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A Muscle Contusion Injury Model

Biomechanics, Physiology, and Histology*

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ABSTRACT

We developed a reproducible muscle contusion injury and studied its effect on contractile function, histology, and passive failure. An instrumented drop-mass technique (mass, 171 g; height, 102 cm; spherical radius, 6.4 mm) delivered a single impact to the posterior surface of the gastrocnemius muscle in one limb of 40 male Wistar rats. On Day 0, the impact significantly ($N = 12$, $P < 0.01$) decreased maximum tetanic tension to 63% of the contralateral control value. Histologic examination demonstrated extravasation of erythrocytes, edema, myofiber disruption, and vacuolation of myofibers. Passive failure initiated at the site of injury. At 2 days, tetanic tension was 75% of controls ($N = 11$, $P < 0.01$). Histologically, acute inflammation and phagocytosis were noted. Tetanic tension at 7 days was 81% of controls ($N = 8$, $P < 0.01$). Vimentin staining indicated a dramatic increase in myoblast activity. Contractile strength was near normal at 24 days. Histologic examination showed complete regeneration of normal striated muscle fibers. No vimentin activity was found. No passive failures initiated at the injury site. Contusion injury produced a significant deficit in contractile function that continually diminished with gross histologic evidence of degeneration, regeneration, and normalization at the injured muscle fibers.

Approximately 90% of all sports-related injuries are muscle contusions and strains.[6,8,10,14] The most frequent traumatic muscle injury is a contusion injury produced by the impact of a blunt nonpenetrating object.[16] The cost of contusion injuries is difficult to assess since hospitalization is typically not required. However, reinjury, muscle atrophy, and contracture can occur, with significant morbidity regarding time lost to training and competition.[3,16,20,29] Although the medical community has made efforts to decrease such morbidity, the best treatment of a muscle contusion injury has yet to be defined.[37] Recommended treatment regimens for quadriceps contusions have varied widely. For example, immobilization in full extension,[20] aggressive full range of motion through passive motion machines and hospitalization,[29] and immediate 24 hours of immobilization at 120° of flexion[3,4] have all been suggested. Other common treatment modalities, for which we have only empirical evidence, include ice, heat, waterpool, and steroids.

An inherent limitation of clinical studies to evaluate treatment of muscle contusions is the variability of the injuries that occur.[5,20] In contrast, basic science has the ability to develop a well-defined and reproducible contusion injury model. Early animal studies yielded important information on cellular responses due to injury and during regeneration.[1,2,15,27,30] Because these injury models were invasive (typically a crush injury with forceps inserted through a skin incision), their ability to model the contusion injuries seen clinically was limited. Noninvasive and reproducible muscle contusion models have used a spring-loaded hammer[18,24,25] and a drop-mass technique.[13,32,35] These studies evaluated the effects of immobilization, mobilization,[19,21–24,26] ultrasound,[32] and nonsteroidal anti-inflammatory drugs[12] by documenting the ultrastructural, biochemical, and vascular responses of regenerating muscle, in addition to its passive failure properties. Although previous studies have estimated the potential energy available for impact,[13,24,25,32,35] the mechanical events that occur during impact and result in injury have not been recorded. Such information is essential to understanding the mechanical failure properties of muscle and the threshold of injury; establishing design criterion for protective sports equipment is one application of such information. Furthermore, the effect of contusion injury and treatment on contractile function, which is perhaps the best indicator of performance, has not been studied.

The purpose of this work was to develop a well-defined and reproducible muscle contusion injury in the rat. Our specific aims were to record the mechanical events during

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impact, to study the effect of injury and healing on contractile properties and passive mechanical behavior, and to compare these findings with histologic observations.

MATERIALS AND METHODS

A total of 40 Wistar male rats (weight, 272.5 to 387.2 g; age, 10 to 12 weeks) (Charles River Company, Wilmington, MA) were studied. All experimental protocols were approved by the Yale Animal Care and Use Committee. Anesthetized animals received a single impact to the midbelly of the gastrocnemius muscle complex (gastrocnemius, soleus, and plantaris). After injury, the animals were allowed to move freely in cages and received laboratory chow and water ad libitum. At 0, 2, 7, and 24 days after injury, the effects of the injury and healing were studied by quantifying maximum tetanic tension and tetanic fatigue time (the time required for the maximum tetanic tension to reach 50% of its original value). After the animals were sacrificed, the gastrocnemius muscle complexes were harvested and subjected either to passive failure tests or histologic study. A schema of the protocol is presented in Figure 1.

Injury production by impact

Before injury, all animals were anesthetized with 0.30 ml ketamine (100 mg/ml) and 0.20 ml xylazine (20 mg/ml) intramuscularly. In the prone animal, the hind limb was positioned by extending the knee and dorsiflexing the foot to 90°. The limb was secured with elastic bands to a plastic support platform. Injury was produced by a mass falling through a clear plastic guide tube. The mass (171 g) was dropped from a height of 102 cm onto the top of an impactor. The impactor (30 g) was a small T-shaped cylinder fitted on the bottom with a spherical tip (radius, 6.4 mm) that directly contacted the skin. The design of the impactor apparatus allowed the impact site on the skin surface over the midportion of the gastrocnemius to be reproducibly defined. The support platform was fixed to a load cell (PCB Piezotronics, Depew, NY). The center of the impactor was a displacement transducer (Schaevitz, Pennsauken, NJ). During impact, the load transmitted through the limb and the displacement of the impactor were continuously recorded as a function of time by computer. The load-time and displacement-time data of each impact were graphed and analyzed by computer.

