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Muscle Fatigue Increases the Probability of Developing Hyperalgesia in Mice

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Abstract

Chronic muscle pain is a major clinical problem that is often associated with fatigue. Conversely, chronic fatigue conditions are commonly associated with muscle pain. We tested the hypothesis that muscle fatigue enhances hyperalgesia associated with injection of acidic saline into muscle. We evaluated mechanical sensitivity of the paw (von Frey) in mice after 2 intramuscular injections of saline (20 μ L; pH 4, pH 5, pH 6, pH 7.2) in a fatigue and a control group. To induce fatigue, mice were run for 2 h/day for 2 days prior to the first injection and 2 h/day for 2 days prior to the second injection. Muscle lactate, pCO₂, pO₂, creatinine kinase, phosphate, and histology were examined after the fatigue task and compared to a control group. Grip force was significantly decreased after 2 h of running indicating fatigue. The fatigue task did not induce muscle damage as there was no difference in muscle lactate, pCO₂, pO₂, creatinine kinase, phosphate, or histology. The fatigue task altered the dose-response relationship to intramuscular acidic saline injections. Mechanical hyperalgesia was observed in both fatigue and control groups after intramuscular injection of pH 4.0, but only the fatigue group after injection of pH 5. Neither the fatigue nor the control group developed hyperalgesia in response to intramuscular injection of pH 6 or pH 7.2. In conclusion, fatigue modified the susceptibility of mice to acid injection of pH 5.0 to result in mechanical hyperalgesia after 2 injections of pH 5.0. The fatigue task did not produce measurable changes in the muscle tissue suggesting a central mechanism mediating the enhancement of hyperalgesia.

Perspective—These data therefore show that muscle fatigue can enhance the likelihood that one develops pain to a mild insult. Clinically, this could relate to the development of pain from such conditions as repetitive strain injury, and may relate to the interrelationship between chronic pain and fatigue.

Keywords

Fatigue; exercise; acid; protons; low-pH saline

Pain arising from musculoskeletal disorders is a major clinical problem globally,⁵⁸ and fibromyalgia affects 2% of population in the United States.⁵⁷ As high as 76% of people with chronic widespread musculoskeletal pain conditions, such as fibromyalgia and arthritis, also report fatigue.²⁶ Conversely, as high as 94% of people with chronic fatigue syndrome report musculoskeletal pain and approximately half of the population has chronic widespread musculoskeletal pain.^{56,60} Pain is increased during and after exercise in people with fibromyalgia and chronic fatigue syndrome.^{20,48,54,55} Similarly, muscle fatigue is increased in people with chronic fatigue syndrome.^{3,10,23} This overlap between muscle fatigue and pain

syndromes suggests an interaction between fatigue and pain such that fatigue may enhance pain.

To assess mechanisms of chronic widespread pain, we developed an animal model that mimics the clinical symptoms. Two injections of acidic saline (pH 4.0), 5 days apart, into 1 muscle results in bilateral long-lasting hyperalgesia of the paw,⁴¹ muscle,⁵⁹ and the viscera²⁸ without muscle tissue damage.⁴¹ The intramuscular pH decreases to a peak of pH 6.0 after intramuscular injection of pH 4.0 saline and is reversed 6 to 7 minutes after injection.⁴¹ After 2 unilateral injections mechanical hyperalgesia develops not only ipsilaterally but also contralaterally, and lasts for 4 weeks. Blockade of NMDA receptors in the spinal cord or local anesthetic delivered to the rostral ventromedial medulla reverses the hyperalgesia once developed.^{38,51} Further, removal of primary afferent input by local anesthetic in the muscle, or ipsilateral dorsal rhizotomy has no effect on the contralateral hyperalgesia.⁴¹ Thus, the hyperalgesia associated with this model is maintained by changes in the central nervous system, and once developed is independent of input from the periphery.^{38,39,41}

