

Inflammatory cells in rat skeletal muscle are elevated after electrically stimulated contractions

THOMAS J. McLOUGHLIN,^{1*} ELENI MYLONA,^{1*} TROY A. HORNBERGER,²
KARYN A. ESSER,² AND FRANCIS X. PIZZA¹

¹Department of Kinesiology, The University of Toledo, Toledo, Ohio 43606;
and ²School of Kinesiology, University of Illinois, Chicago, Illinois 60608

Submitted 20 August 2002; accepted in final form 21 October 2002

McLoughlin, Thomas J., Eleni Mylona, Troy A. Hornberger, Karyn A. Esser, and Francis X. Pizza. Inflammatory cells in rat skeletal muscle are elevated after electrically stimulated contractions. *J Appl Physiol* 94: 876–882, 2003. First published November 1, 2002; 10.1152/jappphysiol.00766.2002.—We determined the effect of muscle contractions resulting from high-frequency electrical stimulation (HFES) on inflammatory cells in rat tibialis anterior (TA), plantaris (Pln), and soleus (Sol) muscles at 6, 24, and 72 h post-HFES. A minimum of four and a maximum of seven rats were analyzed at each time point. HFES, applied to the sciatic nerve, caused the Sol and Pln to contract concentrically and the TA to contract eccentrically. Neutrophils were higher ($P < 0.05$) at 6 and 24 h after HFES in the Sol, Pln, and TA muscles relative to control muscles. ED1⁺ macrophages in the Pln were elevated at 6 and 24 h after HFES and were also elevated in the Sol and TA after HFES relative to controls. ED2⁺ macrophages in the Sol and TA were elevated at 24 and 72 h after HFES, respectively, and were also elevated in the Pln after HFES relative to controls. In contrast to the TA muscles, the Pln and Sol muscles showed no gross histological abnormalities. Collectively, these data indicate that both eccentric and concentric contractions can increase inflammatory cells in muscle, regardless of whether overt histological signs of injury are apparent.

muscle injury; inflammation; neutrophils; ED1 macrophages; ED2 macrophages

SKELETAL MUSCLE RESPONSES to mechanical loading are influenced by not only external load but also contraction type. Specifically, eccentric contractions elicit greater perturbations in signal transduction pathways associated with skeletal muscle hypertrophy and higher rates of protein synthesis relative to concentric contractions (2, 27, 44, 45). In addition, eccentric contractions are more likely to cause muscle dysfunction and histological abnormalities (overt muscle injury) compared with concentric contractions (1, 6, 23, 30). The overt injury after eccentric contractions is accompanied by activation of the acute inflammatory response, as indicated by elevations of neutrophils and/or macrophages in skeletal muscle (15, 33, 41, 43). Whether concentric contractions influence the concen-

tractions of neutrophils and/or macrophages in skeletal muscle, however, is unknown.

In theory, inflammatory cells could accumulate in skeletal muscle after concentric contractions because of an increase in reactive oxygen species production and/or NF- κ B activation (11, 19). Both reactive oxygen species and NF- κ B activation can initiate inflammatory cell chemotaxis via the production of oxidatively modified proteins (32) and chemokines (12), respectively. To date, the function of neutrophils and macrophages in skeletal muscle is generally thought to be limited to the phagocytosis of injured tissue via their arsenal of free radicals and proteases (25, 31). Inflammatory cells, however, can release cytokines, chemokines, and growth factors independent of phagocytosis (4, 29). Because inflammatory cell-derived products are known to influence the transcription of redox-sensitive genes, myoblast proliferation and differentiation, muscle growth, and angiogenesis (9, 10, 39, 46), inflammatory cells may have important biological functions in skeletal muscle that are independent from the phagocytosis of tissue debris after overt injury.

The purpose of the study was to test the hypothesis that both eccentric and concentric contractions elevate muscle concentrations of neutrophils and macrophages (ED1⁺ and ED2⁺). Muscle contractions in rats were elicited via electrical stimulation of the sciatic nerve, which results in maximal contraction of all hindlimb muscles in the distal compartment (2, 20, 21, 27, 44, 45). In this model, the plantar flexors [gastrocnemius, soleus (Sol), and plantaris (Pln)] undergo concentric contractions, and the dorsiflexors [tibialis anterior (TA) and extensor digitorum longus (EDL)] undergo eccentric contractions (44, 45). Therefore, the TA, Pln, and Sol were used to determine changes in neutrophils, ED1⁺ macrophages, and ED2⁺ macrophages after both eccentric and concentric contractions. Because the electrical muscle stimulation model used in the present study is a frequently used animal paradigm to evaluate the time course of changes in molecular, cellular, and tissue level events associated with resistance exercise (2, 20, 21, 27, 44), we also sought to characterize the

*T. J. McLoughlin and E. Mylona contributed equally to this work.
Address for reprint requests and other correspondence: F. X. Pizza, Dept. of Kinesiology, The Univ. of Toledo, 2801 W. Bancroft St., Toledo, OH 43606 (E-mail: fpizza@pop3.utoledo.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

cellular events more completely by quantifying the time course of changes in neutrophils, ED1⁺ macrophages, and ED2⁺ macrophages.