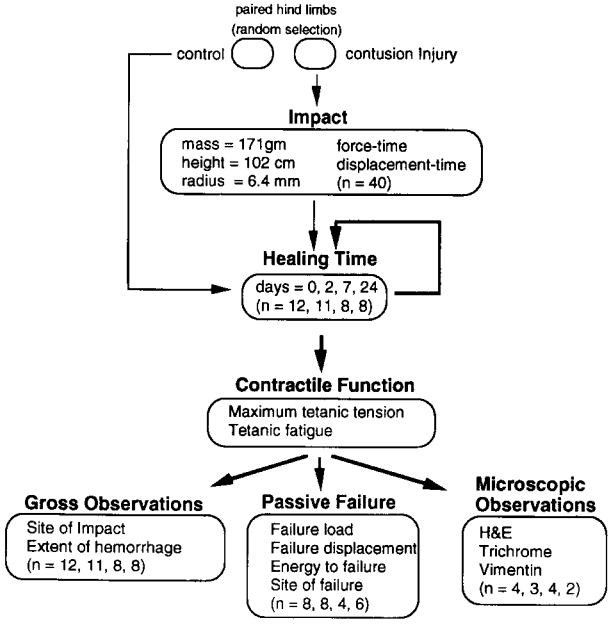

Figure 1. The experimental protocol. Details are provided in the text.

Peak force (in Newtons) was defined as the peak of the load-time curve recorded during impact. Peak displacement (in millimeters) was defined as the peak displacement of the displacement-time curve. Peak velocity (meters per second) was defined as the maximum derivative of the average displacement-time curve. The energy (in joules) absorbed during impact was the integral of the load-displacement curve. Impulse (Newtons times seconds) was the integral of the load-time curve.

Contractile function

After anesthetizing the animal, a Kirschner wire (0.45 mm) was placed transversely through the distal femur 0.5 cm above the knee joint and secured to the testing apparatus. The insertion of the Achilles tendon was exposed and the calcaneus disarticulated. Tourniquet use, excluding the gastrocnemius muscle complex, helped to minimize blood loss. Stainless steel suture wire (22 gauge) was passed through a calcaneal drill hole and attached to a load cell (Entran Devices, Fairfield, NJ). The gastrocnemius muscle belly remained covered by skin throughout the experiment; the exposed Achilles tendon was kept moist with normal saline and paraffin oil. Animal core temperature was maintained between 98°F and 100°F.

The branch of the sciatic nerve innervating the gastrocnemius complex was exposed and isolated through a 1.5-cm incision over the posterior aspect of the thigh. Peak twitch stimulation (40 V, 0.05 msec duration), using a Grass S44 stimulator (Grass Instruments, Quincy, MA) defined the optimal length for maximum tetanic tension measurement. Maximum tetanic tension was obtained with a stimulus (120 V, 0.05 msec, 70 Hz) for 1 second. Fatigue time measurement, defined as the time for a 50% reduction in maximum tetanic tension, followed after a 3-minute rest.

Gross observations

Six other hindlimbs were carefully disected at Day 0 (2 hours after injury); the gastrocnemius muscle complex was isolated and harvested. Specimens were fixed at optimal length by pinning the femur and calcaneus to a wooden block. These specimens were fixed in 10% buffered formalin. After 1 day of fixation, the following macroscopic measurements of the injury were made aided by the use of a magnifying glass and calipers with an estimated of accuracy of 0.1 mm: distance of the injury from the calcaneus, width (transverse) of the hematoma on the surface of the

gastrocnemius, and length (longitudinal) of the hematoma. Specimens were sectioned in the transverse plane with a surgical blade through the center of the injury site. Measurements in the transverse sections included the depth of hematoma and width of hematoma at the deep gastrocnemius surface. Nondestructive observations of the muscle surface were made on all specimens at the time of sacrifice (12 at Day 0, 11 at Day 2, 8 at Day 7, and 8 at Day 24) before preparation for microscopic observation or passive failure.

Microscopic observations

Selected harvested specimens were fixed at optimal length in 10% buffered formalin for 2 days, washed, dehydrated through graded ethanols, cleared in xylene, and embedded in paraffin. Sagittal sections were mounted on glass slides and stained with hematoxylin and eosin (H&E), Masson's trichrome, and immunohistochemical markers for vimentin. Vimentin is a single peptide associated with cells of mesenchymal origin. Each specimen was microscopically evaluated for the extent of hemorrhage, inflammation, tissue disruption and necrosis, collagen formation, myoblastic differentiation, cellular proliferation, and tissue organization.

