Human muscle sarcoplasmic reticulum function during submaximal exercise in normoxia and hypoxia

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Submitted 4 September 2003; accepted in final form 14 March 2004

Duhamel, T. A., H. J. Green, J. G. Perco, S. D. Sandiford, and J. Ouyang. Human muscle sarcoplasmic reticulum function during submaximal exercise in normoxia and hypoxia. J Appl Physiol 97: 180–187, 2004; 10.1152/japplphysiol.00954.2003.—In this study, the response of the sarcoplasmic reticulum (SR) to prolonged exercise, performed in normoxia (inspired O₂ fraction = 0.21) and hypoxia (inspired O₂ fraction = 0.14) was studied in homogenates prepared from the vastus lateralis muscle in 10 untrained men (peak O₂ consumption = 3.09 ± 0.25 l/min). In normoxia, performed at 48 ± 2.2% peak O₂ consumption, maximal Ca²⁺-dependent ATPase activity was reduced by ~25% at 30 min of exercise compared with rest (168 ± 10 vs. 126 ± 8 μmol·g⁻¹ protein⁻¹·min⁻¹), with further reductions observed at 90 min (129 ± 6 μmol·g⁻¹ protein⁻¹·min⁻¹). No changes were observed in the Hill coefficient or in the Ca²⁺ concentration at half-maximal activity. The reduction in maximal Ca²⁺-dependent ATPase activity at 30 min of exercise was accompanied by oxalate-dependent reductions (P < 0.05) in Ca²⁺ uptake by ~20% (370 ± 22 vs. 298 ± 25 μmol·g⁻¹ protein⁻¹·min⁻¹). Ca²⁺ release, induced by 4-chloro-m-cresol and assessed into fast and slow phases, was decreased (P < 0.05) by ~16 and ~32%, respectively, by 90 min of exercise. No differences were found between normoxia and hypoxia for any of the SR properties examined. It is concluded that the disturbances induced in SR Ca²⁺ cycling with prolonged moderate-intensity exercise in human muscle during normoxia are not modified when the exercise is performed in hypoxia.

Evidence has been provided to suggest that the structural alterations that occur to the region of the nucleotide-binding site of Ca²⁺-ATPase, which is induced during contractile activity, occur as a consequence of oxidation and nitrosylation reactions (23). Similarly, oxidation and/or nitrosylation are suspected as being involved in the structural disturbances in the CRC (10). It is of interest that repetitive exercise is known to increase the accumulation of reactive oxygen species (ROS) (8, 37, 40). Although the human studies reported to date involving both submaximal cycling exercise (4, 18) and supramaximal one-leg kicking protocols (16, 21, 25) are all consistent in reporting a disturbance in at least one SR Ca²⁺-cycling property, it is not clear whether the changes in several properties are coordinated in a time-dependent manner. Moreover, studies have been generally incomplete since only limited characterization of a given property has been attempted. For the Ca²⁺-ATPase activity, nothing is known about the Ca²⁺ dependency since only maximal activities have been studied. Although some studies have examined the coupling ratio between Ca²⁺ uptake and Ca²⁺-ATPase, the results are suspect because the ratios are unrealistic given the low Ca²⁺ used to measure Ca²⁺ uptake (4).

It is known that, when the same submaximal absolute intensity of exercise involving large muscle groups is performed in hypoxia, the time to fatigue is decreased (15). Because depression in Ca²⁺ cycling appears to be mechanistically linked to fatigue during prolonged exercise (1), it might be expected that the disturbances in SR Ca²⁺ cycling will be potentiated, given the greater relative percent of peak oxygen consumption (Vo₂ peak) and the increased metabolic stress that occurs (19, 33). It is possible that damage to specific proteins involved in SR Ca²⁺ cycling, such as Ca²⁺-ATPase and the CRC, would be exaggerated during exercise in hypoxia. As a consequence, Ca²⁺ uptake and Ca²⁺ release may be depressed to a greater extent when prolonged exercised is performed in hypoxia compared with normoxia.

In this study, our objective was to determine the effects of prolonged exercise, performed both in normoxia and hypoxia, on SR Ca²⁺-cycling properties in human skeletal muscle. We have hypothesized that prolonged exercise in normoxia would induce a coordinated, time-dependent reduction in Ca²⁺-ATPase activity, Ca²⁺ uptake, and Ca²⁺ release and that these changes would occur in the absence of changes in the coupling ratio between Ca²⁺-uptake and Ca²⁺-ATPase activity. In the case of Ca²⁺-ATPase activity, the exercise-induced effect will be limited to reductions in Vₘₐₓ. Moreover, we have postulated that, when the same absolute exercise protocol is performed in...
hypoxia, the disturbances in the SR Ca\(^{2+}\)-cycling properties would be exaggerated.

