Muscle Na-K-pump and fatigue responses to progressive exercise in normoxia and hypoxia

S. D. Sandiford, H. J. Green, T. A. Duhamel, J. D. Schertzer, J. D. Perco, and J. Ouyang
Department of Kinesiology, University of Waterloo, Ontario, Canada
Submitted 22 September 2004; accepted in final form 22 April 2005

Sandiford, S. D., H. J. Green, T. A. Duhamel, J. D. Schertzer, J. D. Perco, and J. Ouyang. Muscle Na-K-pump and fatigue responses to progressive exercise in normoxia and hypoxia. Am J Physiol Regul Integr Comp Physiol 289: R441–R449, 2005. First published April 28, 2005; doi:10.1152/ajpregu.00652.2004.—To investigate the effects of hypoxia and incremental exercise on muscle contractility, membrane excitability, and maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity, 10 untrained volunteers (age = 20 ± 0.37 yr and weight = 80.0 ± 3.54 kg; ± SE) performed progressive cycle exercise to fatigue on two occasions: while breathing normal room air (Norm; 80.0/11006/H11006) and while breathing a normobaric hypoxic gas mixture (Hypox; FIO\textsubscript{2} = 0.14). Muscle samples extracted from the vastus lateralis before exercise and at fatigue were analyzed for maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase (K\textsuperscript{+}-stimulated 3-O-methylfluorescein phosphate activity) in homogenates. A 32% reduction (P < 0.05) in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity was observed (90.9 ± 7.6 vs. 62.1 ± 6.4 nmol·mg protein\textsuperscript{-1}·h\textsuperscript{-1}) in Norm. At fatigue, the reductions in Hypox were not different (81 ± 5.6 vs. 57.2 ± 7.5 nmol·mg protein\textsuperscript{-1}·h\textsuperscript{-1}) from Norm. Measurement of quadriceps neuromuscular function, assessed before and after exercise, indicated a generalization reduction (P < 0.05) in maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase (K\textsuperscript{+}-stimulated 3-O-methylfluorescein phosphatase activity) in homogenates. A 32% reduction (P < 0.05) in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity was observed (90.9 ± 7.6 vs. 62.1 ± 6.4 nmol·mg protein\textsuperscript{-1}·h\textsuperscript{-1}) in Norm. At fatigue, the reductions in Hypox were not different (81 ± 5.6 vs. 57.2 ± 7.5 nmol·mg protein\textsuperscript{-1}·h\textsuperscript{-1}) from Norm. Measurement of quadriceps neuromuscular function, assessed before and after exercise, indicated a generalization reduction (P < 0.05) in maximal voluntary contractile force (MVC) and in force elicited at all frequencies of stimulation (10, 20, 30, 50, and 100 Hz). In general, no differences were observed between Norm and Hypox. The properties of the compound action potential, amplitude, duration, and area, which represent the electromyographic response to a single, supramaximal stimulus, were not altered by exercise or oxygen condition when assessed both during and after the progressive cycle task. Progressive exercise, conducted in Hypox, results in an inhibition of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity and reductions in MVC and force at different frequencies of stimulation; these results are not different from those observed with Norm. These changes occur in the absence of reductions in neuromuscular excitability.

membrane excitability; Na\textsuperscript{+}-K\textsuperscript{+}-ATPase; peak aerobic power

TO GENERATE THE MECHANICAL power output (PO) necessary to perform exercise of progressive intensity, increased recruitment of motor units must occur in synergistic muscles in conjunction with increased neural discharge frequency to individual motor units (14). The increase in neural discharge frequency results in an increase in the force generated by individual muscle cells and consequently in motor unit force. However, for this to occur, the muscle must be able to translate the increase in neural discharge frequency into an increase in free cytosolic calcium, [Ca\textsuperscript{2+}]	extsubscript{i}, and the increase in [Ca\textsuperscript{2+}]	extsubscript{i} must be translated by the regulatory and contractile proteins into increased force (26). The former processes, collectively referred to as a excitation-contraction coupling (E-C coupling), involve the repetitive generation of action potentials in the sarcolemma and t-tubules, mechanical-chemical coupling between the t-tubules and the calcium release channels (CRC) of the sarcoplasmic reticulum (SR) and the release of Ca\textsuperscript{2+} stored in the lumen of the SR through the CRCs and into the cytosol (18). At some point, the individual is no longer able to respond with increases in PO, and the exercise must be stopped or the PO decreased as a result of fatigue. Evidence is accumulating to suggest that an inability to elicit the desired changes in the [Ca\textsuperscript{2+}]	extsubscript{i}-time integral is closely associated with fatigue (1). The specific process in E-C coupling responsible for the dysregulation in [Ca\textsuperscript{2+}]	extsubscript{i}-time integral is unclear.