Passive failure

The remaining harvested specimens were subjected to passive failure in tension within 2 hours after death. Specimens were mounted for testing by casting the distal femur and proximal tibia in a single plaster of Paris mold. Similarly, the calcaneus was cast in a second mold. One casting was mounted to a load cell and the other to the traveling crosshead of a standard material tester such that the longitudinal axis of the gastrocnemius muscle complex was parallel to the axis of the tester. Specimens were elongated at a rate of 10 mm/sec until failure while a computer recorded the load-displacement behavior. Muscle and tendon were wrapped in gauze and kept moist with normal saline throughout the experiment.

During testing, the specimen was carefully observed for the site of failure initiation. Failure load (in Newtons) was defined as the maximum load recorded. Failure displacement (in millimeters) was the displacement from an initial length (defined at 0.5 N) to the failure load. The energy (in joules) absorbed was the integral of the load-displacement curve to the failure point.

Statistical analysis

Statistical analysis was performed using Student's *t*-test and factorial analysis of variance (ANOVA) with Fisher LSD post hoc test (Statview SE, Abacus Concepts, Inc., Berkeley, CA). Statistical comparisons and significance are reported in the text.

RESULTS

Impact

The average (±) standard deviation of the force transmitted through the hindlimb and the displacement of the impactor as a function of time were determined for all 40 animals (Fig. 2). At a peak force of 246 ± 42 N, the displacement reached 9.0 ± 1.0 mm. The average maximum velocity was 3.8 m/sec. The energy absorbed during impact was 1.22 ± 0.23 J, and the impulse was calculated to be 0.80 ± 0.12 N·sec.

Before beginning animal experimentation, we recorded the mechanics of 10 impacts directly onto the support platform of the apparatus (insert, Fig. 2). The force reached an average peak value of 1471 ± 57 N, with a maximum

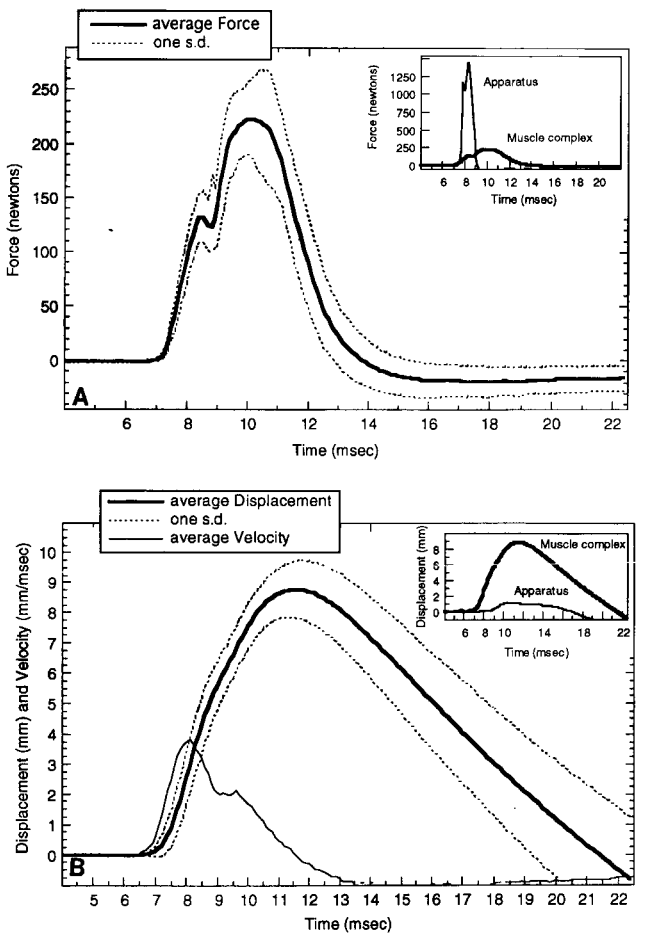

Figure 2. A, the average ($N = 40$) force-time of the impacts to the posterior surface of the gastrocnemius muscle complex. The impacts to the apparatus alone and the gastrocnemius muscle complex are plotted in the inset for comparison. B, the average ($N = 40$) displacement-time and velocity-time of the impacts to the posterior surface of the gastrocnemius muscle complex. The impacts to the apparatus alone and the gastrocnemius muscle complex are plotted in the inset for comparison.

displacement of 1 ±0.1 mm, and an impulse of 1.38 ± 0.04 N·sec. The energy absorbed by the apparatus was 0.41 ± 0.12 J. An estimate of the energy absorbed by the muscle tissue would then be the difference between the impact directly onto the support platform and the impact onto the muscle (approximately 0.8 J). The total energy available was 1.71 J (mass × gravity × drop height).