Muscle fatigue is defined as a temporary loss of muscle force resulting from activity.⁵⁰ It can occur as a result of concentric, eccentric, or isometric contractions.³⁵ However, muscle damage and delayed onset muscle soreness occur predominately with eccentric contractions. Eccentric induced soreness is generally associated with inflammatory changes in exercised muscle that includes infiltration of leukocytes, increases in lactate, increases in phosphate, fatigue, and pain.^{2,8,11,30} In human subjects with experimental muscle pain, there is a decrease in muscle peak force and endurance in painful and in synergistic muscles.⁷ A low-intensity, 60-minute-duration tooth clenching task produces fatigue and pain during the task,⁴⁹ suggesting fatigue produces pain. However, after the tooth clenching task, subjects report continue to report pain and headaches.⁵² Further injection of glutamate into the fatigued muscle enhanced pain sensation and headaches, particularly in female subjects.⁵² However, the role of fatigue as a precipitating factor in the development of clinical pain syndromes is unknown. We hypothesized that muscle fatigue would enhance the hyperalgesia associated with injection of acidic saline into muscle by inducing muscle damage and increasing metabolites.

Materials and Methods

All experiments were approved by the Animal Care and Use Committee at the University of Iowa and are in accordance with the guidelines of the National Institute of Health and the International Association for the Study of Pain policies on the use of laboratory animals.

Animals

Male mice (C57BL/6J, 19–22 g), kept at a 12-hour darklight cycle (7^{AM} to 7^{PM} light; 7^{PM} to 7^{AM} dark) were used for the experiments. Animals were brought to the behavioral testing room 2 hours before the testing to acclimatize them to the testing environment. Behavioral tests were performed between 10^{AM} and 3^{PM}.

Fatigue Task

Before running, mice were acclimatized for 3 days in a mouse running wheel with a 10.3-cm diameter. The number of rotations was calculated with an external sensor and converted to kilometers, based on the size of the wheel. On the first training day, they were placed in the wheel for 10 minutes 3 times with 1-hour intervals between each session. On the second and third training day, mice were required to run for 10 minutes, in 3 separate sessions 1 hour apart. To ensure continuous running in the wheel, the top of the cage was tapped when the mice stopped running for greater than 5 seconds. For the running days, mice were run in the wheel for 2 hours and the total running distance was recorded. Mice ran 2 hours per day for 2 days

immediately before injection 1, and for 2 hours per day for 2 days immediately before injection 2 (Fig 1). The 2-hour duration of running was chosen through preliminary experiments that showed fatigue in response to the running task. Two days of running before each injection were chosen since we hypothesized that the 2-hour running task would induce muscle damage and increase muscle metabolites; and we thus expected to show a greater effect with 2 days of running before each injection.

Injection of Muscle

Immediately after the second day of running (day 0), mice were anesthetized briefly with halothane (2% to 4%) and 1 gastrocnemius muscle was injected with 20 μ L of pH 4.0 (n = 7), pH 5.0 (n = 7), pH 6.0 (n = 7), or pH 7.2 (n = 7) sterile saline.⁴¹ The pH was adjusted with HCl to within 0.1 pH. Five days later, immediately after the fourth day of running (day 5), the animals were re-anesthetized with halothane (2% to 4%) and the same gastrocnemius muscle was re-injected with 20 μ L of the same sterile saline at the same pH as on day 0 (Fig 1). A control group that did not run received 2 injections of pH 4.0 (n = 7), pH 5.0 (n = 6), pH 6.0 (n = 6), or pH 7.2 (n = 6) on day 0 and day 5.

Mechanical Withdrawal Response of the Paw

Mice were tested for response to mechanical stimulation of the paw with von Frey filaments. The animals were placed in lucite cubicles on an elevated screen platform and allowed to acclimate for 45–60 minutes. Von Frey filaments of varying bending forces (1.5, 0.7, 0.3, 0.2, 0.08 mN) were applied 10 times to the each plantar surface of the hindpaw and the number of withdrawals was recorded.³⁶ The mechanical withdrawal threshold was measured before the first run (day -1), before injection 1 (day 0), before the third run (day 4), before injection 2 (day 5), and 24 hours after the second injection (day 6) (Fig 1). In the current study, we interpreted an increase in the number of withdrawals as cutaneous hyperalgesia of the paw.