Failure to generate repetitive action potentials at increasing frequency in the sarcolemma and t-tubules represents a plausible possibility for E-C failure during progressive exercise. The generation of repetitive action potentials is intimately dependent on being able to establish transmembrane gradients for Na\textsuperscript{+} and K\textsuperscript{+}, given the flux of Na\textsuperscript{+} into and K\textsuperscript{+} out of the cell that occurs during excitation (11). Transmembrane gradients for Na\textsuperscript{+} and K\textsuperscript{+} depend on the active pumping of these cations by Na\textsuperscript{+}-K\textsuperscript{+}-ATPase (Na\textsuperscript{+}-K\textsuperscript{+}-pump), an integral membrane protein that uses the energy from ATP hydrolysis to counter transport of 3 Na\textsuperscript{+} and 2 K\textsuperscript{+} across the membrane (54). The catalytic activity of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase is a critical determinant of the rate of ATP hydrolysis and, consequently, the rate of the Na\textsuperscript{+} and K\textsuperscript{+} transport (41). The maximal activity of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase depends on the amount of protein, the isoform composition, and acute regulatory stimulating factors (7). Even under optimal activating conditions, there is evidence that Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity may be insufficient to meet the demands for Na\textsuperscript{+}-K\textsuperscript{+} transport imposed by muscle contractile activity, resulting in a loss of membrane excitability and fatigue (41). Repetitive contractile activity involving large muscle groups, as an example, particularly, if the demands for power are progressively increased, may be more problematic, given the alterations that occur in the intracellular environment. The accumulation of selected by-products of metabolism that occurs during progressive exercise, such as heat, hydrogen ions (H\textsuperscript{+}), inorganic phosphate (Pi), and reactive oxygen species (ROS), (25, 52) could either individually or in combination inhibit the maximal activity of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase similar to what has been shown for other cellular ATPases (13, 42, 57). The results of previous studies using heavy-cycle exercise support the possibility of a loss of membrane excitability, as indicated by disturbances in the properties of the compound action potential in the vastus muscles (2, 31).

Sustained repetitive contractions appear to cause inhibition of muscle Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity. Both our group (19) and

Address for reprint requests and other correspondence: H. J. Green, Dept. of Kinesiology, Univ. of Waterloo, Waterloo, ON Canada N2L 3G1 (E-mail: green@healthy.uwaterloo.ca).

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.
others (22) have reported decreases in maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity, as assessed in vitro by K\textsuperscript{+}-stimulated 3-O-methylfluorocein phosphatase (3-O-MFPase), after exercise. As a consequence, it is possible that the Na\textsuperscript{+}-K\textsuperscript{+}-membrane transport and membrane excitability would be compromised in tasks in which large force levels are required. Moreover, if a similar task is performed under conditions where the muscle metabolic stress is exaggerated as during hypoxia (12, 48), the decrease in maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity and the loss of membrane excitability may be even more pronounced. It is possible that a failure in membrane excitability secondary to reductions in maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity may explain the lower mechanical PO and lower peak aerobic power observed in hypoxia compared with normoxia (46, 47). Support for this possibility comes from a recent study published by our group in which we have shown a greater reduction in maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity during prolonged submaximal exercise when performed in hypoxia compared with normoxia (50).

Although, we could find no evidence for a greater impairment in neuromuscular function during submaximal exercise in hypoxia, such may not be the case with progressive exercise. In contrast to submaximal exercise where force levels and motor unit firing rates are relatively low and VO\textsubscript{2} and muscle energy homeostasis are well protected (24, 50), the generation of increased forces needed to perform progressive exercise exaggerates the demands on membrane excitability and VO\textsubscript{2} requirements and, in the process, greatly elevates metabolic by-product accumulation in muscle (6, 49).

In this study, our objectives were to examine the effect of progressive cycle exercise to fatigue performed under both normoxic and hypoxic conditions on maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity, neuromuscular fatigue, and membrane excitability. We have hypothesized that at fatigue in normoxia, a reduction in maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in the vastus lateralis would occur, accompanied by reductions in neuromuscular force-generating capacity and membrane excitability. Moreover, we have hypothesized that when the exercise is performed in hypoxia, the decrease in muscle maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity, neuromuscular fatigue, and membrane excitability would be more pronounced.

METHODS

Participants. Ten untrained males volunteered to participate in the study after approval from the Office of Research Ethics at the University of Waterloo (Ontario, Canada) and after being informed of the experimental protocols and associated risks. The average age and weight of the volunteers were 20 ± 0.4 yr and 80.0 ± 3.5 kg (0 ± SE), respectively. As a condition of participation, the subjects although occasionally active, did not have a history of regular participation in vigorous muscle activity, occasionally active, did not have a history of regular participation in vigorous muscle activity, and corresponding to the tissue sampling. No tissue samples were obtained from the vastus lateralis muscles were obtained by the needle biopsy technique (5) at rest, before exercise (Pre), and at PO, equivalent to 50% VO\textsubscript{2peak} as determined in Norm (50% VO\textsubscript{2peak} (Norm)). These samples were obtained at the same absolute PO for both conditions. For Norm, additional tissue samples were obtained at 70% VO\textsubscript{2peak} and at fatigue [100% VO\textsubscript{2peak} (Norm) and at Hypox, an additional tissue sample was obtained at fatigue [100% VO\textsubscript{2peak} (Hypox)]. The sample extracted at 70% VO\textsubscript{2peak} in Norm was labeled as 100% VO\textsubscript{2peak} (Hypox) for statistical comparisons. The power outputs at which these tissue samples were obtained was 214 ± 7 W and 250 ± 7 W for normoxia and hypoxia, respectively. Although, it would have been desirable to have matched tissue samples in Norm and Hypox at identical PO at which fatigue was observed in Hypox, this was not possible given the limited number of tissue samples that could safely be extracted and given the fact that the conditions were randomized.

