Hyperbaric oxygen improves contractile function of regenerating rat skeletal muscle after myotoxic injury

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Gregorevic, Paul, Gordon S. Lynch, and David A. Williams. Hyperbaric oxygen improves contractile function of regenerating rat skeletal muscle after myotoxic injury. J Appl Physiol 89: 1477–1482, 2000.—There is growing interest in hyperbaric oxygen (HBO) as an adjunctive treatment for muscle injuries. This experiment tested the hypothesis that periodic inhalation of HBO hastens the functional recovery and myofiber regeneration of skeletal muscle after myotoxic injury. Injection of the rat extensor digitorum longus (EDL) muscle with bupivacaine hydrochloride causes muscle degeneration. After injection, rats breathed air with or without periodic HBO [100% O₂ at either 2 or 3 atmospheres absolute (ATA)]. In vitro maximum isometric tetanic force of injured EDL muscles and regenerating myofiber size were unchanged between 2 ATA HBO-treated and untreated rats at 14 days postinjury but were −11 and −19% greater, respectively, in HBO-treated rats at 25 days postinjury. Maximum isometric tetanic force of injured muscles was −27% greater, and regenerating myofibers were +41% larger, in 3 ATA HBO-treated rats compared with untreated rats at 14 days postinjury. These findings demonstrate that periodic HBO inhalation increases maximum force-producing capacity and enhances myofiber growth in regenerating skeletal muscle after myotoxic injury with greater effect at 3 than at 2 ATA.

INHALATION OF PURE OXYGEN at pressures greater than that experienced at sea level is used as an adjunctive treatment for a variety of serious conditions and is concerned typically with removing gas or air emboli (17), overcoming ischemia and/or hypoxia (9), reducing compartmental edema (31), or neutralizing anaerobic necrotizing bacteria (11). Despite increased interest in the use of hyperbaric oxygen (HBO) as an adjunctive treatment for tendon and muscle injuries in athletes (22, 28), such injuries are not recognized as legitimate indications for the application of HBO according to the Undersea and Hyperbaric Medicine Society, which cites a lack of scientific support (7, 10). Most work on HBO, specifically with regard to skeletal muscle, has focused on ameliorating the effects of experimental ischemia-reperfusion injury or bacterial necroses (8, 14). Published studies of the effect of HBO that are based on other models of muscle injury are comparatively scarce (3, 13, 20, 29, 30), and of those that consider the recovery of functional capacity, the results are equivocal. The purpose of this study was to examine the effects of periodic HBO treatment on functional and structural properties of regenerating skeletal muscle after muscle-specific degeneration. Bupivacaine hydrochloride is a proven myotoxic agent (25) that increases cytosolic calcium (33), subjecting the muscle fibers to the cytotoxic effects associated with high intracellular calcium concentrations (2). Intramuscular injection of bupivacaine to a muscle’s holding capacity causes degeneration of muscle fibers within the first 2 days and a reduction in maximal isometric tetanic force production (Pₒ) of −90% compared with control values from untreated muscles (5). Muscle fiber degeneration is followed by complete regeneration and recovery of functional properties in the rat within 60 days (25). This model of injury is specific for muscle fibers (26), thereby eliminating the complicating factors of damage to motor nerves and associated blood vessels that occur with other models of muscle injury (3, 20). Using the bupivacaine model of muscle injury, we tested the hypothesis that HBO would hasten functional recovery after degeneration by enhancing myofiber growth during regeneration.

METHODS

Experimental injury. All procedures were approved by the Animal Experimentation Ethics Committee of The University of Melbourne and conformed to the guidelines for the care and use of experimental animals as described by the National Health and Medical Research Council of Australia. Adult (350–400 g) male Sprague-Dawley rats were anesthetized with methohexite sodium (60 mg/kg body wt ip; Brietal, Eli Lilly, Indianapolis, IN) such that they were unresponsive to tactile stimuli. The extensor digitorum longus (EDL) muscle of the right hindlimb was surgically exposed and injected with a total volume of ~1,000 μl of 0.5% bupivacaine hydrochloride (Marcain, Astra, North Ryde, New South Wales, Australia) by two injections in each of the proximal, midbelly, and distal regions of the muscle. This was equivalent to, or exceeded, the maximum volume of bupivacaine that each EDL muscle could hold (unpublished observations) and that caused degeneration of the entire muscle mass. After injection, the small skin incision was closed with Michel clips (Aesculap, Tuttingen, Germany) and swabbed with povidone iodine solution.