Contractile function

Contractile function was determined once in each of 39 rats; 1 animal died under anesthesia. On Day 0 (2 hours after impact), the acute effect of the injury was to reduce the maximum tetanic tension by an average of 37.6% ± 6.0% relative to the contralateral control side ($N = 12$; body weight, 317 ± 42 g). A significant deficit ($P < 0.01$, paired Student's t-test) was noted when comparing the values of the control limbs (28.4 ± 12.7 N) to the injured limbs (17.9 ± 8.7 N). On Day 2, maximum tetanic tension was 25.3% ± 12.9% less than the control limb ($N = 11$; body weight, 314 ± 32 g). This deficit was significant ($P < 0.01$) when values were compared (25.3 ± 14.0 N for the controls versus 19.3 ± 11.1 N for the injured). On Day 7, the deficit in maximum tetanic tension was 19.1% ± 14.6% ($N = 8$; body weight, 369 ± 30 g). The difference between control limbs (41.2 ± 10.6 N) and injured (32.8 ± 8.2 N) remained highly significant ($P < 0.01$). On Day 24, we recorded an average deficit in tetanic tension of 9.7% ± 14.1% ($N = 8$; body weight, 415 ± 58 g), but it was not significant when the controls (49.5 ± 3.2 N) were compared with the injured limbs (44.6 ± 7.5 N). The absolute increase in tension of both hindlimbs with time is attributed to the significant increase in the body weights of the animals with maturation.

The return of contractile function with the time of healing is illustrated in Figure 3. The greatest deficit in the maximum tetanic tension was recorded at Day 0, 2 hours after injury. This deficit was significantly larger than the deficit at any other time ($P < 0.05$, factorial ANOVA with Fisher LSD post hoc test). In comparing each of the other times, there was no significant difference between Day 2 and Day 7 or between Day 7 and Day 24. There was a significant difference between Day 2 and Day 24. The fatigue time on average had a response similar to healing, but there were no significant differences with time (Fig. 3).

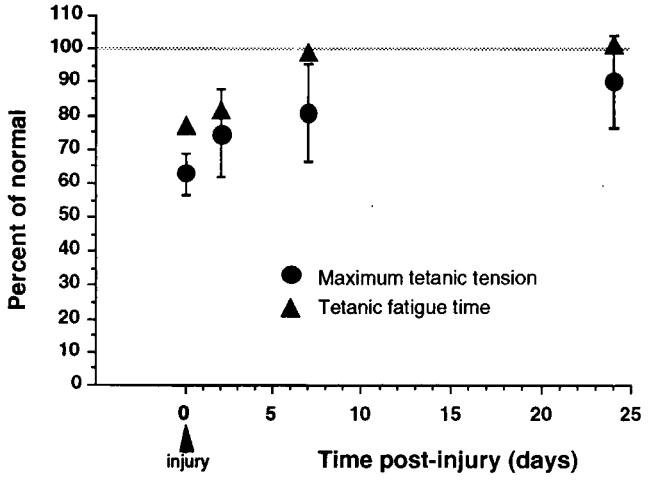

Figure 3. The average maximum tetanic tension, as a percentage of the normal contralateral control limb, significantly decreased with injury and then approached normal values at 24 days. The average tetanic fatigue time had a similar trend but with no significant differences.

Gross observations

After 1 day of formalin fixation, dissection of Day 0 specimens demonstrated a consistent injury above the muscle-tendon junction and below the separation of the two gastrocnemius heads. The mean distance of the center of the injury to the calcaneus was 20.1 ± 1.7 mm. The width of the hematoma on the surface of the gastrocnemius was 7.2 ± 0.8 mm. The length (longitudinal) of the hematoma on the surface of the gastrocnemius was 9.6 ± 2.6 mm. The depth of the hematoma visualized in transverse sections was 5.3 ± 0.5 mm. The width of the hematoma at the gastrocnemius-soleus interface was 5.5 ± 0.7 mm. On dissection, 27% of all impacted legs had a concurrent fibular fracture, apparently resulting from the impact.

Observation of the muscle surface within 2 hours after injury revealed muscle disruption at the center of the impact site surrounded by a large intramuscular-interstitial hematoma. A tissue defect was noted at the site of injury. No damage was noted at either the proximal or distal myotendinous junction or elsewhere on the muscle belly. Two days after injury the degree of muscle disruption was less obvious and the size of the hematoma had diminished to approximately one third to one half of the muscle's diameter. By Day 7 the hematoma had almost completely disappeared, and the muscle grossly appeared normal. At 24 days after injury, no difference from the control side was noted.

The weight of the gastrocnemius muscle complex increased acutely with injury by an average of 11%. This increase was statistically significant ($P < 0.01$, paired Student's t-test) when the control muscle complex (2.36 ± 0.2 g) was compared with the injured muscle complex (2.56 ± 0.6 g). By Day 2, the injured muscle was only 7% heavier, by Day 7 only 2% heavier, and by Day 24 no difference was noted.

Microscopic observations

Specimens from Days 0, 2, 7, and 24 were studied. Slides were examined grossly and microscopically at ×40, ×100, and ×200 power. At Day 0, marked edema and hematoma were observed in all specimens. The injury was localized near the surface of the gastrocnemius muscle, adjacent to the site of impact. From there, it extended in continuity deep into the gastrocnemius muscle complex. Intracellular vacuolation of intact myofibers, as well as gross disruption of myofibers, was noted at the site of injury. Trichrome staining demonstrated no difference in collagen content when compared with uninjured control specimens (Fig. 4).