MATERIALS AND METHODS

Animals. All procedures were approved by the Animal Care Committee at the University of Illinois at Chicago and were in accordance with Guidelines for Care and Use of Laboratory Animals. Six- to seven-week-old female Wistar rats ($n = 18$) (Charles River Laboratories, Wilmington, MA), weighing between 200 and 250 g at the time of death, were maintained on a constant 12:12-h light-dark cycle with food and water available ad libitum. On arrival, animals were allowed to acclimatize for 6 days before commencement of any experimental procedures. The surgical and electrical stimulation procedures were performed under anesthesia (pentobarbital sodium, 50 mg/kg ip; supplemental doses as needed). After electrode implantation, animals were allowed to recover for 5 days before initiation of the stimulation protocol (2).

Stimulation protocol. Muscle contractions were induced by a single bout of high-frequency electrical stimulation (HFES), as previously described (27). Multistrand electrodes (Medwire, Mount Vernon, NY) were implanted on both sides of the right sciatic nerve above the anatomic branching point. Tetanic contractions were delivered with the use of a Grass S5 stimulator (Grass Instruments, Quincy, MA) at 100 Hz, 6–12 V, 1-ms duration, 9-ms delay, for 10 sets of six repetitions, with each repetition lasting 3 s. A 10-s delay was given between repetitions and 1 min between sets. The stimulation protocol lasted a total time of 20 min. This stimulation protocol causes maximal activation of all distal hindlimb muscles and significantly reduces muscle glycogen levels in the TA and Sol (77.9 and 51%, respectively) (27). Training with this protocol has been reported to cause skeletal muscle hypertrophy in the dorsiflexor muscles (2, 45).

Immunohistochemistry of inflammatory cells. Sol, Pln, and TA muscles were excised at 6, 24, and 72 h after HFES, coated with optimum cutting temperature compound, frozen in melting isopentane cooled on dry ice, and stored at -80°C . Muscles from the contralateral limb served as controls. A minimum of four and a maximum of seven rats were analyzed at each time point. Cross sections (10 μm) were cut from the muscle midbelly, adhered to chromium potassium sulfate and gelatin-treated glass slides, and frozen at -20°C . Muscle sections were prepared for immunohistochemistry as previously described (7). The primary antibodies for neutrophils, ED1⁺ macrophages, and ED2⁺ macrophages, which were incubated for 2 h at room temperature, were an anti-rat neutrophil (HIS48; 1:25; PharMingen, Franklin Lake, NJ), mouse anti-rat ED1 (1:100; Serotec; Oxford, UK), and mouse anti-rat ED2 (1:100; Serotec), respectively. The sections were then washed in PBS and incubated with either biotinylated goat anti-mouse IgM (1:200; Vector Laboratories; Burlingame, CA) (HIS48) or biotinylated horse anti-mouse IgG (1:200, Vector Laboratories) (ED1 and ED2) for 30 min. After incubation with the secondary antibody, sections were washed with PBS and incubated with horseradish peroxidase (1:1,000; Vector Laboratories). After three washes, the antibody-antigen complex was developed by using the peroxidase substrate kit 3-amino-9-ethylcarbazole (Vector Laboratories).

Sections were viewed with a light microscope (Olympus IX-70; Olympus, Melville, NY) with Nomarski optics. The number of labeled cells in two entire sections for each muscle was counted manually (magnification $\times 400$), and the mean

was used for statistical analysis. The total area of each section was measured with a calibrated square grid (magnification $\times 200$), and the volume of the muscle sample was calculated as the product of the cross-sectional area of the sections and the section thickness (10 μm). Labeled cells were expressed as number per cubic millimeter (mm^3).

Histological evaluation. Muscle cross sections (10 μm) from exercise and contralateral control muscles were labeled numerically, stained with hematoxylin and eosin, and examined for evidence of muscle injury, as previously described (13). With the use of light microscopy, each muscle section was blindly classified as injured if it exhibited two or more of the following criteria: 1) invasion of myofibers with cells, 2) pale or diffuse staining cytoplasm, and 3) centrally located nuclei. Because these criteria have been shown to be significantly elevated at 3 days after eccentric contractions in mice (13), only the 72-h post-HFES time point was used for the assessment of overt muscle injury. After all muscles were examined, the numeric labeling code was revealed, and the muscles were then classified as exercise or contralateral control.

Statistics. Because the purpose of the study was to determine the effect of HFES on inflammatory cells in the individual muscles and not to compare responses between the muscles, inflammatory cells in Sol, Pln, and TA muscles were analyzed by separate two-way ANOVA tests. The Newman-Keuls post hoc test was used to determine differences between means when the observed F -ratio was significant ($P \leq 0.05$). All data are represented as means \pm SE.

RESULTS

Our results demonstrate that concentric contractions can elicit significant elevations in muscle inflammatory cell concentrations. Specifically, neutrophils were elevated in both the Pln (interaction; Fig. 1A) and Sol (interaction; Fig. 2A) muscles at 6 and 24 h relative to contralateral control muscles. ED1⁺ macrophages in the Pln were elevated at 6 and 24 h (interaction; Fig. 1B). In the Sol, ED1⁺ macrophages were higher after HFES (main effect), but there was no significant interaction (Fig. 2B). ED2⁺ macrophages were elevated in the Pln (main effect; Fig. 1C) and were higher in the Sol at 24 h after HFES relative to controls (interaction; Fig. 2C).