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Postinjection protocol. At the completion of surgery, rats were exposed to either air only (STD), or air with intermittent hyperbaric oxygen (HBO) sessions within a small experimental hyperbaric chamber (Professional Diving Services, Portland, Victoria, Australia), generously loaned by the Hyperbaric Medicine Unit of The Alfred Hospital (Melbourne, Victoria, Australia). STD group rats were returned to their cages after bupivacaine injection, where they remained for the duration of the treatment.

Rats in HBO groups underwent HBO treatment once daily (commencing ~1 h postinjection). Each HBO session consisted of 1) 10 min in the chamber while it was sealed, flushed, and pressurized with 100% oxygen (Industrial-grade oxygen, BOC, Preston, Victoria, Australia) to the desired pressure; 2) inhalation of 100% O₂ for 90 min at 2 ATA or 60 min at 3 ATA with a constant flow rate of 3 l/min; and 3) 10 min in the chamber while it was depressurized and opened.

Where possible, tissue harvesting, testing, processing, and analyzing were performed on pairs of rats or tissue samples (i.e., 1 STD and 1 HBO animal) to minimize interday and intergroup variation in any aspect of the experiment. A total of six experimental groups were used in this study: rats breathing normal air matched with rats breathing and periodic 2 ATA HBO over 14 days, rats breathing normal air matched with rats breathing air and periodic 3 ATA HBO over 14 days, and rats breathing normal air matched with rats breathing air and periodic 2 ATA HBO over 25 days. One injured and one uninjured muscle was tested from each animal in these groups. Daily handling and examination for injured and one uninjured muscle was tested from each animal in these groups. Daily handling and examination for the duration of the study confirmed that no rats exhibited adverse effects to either the initial surgical procedure or the postsurgery treatments.

Contractile properties. At either 14 or 25 days postinjury, rats were anesthetized with pentobarbital sodium (60 mg/kg body wt ip; Nembutal, Rhone Merieux, Pinkenba, Queensland, Australia) with supplemental doses administered to maintain a depth of anesthesia that prevented all responses to tactile stimuli. Once an appropriate depth of anesthesia had been attained, the EDL muscles were surgically excised. Proximal and distal tendons were firmly tied with braided surgical silk (30 USP Davis & Geck, Cyanamid, Dandenong, Victoria, Australia), with the associated nerve and vessel supplies always trimmed last to ensure optimum condition of muscles before entering the organ bath. At the completion of the surgical procedures, the rats were killed by cervical dislocation while deeply anesthetized.

Isometric contractile properties of the EDL muscles were evaluated in vitro. Each muscle was transferred to a custom-built Plexiglas bath filled with Krebs-Ringer solution (137 mM NaCl, 24 mM NaHCO₃, 11 mM d-glucose, 5 mM KCl, 2 mM CaCl₂, 1 mM NaH₂PO₄·H₂O, 487 μM MgSO₄·7H₂O, 293 μM d-tubocurarine chloride) that was bubbled with Carbogen (5% carbon dioxide-95% oxygen, BOC) and thermostatically maintained at 25°C. The muscles were tied directly between a fixed immovable hook and a force transducer (Research grade 60-2999, Harvard Instruments, South Natick, MA). Muscles were field stimulated by supramaximal square-wave pulses (0.2-ms duration; model S88 stimulator, Grass Instruments, Quincy, MA) that were amplified (DC300A Laboratory power amplifier, Crown International, Elkhart, IN) and delivered to two platinum plate electrodes that flanked the length of the muscle to produce a maximum isometric contraction.