**METHODS**

**Subjects.** A total of 10 healthy but untrained male volunteers completed the study. The age, height, and body mass of the participants were 21.4 ± 1.0 yr, 176 ± 3.8 cm, and 76.2 ± 5.5 kg (means ± SE), respectively. VO\(_{2}\)\(_{\text{peak}}\), as assessed during a progressive cycle task to fatigue in normoxia, was 3.09 ± 2.5 L/min. As required, each participant was fully informed of all experimental procedures and risks before signing a consent form. As a condition of entry into the study, none of the volunteers engaged in vigorous exercise more than once per week. This study, including the consent form, was approved by the Office of Research Ethics at the University of Waterloo, where the study was conducted.

**Experimental design.** To investigate the role of exercise and environmental condition on SR function, a randomized cross-over design was employed where the participants exercised in both normoxic and hypoxic conditions. A standardized protocol consisting of 90 min of cycling performed at moderate intensity was used as the exercise challenge. The exercise tests were conducted using two different inspired O\(_2\) fractional (FiO\(_2\)) concentrations, namely normoxia (FiO\(_2\) = 0.21) and hypoxia (FiO\(_2\) = 0.14). The hypoxic condition selected was based on previous research, which demonstrated that prolonged exercise at moderate intensity could be performed despite disturbances in energy metabolism (19). Before and at selected time points during the exercise (30, 60, 90 min), ventilatory, gas exchange, and heart rate responses were measured. As well, tissue samples were obtained from the vastus lateralis muscle from preprepared sites before exercise and at 30 and 90 min of exercise. A period of 3–4 wk separated the exercise tests. All tests were conducted under conditions of temperature and relative humidity, which averaged 24°C for temperature and 50–60% for relative humidity. There were no differences in these conditions between normoxia and hypoxia.

All participants were scheduled for laboratory sessions on at least four different occasions. During the first visit, a progressive cycle exercise protocol to fatigue was performed for measurement of VO\(_{2}\)\(_{\text{peak}}\) and related properties. This test was repeated on another occasion if it appeared, on the basis of representative values of respiratory gas exchange and heart rate, that a maximal response was not observed. During the second visit, the submaximal exercise protocol was performed for a brief period of time (15–20 min) at the exercise intensity prescribed for each individual. The intent was to have each individual perform at power output (PO) designed to elicit ~50% VO\(_{2}\)\(_{\text{peak}}\), based on the VO\(_{2}\)\(_{\text{peak}}\) measured during normoxia. Appropriate adjustments were made to PO on subsequent tests to ensure that this relative percentage of VO\(_{2}\)\(_{\text{peak}}\) occurred. During hypoxia, subjects cycled at the same absolute PO as in normoxia. It was estimated that the relative percentage of VO\(_{2}\)\(_{\text{peak}}\) in hypoxia was ~64% (19). During the third and fourth visits, participants performed the 90 min of cycling during normoxia and hypoxia in randomized order.

For all exercise tests, the participants sat in the upright position on an electrically braked cycle ergometer (Quinton 870), which was calibrated on a daily basis. The protocol used to measure VO\(_{2}\)\(_{\text{peak}}\) consisted of 4 min of a baseline period of cycling at 25 W followed by 15-W step increases in PO each minute. Volunteers pedalled at ~60 cycles/min until volitional fatigue. Fatigue was defined as an inability to maintain at least 50 cycles/min. Ventilation, gas exchange, and heart rate were monitored both before exercise and throughout exercise by previous published methods (22). Ventilatory volume and gas-fraction signals were integrated to produce 30-s windows of minute ventilation (V\(_{E}\)), oxygen uptake (VO\(_{2}\)), and CO\(_{2}\) uptake (VCO\(_{2}\)).

For the prolonged exercise tests, volunteers reported to the laboratory ~60 min before the beginning of exercise. During this period, preparations were made for tissue sampling. For tissue sampling, three sites over the vastus lateralis (randomized between legs) were prepared during each exercise test. The preparation for tissue sampling included making three separate incisions after local anaesthesia was applied. These incisions were used to extract samples using the biopsy technique (3) during rest before exercise and at 30 and 90 min of exercise. Two separate samples were extracted from each site to secure sufficient tissue (~100 mg) for the analytical measurements. Ventilation and gas-exchange measurements were made over a 4–5 min period during rest and beginning at 25, 55, and 85 min of exercise with techniques identical to those described previously (22). Heart rate was also recorded during the gas-collection periods with standard electrocardiographic techniques.