Eccentric contractions of the TA muscles resulted in higher neutrophil concentrations at 6 and 24 h after HFES relative to control muscles (interaction; Fig. 3A). ED1⁺ macrophages were higher in the TA muscles after HFES (main effect), but there was no significant interaction (Fig. 3B). ED2⁺ macrophages in the TA muscles were higher 72 h after HFES relative to control muscles (interaction; Fig. 3C).

Histological evaluation of muscle cross sections by hematoxylin and eosin staining revealed that myofibers in the Pln (Fig. 4A) and Sol (Fig. 4B) muscles showed no gross histological signs of injury. In contrast to the Pln and Sol, the eccentrically contracted TA muscles exhibited gross histological abnormalities, evidenced by altered cytoplasmic staining and profound invasion by cells (Fig. 4C). None of the control muscles exhibited histological characteristics of overt muscle injury.

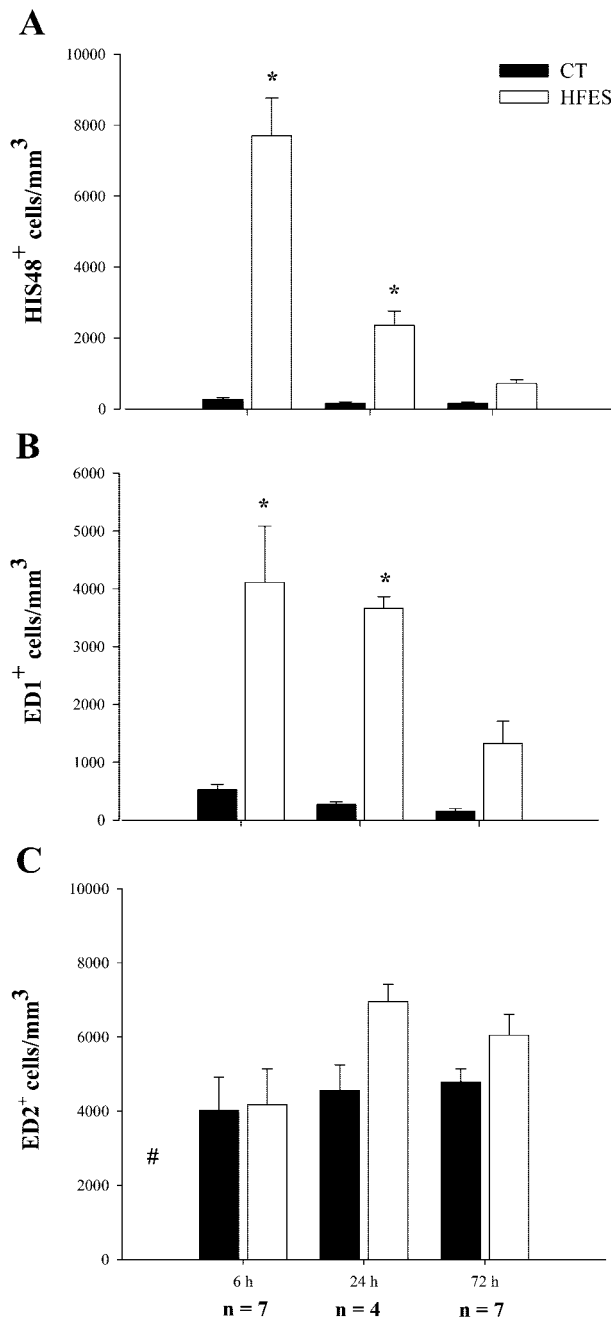


Fig. 1. Inflammatory cell concentrations after electrically stimulated concentric contractions of the plantaris muscles: neutrophils (HIS48⁺; A), ED1⁺ macrophages (B), and ED2⁺ macrophages (C). HFES, high-frequency electrical stimulation; CT, contralateral controls. Values are means \pm SE; *n*, no. of rats. *Significantly higher for HFES relative to CT at specified time points, $P \leq 0.05$. # Significantly elevated after HFES relative to CT (main effect), $P \leq 0.05$.

DISCUSSION

The novel observations in the present study were the elevations in neutrophils, ED1⁺ macrophages, and ED2⁺ macrophages in the Pln and Sol after concentric contractions (Figs. 1 and 2, respectively). As expected, inflammatory cells were also increased after eccentric contractions of the TA (Fig. 3). Because both the Pln and Sol showed no gross histological abnormalities

(Fig. 4, A and B, respectively), the observed changes in these muscles may indicate that overt injury is not the sole determinant for the accumulation of neutrophils and macrophages in skeletal muscle. These observations may also indicate that inflammatory cells have biological functions in skeletal muscle that extend beyond their proposed role in the phagocytosis of tissue debris after overt muscle injury. Because of the vast array of inflammatory cell-derived products (23, 35), inflammatory cells in skeletal muscle (injured or non-injured) may also function as a source of cellular sig-