Optimum muscle length (L₀) and optimum stimulation voltage were determined from micromanipulation of muscle length and a series of twitch contractions. Optimum fiber length (L₁) was calculated by using the previously determined L₁-to-L₀ ratio of 0.44 for the EDL muscle (4). L₁/L₀ is not affected by bupivacaine-induced degeneration and regeneration (25) because new fibers grow into the spaces of the preexisting basal laminae (which are preserved despite bupivacaine-induced fiber degeneration). P₀ was determined from the plateau of the frequency-force relationship after stimulation at 10, 30, 50, 80, 100, 120, and 150 Hz with 3 min of rest between recordings to prevent fatigue. Contractile measurements were recorded by using a four-channel MacLab recorder (MacLab 4/s, ADInstruments, Castle Hill, New South Wales, Australia) run by an Apple Macintosh 7220/200 computer (Apple Computer, Cupertino, CA) operating Chart data acquisition software (v3.5.6/s, ADInstruments). After force testing, muscles were removed from the bath, trimmed of their tendons and any adhering nonmuscle tissue, blotted on filter paper, and weighed on an analytic balance. Muscle mass, L₁, and P₀ were used to calculate maximum specific isometric tetanic force (fPo) or force normalized per total muscle fiber cross-sectional area (CSA; in kN/m²). Values for fPo obtained from the uninjured muscles of air treated rats were consistent with the accepted values in the literature (5, 25). However, the most important measure of muscle regeneration was derived from the deficit in P₀ between the injured and uninjured muscles.

Morphological properties. After weighing, each muscle was tied at L₀ to a small wooden stick, snap frozen in isopentane cooled in liquid nitrogen, and stored at −80°C. From as close to the middle of each muscle midbelly as possible, 8-μm-thick serial transverse sections were cut (CTI cryostat, IEC, Needham Heights, MA; operating at −20°C) and placed on uncoated glass slides. Muscle sections were stained with hematoxylin and eosin and coverslipped.

Digitized images (12-bit gray scale) of muscle sections were acquired using an upright microscope (BH-2, Olympus, Toyoko, Japan) with camera (Spot model 1.3.0, Diagnostic Instruments, Sterling Heights, MI), driven by Spot diagnostic software (v2.1, Diagnostic Instruments). Image files were analyzed with Image Pro (v4.0, Media Cybernetics, Silver Spring, MD) whereby the mean CSA of muscle fibers was calculated by interactive determination of the circumference of at least 100 adjacent cells from the center of every muscle section examined.

Statistical analyses. All values in the text and tables are reported as means ± SE. Experimental groups from each time point were compared by using a general linear model, two-factor ANOVA for influences of treatment type (STD vs. HBO) and limb tested (control vs. injured), by using Fisher’s least significant difference post hoc multiple-comparison procedure to identify differences between specific groups. Parameters describing injured muscle relative to uninjured muscle (injured/control) were analyzed for time or treatment effects by using Fisher’s least significant difference post hoc multiple-comparison procedure. In all cases, differences between groups were considered significant when P < 0.05.

RESULTS

Contractile properties. No differences were observed in uninjured muscles between treatment groups at either time point (Tables 1–3). P₀ and fPo of injured muscles from untreated animals at 14 days after bupivacaine injection (14–2STD group in Table 1) were 47.8 and 47.4%, respectively, of contralateral control muscles. At 14 days postinjury, there were no differences between rats receiving periodic 2 ATA HBO and rats receiving air only, with the exception of the mean
mass for injured muscles, which was ~8% lower in HBO-treated rats. P o and sP o of injured muscles from rats breathing air only for 14 days postinjury (14–3STD group in Table 2) were 51.5 and 44.7% of contralateral control values, respectively. P o and sP o of injured muscles after treatment with 3 ATA HBO for 14 days were 60.8 and 58.8% of control values, respectively. Mean P o and sP o of injured muscles were higher (P < 0.05) for rats treated with 3 ATA HBO than for rats breathing air only. Furthermore, the deficit in P o between injured and uninjured muscles was significantly smaller in rats treated with 3 ATA HBO than in rats treated with 2 ATA HBO at the same time.