To determine the effects of exercise and oxygen conditions on muscle mechanical properties and membrane excitability, isometric knee extension was used using a subset of the group (n = 7). These measurements were obtained 30–60 min before and within 4–5 min after the progressive tests while the participants were breathing room air. Mechanical characteristics were assessed using both voluntary contractions and contractions induced by electrical stimulation. Familiarization with the measurement protocols and standardization of conditions (see Mechanical function and membrane excitability protocols) were completed during a separate visit to the laboratory on the days before the performance of the progressive tests in Norm and Hypox.

The period after exercise represented the amount of time needed to instrument the subjects for the neuromuscular measurements. Previous research has demonstrated that alterations in muscle membrane excitability as measured by the amplitude of the muscle compound action potential (M wave) showed no recovery for and least 10 min after progressive exercise (31).

In the separate set of experiments, employing untrained males of similar age (21.5 ± 0.89 yr) and VO\textsubscript{2peak} (3.20 ± 0.17 l/min), we have recorded the M waves during the cycling itself. The properties of the M waves were assessed during Norm and Hypox at power outputs corresponding to the tissue sampling. No tissue samples were obtained, and no mechanical measurements were performed on these participants.

Progressive exercise protocol. For the progressive cycle tests, the subjects sat in an upright position on an electrically braked cycle ergometer (Quinton 870) that had been calibrated on a daily basis. Calibration was accomplished using standardized weights. The exercise protocol consisted of a 4-min baseline period of cycling at 25 W followed by 15-W step increases in PO each minute until fatigue. Fatigue was defined as an inability to sustain pedal frequency at 60 revolutions/min. Ventilation and gas exchange were monitored continuously beginning prior to exercise and until fatigue by previously described methods (29). The ventilatory volume and gas fraction signals were integrated to produce 30-s windows of VE, VO\textsubscript{2}, and VCO\textsubscript{2}. Only the VO\textsubscript{2} values are presented in this paper. During the
exercise tests, arterial oxygen saturation (SpO₂) was monitored from the fingertip using oximetry (Ohmeda model 3700). The validity of this method has been previously established by our group (unpublished data) and by others (44). Measurements were obtained over a 15-s period, and the values recorded represented the average of three determinations. The finger was carefully cleaned with alcohol before attaching the probe.

For Norm, the four tissue samples were obtained from separate sites distributed between the two legs. For Hypox, only three samples were obtained. For Hypox, one sample was obtained from one leg and two from the other leg. All sampling sites were prepared during the preparatory period before exercise. As a consequence, during the exercise, only a brief interruption in cycling (15–20 s) was needed to rapidly secure the tissue samples. For the measurement of Na⁺-K⁺-ATPase activity, the tissue sample was frozen in liquid N₂ and stored at −80°C.

Mechanical function and membrane excitability protocols. For muscle force and membrane excitability measurements, the subject sat upright in a straight-backed chair with hips and legs firmly secured by velcro straps, the knee at 90° to the thigh, and the arms folded across the chest. The variables measured included maximal voluntary contraction (MVC), the %activation (%ACT) using the interpolated twitch technique (3), and the forces generated during a twitch (Ft) obtained with supramaximal voltage, and during stimulation frequencies (Hz) of 10(P₁₀), 20(P₂₀), 30(P₃₀), 50(P₅₀), and 100(P₁₀₀). The interpolated twitch, which is obtained by application of a single supramaximal stimulus during an MVC, is a measure of central or neural inhibition. For all of these frequencies, the maximal rate of force development (+dP/dtmax) and the maximal decline in force development (−dP/dtmax) were assessed. In addition, contraction time (CT) and one-half relaxation time (1/2 RT) were measured assessed for the twitch only. The potentiated twitch, which is a measure of the increased Pt, that occurs as a consequence of a 10-s conditioning MVC applied just before measurement, was assessed in conjunction with Pt. The potentiated twitch force, which can be isolated to the muscle, is used to adjust the calculation of %ACT of muscle by the neural system. All of these procedures have been previously described in detail by our group (21, 55).

For measurement of force behavior, a 5-cm-wide plastic cuff placed around the lower right leg just proximal to the ankle malleoli was tightly attached to a linear variable differential transducer anchored at a level just below the malleoli. The linear variable differential transducer was amplified by a Dayton carrier preamplifier at 1 kHz, converted to a digital signal and fed into a 12-bit analog-to-digital converter and collected at 2,028 Hz. Custom-modified National Institutes of Allergy and Infectious Diseases software (National Instruments) was used to acquire EMG and force records and analyze raw data (Labview 5–1 software routine). Additional details, including the calculation of specific properties, are as previously published from our laboratory (21). It should be noted that M-wave properties were collected from the vastus medialis, while tissue was sampled from the vastus lateralis. The possibility exists that different response patterns may occur between the two muscles.

In an additional group, M waves were recorded during the progressive cycling, according to the procedures of Jannes et al. (31). In this procedure, the M wave was obtained from the vastus medialis after stimulation of the vastus lateralis. Both the location of the stimulating and recording electrodes and the pulse characteristics were as described for the mechanical measurements. The M wave was collected from the right leg at the beginning of eight consecutive leg extensions. A signal generated from an electronic sensor was used to standardize the joint angle at which the twitch was induced (31).

Analytical procedures. The maximal activity of the Na⁺-K⁺-ATPase was assessed in whole muscle homogenates and collected at 2,028 Hz.

The homogenate was collected from the right leg at the beginning of eight consecutive leg extensions. A signal generated from an electronic sensor was used to standardize the joint angle at which the twitch was induced (31).