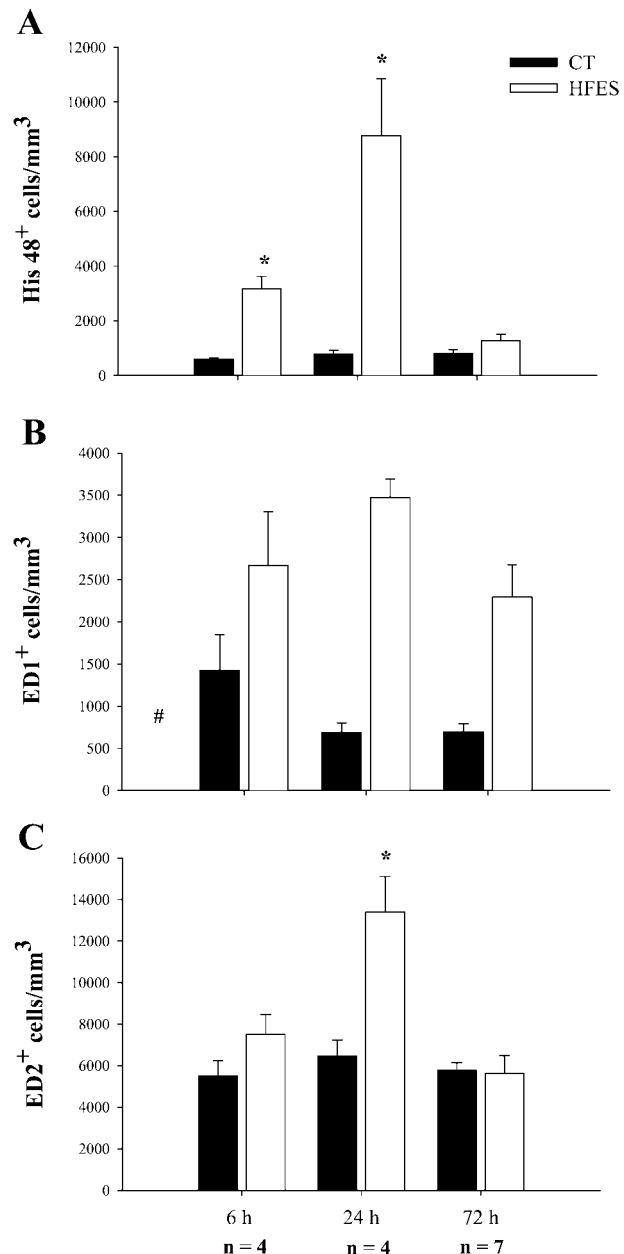


Fig. 2. Inflammatory cell concentrations after electrically stimulated concentric contractions of the soleus muscles: neutrophils (HIS48⁺; A), ED1⁺ macrophages (B), and ED2⁺ macrophages (C). Values are means \pm SE; *n*, no. of rats. *Significantly higher for HFES relative to CT at specified time points, $P \leq 0.05$. # Significantly elevated after HFES relative to CT (main effect), $P \leq 0.05$.

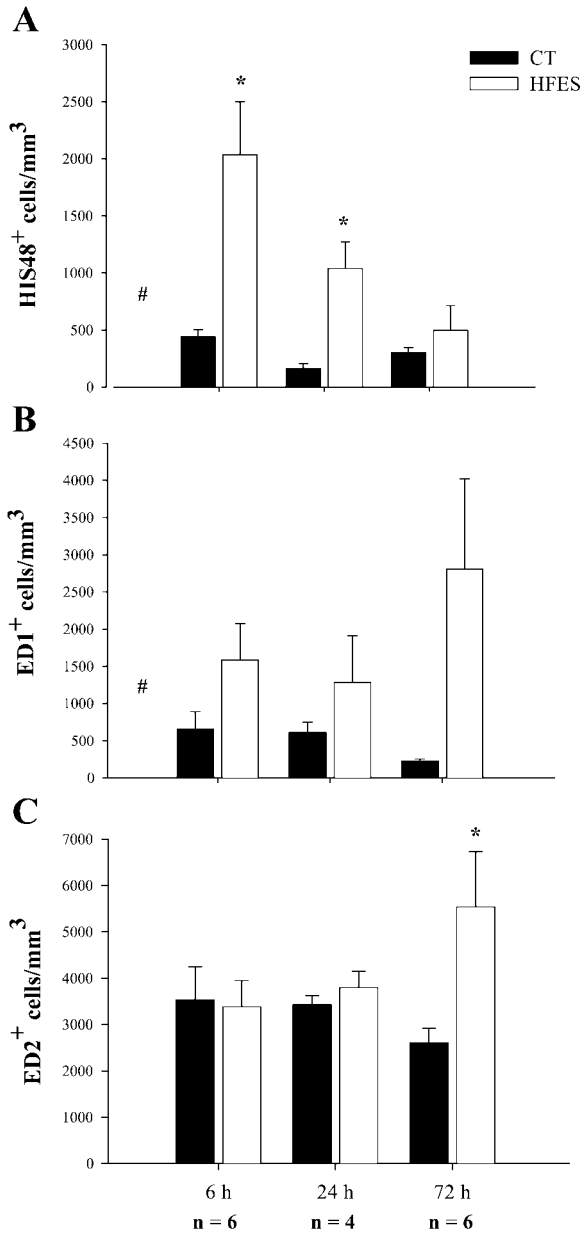


Fig. 3. Inflammatory cell concentrations after electrically stimulated eccentric contractions of the tibialis anterior muscles: neutrophils (HIS48⁺; A), ED1⁺ macrophages (B), and ED2⁺ macrophages (C). Values are means \pm SE; *n*, no. of rats. *Significantly higher for HFES relative to CT at specified time points, $P \leq 0.05$. # Significantly elevated after HFES relative to CT (main effect), $P \leq 0.05$.

nals that contribute to skeletal muscle adaptations after mechanical loading.

