can be stimulated by injury to proliferate or differentiate into mononucleated myoblasts, which then fuse to form multinucleated myotubes, which then fuse to form new skeletal muscle, replacing damaged or lost tissue.^{4,16,34}

Platelet-rich plasma contains up to 8 times the concentration of platelets found in whole blood,⁷ and these platelets contain α -granules, which can release a multitude of growth factors, such as PDGF, IGF-1, TGF- β , FGF, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and HGF.^{7,8,32} The fact that PRP contains several different growth factors, present in physiological proportions, is an appealing benefit compared with using isolated growth factors. Other advantages are that it is relatively simple and easy to obtain PRP from a human blood sample, and there is little risk of developing an immune response from autologous PRP.

Given the common nature of muscle strain injuries, a treatment that can improve recovery time could have a tremendous effect in athletics. Platelet-rich plasma can be isolated from a centrifuge about the size of a small microwave oven; this can increase the convenience and feasibility of using this treatment method in an athletic training room. After an injury, whole blood could be drawn from an athlete and centrifuged, and autologous PRP could be retrieved and injected at the site of injury. Conditioning of the PRP could be performed via sonication but necessarily under sterile conditions to avoid infection. This would be a convenient and cost-effective way to administer concentrated growth factors locally. Injection of isolated growth factors, even if effective, could be prohibitively expensive. A potential limitation to the use of PRP in the clinical setting, at least with elite athletes, is the concern expressed by the World Anti-Doping Association (WADA) regarding the use of growth factors in sports.⁷ One possible solution for professional athletes is to obtain an exemption from a WADA-approved anti-doping organization for therapeutic use.

The mechanisms for the improved muscle recovery resulting from the use of PRP after injury need to be elucidated. We have shown via RT-PCR analysis and counts of CNFs that myogenesis is enhanced with the use of PRP; however, this does not preclude other possibilities. Platelet-rich plasma may alter cytokine release, limit inflammation, or have other effects not yet examined. Although we did not attempt to quantify inflammation, the inflammatory process is likely altered in the presence of PRP. This may explain the improvement seen only at day 3 of recovery after the single-repetition protocol, as this is when inflammation peaks after a muscle strain.²⁹ Another possibility is that muscle fiber membrane damage or repair is altered or that damage or repair of contractile and/or structural proteins is altered by the use of PRP. These and other hypotheses have not been rigorously tested.

We operationally defined muscle injury as a loss in the ability of the muscle to produce force. Torque of a muscle is represented by the equation $T = F * d$, where T is torque, F is muscle force, and d is the moment arm of the muscle. Because we use a maximal tetanic contraction and we measured torque at a fixed ankle position, our measure of torque ultimately reflects muscle force.^{2,21,22,35} After the initial injury, there is sometimes another decrease in force, as seen after our single-repetition protocol (Figure 3A). This secondary injury is thought to be attributable to the increase in inflammation that occurs several days after the muscle strain and is also the presumed cause of delayed-onset muscle soreness.⁹ We did not see a secondary injury with the multiple-repetition protocol. Although it is not clear why, it could be that we missed a further decline in force production because of our selected time points. For example, force in the multiple-repetition protocol may have decreased even further than the initial injury, but within 24 or even 48 hours, well before our day 3 measurement.

An animal model provides several obvious advantages, such as control over force of contraction, type of contraction, lengthening velocity, diet, activity level, and access to tissues for analysis. Animal models also provide control over many threats to internal validity of a study, such as history, selection bias, and maturation. Yet, this study has several limitations. The first and most obvious is that findings from animal studies are not always applicable to humans. Second, the high level of control over experimental parameters (timing of contraction, arc of motion, etc) is less representative of the wide array of injuries that occur in humans. For example, we use maximal stimulation to recruit all motor units within the muscle (to obtain a consistent injury), but this is not representative of the graded recruitment of motor units that occurs in humans. A third possible limitation is that we did not identify which components of PRP are responsible for the improvements we found. However, because PRP is easily obtainable through several commercial centrifuge devices and no negative side effects have been reported, it may not be necessary to isolate the specific growth factors within PRP that account for the enhanced recovery from injury.