Measurement of Grip Force

In a separate group of animals, fatigue was confirmed by measurement of grip force immediately after running and comparing to the grip force before running for both the hind limbs and the forelimbs (n = 8). This was compared with a control group of mice that did not perform the running task (n = 8). Mice were trained 3 days before data collection to the grip force device. The mice were familiarized twice per day to the apparatus by performing the grip force task. Mice were pulled by the tail to the right to read grip force on the forelimb and then pulled to the left to read grip force on hind limb. Mice were pulled until a good grip was felt. Grip force was measured before and after every run: Day 0, day 1, day 4, day 5. Grip force was analyzed on both forelimb and hind limb and an average of 5 trials recorded. Each mouse had forelimb and hind limb measured before moving on to the next mouse. A decrease in grip force after running was interpreted as muscle fatigue.

Muscle Histology

To test for potential tissue damage, muscle histology was performed after the second and fourth day of 2 hours running (n = 4) and compared with a control group of mice that did not run (n = 4). Evans blue dye was used to assess for potential myocyte membrane damage. The gastrocnemius muscle was removed and frozen in isopentane cooled to -160°C . Muscles were step-sectioned at 100- μ m intervals. Cross sections were cut on a cryostat at 7 to 10 μ m, stained with hematoxylin and eosin, and examined by light microscopy.

Metabolic Changes in Muscle

Some animals (n = 8 fatigue and n = 8 control) were anesthetized with halothane (2% to 5%) and the gastrocnemius muscle was removed immediately after the second session of running

on day 0. The muscles were analyzed for p_{CO_2} and p_{O_2} with an ABL5 radiometer (GMI Inc, Ramsey, MN) and for lactate with YSI 2300 STAT Plus (YSI Inc, Yellow Springs, OH). In a separate group of animals after the 4th day of running, ($n = 4$ fatigue, $n = 4$ control) mice were deeply anesthetized, guillotined, and the gastrocnemius muscle was removed and placed into cold buffer containing 1% Triton X, 10 mmol/L Tris pH 4, 20 mmol/L EDTA, 10 mmol/L EGTA, 150 mmol/L NaCl, leupeptin, pepstatin A, and PMSF. Samples were homogenized, centrifuged, and filtered through a 0.2- μ m filter. CK-MB was measured with an ELISA kit obtained from Diagnostic Automotion, Inc (Calabasas, CA), and phosphate was measured using a kit obtained from Biomedical Research Service Center (Buffalo, NY). CK-MM was also measured by ELISA with a method developed in our laboratory. For CK-MM ELISA, the 1:200 primary antibody mouse anti-human CK-MM obtained from Scripts Laboratories (San Diego, CA) was loaded into PVC microtiter plate. The plate was covered and stored overnight at 4°C. The wells were washed twice with phosphate-buffered saline (PBS) and blocked with 3% bovine serum albumin/PBS for 2 hours. The wells were washed twice with PBS. The wells were loaded with 50 μ L of standard or sample. 50 μ L of 1:250 biotinylated anti mouse IgG was added to each of the wells and incubated for 1 hour, then the plate was washed twice with PBS. 50 μ L 1:500 streptavidin-horse-radish peroxidase was added to each well. The plates were then washed 3 times with PBS. 50 μ L of TMB was added and the plate incubated at room temp for 15 minutes. The reaction was stopped with 50 μ L of 0.1N sulfuric acid. The CK-MM and CK-MB plates were read at 490 nm and the phosphate plate was read at 650 nm on a spectromax (Molecular Devices, Sunnyvale, CA), all samples except blood gases and lactate were measured in duplicate.

Statistical Analysis

A repeated-measures ANOVA tested for changes in the number of responses to repeated mechanical withdrawal threshold across time and between groups for each individual von Frey filaments. A repeated-measures ANOVA also tested for changes in grip force across time and between groups. A one-way ANOVA for group tested for differences between groups (fatigue vs control) for p_{O_2} , p_{CO_2} , lactate, phosphate, CK-MM, and CK-MB. Data were considered significant if $P < .05$. Data are represented as mean $\pm$ SEM.