The prolonged exercise tests were performed at approximately the same time of day for each participant and 3–4 h after the ingestion of a liquid supplement consisting of one can of Ensure (1.045 kJ, 14.8% protein, 3.15% fat, and 53.7% carbohydrates; Ross Laboratories, Montreal, Canada). All subjects were requested to refrain from any supplement, including coffee, on the day of testing. Moreover, coffee or alcohol was not permitted the day before testing, and vigorous exercise was not allowed during the 3–4 days before the submaximal tests.

**SR properties.** Immediately after extraction of the tissue samples, whole muscle homogenates were prepared, rapidly frozen in liquid N\(_2\), and stored at ~80°C, pending measurements of Ca\(^{2+}\)-ATPase activity, Ca\(^{2+}\) uptake, and Ca\(^{2+}\) release. Homogenates were prepared in ice-cold homogenizing buffer (11:1 vol/wt dilution) containing (in mM) 250 sucrose, 5 HEPES, 10 Na\(_{2}\)ATP, and 0.2 PMSF (pH 7.5) by using a hand-held glass homogenizer (Kontes, Dual 20) (30). Each homogenate was divided into a number of aliquots before freezing and storage. Protein content in homogenates was measured by the method of Lowry, as modified by Schactele and Pollock (34).

Ca\(^{2+}\) uptake and Ca\(^{2+}\) release measurements were performed during a single assay according to procedures previously detailed from our laboratory (35, 41). These measurements involve assessing the changes in free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{i}\)) in the buffer. The measurement of [Ca\(^{2+}\)]\(_{i}\) is obtained using the fluorescent Ca\(^{2+}\) indicator indo 1 as described earlier (7, 32) with a spectrophotometer (Ratiomaster, Photon Technology Internationale, Brunswick, NJ) equipped with dual-emission monochromators. The measurement of [Ca\(^{2+}\)]\(_{i}\) using this procedure is based on the difference in maximal emission wavelengths between the Ca\(^{2+}\) bound to indo1 and the free form. The excitation wavelength was 355 nm, and the Ca\(^{2+}\) free and Ca\(^{2+}\) bound to indo1 have emission maxima of 485 and 405 nm, respectively. Changes in the ratio are used to calculate [Ca\(^{2+}\)]\(_{i}\) according to method at Grynkiewicz et al. (20). For whole muscle homogenates, we have used a dissociation constant of 250 nM for the binding of indo1 and Ca\(^{2+}\) (20). It should be emphasized that in homogenates the actual dissociation constant is significantly affected by protein composition, and, consequently, the values obtained for Ca\(^{2+}\) uptake and Ca\(^{2+}\) release may vary between experimental conditions when alterations in protein composition occur. For these reasons, the dissociation constant employed is not a true value but only an estimate, and, therefore, the pCa values indicated throughout the paper are only apparent.

For the measurements of Ca\(^{2+}\) uptake and Ca\(^{2+}\) release, the reaction buffer contained (in mM) 200 KC1, 20 HEPES, 10 Na\(_{2}\), 0.005 N,N,N’,N’-tetrakis(2-pyridylmethyl)-ethylenediamine, 5 oxalate, 15 MgCl\(_2\), and 10 phosphonoctylpyruvate (pH 7.0). Before emission spectra were collected, 18 U/ml lactate dehydrogenase, 18 U/ml pyruvate kinase, and 1.5 µM of indo 1 were added to the cuvette containing 2 ml of the reaction buffer. In addition, 3 µl of CaCl\(_{2}\) (10 mM) was added at each trial to achieve an initial [Ca\(^{2+}\)]\(_{i}\) of ~3.0 µM. Immediately after data collection was initiated, 90 µl of homogenate were added to the cuvette. The reaction was initiated by the addition of 5 mM ATP. Ca\(^{2+}\) uptake was followed until it plateaued. Average
rates of Ca\textsuperscript{2+} uptake were determined at 500, 1,000, 1,500, and 2,000 nM of [Ca\textsuperscript{2+}], obtained during a single assay. After active loading of the SR when [Ca\textsuperscript{2+}] decreased to a plateau, Ca\textsuperscript{2+} release was initiated by adding 10 μM of 4-chloro-m-cresol (4-CMC) to the cuvette. We have found that 4-CMC induces a biphasic Ca\textsuperscript{2+}-release pattern, which is characterized by a rapid early release, which we have labeled phase 1, and a slower, more delayed release, which we have labeled phase 2 (41).