Like any experiment, this work raises new questions, which we hope to address in future work. We did not examine the effects of conditioned PRP. The ELISAs indicate no discernible difference with conditioning (Figure 2), but the ELISA results are based on only 2 representative growth factors. It is possible that a small fraction of platelets persisted in the PRP, containing other growth factors that could affect recovery from injury. Because of the significant increase in growth factors with high-frequency ultrasound (conditioning), our PRP treatment consisted of conditioned PRP. This allowed us to minimize the number of animals needed for the study and improved our chances of finding an effect. It is likely that the platelets from the unconditioned PRP would rupture during injection and still release their contents, either immediately or soon after injection, but we did not test this.

Another question is what type of strain injury PRP is appropriate for. Our results do not determine whether PRP

would be beneficial for an acute strain versus an overuse injury in humans. It is clear that injuries that require myogenesis are better candidates for PRP therapy, but muscle injury is highly variable between subjects. Finally, we do not know what dose and delivery methods are optimal. We did not test various permutations of frequency, amount, and duration of injections, and it is likely that other delivery methods may work as well as or better than repeated injections.

To our knowledge, this is the first study to use PRP in a model of muscle strain injury. We demonstrated that PRP extract can hasten recovery from a muscle strain injury and that enhanced myogenesis is the probable mechanism underlying this effect. Delivery of growth factors at the site of injury is a potential therapy to treat muscle injuries, and because autologous blood products are safe, PRP may be a useful product in treatment of muscle injuries.

ACKNOWLEDGMENT

Funding was provided by the National Institutes of Health (K01AR053235 to RML) and the Muscular Dystrophy Association (MDA 4278 to RML).