The potentiated twitch, which is a measure of the increased Pt, that occurs as a consequence of a 10-s conditioning MVC applied just before measurement, was assessed in conjunction with Pt. The potentiated twitch force, which can be isolated to the muscle, is used to adjust the calculation of %ACT of muscle by the neural system. All of these procedures have been previously described in detail by our group (21, 55).

For measurement of force behavior, a 5-cm-wide plastic cuff placed around the lower right leg just proximal to the ankle malleoli was tightly attached to a linear variable differential transducer anchored at a level just below the malleoli. The linear variable differential transducer was amplified by a Dayton carrier preamplifier at 1 kHz, converted to a digital signal and fed into a 12-bit analog-to-digital converter and collected at 2,028 Hz. Custom-modified National Institutes of Allergy and Infectious Diseases software (National Instruments) was used to acquire EMG and force records and analyze raw data (Labview 5–1 software routine). Additional details, including the calculation of specific properties, are as previously published from our laboratory (21). It should be noted that M-wave properties were collected from the vastus medialis, while tissue was sampled from the vastus lateralis. The possibility exists that different response patterns may occur between the two muscles.

In an additional group, M waves were recorded during the progressive cycling, according to the procedures of Jannes et al. (31). In this procedure, the M wave was obtained from the vastus medialis after stimulation of the vastus lateralis. Both the location of the stimulating and recording electrodes and the pulse characteristics were as described for the mechanical measurements. The M wave was collected from the right leg at the beginning of eight consecutive leg extensions. A signal generated from an electronic sensor was used to standardize the joint angle at which the twitch was induced (31).

The potentiated twitch, which is a measure of the increased Pt, that occurs as a consequence of a 10-s conditioning MVC applied just before measurement, was assessed in conjunction with Pt. The potentiated twitch force, which can be isolated to the muscle, is used to adjust the calculation of %ACT of muscle by the neural system. All of these procedures have been previously described in detail by our group (21, 55).

For measurement of force behavior, a 5-cm-wide plastic cuff placed around the lower right leg just proximal to the ankle malleoli was tightly attached to a linear variable differential transducer anchored at a level just below the malleoli. The linear variable differential transducer was amplified by a Dayton carrier preamplifier at 1 kHz, converted to a digital signal and fed into a 12-bit analog-to-digital converter and collected at 2,028 Hz. Custom-modified National Institutes of Allergy and Infectious Diseases software (National Instruments) was used to acquire EMG and force records and analyze raw data (Labview 5–1 software routine). Additional details, including the calculation of specific properties, are as previously published from our laboratory (21). It should be noted that M-wave properties were collected from the vastus medialis, while tissue was sampled from the vastus lateralis. The possibility exists that different response patterns may occur between the two muscles.

In an additional group, M waves were recorded during the progressive cycling, according to the procedures of Jannes et al. (31). In this procedure, the M wave was obtained from the vastus medialis after stimulation of the vastus lateralis. Both the location of the stimulating and recording electrodes and the pulse characteristics were as described for the mechanical measurements. The M wave was collected from the right leg at the beginning of eight consecutive leg extensions. A signal generated from an electronic sensor was used to standardize the joint angle at which the twitch was induced (31).

The potentiated twitch, which is a measure of the increased Pt, that occurs as a consequence of a 10-s conditioning MVC applied just before measurement, was assessed in conjunction with Pt. The potentiated twitch force, which can be isolated to the muscle, is used to adjust the calculation of %ACT of muscle by the neural system. All of these procedures have been previously described in detail by our group (21, 55).

For measurement of force behavior, a 5-cm-wide plastic cuff placed around the lower right leg just proximal to the ankle malleoli was tightly attached to a linear variable differential transducer anchored at a level just below the malleoli. The linear variable differential transducer was amplified by a Dayton carrier preamplifier at 1 kHz, converted to a digital signal and fed into a 12-bit analog-to-digital converter and collected at 2,028 Hz. Custom-modified National Institutes of Allergy and Infectious Diseases software (National Instruments) was used to acquire EMG and force records and analyze raw data (Labview 5–1 software routine). Additional details, including the calculation of specific properties, are as previously published from our laboratory (21). It should be noted that M-wave properties were collected from the vastus medialis, while tissue was sampled from the vastus lateralis. The possibility exists that different response patterns may occur between the two muscles.

In an additional group, M waves were recorded during the progressive cycling, according to the procedures of Jannes et al. (31). In this procedure, the M wave was obtained from the vastus medialis after stimulation of the vastus lateralis. Both the location of the stimulating and recording electrodes and the pulse characteristics were as described for the mechanical measurements. The M wave was collected from the right leg at the beginning of eight consecutive leg extensions. A signal generated from an electronic sensor was used to standardize the joint angle at which the twitch was induced (31).

The potentiated twitch, which is a measure of the increased Pt, that occurs as a consequence of a 10-s conditioning MVC applied just before measurement, was assessed in conjunction with Pt. The potentiated twitch force, which can be isolated to the muscle, is used to adjust the calculation of %ACT of muscle by the neural system. All of these procedures have been previously described in detail by our group (21, 55).
the average of three trials. To control for between-day variability in the assay, all samples for a given individual were analyzed during the same analytical session. The assay is a highly sensitive measurement of 3-O-MFase, as indicated by the complete elimination of the slope with the addition of KCl by ouabain (4, 23).