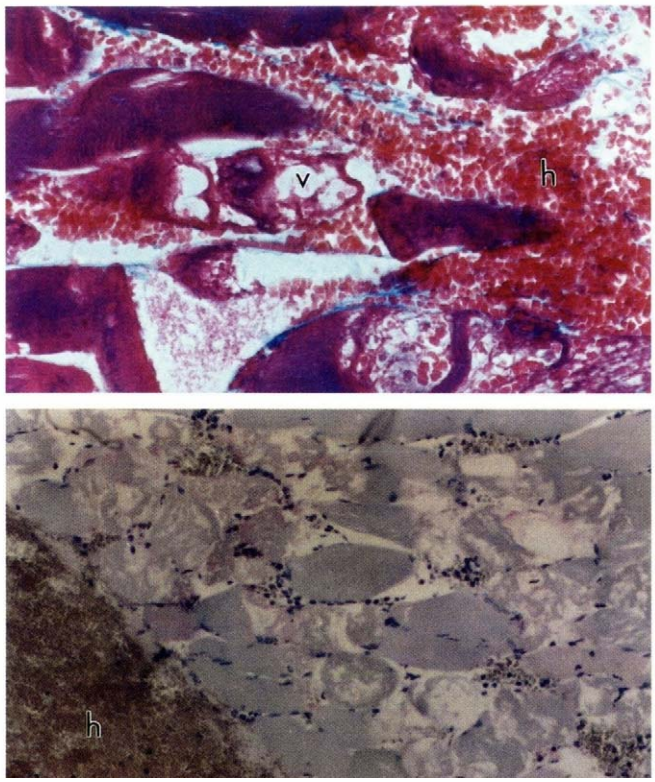

Figure 4. Histologic sections (×200) at Day 0 within 2 hours after injury. A, trichrome stain. Note marked hematoma (h). Gross disruption of muscle fibers and intracellular vacuolation (v) of myofibers can be seen. B, vimentin stain. No vimentin activity is noted. Hematoma is stained green-brown (h).

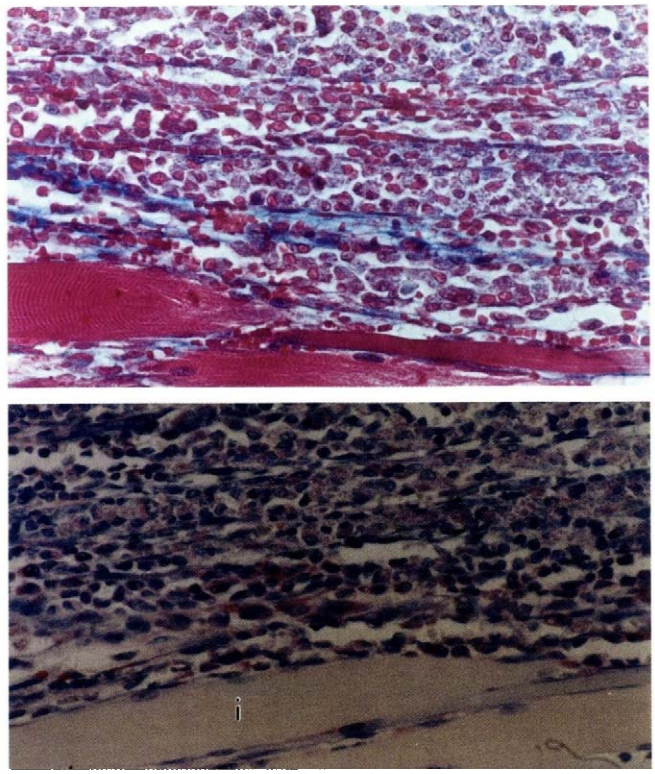

Figure 5. Histologic sections (×200) at Day 2 after injury. A, trichrome stain. Note intense inflammatory response with phagocytosis. Intact basement membranes are seen as thin lines stained blue. B, vimentin stain. Slight activity (red) is noted at the periphery of the injury adjacent to intact fibers (i).

There was no vimentin activity demonstrable in any Day 0 specimen. The rare presence of inflammatory cells in the interstitial spaces was noted.

Two days after injury, H&E staining revealed an intense inflammatory response. Macrophages, polymorphonucleocytes (PMN), and degenerating contractile proteins were evident. Phagocytosis of decomposing contractile elements was in progress. Hematoma persisted but was resolving. Basement membranes were frequently left intact and could easily be recognized in areas of severe injury and inflammation. Spindle-shaped cells, presumptively identified as fibroblasts, were present in modest numbers. Trichrome staining demonstrated the uptake of proteinaceous material within macrophages (Fig. 5). The presence of intact basement membranes was confirmed. Slight vimentin activity was noted at the periphery of the injury indicating the differentiation of myoblastic precursor cells from satellite stem cells.