REFERENCES

- Allen RE, Sheehan SM, Taylor RG, Kendall TL, Rice GM. Hepatocyte growth factor activates quiescent skeletal muscle satellite cells in vitro. *J Cell Physiol*. 1995;165:307-312.
- Ashton-Miller JA, He Y, Kadhiresan VA, McCubbrey DA, Faulkner JA. An apparatus to measure in vivo biomechanical behavior of dorsi- and plantarflexors of mouse ankle. *J Appl Physiol*. 1992;72:1205-1211.
- Barash IA, Mathew L, Ryan AF, Chen J, Lieber RL. Rapid muscle-specific gene expression changes after a single bout of eccentric contractions in the mouse. *Am J Physiol Cell Physiol*. 2004;286:C355-C364.
- Best TM, Hunter KD. Muscle injury and repair. *Phys Med Rehabil Clin N Am*. 2000;11:251-266.
- Chan YS, Li Y, Foster W, Huard J. The use of suramin, an antifibrotic agent, to improve muscle recovery after strain injury. *Am J Sports Med*. 2005;33:43-51.
- Charge SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. *Physiol Rev*. 2004;84:209-238.
- Creaney L, Hamilton B. Growth factor delivery methods in the management of sports injuries: the state of play. *Br J Sports Med*. 2008;42:314-320.
- El-Sharkawy H, Kantarci A, Deady J, et al. Platelet-rich plasma: growth factors and pro- and anti-inflammatory properties. *J Periodontol*. 2007;78:661-669.
- Faulkner JA, Brooks SV, Opitck JA. Injury to skeletal muscle fibers during contractions: conditions of occurrence and prevention. *Phys Ther*. 1993;73:911-921.
- Garrett WE Jr. Muscle strain injuries. *Am J Sports Med*. 1996;24:S2-S8.
- Hakim M, Hage W, Lovering RM, Moorman CT III, Curl LA, De Deyne PF. Dexamethasone and recovery of contractile tension after a muscle injury. *Clin Orthop Relat Res*. 2005;439:235-242.
- Huard J, Li Y, Fu FH. Muscle injuries and repair: current trends in research. *J Bone Joint Surg Am*. 2002;84:822-832.
- Hunter KD, Faulkner JA. Pliometric contraction-induced injury of mouse skeletal muscle: effect of initial length. *J Appl Physiol*. 1997;82:278-283.
- Husmann I, Soulet L, Gautron J, Martelly I, Barritault D. Growth factors in skeletal muscle regeneration. *Cytokine Growth Factor Rev*. 1996;7:249-258.
- Ingalls CP, Warren GL, Zhang JZ, Hamilton SL, Armstrong RB. Dihydropyridine and ryanodine receptor binding after eccentric contractions in mouse skeletal muscle. *J Appl Physiol*. 2004;96:1619-1625.
- Jarvinen TA, Jarvinen TL, Kaariainen M, Kalimo H, Jarvinen M. Muscle injuries: biology and treatment. *Am J Sports Med*. 2005;33:745-764.
- Kirkendall DT, Garrett WE Jr. Clinical perspectives regarding eccentric muscle injury. *Clin Orthop Relat Res*. 2002;403:S81-S89.
- LaStayo PC, Woolf JM, Lewek MD, Snyder-Mackler L, Reich T, Lindstedt SL. Eccentric muscle contractions: their contribution to injury, prevention, rehabilitation, and sport. *J Orthop Sports Phys Ther*. 2003;33:557-571.
- Lefaucheur JP, Sebillé A. Muscle regeneration following injury can be modified in vivo by immune neutralization of basic fibroblast growth factor, transforming growth factor beta 1 or insulin-like growth factor I. *J Neuroimmunol*. 1995;57:85-91.
- Lieber RL, Friden J. Muscle damage is not a function of muscle force but active muscle strain. *J Appl Physiol*. 1993;74:520-526.
- Lieber RL, Schmitz MC, Mishra DK, Friden J. Contractile and cellular remodeling in rabbit skeletal muscle after cyclic eccentric contractions. *J Appl Physiol*. 1994;77:1926-1934.
- Lieber RL, Shah S, Friden J. Cytoskeletal disruption after eccentric contraction-induced muscle injury. *Clin Orthop*. 2002;403:S90-S99.
- Lovering RM, De Deyne PG. Contractile function, sarcolemma integrity, and the loss of dystrophin after skeletal muscle eccentric contraction-induced injury. *Am J Physiol Cell Physiol*. 2004;286:C230-C238.
- Lovering RM, Hakim M, Moorman CT III, De Deyne PG. The contribution of contractile pre-activation to loss of function after a single lengthening contraction. *J Biomech*. 2005;38:1501-1507.
- Lovering RM, Roche JA, Bloch RJ, De Deyne PG. Recovery of function in skeletal muscle following 2 different contraction-induced injuries. *Arch Phys Med Rehabil*. 2007;88:617-625.
- Meijer H, Reinecke J, Becker C, Tholen G, Wehling P. The production of anti-inflammatory cytokines in whole blood by physico-chemical induction. *Inflamm Res*. 2003;52:404-407.
- Menetrey J, Kasemkijwattana C, Day CS, et al. Growth factors improve muscle healing in vivo. *J Bone Joint Surg Br*. 2000;82:131-137.
- Nilsen-Hamilton M. Transforming growth factor-beta and its actions on cellular growth and differentiation. *Curr Top Dev Biol*. 1990;24:95-136.
- Pizza FX, Peterson JM, Baas JH, Koh TJ. Neutrophils contribute to muscle injury and impair its resolution after lengthening contractions in mice. *J Physiol*. 2005;562:899-913.
- Proske U, Allen TJ. Damage to skeletal muscle from eccentric exercise. *Exerc Sport Sci Rev*. 2005;33:98-104.
- Ross R, Raines EW, Bowen-Pope DF. The biology of platelet-derived growth factor. *Cell*. 1986;46:155-169.
- Schnabel LV, Mohammed HO, Miller BJ, et al. Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. *J Orthop Res*. 2007;25:230-240.
- Stone MR, O'Neill A, Lovering RM, et al. Absence of keratin 19 in mice causes skeletal myopathy with mitochondrial and sarcolemmal reorganization. *J Cell Sci*. 2007;120:3999-4008.
- Wagers AJ, Conboy IM. Cellular and molecular signatures of muscle regeneration: current concepts and controversies in adult myogenesis. *Cell*. 2005;122:659-667.
- Willems ME, Stauber WT. Force deficits after repeated stretches of activated skeletal muscles in female and male rats. *Acta Physiol Scand*. 2001;172:63-67.
- Wright-Carpenter T, Opolon P, Appell HJ, Meijer H, Wehling P, Mir LM. Treatment of muscle injuries by local administration of autologous conditioned serum: animal experiments using a muscle contusion model. *Int J Sports Med*. 2004;25:582-587.
- Zarins B, Ciullo JV. Acute muscle and tendon injuries in athletes. *Clin Sports Med*. 1983;2:167-182.