Results

Induction of Fatigue

Running distance averaged 2.16 ± 0.52 km/2 h for all groups and the distance was similar at each time and between groups. In the running group, the grip force was significantly less than the baseline grip force immediately after the running task and from the control non-running group for both the forelimbs and the hind limbs (Fig 2). For the forepaws, the grip force after the 4th run was increased in 4 of 8 animals and decreased in another 4 of 8 animals, which resulted in a net effect of no change.

Cutaneous Hyperalgesia

For the mice injected intramuscularly with pH 4.0 saline, both the fatigued and the control groups showed a bilateral increase in the number of responses to mechanical stimuli 24 hours after injection (Fig 3). The pH 5 group responded differently between groups; there was a significant increase in the number of responses to mechanical stimuli 24 hours bilaterally after the second injection of acidic saline for mice that were fatigued when compared with control animals (Fig 3). This increase in mechanical sensitivity after injection of pH 5.0 into fatigued mice was of the same magnitude observed after injection of pH 4.0 (Fig 3). On the other hand, injections of pH 6 and pH 7.2 did not produce significant change in the number of responses to mechanical stimuli in the both groups when compared with baseline values. There were no

changes at other time points tested for either the ipsilateral or the contralateral hindpaw (data not shown).

Local Muscle Histology and Metabolism

No signs of muscle damage were observed in the fatigue group. No visual histological changes were observed in the muscle after 2 days of 2-hour running (Fig 4), or after 4 days of 2-hour running (data not shown). Similarly, there was no difference in muscle lactate, p_{CO_2} , and p_{O_2} after 2 days of 2-hour running; or for phosphate and creatinine kinase (CK-MB or CK-MM) after 4 days of 2-hour running when comparing the fatigue group with the nonfatigue group (Fig 5).

Discussion

The current study shows there was a similar increase in sensitivity to mechanical stimuli after 2 injections of pH 4.0 in animals with muscle fatigue and in control mice that were not fatigued. In control mice, 2 intramuscular injections of pH 5.0, 6.0, or 7.2 had no effect on the sensitivity to mechanical stimuli. However, in animals with muscle fatigue, 2 injections of pH 5.0 increased the mechanical sensitivity of the paw similar to that observed after 2 injections of pH 4.0. These data suggest that the fatigue task primed the nervous system to enhance nociceptive responses to acidic pH that were previously subthreshold.

Fatigue is defined as a temporary loss in force as a result of recent muscle contraction.⁵⁰ The current study shows an approximately 10% decrease in force after 2 hours of wheel running and is thus defined as a fatigue task. Since fatigue recovers quickly this loss of force probably is a conservative estimate. There are numerous mechanisms of fatigue that include changes in the central nervous system, changes in the microenvironment of the muscle (peripheral fatigue), or changes in excitation-contraction coupling (peripheral fatigue).^{1,2,50}

Peripheral Mechanisms for Enhanced Nociception After Fatigue

One peripheral mechanism by which an enhanced response to acidic pH could occur is through metabolic changes in the muscle as a result of fatigue. Peripherally, fatiguing exercise can result in changes in the muscle tissue that includes increases in lactate, phosphate and creatinine kinase, decreases in pH, and infiltration of neutrophils.^{2,8,11,30} Changes in these substances could directly activate nociceptors through acid sensing ion channels and increased release of cytokines and prostaglandins.^{5,22,27,31,42,43,47} Alternatively, there could be changes in the buffering capacity of the muscle to maintain pH such that a pH of 5 is buffered slower than under nonfatigue conditions.^{13,33} However, the current study was unable to detect changes in lactate, phosphate, creatinine kinase, or to detect infiltration of neutrophils in the muscle, suggesting that metabolic changes in the muscle associated with damage are unlikely to contribute to the enhanced nociceptive response to pH. Changes in blood lactate clearly occur during a fatiguing exercise bout,^{12,16,34,37} and these lactate changes can last up to 15 minutes after a high-intensity exercise bout.^{16,17,37} In the current study, the animals spontaneously ran in the running wheel and thus were not likely exercising at a strong intensity typically observed in studies that measure lactate. It is further possible that increases in lactate occurred during the 2-hour fatigue task, even though we were unable to measure the changes after the fatigue task. Regardless, we measured metabolic changes in the muscle at the same time as we would have given the intramuscular injection of saline. Thus, if metabolic changes occurred they were resolved quickly and were not present at the time of injection. It should be also pointed out that there are minimal changes in the periphery after injection of acidic saline. The intramuscular pH after injection of pH 4 decreases to an average of pH 6.5 and after injection of pH 5 to an average of pH 6.9. There is also no change in muscle after injection of pH 4 examined histologically. Therefore, a peripheral mechanism is unlikely since the induction and