Data analyses. Statistical analysis was performed on Statistica for Windows R.4.5 software (1993; Statsott A, Tulsa, OK). Both one- and two-way ANOVA procedures for repeated measures were used to analyze 3-O-MFase changes with condition and with time. One-way ANOVA was used for each condition while two-way ANOVA was used to compare conditions using matched samples. For mechanical and membrane excitability, only two-way ANOVA procedures were employed. Post hoc analyses of main and interactive effects were performed using the Tukey test. The probability for statistical significance was set at \( P < 0.05 \). Throughout the test, all group values are represented as \( 0 \pm SE \).

RESULTS

\( \dot{V}O_2 \) and arterial O\(_2\) saturation. In Norm, progressive increases in PO to fatigue resulted in progressive increases in \( \dot{V}O_2 \) (l/min) (Fig. 1). A similar response was observed during progressive exercise in Hypox. The primary effect of Hypox was to reduce the peak mechanical power output and peak \( \dot{V}O_2 \) that could be realized. In Norm compared with Hypox, peak PO was \( 292 \pm 7.5 \) and \( 238 \pm 7.0 \) W and \( \dot{V}O_2 \) peak was \( 3.95 \pm 0.18 \) vs. \( 3.20 \pm 0.10 \) l/min. The reductions amounted to \( \sim 18\% \) and \( 19\% \) for mechanical PO and \( \dot{V}O_2 \) peak, respectively. No differences were observed between Norm and Hypox in \( \dot{V}O_2 \) at any of the exercise intensities examined.

Before exercise and throughout exercise, Hypox resulted in a persistently lower SpO\(_2\) (Fig. 2). Exercise in Hypox also resulted in a lower SpO\(_2\), compared with preexercise. The lower SaO\(_2\) was observed earlier in exercise and persisted throughout the progressive workload protocol until fatigue. In contrast, no reduction in SpO\(_2\) from Pre regardless of the PO, was observed in Norm.

Mechanical function. Progressive exercise in both Norm and Hypox resulted in a pronounced neuromuscular fatigue in the quadriceps muscle as assessed isometrically based on measurements made before and after the cycle exercise for each condition. When assessed using MVC, a depression of \( \sim 12\% \) in force was observed in Norm (Table 1). The reduction in MVC was not accompanied by reductions in %ACT as assessed by the interpolated twitch technique. No differences were observed between Norm and Hypox in MVC and %ACT. As with MVC, reductions in AEMG were observed with exercise, regardless of condition.

Exercise also resulted in reductions in several properties assessed by a single supramaximal twitch response (Table 2). For both Norm and Hypox, reductions between 26 and 38% were observed in Pt. The reduction in Pt was accompanied by reduced rates in \( +dP/dt_{\text{max}} \) of between 22 and 36% and in \( -dP/dt_{\text{max}} \) of between 29 and 33% for Norm and Hypox. Neither CT nor 1/2 RT was altered with exercise. As with the MVC, no differences were observed between Norm and Hypox for any of these properties.

Exercise in both Norm and Hypox resulted in a frequency-dependent loss of isometric force (Fig. 3). The loss of force with exercise in Norm amounted to 51, 40, 26, and 21% for 10, 20, 30, and 50 Hz, respectively. At the higher frequency of 100 Hz, the reductions observed in force after exercise were also significant. Exercise in Hypox also resulted in reductions in force at 10, 20, 30, and 50 Hz. At 100 Hz, the reduction in force that occurred with exercise was a main effect and not restricted to a condition. With the exception of 50 Hz, no differences were observed between Norm and Hypox at any of these frequencies.

Table 1. Maximal voluntary contraction and interpolated twitch force before and after progressive exercise in normoxic and hypoxic conditions

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>MVC (N)*</td>
<td>767 ± 69</td>
<td>695 ± 67</td>
</tr>
<tr>
<td>%ACT</td>
<td>96.6 ± 1.3</td>
<td>95.9 ± 1.5</td>
</tr>
<tr>
<td>AEMG*</td>
<td>0.862 ± 0.12</td>
<td>0.751 ± 0.12</td>
</tr>
</tbody>
</table>

Values are \( 0 \pm SE \) \((n = 7)\). MVC, maximal voluntary contraction; %ACT, percentage activation of motor units in an MVC (see text). AEMG, average integrated electromyogram in an MVC. Pre, preexercise; Post, postexercise. *Main effects \((P < 0.05)\) of exercise were observed for both MVC and AEMG. For MVC and AEMG, Pre > Post.
Exercise in normoxic and hypoxic conditions

Table 2. Quadriceps twitch characteristics elicited by a single impulse before and after progressive development; 1/2 RT, one-half relaxation time. *Main effects (Table 3). For 10 and 20 Hz, exercise induced a reduced rate in main effect (*P < 0.05) of exercise were observed for $P_{\text{max}}$, $+dP/d_{\text{max}}$, and $-dP/d_{\text{max}}$. For all measures, Pre > Post.