The maximal rate of Ca\textsuperscript{2+} uptake at each of the four [Ca\textsuperscript{2+}]-concentrations as well as the maximal rate of Ca\textsuperscript{2+} release for phase 1 and phase 2 were obtained by smoothing the [Ca\textsuperscript{2+}] vs. time curve over 21 points (Savitsky-Golay algorithm) and differentiating the linear-fit curves to determine maximal rates. Measurement of Ca\textsuperscript{2+}-dependent SR Ca\textsuperscript{2+}-ATPase activity in whole muscle homogenates was performed using spectrophotometric techniques as described by Simonides and van Hardeveld (39) with minor modification by our laboratory (43). In this procedure, total Ca\textsuperscript{2+}-Mg\textsuperscript{2+}-ATPase activity and basal ATPase activity are assessed, and Ca\textsuperscript{2+}-ATPase is calculated as the difference between the two measures. Other potentially contaminating ATPases are selectively inhibited by different pharmacological agents. It has been shown that this measurement is highly specific to Ca\textsuperscript{2+}-ATPase as indicated by the near total loss of activity with the addition of cyclosporine acid, known to selectively inhibit the enzyme (32, 39). We have also been able to confirm this result (43). The reaction buffer contained (in mM) 200 KC1, 20 HEPES, 15 MgCl\textsubscript{2}, 1 EGTA, 10 NaN\textsubscript{3}, and 5 ATP (pH 7.0). In addition, 18 U/ml lactate dehydrogenase, 18 U/ml pyruvate kinase, 0.3 mM NADH, and 1 μM of the ionophore A-23187 (Sigma C-7522) were added. Assays were performed at 37°C and at 340 nm (Shimadzu UV 160) using ~1 mg wet weight of tissue (25-μl homogenate) in 1 ml of reaction buffer. After the baseline absorbance of NADH was recorded for ~1 min, the reaction was initiated by adding 1 μl of 100 mM CaCl\textsubscript{2} and monitored for 2 min. Maximal Ca\textsuperscript{2+}-ATPase activity and Ca\textsuperscript{2+}-dependent Ca\textsuperscript{2+}-ATPase activity was measured using 0.5-μl additions of 100 mM CaCl\textsubscript{2}. Basal or background ATPase activity was determined in the presence of 40 μM of cyclosporin acid. Measurement of [Ca\textsuperscript{2+}] used to assess Ca\textsuperscript{2+} dependency of the Ca\textsuperscript{2+}-ATPase reaction was assessed as described earlier (44).

Because the measurement of Ca\textsuperscript{2+}-ATPase activity is based on the regeneration of ATP, a potential limitation is the loss of ADP to AMP via the myokinase reaction. However, we have shown using a myokinase inhibitor (APSA) that Ca\textsuperscript{2+}-ATPase activity is unaffected (unpublished observations).

The kinetic properties of the Ca\textsuperscript{2+}-ATPase activity that were measured included V\textsubscript{max}, the [Ca\textsuperscript{2+}]\textsubscript{0} needed to obtain half-V\textsubscript{max} (Ca\textsubscript{50}) and the Hill coefficient (nH). These properties were obtained by plotting Ca\textsuperscript{2+}-ATPase activity against the negative logarithm of [Ca\textsuperscript{2+}]\textsubscript{0} (pCa). V\textsubscript{max} represented the peak value, Ca\textsubscript{50} represented the [Ca\textsuperscript{2+}] obtained from a sigmoid fit of the data that yields 50% of V\textsubscript{max}, and nH was obtained through nonlinear regression with computer software (Graph Pad Software) by using a portion of the curve that corresponded to between 20 and 80% of V\textsubscript{max}.

All SR properties assessed were measured in duplicate. For any given analytical session, care was taken to ensure that all samples for a given subject for both conditions were analyzed together. All measurements were made in aliquots that had been allowed to thaw only once. The coefficient of variation for duplicate trials for Ca\textsuperscript{2+}-ATPase and Ca\textsuperscript{2+} uptake was 7.9 ± 0.9 and 7.6 ± 0.9, respectively. Only a single trial was used for Ca\textsuperscript{2+} release.

