Human neuromuscular fatigue is associated with altered Na\(^+\)-K\(^+\)-ATPase activity following isometric exercise

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Fowles, J. R., H. J. Green, R. Tupling, S. O’Brien, and B. D. Roy. Human neuromuscular fatigue is associated with altered Na\(^+\)-K\(^+\)-ATPase activity following isometric exercise. J Appl Physiol 92: 1585–1593, 2002.—The purpose of this study was to investigate the hypothesis that reductions in Na\(^+\)-K\(^+\)-ATPase activity are associated with neuromuscular fatigue following isometric exercise. In control (Con) and exercised (Ex) legs, force and electromyogram were measured in 14 volunteers [age, 23.4 ± 0.7 (SE) yr] before and immediately after (PST0), 1 h after (PST1), and 4 h after (PST4) isometric, single-leg extension exercise at ~60% of maximal voluntary contraction for 30 min using a 0.5 duty cycle (5-s contraction, 5-s rest). Tissue was obtained from vastus lateralis muscle before exercise in Con and after exercise in both the Con (PST0) and Ex legs (PST0, PST1, PST4), for the measurements of Na\(^+\)-K\(^+\)-ATPase activity, as determined by the 3-O-methylfluorescein phosphatase (3-O-MFPase) assay. Voluntary (maximal voluntary contraction) and elicited (10, 20, 50, 100 Hz) force was reduced 30–55% (P < 0.05) at PST0 and did not recover by PST4. Muscle action potential (M-wave) amplitude and area (measured in the vastus medialis) and 3-O-MFPase activity at PST0-Ex were less than that at PST0-Con (P < 0.05) by 37, 25, and 38%, respectively. M-wave area at PST1-Ex was also less than that at PST1-Con (P < 0.05). Changes in 3-O-MFPase activity correlated to changes in M-wave area across all time points (r = 0.38, P < 0.05, n = 45). These results demonstrate that Na\(^+\)-K\(^+\)-ATPase activity is reduced by sustained isometric exercise in humans from that in a matched Con leg and that this reduction in Na\(^+\)-K\(^+\)-ATPase activity is associated with loss of excitability as indicated by M-wave alterations.

A number of intracellular sites have been mechanistically linked to depressed force after activity. Accumulating evidence suggests that an impairment in excitation-contraction coupling can be a primary source of force failure with severe fatigue (49); however, other sites may contribute to varying degrees (19). More specifically, the role of the sarcolemma and t-tubular membranes in conducting repetitive action potentials has been identified as a possible contributor to fatigue in humans during voluntary exercise (20, 43). Failure at the level of the sarcolemma could occur because the reduction in the transmembrane gradients for Na\(^+\) and K\(^+\) during exercise reduces the ionic membrane potential. This would be reflected in decreases in the amplitude and area of the muscle compound action potential (M wave) and force (37).

Previous work studying the sarcolemma as a site in fatigue has relied primarily on properties of the electromyogram (EMG) and, subsequently, the M wave, as an indirect measure of sarcolemmal excitability. Reduced M-wave amplitude and/or area has been directly related to reduced force in experiments employing sustained muscle stimulation in both animals (2, 40) and humans (26). However, acute decreases in M-wave amplitude and area are not commonly observed during volitional exercise, especially if the fatigue is induced by high-intensity, short-duration (<5 min) activity (3, 9). This may be due to M-wave potentiating effects that counteract or supersede the effects of fatigue directly after these types of contraction schedules (34).

In contrast, contractions repeated for longer durations appear to induce greater M-wave depressions than brief, high-force, fast-fatiguing contractions (3, 18). In studies by Arnaud et al. (1) and Jammes et al. (27), the depression in M-wave amplitude persisted for a minimum of 15 min after bouts of supramaximal cycling and progressive cycling to fatigue, respectively. Persistent reductions in M-wave properties have also been observed by others (35) during recovery after sustained, intermittent contractile activity. Collectively, these findings suggest that reduced excitability may have a more significant role in fatigue induced by sustained exercise than previously believed. It is possible that acute alterations of membrane proteins may be the basis for sustained M-wave changes and, consequently, contribute to the long-lasting fatigue of postcontractile depression (PCD) that is commonly observed after sustained isometric exercise (45, 47).