Previous investigators have examined muscle neutrophils and/or macrophages after eccentric contractions (15, 33, 41, 43) and in the hindlimb-suspension reloading model of muscle injury (7, 42). In the present study, the elevations in neutrophils in the TA are consistent with our observations of rat Sol after downhill running (43) and with our findings in mice after in situ eccentric contractions of the EDL (33). Previous investigators using the hindlimb-suspension model of muscle injury have also reported elevations in neutro-

phils in rat Sol at 6 and 24 h after muscle reloading (7, 42). These studies, however, are in contrast to a recent study by Lapointe et al. (15), who reported that neutrophils are not elevated in the EDL muscles of young rats after in situ eccentric contractions. Conflicting neutrophil results are not easily reconciled but may be attributable to the age of the female Wistar rats (50–70 g) in the study by Lapointe et al. relative to ours

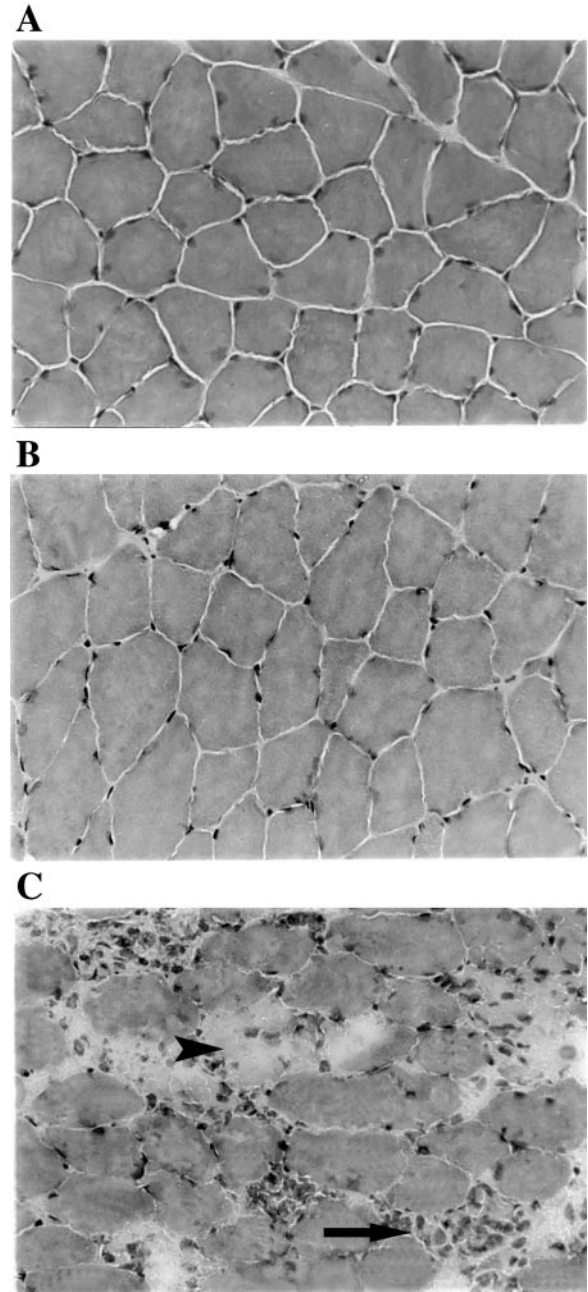


Fig. 4. Muscle cross sections obtained 72 h after HFES and stained with hematoxylin and eosin (magnification $\times 400$). Concentric contractions of the plantaris (A) and soleus (B) muscles showed no gross histological signs of overt injury. In contrast to the plantaris and soleus, eccentric contractions of the tibialis anterior (C) muscles resulted in overt injury as indicated by a substantial number of myofibers that had a pale cytoplasm (arrowhead) and/or were invaded by cells (arrow).

(200–250 g). In addition, Lapointe et al. used an antibody against leukosialin (CD43; clone W3/13; Serotec) to identify neutrophils in cross sections. This antibody may not be an appropriate marker of neutrophils in skeletal muscle, because CD43 is expressed by other leukocytes and is shed from neutrophils when activated and when they adhere to endothelial cells (3, 35). The time course of elevations in ED1⁺ and ED2⁺ macrophages after eccentric contractions in the present study is consistent with the observations of Lapointe et al. (15) and with previous hindlimb-suspension studies (7, 42).