On Day 7, H&E staining revealed an intense cellular proliferation of myoblasts and fibroblasts. Residual inflammatory elements were rare. No hematoma or edema was present. Extracellular ground substance consisting of proteinaceous and collagenous material was present. Trichrome staining differentiated collagen from protein and showed increased scarring in the superficial fascia of the gastrocnemius complex. Marked increase in vimentin activity was noted, most intensely at the periphery but also toward the center of the injury (Fig. 6).

By the 24th day, H&E, trichrome, and vimentin staining of injured specimens were indistinguishable from controls. No vimentin activity was observed. Superficial remnants of bands of scar tissue were noted in some specimens.

Site of passive failure

When the harvested gastrocnemius complexes were passively pulled to failure, all acute and 2-day injuries ($N = 8$) failed at the site of the injury in the midbelly of the gastrocnemius muscle. At 7 days, 2 of 4 specimens failed at the injury site, while the remaining two failed at the distal myotendinous junction. By Day 24, no failures initiated in the region of the injury ($N = 6$). In these specimens and in all controls, failure initiated at the proximal myotendinous junction, distal myotendinous junction, tendon-calcaneus insertion, and the distal femoral growth plate. The frequency of failures initiating at the injury site and at the general region of the myotendinous junction are plotted against the time of healing in Figure 7.

Passive failure properties

At Day 0, the average ($N = 4$) failure load of the injured complex (72.4 ± 8.6 N) was less than the controls (83.3 ±

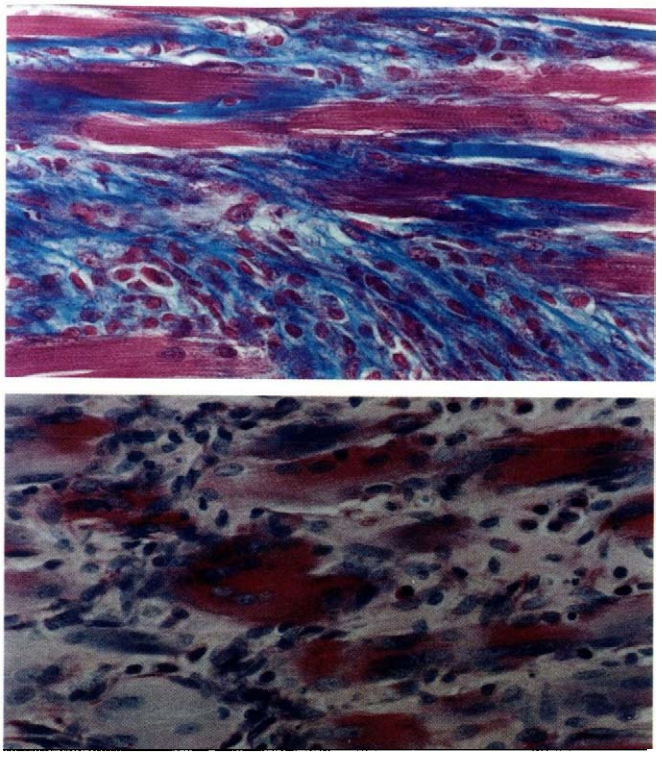

Figure 6. Histologic sections (×200) at Day 7. A, trichrome stain. Collagenous (blue) and proteinaceous (red) ground substance can be differentiated. B, vimentin stain. Intense vimentin activity (red) is noted at the periphery of the injury and extends centrally.

14.7 N). Although this decrease was not significant, the displacement at failure was significantly ($P < 0.05$) less in the injured complex (24.3 ± 2.8 mm) than in the controls (28.9 ± 6.1 mm). There was a more significant decrease ($P < 0.01$) in the energy absorbed to failure. The average of the injured complex was 776 ± 104 J as compared with 1125 ± 190 J for the controls. At Day 2 ($N = 3$) there were no differences between the controls and injured complexes. We note that data on four and five specimens from Day 0 and Day 2, respectively, were discarded because of technical problems.

At Day 7, the average ($N = 4$) decreases in failure load (80.8 ± 11 N versus 69.2 ± 7 N) were significant ($P < 0.05$). There were no differences at 24 days (Fig. 8).

DISCUSSION

The muscle contusion injury model developed in this work enabled us to record the mechanics of a nonpenetrating impact and to demonstrate the effect of injury and healing on contractile function. We also performed gross and microscopic observations and determined the passive failure properties, as previous studies have done. These data indicate that a significant muscle injury was produced in our model. From our gross observation, we would classify this injury model as a "moderate" muscle contusion injury. There was edema and hemorrhage with limited disruption