<table>
<thead>
<tr>
<th>Twitch Response</th>
<th>Property</th>
<th>Normoxia</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P_{\text{max}}$, N</td>
<td>$+dP/d_{\text{max}}$, N/s</td>
<td>$-dP/d_{\text{max}}$, N/s</td>
</tr>
<tr>
<td>Pre</td>
<td>177±17</td>
<td>4,940±442</td>
<td>$-2,861±334$</td>
</tr>
<tr>
<td>Post</td>
<td>109±13</td>
<td>2,960±325</td>
<td>$-2,030±421$</td>
</tr>
<tr>
<td>Pre</td>
<td>165±12</td>
<td>3,979±317</td>
<td>$-2,246±184$</td>
</tr>
<tr>
<td>Post</td>
<td>122±12</td>
<td>3,018±312</td>
<td>$-1,771±199$</td>
</tr>
</tbody>
</table>

Values are $0±SE (n = 7)$. $P_{\text{max}}$, peak twitch force; $+dP/d_{\text{max}}$, maximal rate of force development; CT, contraction time; $-dP/d_{\text{max}}$, maximal decline in force development; 1/2 RT, one-half relaxation time. *Main effects ($P < 0.05$) of exercise were observed for $P_{\text{max}}$, $+dP/d_{\text{max}}$, and $-dP/d_{\text{max}}$. For all measures, Pre > Post.

In addition to the measurements of force at the different frequencies of stimulation, we have also examined both $+dP/d_{\text{max}}$ and $-dP/d_{\text{max}}$ at the lower stimulation frequencies (Table 3). For 10 and 20 Hz, exercise induced a reduced rate in both $+dP/d_{\text{max}}$ and $-dP/d_{\text{max}}$. The reductions in rate that were observed in these measures were not dependent on whether the exercise was performed in Norm or Hypox. Exercise also resulted in comparable reductions in $+dP/d_{\text{max}}$ at 30 Hz for both Norm and Hypox. For $-dP/d_{\text{max}}$, a reduced rate was observed with exercise in Norm but not in Hypox. At both 20 and 30 Hz, $+dP/d_{\text{max}}$ was higher in Norm compared with Hypox before exercise. Similarly, at both of these frequencies, $-dP/d_{\text{max}}$ was higher in Hypox compared with Norm after exercise.

Membrane excitability. To examine for changes in membrane excitability, we have measured the properties of the M-wave, namely, the amplitude, duration, and area, before and after the cycle exercise (Table 4). No effect of either exercise or condition was observed for any of the properties examined.

In an additional set of experiments, we monitored the M-wave during the cycle exercise itself at POs corresponding to the tissue sampling. With Norm, no changes were observed in any of the M-wave properties at power outputs corresponding to 50% $\dot{V}O_2\text{peak}$ (Norm), 100% $\dot{V}O_2\text{peak}$ (Hypox), and 100% $\dot{V}O_2\text{peak}$ (Norm) (Fig. 4). In contrast, both the amplitude and the area of the M-wave were increased with Hypox. At both 50% $\dot{V}O_2\text{peak}$ (Norm) and 100% $\dot{V}O_2\text{peak}$ (Hypox), these properties were higher in Hypox compared with Norm.

$Na^+\text{-}K^+\text{-}ATPase$ activity. Progressive exercise whether performed in Norm or Hypox resulted in pronounced reductions in

Table 3. Frequency-dependent alterations in human quadriceps force development and decay before and after progressive exercise in normoxic and hypoxic conditions

<table>
<thead>
<tr>
<th>Stimulation, Hz</th>
<th>$+dP/d_{\text{max}}$, N/s</th>
<th>$-dP/d_{\text{max}}$, N/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Normoxia</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>Pre</td>
<td>1,540±250</td>
<td>2,587±193</td>
</tr>
<tr>
<td>Post</td>
<td>823±118</td>
<td>1,460±214</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>1,603±233</td>
<td>2,331±160</td>
</tr>
<tr>
<td>Post</td>
<td>1,020±126</td>
<td>1,545±221</td>
</tr>
<tr>
<td>30</td>
<td>Normoxia</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>Pre</td>
<td>$-657±110$</td>
<td>$-3,814±502$</td>
</tr>
<tr>
<td>Post</td>
<td>$-383±135$</td>
<td>$-1,831±340$</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>$-687±82$</td>
<td>$-3,526±502$</td>
</tr>
<tr>
<td>Post</td>
<td>$-518±97$</td>
<td>$-2,300±389$</td>
</tr>
</tbody>
</table>

Values are $0±SE (n = 7)$. Stimulation, stimulation frequency. $+dP/d_{\text{max}}$, maximal rate of force development in Newtons per second; $-dP/d_{\text{max}}$, maximal rate of force decline in Newtons per second. Main effects ($P < 0.05$) were found for exercise. For $+dP/d_{\text{max}}$, at 10, 20, 30 Hz, Pre > Post. For $-dP/d_{\text{max}}$ at 10 and 20 Hz, Pre > Post. †Significantly different from Prenormoxia ($P < 0.05$). †Significantly different from Postnormoxia ($P < 0.05$).
Na\(^+\)-K\(^+\)-ATPase activity (Fig. 5). For both conditions, the effect of exercise was evident by the first sampling point, representing only 50% of normoxic \(\dot{V}O_2\) peak. For both Norm and Hypox, the magnitude of the inactivation of Na\(^+\)-K\(^+\)-ATPase activity was not different, amounting to between 29 and 32%. Nonspecific activity was also observed to decrease with exercise. This was not unique to condition. As with the specific Na\(^+\)-K\(^+\)-ATPase activity, the decline was fully manifested by the first measurement point. For Norm, the nonspecific values (nmol mg protein\(^{-1}\) h\(^{-1}\)) were 935\(\pm\)66, 771\(\pm\)47, 719\(\pm\)32 and 767\(\pm\)34 for 0, 137, 250 and 300 W, respectively. For Hypox, the values were 779\(\pm\)20, 646\(\pm\)22 and 751\(\pm\)42, for 0, 137 and 214 W, respectively.