When the relationship between inflammatory cells and overt signs of muscle injury is based on temporal analysis, the accumulation of inflammatory cells in skeletal muscle has traditionally been attributed to events associated with muscle injury and the subsequent regeneration (9, 36). This interpretation is a reasonable explanation for the elevated inflammatory cells in the TA (Fig. 3) because 1) all of the exercised TA muscles in the present study showed gross histological abnormalities at 72 h post-HFES (Fig. 4C), and 2) the same electrical muscle stimulation model used in the present study has been reported to cause overt injury to the TA, as indicated by a 35% force deficit 2 days after the stimulation protocol (20, 21). In contrast, concentric contractions of the Pln (Fig. 1) and Sol (Fig. 2) caused an elevation in neutrophils, ED1⁺ macrophages, and ED2⁺ macrophages in the absence of gross histological abnormalities (Fig. 4, A and B, respectively). The lack of histological abnormalities in the Pln and Sol is consistent with previous studies that have established that concentric contractions do not cause overt muscle injury (6, 23, 30). Taken together, these observations may indicate that overt muscle injury is not the sole prerequisite for the accumulation of inflammatory cells in skeletal muscle after mechanical loading. This interpretation is consistent with our laboratory's recent report (33) of elevated neutrophils, but not macrophages, in the absence of overt injury in mice after isometric contractions and passive stretches. An alternative explanation of our findings is that some degree of minor injury (i.e., injury that does not result in a functional impairment or gross histological abnormalities) may have occurred after concentric contractions, and thus one or more chemoattractant(s) for inflammatory cells may have been produced and/or released.

Because the amount of force produced by the TA, Sol, and Pln in this model is unknown, the influence of force development on the accumulation of inflammatory cells in these muscles cannot be addressed. Our laboratory has previously demonstrated that passive stretching elevates neutrophils, but not macrophages, to the same level as maximal isometric contractions (33). These observations may indicate that the amount of force produced by a muscle per se is not a critical determinant for the accumulation of inflammatory cells in skeletal muscle.

Unfortunately, because very little is known about factors that orchestrate the accumulation of inflamma-

tory cells in skeletal muscle, it is difficult to speculate as to which chemoattractants may have been produced and/or released after eccentric and concentric contractions. Potential candidates include fragments of complement proteins (e.g., C5a) and chemoattractants derived from endothelial cells, skeletal muscle cells, and/or resident cells in skeletal muscle (reviewed in Refs. 4, 9, 14, 18, 40). The vascular endothelium is a potential source of chemoattractants because it represents an initial barrier that neutrophils and monocytes must cross before entering skeletal muscle, and because the vascular endothelium is capable of producing numerous chemoattractants for neutrophils and macrophages, including chemokines (e.g., IL-8 and monocyte chemoattractant protein-1) and lipid mediators (e.g., platelet-activating factor and leukotriene B₄) (14). Recent evidence indicates that skeletal muscle cells are also capable of producing chemoattractants for either neutrophils and/or macrophages under basal and/or proinflammatory conditions, after injury, and in inflammatory myopathies. Such factors include IL-8 (5), monocyte chemoattractant protein-1 (5), complement proteins (8, 16, 17), transforming growth factor- β (28), and lipopolysaccharide-inducible CXC chemokine (38). Finally, resident fibroblasts and/or inflammatory cells in skeletal muscle may also be a source of chemoattractants for inflammatory cells (4, 40), with the latter source possibly serving as a positive feedback mechanism whereby greater numbers of neutrophils and macrophages could be attracted to skeletal muscle. Which, if any, of the above chemoattractants and/or sources contributed to the accumulation of neutrophils and/or macrophages in the present study remains to be determined.

Interestingly, some chemoattractants for inflammatory cells have been shown to activate and/or enhance their function, whereas others do not. For example, IL-8, a chemoattractant for neutrophils but not monocytes, has been shown to be capable of causing and enhancing the release of reactive oxygen species and proteases from neutrophils (4). Theoretically, the release of reactive oxygen species from neutrophils after mechanical loading could cause minor or overt injury to skeletal muscle, because they have been demonstrated to damage skeletal muscle during the reperfusion of ischemic tissue (37) and have been reported to injure cultured myotubes (26). On the other hand, transforming growth factor- β ₁, a potent chemoattractant for both neutrophils and monocytes, does not appear to influence reactive oxygen species production from neutrophils (4, 34) but stimulates monocytes to produce cytokines (e.g., tumor necrosis factor- α , platelet-derived growth factor BB, and fibroblast growth factor) (22) that could influence skeletal muscle growth (9, 10, 46). Thus, depending on the chemoattractants produced within skeletal muscle (injured or noninjured), inflammatory cells and their derived products may have dichotomous actions in skeletal muscle after mechanical loading.

Neutrophils and ED1⁺ macrophages are believed to be responsible for the removal of cellular debris after

overt injury based on their ability to perform phagocytosis and on limited qualitative observations (25, 31). ED2⁺ macrophages, which have a limited phagocytic capacity (24), have been hypothesized to contribute to the early events of muscle regeneration after overt injury by causing satellite cell activation and myoblast proliferation (9, 10). Thus the function of inflammatory cells in skeletal muscle has traditionally been limited to events that follow overt injury. The accumulation of neutrophils, ED1⁺ macrophages, and ED2⁺ macrophages in the Sol and Pln after concentric contractions, however, may indicate that these cells influence skeletal muscle in the absence of overt injury. Because inflammatory cells are capable of producing reactive oxygen species, reactive nitrogen species, cytokines, and growth factors that individually are known to influence skeletal muscle, the presence of inflammatory cells in noninjured muscle may serve as an additional source of cellular signals for muscle adaptations to mechanical loading. Such adaptations may include protection from injury, muscle hypertrophy, and angiogenesis. Further work is needed before the potential implications of our results can be fully appreciated.