At 25 days postinjury, P o and sP o were reduced by 12 and 36%, respectively, in injured muscles compared with contralateral controls for rats breathing air only, although the deficit between injured and uninjured muscles was less than that in rats treated at 14 days (P < 0.05). The P o of injured muscles from rats receiving 2 ATA HBO for 25 days was increased 11% compared with muscles from rats receiving air only and not different from P o of uninjured contralateral muscles.

The sP o of injured muscles was not different for rats receiving 2 ATA HBO for 25 days vs. untreated rats, but it was reduced by 28.5% compared with the sP o of uninjured contralateral muscles. Injured muscle P o and sP o, expressed as a percentage of uninjured muscle P o and sP o, were increased 12.7 and 11%, respectively, in HBO-treated rats at 25 days postinjury (P < 0.05).

The mass of injured muscles relative to the mass of uninjured contralateral muscles was not different for treated and untreated rats at 25 days postinjury.

### Morphological properties

HBO treatment had no effect on the mean CSA of myofibers in uninjured muscles. Sections from injured muscles were characterized by entire populations of multinucleated fibers, with a high proportion of centrally located nuclei (Fig. 1, B and C). Mean CSA of fibers in injured muscles was reduced by ~27% in untreated rats examined at 14 days postinjury.

### Table 1. Summary of data for in vitro evaluation of contractile and morphological properties of control and injured EDL muscles from rats receiving air only or periodic hyperbaric oxygen at 2 ATA tested at 14 days postinjury

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Injured</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM, g</td>
<td>415 ± 4</td>
<td>403 ± 5</td>
</tr>
<tr>
<td>MM, mg</td>
<td>176.1 ± 2.7</td>
<td>184.1 ± 3.3†</td>
</tr>
<tr>
<td>L o, mm</td>
<td>34.4 ± 0.4</td>
<td>35.6 ± 0.3†</td>
</tr>
<tr>
<td>Cell CSA, µm²</td>
<td>2,337 ± 110</td>
<td>1,614 ± 56*</td>
</tr>
<tr>
<td>P o, mN</td>
<td>3,139 ± 86*</td>
<td>1,483 ± 83*</td>
</tr>
<tr>
<td>sP o, kN/m²</td>
<td>286.6 ± 8.4*</td>
<td>154.2 ± 8.6*</td>
</tr>
<tr>
<td>Inj/Con (%)</td>
<td>104.8 ± 2.4</td>
<td>97.4 ± 2.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. 14-2STD, rats breathing air only (STD) for 14 days postinjury; 14-2HBO, rats breathing air and periodic hyperbaric oxygen (HBO) at 2 atmospheres absolute (2 ATA) for 14 days postinjury; MM, muscle mass; L o, optimal muscle length; P o, absolute maximum tetanic force; sP o, specific maximum tetanic force; cell CSA, myofiber cross-sectional area. Inj/Con, parameters of the injured limb expressed as a percentage of the respective control (uninjured) limb values. *P < 0.05 for injured vs. respective control by 2-tailed Student’s t-test. †P < 0.05 for HBO treated vs. STD and §P < 0.05 for HBO vs. STD, by Fisher’s post hoc multiple-comparison procedure.

### Table 2. Summary of data for in vitro evaluation of contractile and morphological properties of control and injured EDL muscles from rats receiving air only or periodic hyperbaric oxygen at 3 ATA tested at 14 days postinjury