**Statistics.** The data were analyzed by two- and three-way ANOVA procedures for repeated measures. For two-way ANOVA applications, exercise time and oxygen condition represented the independent variables. These procedures were applicable to the gas exchange, ventilation, and heart rate data, and all SR properties were assessed with the exception of Ca\textsuperscript{2+} uptake. For Ca\textsuperscript{2+} uptake, a three-way ANOVA was utilized (Ca\textsuperscript{2+} concentration, exercise time and, oxygen condition). Where significant differences were found, the Newman-Keuls technique was applied to determine which means were different. Significance was set at the 0.05 level. Throughout the text, data are expressed as means ± SE.

**RESULTS**

**Respiratory gas exchange.** Exercise resulted in an approximate 4.4-fold increase in VO\textsubscript{2} (Table 1). The steady-state VO\textsubscript{2} that was achieved represented 47.5 ± 2.2% of the VO\textsubscript{2} peak obtained during normoxia. Although VO\textsubscript{2} increased from rest to exercise, no changes in VO\textsubscript{2} were observed throughout the 90-min exercise protocol. Similarly, no differences were observed between normoxia and hypoxia either before exercise or during exercise. As with VO\textsubscript{2}, VO\textsubscript{2CO2} increased from rest to exercise and then remained stable as time progressed. As well, like VO\textsubscript{2}, a main effect of condition was not observed for VO\textsubscript{2CO2}. The respiratory exchange ratio (RER), defined as the ratio of VO\textsubscript{2} to VO\textsubscript{2CO2}, was different during exercise in both conditions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pre</th>
<th>30</th>
<th>60</th>
<th>90</th>
</tr>
</thead>
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<tr>
<td>VO\textsubscript{2}, l/min</td>
<td>N</td>
<td>0.33±0.03</td>
<td>1.50±0.15</td>
<td>1.52±0.14</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>0.31±0.03</td>
<td>1.55±0.17</td>
<td>1.48±0.14</td>
</tr>
<tr>
<td>VCO\textsubscript{2}, l/min</td>
<td>N</td>
<td>0.28±0.03</td>
<td>1.38±0.13</td>
<td>1.35±0.13</td>
</tr>
<tr>
<td></td>
<td>H</td>
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<td>1.52±0.17</td>
<td>1.47±0.14</td>
</tr>
<tr>
<td>VE, l/min, BTPS</td>
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<td>32.2±2.4*</td>
<td>32.4±2.5*</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>10.1±1.2</td>
<td>39.2±4.3*</td>
<td>39.7±3.4*</td>
</tr>
<tr>
<td>RER</td>
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<td>0.92±0.01*</td>
<td>0.89±0.01</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>1.05±0.03</td>
<td>0.99±0.03*</td>
<td>1.00±0.03*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>N</td>
<td>76±2.6</td>
<td>140±6*</td>
<td>141±6*</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>89±3.4</td>
<td>154±4*</td>
<td>156±4*</td>
</tr>
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</table>

Values are means ± SE; n = 10. Pre, preexercise; N, normoxia; H, hypoxia; VO\textsubscript{2}, oxygen consumption; VCO\textsubscript{2}, carbon dioxide production; VE, expired ventilation; RER, respiratory exchange ratio; HR, heart rate. For VO\textsubscript{2} and VCO\textsubscript{2}, a main effect (P < 0.05) of condition was found. For VO\textsubscript{2} and VCO\textsubscript{2}, Pre < 30, 60, and 90 min. For RER and HR, a main effect (P < 0.05) of condition was found. For both RER and HR, H > N. *Significantly different from Pre (P < 0.05). †Significantly different from 30 min (P < 0.05). ‡Significantly different from 60 min (P < 0.05). §Significantly different from N (P < 0.05).

**Table 1. Respiratory gas exchange during prolonged exercise in normoxia and hypoxia**
compared with rest. In the case of exercise in normoxia, the increase in RER was only noted at 15 min. For hypoxia, RER was lower than before exercise at all exercise time points. Higher RER levels were also observed in hypoxia compared with normoxia during both rest and exercise. Hypoxia also resulted in a higher \( V_{\text{E}} \) during exercise compared with normoxia but not at rest. As expected, \( V_{\text{E}} \) increased with exercise in normoxia and then remained stable. The same pattern was observed with hypoxia, except that \( V_{\text{E}} \) at 90 min was greater than at 30 min. Heart rate was persistently elevated during hypoxia compared with normoxia both before and during exercise. For both normoxia and hypoxia, a drift in heart rate was observed during exercise. For normoxia, heart rate was higher at 90 min of exercise compared with 30 and 60 min. For hypoxia, heart rate was higher at 90 min compared with 30 min.