The authors thank Susan K. Tsivitsse for assistance with muscle sectioning.

This project was partially funded through National Institute of Arthritis and Musculoskeletal and Skin Diseases Grants AR-47599-02 (to F. X. Pizza) and AR-45617 (to K. A. Esser).

REFERENCES

1. **Armstrong RB, Ogilvie RW, and Schwane JA.** Eccentric exercise-induced injury to rat skeletal muscle. *J Appl Physiol* 54: 80–93, 1983.
2. **Baar K and Esser K.** Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol Cell Physiol* 276: C120–C127, 1999.
3. **Bazil V and Strominger JL.** CD43, the major sialoglycoprotein of human leukocytes, is proteolytically cleaved from the surface of stimulated lymphocytes and granulocytes. *Proc Natl Acad Sci USA* 90: 3792–3796, 1993.
4. **Cassatella MA.** Neutrophil-derived proteins: selling cytokines by the pound. *Adv Immunol* 73: 369–509, 1999.
5. **De Rossi M, Bernasconi P, Baggi F, de Waal Malefyt R, and Mantegazza R.** Cytokines and chemokines are both expressed by human myoblasts: possible relevance for the immune pathogenesis of muscle inflammation. *Int Immunol* 12: 1329–1335, 2000.
6. **Faulkner JA, Jones DA, and Round JM.** Injury to skeletal muscles of mice by forced lengthening during contractions. *Q J Exp Physiol* 74: 661–670, 1989.
7. **Frenette J, St-Pierre M, Cote CH, Mylona E, and Pizza FX.** Muscle impairment occurs rapidly and precedes inflammatory cell accumulation after mechanical loading. *Am J Physiol Regul Integr Comp Physiol* 282: R351–R357, 2002.
8. **Gasque P, Morgan BP, Legoedec J, Chan P, and Fontaine M.** Human skeletal myoblasts spontaneously activate allogeneic complement but are resistant to killing. *J Immunol* 156: 3402–3411, 1996.
9. **Grounds MD and Davies MJ.** Chemotaxis in myogenesis. *Basic Appl Myol* 6: 469–483, 1996.
10. **Hawke TJ and Garry DJ.** Myogenic satellite cells: physiology to molecular biology. *J Appl Physiol* 91: 534–551, 2001.
11. **Hollander J, Fiebig R, Gore M, Ookawara T, Ohno H, and Ji LL.** Superoxide dismutase gene expression is activated by a single bout of exercise in rat skeletal muscle. *Pflügers Arch* 442: 426–434, 2001.
12. **Jobin C and Sartor RB.** The I κ B/NF- κ B system: a key determinant of mucosal inflammation and protection. *Am J Physiol Cell Physiol* 278: C451–C462, 2000.
13. **Koh TJ and Brooks SV.** Lengthening contractions are not required to induce protection from contraction-induced muscle injury. *Am J Physiol Regul Integr Comp Physiol* 281: R155–R161, 2001.
14. **Krishnaswamy G, Kelley J, Yerra L, Smith JK, and Chi DS.** Human endothelium as a source of multifunctional cytokines: molecular regulation and possible role in human disease. *J Interferon Cytokine Res* 19: 91–104, 1999.
15. **Lapointe BM, Frenette J, and Cote CH.** Lengthening contraction-induced inflammation is linked to secondary damage but devoid of neutrophil invasion. *J Appl Physiol* 92: 1995–2004, 2002.
16. **Legoedec J, Gasque P, Jeanne JF, and Fontaine M.** Expression of the complement alternative pathway by human myoblasts in vitro: biosynthesis of C3, factor B, factor H and factor I. *Eur J Immunol* 25: 3460–3466, 1995.
17. **Legoedec J, Gasque P, Jeanne JF, Scotte M, and Fontaine M.** Complement classical pathway expression by human skeletal myoblasts in vitro. *Mol Immunol* 34: 735–741, 1997.
18. **Matsukawa A, Hogaboam CM, Lukacs NW, and Kunkel SL.** Chemokines and innate immunity. *Rev Immunogenet* 2: 339–358, 2000.
19. **McArdle A, Pattwell D, Vasilaki A, Griffiths RD, and Jackson MJ.** Contractile activity-induced oxidative stress: cellular origin and adaptive responses. *Am J Physiol Cell Physiol* 280: C621–C627, 2001.
20. **McBride TA, Gorin FA, and Carlsen RC.** Prolonged recovery and reduced adaptation in aged rat muscle following eccentric exercise. *Mech Ageing Dev* 83: 185–200, 1995.
21. **McBride TA, Stockert BW, Gorin FA, and Carlsen RC.** Stretch-activated ion channels contribute to membrane depolarization after eccentric contractions. *J Appl Physiol* 88: 91–101, 2000.
22. **McCartney-Francis N, Mizel D, Wong H, Wahl L, and Wahl S.** TGF-beta regulates production of growth factors and TGF-beta by human peripheral blood monocytes. *Growth Factors* 4: 27–35, 1990.
23. **McCully KK and Faulkner JA.** Injury to skeletal muscle fibers of mice following lengthening contractions. *J Appl Physiol* 59: 119–126, 1985.
24. **McLennan IS.** Resident macrophages (ED2- and ED3-positive) do not phagocytose degenerating rat skeletal muscle fibres. *Cell Tissue Res* 272: 193–196, 1993.
25. **McLennan IS.** Degenerating and regenerating skeletal muscles contain several subpopulations of macrophages with distinct spatial and temporal distributions. *J Anat* 188: 17–28, 1996.
26. **McLoughlin TJ and Pizza FX.** Hydroxyl radical injures and nitric oxide protects cultured myotubes from neutrophil-mediated injury (Abstract). *FASEB J* 15: A1166, 2002.
27. **Nader GA and Esser KA.** Intracellular signaling specificity in skeletal muscle in response to different modes of exercise. *J Appl Physiol* 90: 1936–1942, 2001.
28. **Nagaraju K, Raben N, Merritt G, Loeffler L, Kirk K, and Plotz P.** A variety of cytokines and immunologically relevant surface molecules are expressed by normal human skeletal muscle cells under proinflammatory stimuli. *Clin Exp Immunol* 113: 407–414, 1998.
29. **Nathan CF.** Secretory products of macrophages. *J Clin Invest* 79: 319–326, 1987.
30. **Newham DJ, McPhail G, Mills KR, and Edwards RH.** Ultrastructural changes after concentric and eccentric contractions of human muscle. *J Neurol Sci* 61: 109–122, 1983.
31. **Papadimitriou JM, Robertson TA, Mitchell CA, and Grounds MD.** The process of new plasmalemma formation in focally injured skeletal muscle fibers. *J Struct Biol* 103: 124–134, 1990.
32. **Petrone WF, English DK, Wong K, and McCord JM.** Free radicals and inflammation: superoxide-dependent activation of a neutrophil chemotactic factor in plasma. *Proc Natl Acad Sci USA* 77: 1159–1163, 1980.