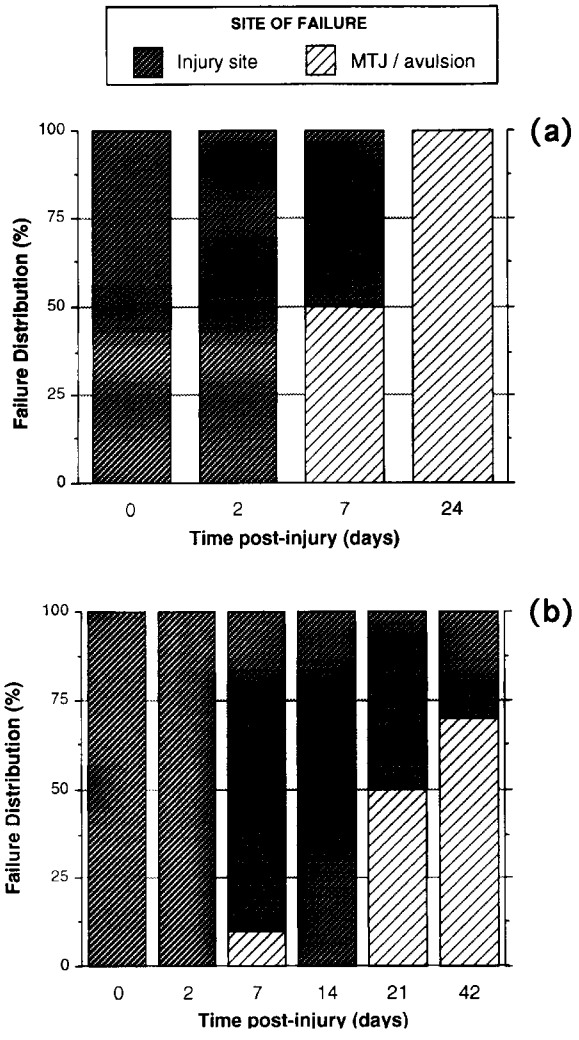

Figure 7. In the normal control muscles, 100% of the passive failures occurred with some bony avulsions at the proximal and distal myotendinous junctions. A, after injury (Day 0), all passive failures initiated at the site of injury in the muscle belly. With healing, the percentage of failures at the injury site decreased. B, Jarvinen[22] reported a similar trend with a slower rate of return to normal, suggesting a more severe injury in his model.

of muscle tissue.[5,20] Even with this moderate contusion injury there was an immediate loss of 38% in the maximum tetanic tension.

Correlating the severity of this injury with those seen clinically is difficult. In clinical studies that have graded contusion injuries, decreased range of motion was the only parameter defining injury severity.[3,4,20,29] A limitation of our animal model was the inability to measure range of motion. Extrapolating the relationship between injury severity and functional loss to clinical situations is also limited since contractility was measured during maximal tetanus in an anesthetized animal. Maximum tetanus, the state in which all motor units are maximally active, is a necessary experimental procedure that ensures repeatability but is probably rarely, if ever, obtained in vivo. The use

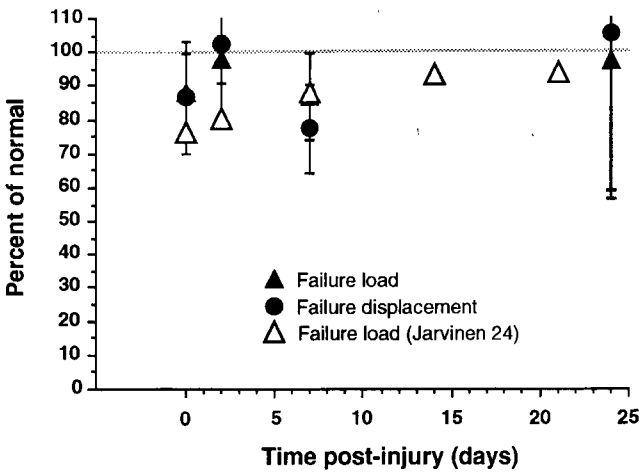

Figure 8. Although some significant differences were observed in the passive failure properties, we could not identify any overall trends with healing. In contrast to these findings, Jarvinen[22] reported that values of failure load continually returned to normal.

of anesthesia is unavoidable in this type of investigation; xylazine and ketamine were chosen because it is believed that they do not influence muscle function. The regenerative capabilities of the rat are known to be seven times faster than those of the cat.[37] Although these mechanisms of regeneration are believed to be similar to those of humans,[2] comparable rates and specific injury mechanisms for humans are not clearly defined. Comparison of our results with other experimental studies is precluded by the lack of work quantifying contractility as a function of contusion injury.

Only two previous studies using animal models have used a single impact to produce a contusion injury.[13,23] The injury model developed by Jarvinen and Sorvari[24] described the acute injury as containing three different zones: a central zone or gap due to the contraction of disrupted muscle fibers that extended about one-half the thickness of the muscle, a regeneration zone bordering the central zone, and a surviving zone of normal tissue. This amount of fiber disruption would indicate a more severe injury than in our model. Based on histologic description, our acute injury appears to be similar in severity to that reported by Fisher et al.[13]

The severity of a muscle contusion has been compared with an unrepaired partial muscle laceration in a mouse model by McGeachie and Grounds.[27] They found the laceration injury to be more severe in terms of prolonged myogenesis than the contusion injury. Considering the wide variation in the severity of contusion injuries, as well as potential injury to neurovascular structures, such conclusions should be limited to the specific injury model studied. It is also worth noting that multiple impacts to muscle tissue have been necessary to produce myositis ossificans using animal models.[9,35]