maintenance of the hyperalgesia associated with this model involves changes in the central nervous system^{38,39,41,51} (outlined below) and we were unable to detect long-lasting changes in muscle metabolism or muscle damage after the fatigue task.

Central Mechanisms for Enhanced Nociception After Fatigue

An alternate mechanism could be that the enhanced response to pH after fatigue is mediated by changes in the central nervous system. Indeed, in this model of muscle-induced hyperalgesia there is a long-lasting increased mechanical sensitivity that is maintained by changes in the central nervous system.^{38–41,44,51} The contralateral hyperalgesia that develops after the second intramuscular acid injection is independent of input from the periphery⁴¹ and prevented or reversed by blockade of receptors in the spinal cord.³⁸ There is also an enhanced release of the excitatory neurotransmitter glutamate in the spinal cord in response to the second injection of acidic saline.³⁹ Recently, we showed that anesthetic blockade of supraspinal sites in the medulla 1) prevent the development of the hyperalgesia that occurs in response to the second injection of acidic saline and 2) reverse the mechanical hyperalgesia once developed.⁵¹ These sites in the rostral ventromedial medulla integrate both sensory and motor systems and thus changes in these sites could enhance nociceptive responses after a fatigue task. Serotonergic neurons in the nucleus raphe obscurus and pallidus in the medulla are implicated in fatigue since there is a strong correlation between neuronal activity and motor activity.¹⁹ Both the nucleus raphe obscurus and pallidus, in addition to the nucleus raphe magnus, have also been shown to facilitate nociception⁶¹ specifically in this muscle-induced model of hyperalgesia.⁵¹

Exercise Training and Analgesia

Chronic running could also result in decreased endogenous inhibition. Allowing rats free access to a running wheel for 3 weeks resulted in lower tail flick latencies compared with nonrunning rats.^{9,21,24,25,45,46} The active rats showed a lower analgesic response to opioid analgesics administered peripherally, systemically, or into the periaqueductal gray in the midbrain.^{24,25} These data suggest that running wheel activity increases release of endogenous opioid peptides. With repeated running and continued opioid release tolerance occurs at opioid receptors and thus there is less endogenous inhibition. Noxious stimulation clearly increases activity in the descending inhibitory systems and removal of this inhibition enhances nociception.⁵³ In the current study, however, baseline withdrawal thresholds were similar between groups suggesting that opioid tolerance did not occur with 4 days of running.

On the other hand, low intensity, short duration treadmill exercise (15–30 min/d for 5 days) reduces hyperalgesia in rats after repeated intramuscular injections of pH 4.0 saline.⁴ Similarly, in rats with spinal cord injury, low-intensity treadmill exercise (20–25 min/d for 7 weeks) reduces the enhanced sensitivity to mechanical stimuli. Naloxone prevents the analgesic effects of low intensity exercise suggesting opioid mechanisms are responsible for the reduction in hyperalgesia.⁴ However, decreases in brain-derived neurotrophic factor (BDNF) mRNA in the spinal cord and muscle that normally occur after spinal cord injury are normalized by exercise, and neurotrophin-3 mRNA increases in the soleus after exercise training.¹⁸ Similarly, mice that overexpress neurotrophin-3 in muscle have decreased mechanical hyperalgesia to acidic saline¹⁵ and BDNF in the periphery or spinal cord reduces hyperalgesia.³² Together these studies suggest there may be both peripheral and central mechanisms underlying the responses to exercise, fatigue and enhanced nociception.