**DISCUSSION**

As hypothesized, we have found that the untrained progressive cycle exercise to voluntary fatigue in Norm resulted in decreased muscle mechanical function and reductions in maximal Na\(^+\)-K\(^+\)-ATPase activity. However, contrary to our expectations, we found no evidence for a loss of membrane excitability as assessed by measurement of M wave properties, namely, the amplitude, area, and duration, when measured both during and after the exercise or when measured during the exercise itself. Moreover, even though the performance of the progressive exercise protocol to fatigue in Hypox resulted in a blunting of the mechanical PO and \(\dot{V}O_2\) peak that could be achieved, in general, neuromuscular performance and membrane excitability were not negatively affected compared with Norm. These findings suggest that the impairment in neuromuscular function, measured before and after the progressive exercise, is attributable to sites other than the sarcolemma. In this regard, our findings confirm our earlier research using a prolonged exercise protocol, namely that the neuromuscular deficit that occurs with Norm and Hypox cannot be explained by a failure in membrane excitability (50). The results of the current study indicate that although using a test protocol in hypoxia severely blunts power output, compromises \(\dot{V}O_2\) peak, disrupts energy homeostasis, and depresses Na\(^+\) and K\(^+\) membrane transport potential, membrane excitability does not appear to be limiting.

**Muscular fatigue.** What is clear from our measurements is that the progressive exercise protocol resulted in a neuromuscular deficit in force-generating capacity. Measurements of MVC indicated that reductions of \(~92\) N, or 12%. To isolate
The frequency-dependent nature of fatigue, induced by the progressive exercise protocol, was examined by brief submaximal stimulation of the muscle at frequencies ranging from 10 to 100 Hz. Reductions in force output of between 16% and 51% were observed across the frequencies examined. The reductions observed are also consistent with a depression in $\text{Ca}^{2+}$ release (1). The fact that force is increased with increasing stimulation frequency after exercise, similar to preexercise, suggests that increasing activation can increase $\text{Ca}^{2+}$ release and $[\text{Ca}^{2+}]_i$. However, with a given stimulation condition, $\text{Ca}^{2+}$ release and, consequently, $[\text{Ca}^{2+}]_i$, is impaired (10).

To gain further insight into the contractile processes that might be altered with progressive exercise, we have also measured the rates of force development and relaxation. After exercise, the rate of twitch for both $+\text{dP}/\text{d}t_{\text{max}}$ and $-\text{dP}/\text{d}t_{\text{max}}$ decreased, while CT and 1/2 RT were unaltered. The decreases in $+\text{dP}/\text{d}t_{\text{max}}$ and $-\text{dP}/\text{d}t_{\text{max}}$ are suggestive of both a slower maximal rate of weak to strong binding and a slower maximal strong-to-weak binding and dissociation of cross bridges, respectively (39). At least for $-\text{dP}/\text{d}t_{\text{max}}$, reductions in SR $\text{Ca}^{2+}$ uptake and $\text{Ca}^{2+}$-ATPase activity occur after progressive exercise to fatigue (16). It should be emphasized that when the decrease in $P_t$ was taken into account, neither $+\text{dP}/\text{d}t_{\text{max}}$ nor $-\text{dP}/\text{d}t_{\text{max}}$ were altered after exercise. This would suggest that the putative reductions in $[\text{Ca}^{2+}]_i$ that occur with fatigue does not impair the maximal kinetics of force development and relaxation when adjusted for peak force (43).

Although force at different frequencies of stimulation after exercise was generally not differentially affected by Hypox, we found evidence that $-\text{dP}/\text{d}t_{\text{max}}$ was not as disturbed. At 20 and 30 Hz, reductions in $-\text{dP}/\text{d}t_{\text{max}}$ with Hypox were not as pronounced. This could suggest less of an impairment in actin-myosin dissociation and/or $\text{Ca}^{2+}$ uptake in Hypox compared with Norm, during relaxation. At present, it is unclear whether these differences are due to Hypox per se on the shorter exercise time or PO realized in Hypox.

The reduction in the absolute maximal rate of relaxation could have important consequences to force levels, particularly at the lower frequency of stimulation where unfused tetani occurs. The effect of the slower rate of relaxation would be to attenuate the force loss, observed with exercise, given the higher force level that would persist between each impulse (20, 56).

$M$-wave properties. A particularly surprising finding was the lack of an impairment in the properties of the $M$ wave, considering the approximate 32% reduction that we have observed in maximal $\text{Na}^{+}-\text{K}^{+}$-ATPase activity with progressive exercise to fatigue in Norm. Reductions in membrane excitability have been shown to parallel reductions in force in a variety of studies using repetitive contractions where the $\text{Na}^{+}-\text{K}^{+}$-ATPase activity has been partially inhibited by ouabain (41). However, in contrast to the previous studies where $M$-wave characteristics were measured concurrently with fatigue, initially, we assessed these properties only 4–5 min after exercise. It would be expected that some recovery in $M$-wave properties would occur during the period after exercise when the volunteers were being prepared for stimulation, as a result of recovery or partial recovery of selected intracellular metabolic by-products (40) and restoration of transmembrane $\text{Na}^{+}$ and $\text{K}^{+}$ gradients (41). To investigate whether or not significant recovery had occurred, we have performed a...
separate set of experiments measuring the M-wave properties during the progressive exercise task itself. During the progressive exercise in Norm, although a trend was evident, suggesting decreased levels of the M-wave properties, none of the changes were significant.