**SR properties.** A typical tracing illustrating \( \text{Ca}^{2+} \) release and \( \text{Ca}^{2+} \) uptake is provided in Fig. 1. Exercise in normoxia resulted in a reduction in \( \text{Ca}^{2+} \) uptake at all of the \( \text{Ca}^{2+} \) concentrations examined (Fig. 2). The reductions that were observed, amounting to \( \sim 20\% \), were fully manifested by 30 min of exercise. Exercise in hypoxia did not have any additional effect on \( \text{Ca}^{2+} \) uptake over that observed in normoxia. Two phases of \( \text{Ca}^{2+} \)-release kinetics were assessed, a fast phase (phase 1) and a slow phase (phase 2) (Fig. 1). Both phase 1 and phase 2 were reduced with exercise (Fig. 3). In the case of phase 1, the reduction, which amounted to \( \sim 16\% \), was only found at the end of exercise. For phase 2, the decrease was progressive, amounting to \( \sim 18\% \) at 30 min and \( \sim 32\% \) at 90 min. As with \( \text{Ca}^{2+} \) uptake, we could find no additional effect of hypoxia compared with normoxia.

For \( \text{Ca}^{2+} \)-ATPase activity, reductions of 25% in \( V_{\text{max}} \) were observed by 30 min of exercise in normoxia with no further changes observed during the additional 60 min of exercise (Table 2). Basal ATPase, determined as the difference between total \( \text{Ca}^{2+} \)-Mg\(^{2+} \)-ATPase activity and \( \text{Ca}^{2+} \)-ATPase activity was \( 45 \pm 5, 38 \pm 3, \) and \( 43 \pm 2 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \) in normoxia before exercise and after 30 and 90 min of exercise, respectively. No differences were observed between normoxia and hypoxia in either \( V_{\text{max}} \) or basal ATPase either during rest or exercise. There was no effect of either exercise or oxygen conditions on either \( n_H \) or \( C_{50} \), both measures of the binding affinity of the \( \text{Ca}^{2+} \)-ATPase enzyme for \( \text{Ca}^{2+} \). The \( \text{Ca}^{2+} \)-dependent changes in \( \text{Ca}^{2+} \)-ATPase activity for a typical subject are displayed in Fig. 4.

In addition, coupling ratios, defined as the ratio of \( \text{Ca}^{2+} \) uptake (2,000 nM) to \( V_{\text{max}} \) were also unaffected by exercise whether performed in normoxia or hypoxia (Fig. 5).

**DISCUSSION**

Our results partially support our first hypothesis, namely that prolonged exercise in normoxia would elicit disturbances in SR \( \text{Ca}^{2+} \)-cycling function in the vastus lateralis muscle as assessed in vitro. As postulated, we have found reductions in \( \text{Ca}^{2+} \) uptake, \( \text{Ca}^{2+} \) release, and \( \text{Ca}^{2+} \)-ATPase activity. These changes occurred in the absence of changes in the coupling ratios and in the other kinetic properties of the \( \text{Ca}^{2+} \)-ATPase, namely \( n_H \) and \( C_{50} \). Unexpected, however, was the discordant time-course changes between \( \text{Ca}^{2+} \) uptake and \( \text{Ca}^{2+} \) release during the exercise. Contrary to our second hypothesis, we could find no additional effect on the SR properties that were examined when the same exercise was performed in hypoxia.

A number of previous studies on humans using a variety of exercise protocols other than prolonged cycling have reported
reductions in Ca\textsuperscript{2+}-uptake (12, 16, 21, 42), Ca\textsuperscript{2+}-release (21, 25), and Ca\textsuperscript{2+}-ATPase activity (25, 42) in the SR of vastus lateralis. Prolonged submaximal exercise has also been reported to alter SR Ca\textsuperscript{2+} cycling in human skeletal muscle. As an example, Booth et al. (4) have found that cycling exercise performed at 75% \(\dot{V}O_2\) peak to exhaustion (~72-min duration) resulted in a reduction in Ca\textsuperscript{2+}-uptake and Ca\textsuperscript{2+}-ATPase activity of 17 and 21%, respectively. Our group has reported reductions in Ca\textsuperscript{2+}-ATPase activity of 14% during 30 min of cycle exercise at 58% \(\dot{V}O_2\) peak, which was followed by another 19% reduction during exercise at 72% \(\dot{V}O_2\) peak (18). Several studies using prolonged treadmill exercise in rats have also detected reductions in muscle Ca\textsuperscript{2+} uptake (5, 14), Ca\textsuperscript{2+}-ATPase activity (2, 5, 26, 49), and Ca\textsuperscript{2+} release (11, 31). These studies in conjunction with a variety of other studies using different species and different contractile schedules (46, 47) contribute to the growing consensus that exercise can substantially disrupt SR Ca\textsuperscript{2+}-cycling homeostasis.