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Injured</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM, g</td>
<td>224.4 ± 8.5</td>
<td>264.9 ± 22.5</td>
</tr>
<tr>
<td>MM, mg</td>
<td>36.6 ± 0.4</td>
<td>37.2 ± 0.4</td>
</tr>
<tr>
<td>L o, mm</td>
<td>2,918 ± 325</td>
<td>2,146 ± 263*</td>
</tr>
<tr>
<td>P o, mN</td>
<td>3,467 ± 140</td>
<td>1,799 ± 192*</td>
</tr>
<tr>
<td>sP o, kN/m²</td>
<td>265.5 ± 13.1</td>
<td>117.1 ± 4.9*</td>
</tr>
<tr>
<td>Inj/Con, %</td>
<td>117.6 ± 7.4</td>
<td>105.2 ± 2.2†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. 14-3STD, rats breathing air only for 14 days postinjury; 14-3HBO, rats breathing air and periodic HBO at 3 ATA for 14 days postinjury. *P < 0.05 for injured vs. respective control by 2-tailed Student’s t-test. †P < 0.05 for injured muscles HBO treated vs. STD; §P < 0.05 for HBO vs. STD; and §P < 0.05 for 14-3HBO vs. 14-2HBO, by Fisher’s post hoc multiple-comparison procedure.

### Table 3. Summary of data for in vitro evaluation of contractile and morphological properties of control and injured EDL muscles from rats receiving air only or periodic hyperbaric oxygen at 2 ATA tested at 25 days postinjury

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Injured</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM, g</td>
<td>538 ± 25</td>
<td>539 ± 27</td>
</tr>
<tr>
<td>MM, mg</td>
<td>239.9 ± 11.2</td>
<td>351.1 ± 15.5*</td>
</tr>
<tr>
<td>L o, mm</td>
<td>36.6 ± 0.2</td>
<td>37.5 ± 0.8</td>
</tr>
<tr>
<td>Cell CSA, µm²</td>
<td>2,897 ± 157</td>
<td>2,420 ± 199</td>
</tr>
<tr>
<td>P o, mN</td>
<td>3,417 ± 82</td>
<td>2,997 ± 53*</td>
</tr>
<tr>
<td>sP o, kN/m²</td>
<td>243.0 ± 5.4</td>
<td>151.0 ± 7.6*</td>
</tr>
<tr>
<td>Inj/Con, %</td>
<td>143.3 ± 2.5</td>
<td>141.3 ± 1.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. 25-2STD, rats breathing air only for 25 days postinjury; 25-2HBO, rats breathing air and periodic HBO at 2 ATA for 25 days postinjury; *P < 0.05 for injured vs. respective control by 2-tailed Student’s t-test. †P < 0.05 for injured muscles HBO treated vs. STD and §P < 0.05 for HBO vs. STD, by Fisher’s post hoc multiple-comparison procedure. §P < 0.05 for HBO vs. STD by 2-tailed Student’s t-test.
days postinjury (Tables 1 and 2). Mean CSA of fibers in injured muscles from 2 ATA HBO-treated rats was also reduced at 14 days after bupivacaine injection, and there was no difference in the mean CSA of regenerating muscle fibers from 2 ATA HBO-treated and untreated rats at this time point. At 14 days postinjury, the mean CSA of fibers from the contralateral limb muscles and greater than the CSA of fibers from the injured muscles (expressed relative to fibers from uninjured muscles) of rats breathing air only \( (P < 0.05) \). At 14 days postinjury, the CSA of regenerating fibers (relative to uninjured fibers) was significantly greater in rats treated with 3 ATA HBO than in rats treated with 2 ATA HBO (Tables 1 and 2).

At 25 days postinjury, the CSA of regenerating myofibers was \( \sim15\% \) smaller in untreated rats, and 13\% larger in 2 ATA HBO-treated rats, than the CSA of myofibers from the respective contralateral muscles. At 25 days postinjury, the relative (%) CSA of regenerating muscle fibers compared with contralateral control muscle myofibers, was \( \sim32\% \) larger in the 2 ATA HBO-treated group \( (P < 0.05) \).

**DISCUSSION**

The most important finding of this study was that bupivacaine-injected EDL muscles produced more force after exposure to HBO, with greater effect after treatment over 14 days at 3 than at 2 ATA. Injured muscles from rats treated for 25 days with periodic HBO at 2 ATA produced more force than bupivacaine-injected muscles from rats breathing air only and were composed of regenerating muscle fibers of larger relative CSA. Periodic treatment for 14 days with 2 ATA HBO did not increase the functional capacity or size of regenerating fibers in bupivacaine-injected muscles. In contrast, periodic treatment for 14 days with 3 ATA HBO increased the maximum force-producing capacity of bupivacaine-injected muscles, and the regenerating myofibers were of similar size compared with uninjured fibers from the contralateral EDL muscle. The findings of the present study demonstrate that HBO enhances the recovery of contractile function in regenerating skeletal muscle previously subjected to a muscle-specific mode of degeneration.