Clinical Implications

Clinically, fatigue is common in many musculoskeletal conditions; people with chronic fatigue have significant musculoskeletal pain. There is a clear association between the severity of musculoskeletal pain and disability as well as between the severity of fatigue and disability in people with chronic fatigue syndrome.²⁹ This fatigue is described by subjects as a reduced

amount of energy needed to perform normal activities of daily living. However, in people with musculoskeletal pain or chronic fatigue syndrome there is decreased muscle force compared with normal subjects,^{3,10,23} and there is commonly pain on or after exercise.^{20,48,54,55} Clearly, exercise is an effective treatment for people with musculoskeletal pain conditions such as fibromyalgia and chronic fatigue syndrome^{6,14} decreasing pain, fatigue, and/or improving function. This exercise, however, must be carefully monitored to avoid worsening of symptoms. In people with fibromyalgia and chronic fatigue syndrome ‘pacing’ is a commonly utilized strategy that balances the amount of physical activity with rest to achieve optimal physical function without exacerbation of symptoms. This type of strategy is utilized not only for developing an appropriate exercise program but also for all activities of daily living. Importantly, pacing suggests that there is a dose of exercise which is analgesic and one in which you can exacerbate the pain. It is therefore imperative to find a level of exercise that does not produce excessive fatigue and enhance or promote pain.

Summary

In summary, the current study shows that muscle fatigue enhances the probability of the development of mechanical hyperalgesia in mice in response to acidic saline without histological change. This increased mechanical hyperalgesia occurs in mice intramuscularly injected with pH 5 saline, a pH that does not produce hyperalgesia in nonfatigued mice. The fatigue task did not produce measurable changes in the muscle tissue suggesting muscle damage did not occur not contribute to the hyperalgesia observed with pH 5. Thus, fatigue could play a critical role in the development of chronic musculoskeletal pain.

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Figure 1.

Time line for the experiment is shown. The running task was performed on day -1, day 0, day 4, and day 5. Before the running task, the mechanical withdrawal threshold of the paw (MWT) was tested on each day and again 24 hours after the second intramuscular acid injection. Injection of acid was delivered immediately after the running tasks on day 0 and day 5.

Figure 2.

Line graphs show the withdrawal force in grams before (*B*) and after (*A*) each 2-hour running session (1, 2, 3, 4) for both the hindpaws and the forepaws. There was a significant decrease in force immediately after each run ($*P < .05$) in the running group (fatigue; closed symbols) compared with the group that did not run (control; open symbols). Data are mean SEM.

Figure 3.

Line graphs show the number of withdrawals (of 10) to von Frey filaments (0.08, 0.2, 0.4, 0.7, 1.5 mN) for the ipsilateral (top panels) and contralateral (bottom panels) paws from animals that ran (fatigue; squares) and control animals that did not run (control; circles). Each panel shows data for rats that received 2 intramuscular injections of different pH (4.0, 5.0, 6.0, 7.2). Open symbols represent data before the first intramuscular injection of saline and the closed symbols represent data 24 hours after the second injection of saline. Significant difference between the fatigue and control groups was observed 24 hours after the second intramuscular injection of pH 5.0 saline (* $P < .05$). Data are mean $\pm$ SEM.

Figure 4. Representative sections from muscle from an animal that was fatigued compared with a nonfatigued animal. There was no visible tissue damage in the fatigue group (fatigue; $n = 4$) when compared with control group (control; $n = 4$).

Figure 5.

Bar graphs depict muscle concentrations of lactate, pCO₂, pO₂, CK-MM, CK-MB, and phosphate for the control (C) and the fatigue (F) groups. There was no significant difference in any measure. Data are mean $\pm$ SEM.