In a drop-mass technique, the impact is delivered by a mass that is dropped from a given height. This mass has a specific amount of potential energy available (potential energy = mass × gravity × height). Others have incorrectly stated that this potential energy is proportional to the instantaneous force delivered during an impact.[13] When we dropped the same mass from the same height onto the plastic apparatus and onto muscle tissue, there was clearly a difference in the impact mechanics (Fig. 2). To avoid an extraneous theoretical discussion, suffice it to say that the mechanics of the impact (i.e., force, displacement, and duration) are highly dependent on the material properties of the structure being impacted and how the structure is being supported. Furthermore, the radius of curvature of the impactor, the height (which determines the velocity), and the mass all contribute to defining the mechanics of the impact in a drop-mass technique. One advantage of our model as compared with those of Fisher et al.[13] and Jarvinen and Sorvari[24] was the ability to record the force-displacement-time of the impact. In our experimental impacts, we recorded variations (refer to the SD curves in Fig. 2) in the impacts. The source of these variations is most likely due to subtle differences in muscle size, mass, tone, and the site of impact.

Impact produces pressures on the surface of the skin. These pressures give rise to stresses within the muscle tissue. When the stresses reach some critical value, damage to muscle tissue results. Although we hypothesize that this is the mechanism of a contusion injury and that such critical stresses do exist, the design of this experimental protocol was not sufficient to specifically test this hypothesis. At present there are no quantitative studies of muscle impact to which we can compare our results. Impact injury has been studied in numerous other soft tissues including the spinal cord,[17,28] lung tissue,[34,38] and cardiac muscle.[31] In these experiments, a variety of models were employed to determine which impact parameters (e.g., peak force, displacement, impulse, velocity) correlated best with injury. In general, these experiments defined a threshold of injury in terms of a critical velocity and the product of velocity and displacement. The threshold of injury was not addressed in terms of pressures and stresses because the internal locations of these organs protects them from direct nonpenetrating blows. Such is not the case with muscle tissue. We propose that not only is the velocity and mass of the impact object critical but also the size and shape of the object. Increasing the size and shape of the impacting surface would reduce the surface pressures generated during impact.

Our stains with standard H&E and trichrome corroborate previously documented findings.[2,4,21-23,26] Immunohistochemical staining has previously identified vimentin expression in studies ranging from undifferentiated carcinomas[33] to bronchial epithelial cells.[11] Although vimentin is a component of the intermediate filaments that comprise the exosarcomeric cytoskeleton,[36] we believe that it is not in sufficient quantity to test positive with our immunohistochemical technqiues in normal and regenerated muscle tissue. Only recently have these techniques identified vimentin during muscle regeneration after experimentally induced necrosis.[7] Our findings suggest that vimentin staining documents myoblastic differentiation

in a regenerating zone at the periphery of the injury; the mechanisms of the process remain to be determined.

We documented an increase in muscle weight after injury, which we attributed to an accumulation of edema and hemorrhage. Jarvinen[22] recorded an increase in the weight of the injured muscle 2 days after injury, with a slight decrease in weight thereafter. Fisher et al.[13] found no increase in the weight of the injured muscle and suggested protein loss equaled the increased weight of the edema and hemorrhage. They also showed that there was a significant decrease in total protein content at 2 days, probably due to reflex disuse atrophy and protein catabolism at the injury site. Muscle protein accumulation commenced at Day 3, and regeneration was not complete until 21 days.[13]

The effect of contusion injury and healing has been quantified by the passive tensile failure properties of the rat gastrocnemius muscle.[22] They showed that passive failure load significantly decreased after impact and continually improved with healing but at 42 days after injury remained 95% of normal (Fig. 8). We were unable to demonstrate any significant trends in the failure load or failure displacement. The differences with our results may be due to the more serious injury model of Jarvinen and Sorvari,[24] with its greater amount of disrupted muscle fibers, and to the limited number of specimens in our mechanical tests. However, in agreement with their studies, we also observed that failure initiated at the site of injury acutely and during the early stages of healing. The percentage of failures occurring at the injury site decreased sooner with healing in our model, further suggesting our injury was less severe than that of Jarvinen and Sorvari[24] (Fig. 7). Given that passive failure is an indication of tissue strength,[14] these results suggest that acute contusion injuries may be more susceptible to subsequent strain injuries at the site of injury.[20,29] This assumes, however, that the relatively slow extension rate used herein and by others[14] is an appropriate simulation of the mechanical behavior at the higher extension rates at which strain injuries occur.

In summary, we developed and quantified a reproducible muscle contusion injury. We determined that the mechanics of the impact (force, displacement, velocity, energy absorbed, and impulse) were not defined by the available potential energy in a drop-mass but depended on several factors including the physical properties of the impacted structure. We microscopically confirmed four histologic stages at the level of the myofiber: acute injury, degeneration, regeneration, and normalization. Regeneration was confirmed with the novel staining for vimentin. We determined that contractile function, an indicator of muscle performance, was significantly compromised by contusion injury and improved continuously with each histologic stage.

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