33. **Pizza FX, Koh TJ, McGregor SJ, and Brooks SV.** Muscle inflammatory cells after passive stretches, isometric contractions, and lengthening contractions. *J Appl Physiol* 92: 1873–1878, 2002.
34. **Reibman J, Meixler S, Lee TC, Gold LI, Cronstein BN, Haines KA, Kolasinski SL, and Weissmann G.** Transforming growth factor beta 1, a potent chemoattractant for human neutrophils, bypasses classic signal-transduction pathways. *Proc Natl Acad Sci USA* 88: 6805–6809, 1991.
35. **Rieu P, Porteu F, Bessou G, Lesavre P, and Halbwachs-Mecarelli L.** Human neutrophils release their major membrane sialoprotein, leukosialin (CD43), during cell activation. *Eur J Immunol* 22: 3021–3026, 1992.
36. **Robertson TA, Maley MA, Grounds MD, and Papadimitriou JM.** The role of macrophages in skeletal muscle regeneration with particular reference to chemotaxis. *Exp Cell Res* 207: 321–331, 1993.
37. **Rubin BB, Romaschin A, Walker PM, Gute DC, and Korthuis RJ.** Mechanisms of postischemic injury in skeletal muscle: intervention strategies. *J Appl Physiol* 80: 369–387, 1996.
38. **Sachidanandan C, Sambasivan R, and Dhawan J.** Tristetraprolin and LPS-inducible CXC chemokine are rapidly induced in presumptive satellite cells in response to skeletal muscle injury. *J Cell Sci* 115: 2701–2712, 2002.
39. **Sen CK and Packer L.** Antioxidant and redox regulation of gene transcription. *FASEB J* 10: 709–720, 1996.
40. **Smith RS, Smith TJ, Blieden TM, and Phipps RP.** Fibroblasts as sentinel cells. Synthesis of chemokines and regulation of inflammation. *Am J Pathol* 151: 317–322, 1997.
41. **St. Pierre Schneider B, Correia LA, and Cannon JG.** Sex differences in leukocyte invasion in injured murine skeletal muscle. *Res Nurs Health* 22: 243–250, 1999.
42. **Tidball JG, Berchenko E, and Frenette J.** Macrophage invasion does not contribute to muscle membrane injury during inflammation. *J Leukoc Biol* 65: 492–498, 1999.
43. **Tsivitse SK, McGregor SJ, Peterson JM, McLoughlin TJ, Mylona E, and Pizza FX.** Muscle inflammatory cell concentrations following downhill running (Abstract). *Med Sci Sports Exerc* 33: S120, 2001.
44. **Wong TS and Booth FW.** Protein metabolism in rat gastrocnemius muscle after stimulated chronic concentric exercise. *J Appl Physiol* 69: 1709–1717, 1990.
45. **Wong TS and Booth FW.** Protein metabolism in rat tibialis anterior muscle after stimulated chronic eccentric exercise. *J Appl Physiol* 69: 1718–1724, 1990.
46. **Zoico E and Roubenoff R.** The role of cytokines in regulating protein metabolism and muscle function. *Nutr Rev* 60: 39–51, 2002.