However, not all studies report exercise-induced disturbances in SR function with prolonged exercise in running rats. Work from our laboratory (6, 35) as well as others (13, 14) has observed...
failed to find reductions in \( \text{Ca}^{2+} \)-ATPase activity after treadmill exercise in rats. Although the underlying reasons for the contradictory results remain unclear, differences in exercise protocol, the tissues examined, and fractionation procedures all remain of possible importance. Time of tissue sampling after the exercise has also emerged as another potentially significant consideration. Compared with preexercise and immediate post-exercise samples, large increases in maximal \( \text{Ca}^{2+} \)-ATPase activity have been described relatively early in the recovery period (13). We have recently confirmed this finding, and we have been able to demonstrate that the increase in \( V_{\text{max}} \) is accompanied by increases in \( \text{Ca}^{2+} \) uptake but not in \( \text{Ca}^{2+} \) release (35).

A distinguishing feature of our present study, in contrast to most earlier studies investigating exercise in both humans and animals, which have generally only measured selected SR properties, is the comprehensive nature of our analytical assessment. The collective measurements that we have made, in addition to providing \( \text{Ca}^{2+} \)-dependent examination of \( \text{Ca}^{2+} \) uptake and \( \text{Ca}^{2+} \)-ATPase activity, have also allowed examination of the interrelations between \( \text{Ca}^{2+} \) uptake, \( \text{Ca}^{2+} \)-ATPase, and \( \text{Ca}^{2+} \) release characteristics. These measurements are important for a number of reasons but particularly because a dissociation between changes in \( \text{Ca}^{2+} \)-ATPase activity and \( \text{Ca}^{2+} \) uptake during prolonged exercise has previously been reported (2, 14). Based on these properties, we have been able to conclude that prolonged moderate exercise in humans resulted in reductions in \( \text{Ca}^{2+} \) uptake that were independent of \([\text{Ca}^{2+}]_i\). In addition, we found that, although \( \text{Ca}^{2+} \)-ATPase activity was depressed, no changes were found in the affinity of the enzyme for \([\text{Ca}^{2+}]_i\), as indicated by a lack of change in \( n_H \) and \( \text{Ca}_{\text{SR}} \). Moreover, the efficiency of \( \text{Ca}^{2+} \) transport was unchanged, as shown by the coupling ratio, which is defined as the ratio between \( \text{Ca}^{2+} \)-uptake and \( \text{Ca}^{2+} \)-ATPase activity. These results have important functional implications, because repetitive sustained contractile activity, given the changes in intracellular temperature, substrate, and metabolite milieu, could conceivably alter SR membrane composition and fluidity in addition to enzyme behavior (17, 24, 36).

Based on previous studies employing prolonged exercise in rats (26) and chronic low-frequency stimulation in rats and rabbits (9), it would appear that the reduction in \( V_{\text{max}} \) we have observed with exercise is due to structural alterations in the region of the nucleotide binding site of the enzyme. Moreover, it appears that the structural alterations in enzymes occur as a result of oxidation and nitrosylation, which occur as a consequence of elevations in ROS (23). If such is the case, recovery of normal enzyme catalytic activity may take 2–3 days (27).