Studies that have considered HBO as a treatment for muscle injury per se (as opposed to ischemia-reperfusion injuries or bacterial necroses) have used either a contusion or contraction-induced protocol to cause muscle damage \( (3, 20, 29, 30) \). A limited number of these studies have reported mixed results on the functional recovery of regenerating skeletal muscle with HBO treatment \( (3, 20, 30) \). Although these studies provide information on the consequences of HBO exposure for the particular models, it is difficult to identify the mechanisms associated with specific effects of HBO application in each instance. One complicating factor of these studies is that contusion and contraction-induced injury models both involve a degree of hemorrhage arising from the initial injury \( (3, 20) \), with deleterious consequences for circulatory delivery to and from the site of injury. It is well established that HBO application can deliver greater than typical oxygen concentrations to peripheral tissues, which can override the regional ischemia and/or hypoxia that occurs when vasculature is compromised \( (12) \). Thus it is important to determine whether HBO application simply compensates for the reduction in peripheral oxygen partial pressures during the hypoxic interim postinjury or whether it also involves a muscle-specific effect.

![Fig. 1. Photomicrographs of cross sections of extensor digitorum longus muscles from rats treated with air only. A: uninjured muscle. B: injured muscle examined 14 days postinjury. C: injured muscle examined 25 days postinjury. Note presence of centrally nucleated fibers (examples indicated by closed-head arrows) and multinucleated fibers (example indicated by open-head arrow) in B and less frequently in C. Scale bar = 100 μm.](image-url)
This study examined the structure and function of regenerating rat skeletal muscle to determine the influence of HBO exposure specifically on the regenerative process. The well-characterized degeneration of skeletal muscle after intramuscular injection with 0.5% bupivacaine injection enabled this study to focus on the effects of the HBO intervention on muscle without the complication of nerve injury or hypoxia (26). Injection of a muscle to its holding capacity with bupivacaine causes almost total degeneration of the muscle mass and a concomitant ~90% reduction in force-producing capacity (5, 25), unlike the focal injury after contusion or contraction-induced injury. The damaged muscle fibers are replaced through regeneration of new fibers within the preexisting basal laminae structures, and full functional recovery is attained within 60 days. Studying the effect of HBO on a focal injury complicates the investigation because it becomes unclear whether the intervention is acting to ameliorate secondary tissue damage postinjury and so reduce the magnitude of damage caused or augmenting the regeneration of new muscle tissue. With complete degeneration of the muscle after injection of bupivacaine, the mode of action for HBO is focused on the processes associated with skeletal muscle regeneration (26).

Aside from the well-demonstrated effects of HBO on gas embolism and compartment syndrome conditions, current theory suggests that the potential benefits of treatment with HBO are limited to models of injury associated with the reduced partial pressure of oxygen within the wound site (14, 23, 28). Restoring oxygen delivery in wounds of this type to normal levels assists with the reestablishment of a previously compromised blood supply or with the repair of associated nerve components. Furthermore, it is evident that the improved functional recovery of regenerating muscles after HBO treatment in rats was affected by the pressure of oxygen inspired, at concentrations above that experienced breathing air at sea level. We conclude that HBO treatment at 2 or 3 ATA increases the maximum force-producing capacity of regenerating muscles and the size of the regenerating muscle fibers after myotoxic injury, with greater effect following 14 days of HBO treatment at 3 ATA than at 2 ATA.

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REFERENCES

10. Hirn M.
11. Jain KK.
12. James PB, Scott B, and Allen MW.
13. Kindwall EP.
15. Narkowicz CK, Vial JH, and McCartney PW.
17. Cianci P.
18. McDermott JJ, Dutka AJ, Koller WA, and Flynn ET.