The failure to detect reductions in M-wave properties was not expected since Jammes et al. (31) have shown that the pronounced decline in vastus lateralis M-wave amplitude persists for at least 10 min in recovery. Interestingly, in the Jammes et al. study (31), the apparent disturbance in membrane excitation only occurred in untrained and not trained volunteers. Our study also employed untrained participants. However, in the our study, the volunteers were ~20 years younger. It is possible that other factors such as differences in methodology may be involved in explaining the different results obtained between the two studies. Because Jammes et al. (31) also used progressive exercise, with the same general conditions—both with respect to time of exercise and the incremental power output—the differences in the exercise protocol would not appear to be important.

Na\(^{+}\)-K\(^{+}\)-ATPase. Contrary to our hypothesis, we found no additional inactivation of the Na\(^{+}\)-K\(^{+}\)-ATPase activity in Hypox at a comparable PO to Norm or when the progressive cycle exercise was performed to fatigue in Hypox. This was unexpected, as the exercise at comparable POs in Hypox compared with Norm results in a greater metabolic stress, including elevation of selected metabolic by-products such as Pi and H\(^{+}\) (36, 48) and conceivably in ROS accumulation (48). Previous studies have demonstrated a susceptibility of ion channel pumps, including the Na\(^{+}\)-K\(^{+}\)-ATPase, to damage caused by ROS (33). Although, it is known that exhaustive exercise causes ROS accumulation (45), it remains to be definitively established that progressive exercise in Hypox results in a more emphasized ROS accumulation. Unexpectedly, the reduction in Na\(^{+}\)-K\(^{+}\)-ATPase activity was observed early in the exercise protocol for both conditions. The inactivation that was observed would appear to involve an intracellular change related to the adjustment to exercise, possibly a rapid increase in ROS. It should be emphasized that our measurements of Na\(^{+}\)-K\(^{+}\)-ATPase activity were performed under optimal in vitro conditions. In vivo, the catalytic activity of the Na\(^{+}\)-K\(^{+}\)-ATPase would be expected to be lower (32) given the accumulation of metabolic by-products in the intracellular environment with progressive exercise. In light of this, it is even more remarkable that the Na\(^{+}\)-K\(^{+}\)-ATPase activity that remains is sufficient to support membrane excitability. At fatigue, no differences existed between Norm and Hypox in Na\(^{+}\)-K\(^{+}\)-ATPase activity. This finding was also unexpected given the longer exercise time at greater PO achieved in Norm compared with Hypox.

The possibility remains that the dissociation that we have observed between changes in Na\(^{+}\)-K\(^{+}\)-ATPase activity and membrane excitability reflects our sampling sites. Membrane excitability was measured in the vastus medialis muscle, while Na\(^{+}\)-K\(^{+}\)-ATPase activity was measured in the vastus lateralis. Previous studies, however, have reported generally similar M-wave responses between these muscles (34, 35).

\textit{V}O\textsubscript{2}\textit{peak}. The reduction in \textit{V}O\textsubscript{2}\textit{peak} observed in Hypox compared with Norm during the progressive exercise does not appear to be due to a failure in membrane excitability as a cause of the limited PO, which potentially could also limit oxidative phosphorylation. During the progressive exercise in Hypox, we have found increases in M-wave amplitude and area, not decreases as postulated. The increases in these properties have been observed earlier in a variety of exercise protocols and have been labeled pseudofacilitation (38). The increases have been attributed to hyperpolarization, secondary to activation of the electrogenic Na\(^{+}\)-K\(^{+}\)-ATPase (38). Interestingly, with hypoxemia, no evidence of pseudofacilitation during sustained contractile activity has been observed (15). Differences in muscle mass, type of muscle, and the task may be important in explaining the differences between the two studies.

In summary, the results of this study indicate that progressive cycle exercise to fatigue in Hypox compared with Norm results in a blunting of the peak mechanical PO and \textit{V}O\textsubscript{2} peak. Progressive exercise to fatigue also results in an approximate 30% reduction in maximal Na\(^{+}\)-K\(^{+}\)-ATPase activity, regardless of condition. Mechanical function when measured after the exercise indicates a reduction in maximal force-generating capacity, possibly by disturbances at one or more sites in the muscle contractility. The intracellular processes involved in fatigue measured after exercise appear not to involve a failure in membrane excitability, since no alterations in the properties of the M wave occurred either in Norm or Hypox. A failure in the sarcolemma and t-tubule to conduct repetitive action potentials also does not remain as a viable site of fatigue during progressive exercise itself in the untrained. As such, a failure in membrane excitability cannot explain the \textit{V}O\textsubscript{2} peak attained in Norm or the blunting in \textit{V}O\textsubscript{2} peak observed in Hypox. It is possible that the intracellular site involved in muscle fatigue during Norm and Hypox is the same, the differences being that the Hypox stress precipitates the failure at a lower PO and \textit{V}O\textsubscript{2} peak.

ACKNOWLEDGMENTS

Special appreciation is extended to the Natural Sciences and Engineering Research Council for financial support.

REFERENCES