Accompanying the exercise-induced reductions in \( \text{Ca}^{2+} \) uptake we have observed were reductions in \( \text{Ca}^{2+} \) release. Unlike many previous studies, which have employed \( \text{AgNO}_3 \) to measure functional CRC, we have employed 4-CMC. The use of 4-CMC instead of \( \text{AgNO}_3 \) was based on a recent study by our group (41), which demonstrated that \( \text{AgNO}_3 \) but not 4-CMC induced \( \text{Ca}^{2+} \) release from the lumen of the SR by acting both on the CRC and \( \text{Ca}^{2+} \)-ATPase. We have also found that two kinetic phases of \( \text{Ca}^{2+} \) release can be determined with 4-CMC, a rapid phase (phase 1) and a more delayed phase (phase 2). Although the characteristics of \( \text{Ca}^{2+} \) release would be expected to be influenced by the technique employed, a multiphase release pattern has also been demonstrated previously in other types of preparations (29, 38). Prolonged exercise resulted in reductions in both phase 1 and phase 2. It should be emphasized that the physiological significance of each of these phases remains unclear. In our system, those phases were measured in homogenates prepared from whole muscle. It is to be expected, given the protein complexity of the homogenate preparation, that differences in both the time and magnitude of the \( \text{Ca}^{2+} \)-release phases would differ between enriched SR fractions and single-channel preparations (10). It is of interest that previous studies using \( ^3 \text{H} \text{ryanodine binding to assess channel integrity} \) have shown that exercise-induced reductions in \( \text{Ca}^{2+} \) release are also accompanied by reductions in \( ^3 \text{H} \text{ryanodine binding} \) (11). Although definitive evidence is lacking, there is a consensus that the structural alterations in CRC are mediated by oxidation secondary to ROS accumulation (10). It is noteworthy that the reductions in \( \text{Ca}^{2+} \)-cycling behavior we have found, both \( \text{Ca}^{2+} \) uptake and \textit{phase 2} \( \text{Ca}^{2+} \) release, were both initially observed early in exercise, suggesting a common mechanism. However, unlike \( \text{Ca}^{2+} \) uptake, which remained unchanged over the remainder of the exercise, the impairment is \( \text{Ca}^{2+} \) release increased. In addition, \textit{phase 1} was only reduced at 90 min of exercise. It is unclear whether the impairment in \( \text{Ca}^{2+} \) uptake and \( \text{Ca}^{2+} \) release are mediated by common mechanisms.

We could find no additional effect of hypoxia on SR function during exercise. This was unexpected, given the significance of altered SR \( \text{Ca}^{2+} \) cycling in fatigue in submaximal exercise (1) and the effects of hypoxia on increasing fatigue (15). As shown by our \( V_{\text{E}} \), RER, and heart rate data, exercise in hypoxia compared with normoxia clearly increased the responses. The increases in \( V_{\text{E}} \), RER, and heart rate that we have observed during exercise in hypoxia compared with normoxia have all been previously documented (45, 48). Although greater increases in \( V_{\text{CO}_2} \) appeared to have occurred in hypoxia compared with normoxia, as might be expected (45), these differences were not significant. Hypoxia did not result in an altered \( V_{\text{O}_2} \) during either rest or exercise compared with normoxia. These results indicate that, for the submaximal exercise intensity employed, compensatory adjustments are capable of protecting oxidative phosphorylation during steady-state exercise. Based on previous research (48), it would appear that oxidative phosphorylation is protected primarily via increases in blood flow to the working muscle, which maintains arterial oxygen delivery in the face of decrease in arterial content.

The maintenance of oxidative phosphorylation during hypoxia may be important in preventing additional disturbances in SR \( \text{Ca}^{2+} \)-cycling properties because the intracellular environment would not be dramatically altered even though the volunteers were working at a greater percentage of \( \dot{V}_{\text{O}_2} \) peak. It has been estimated that \( 2–4\% \) of oxygen transport through the mitochondria escapes in the form of ROS (37). However, increased ROS accumulation would be expected to occur as a result of a greater dependence on AMP-to-inosine 5'-monophosphate formation in hypoxia (19, 33) and consequent degradation to inosine via the xanthine oxidase system (37). Alternatively, given the relatively light nature of the prolonged exercise protocol (i.e., \( 48\% \) of \( \dot{V}_{\text{O}_2} \) peak), ROS scavenger
mechanisms may have been sufficient to minimize further changes in the oxidative environment during exercise in hypoxia. In this regard, it would appear productive to examine the effects of heavier exercise, which is thought to result in greater production of ROS (40) on the SR Ca\textsuperscript{2+}-cycling function in working muscle during both normoxia and hypoxia.

In summary, we have found that prolonged exercise of moderate intensity results in a disturbance in SR Ca\textsuperscript{2+} homeostasis, as indicated by the reductions in Ca\textsuperscript{2+} to be due to the inhibition of Ca\textsuperscript{2+} uptake and Ca\textsuperscript{2+} release that occurs. The reduction in Ca\textsuperscript{2+} uptake appears to be due to the inhibition of Ca\textsuperscript{2+}-ATPase activities because coupling ratios were unaltered by exercise. Published evidence suggests that the reduction in in vitro Ca\textsuperscript{2+} uptake and Ca\textsuperscript{2+} release activity is mediated by structural alterations in the region of the nucleotide binding region of the enzyme, an effect mediated by ROS accumulation. The performance of the same exercise protocol in hypoxia (Fi\textsubscript{O} = 0.14) does not further increase the disturbance in SR Ca\textsuperscript{2+} homeostasis.

GRANTS
This study received financial support from the National Sciences and Engineering Research of Canada.

REFERENCES