The Na\(^+\)-K\(^+\)-ATPase is an integral membrane protein that maintains ionic gradients at the sarcolemma by pumping 3Na\(^+\) out and 2K\(^+\) into the cell during each cycle of the pump. The activity of the pump is increased in response to repetitive action potentials. This pump activity is also electrogenic in nature and, therefore, contributes to membrane potential and the maintenance of sarcolemmal excitability (11). Changes in the transmembrane gradients for Na\(^+\) and K\(^+\) during exercise would be reflected in changes in the amplitude and area of the compound muscle action potential (M wave) and force (37).

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in M wave result primarily from a loss in muscle excitability, secondary to a loss in Na\(^+\) and K\(^+\) gradients (40). Consequently, the catalytic activity of the Na\(^+-\)K\(^+\) pump to maintain ion gradients is believed to directly influence the magnitude of the M wave (24, 39). Although the Na\(^+-\)K\(^+\)-ATPase has a wide dynamic range of activity to maintain excitability in vivo (37), there is little evidence to indicate that exercise intrinsically alters the enzymatic activity of the Na\(^+-\)K\(^+\)-ATPase in skeletal muscle.

A recent study by Verburg et al. (48) supports this possibility. These investigators reported that, during sustained submaximal exercise, an increased loss of K\(^+\) from muscle was observed late in the exercise, which was attributed to an “insufficient activation of pumps.” The “insufficient activation” could be due to an exercise volume-dependent inactivation of the Na\(^+-\)K\(^+\)-ATPase, resulting in a loss of enzymatic activity and an inability to maintain K\(^+\) homeostasis, membrane excitability, and, consequently, force. Evidence from cardiac muscle Na\(^+-\)K\(^+\)-ATPase indicates that the pump may be susceptible to damage by free radicals (29, 30), which are produced during contractile activity (44). Recently, our laboratory has demonstrated that moderate-intensity isometric contractions for 30 min in humans were associated with prolonged reductions in sarcoplasmic reticulum (SR) Ca\(^{2+}\)-ATPase pump function in vitro, which could have contributed to PCD (47). PCD is a type of fatigue characterized by a depression in force across low and high frequencies of stimulation after exercise (47). It is possible, particularly with the high level of activation required to sustain this type of isometric exercise schedule, that the Na\(^+-\)K\(^+\)-ATPase may be similarly affected and result in reduced enzyme activity measured in vitro after exercise.

The purpose of this study was to determine whether Na\(^+-\)K\(^+\)-ATPase activity is affected by sustained, moderate-intensity, isometric exercise and to investigate the interrelationship among Na\(^+-\)K\(^+\)-ATPase activity, muscle excitability, and neuromuscular fatigue. We have hypothesized that Na\(^+-\)K\(^+\)-ATPase activity and muscle excitability will be reduced during exercise and will remain depressed during recovery, as was observed for the Ca\(^{2+}\)-ATPase using a similar protocol.

**METHODS**

**Subjects.** Fourteen healthy, active volunteers [7 men, 7 women, age 23.4 ± 0.7 (SE) yr] participated in the study. Nine of these subjects volunteered for the biopsy component of the study. The study carried the approval of the Office of Human Research at the University of Waterloo, and all participants were fully informed of all experimental procedures and associated risks before written consent was obtained.

**Experimental design.** To investigate the relationship between Na\(^+-\)K\(^+\) pump function and PCD, subjects completed a moderately intense isometric exercise protocol as previously described (47), with minor modifications. Subjects performed isometric single-leg extension at a target force of ~60% of maximum voluntary contraction (MVC) for 30 min using a 0.5 duty cycle (5-s contraction, 5-s rest) to induce PCD.

Force and EMG were measured before (Pre), immediately after (PST0), 1 h after (PST1), and 4 h after (PST4) exercise, both in the control (Con) and exercise (Ex) legs. Muscle tissue samples were obtained using the muscle biopsy technique under suction at Pre in a rested Con leg and at PST0 in both the Con and Ex legs and at PST1 and PST4 in the Ex leg only. The Ex leg was randomized among subjects. Tissue sampling sites were prepared before initial mechanical measurements, and a total of two biopsies were extracted from each site at each time point, according to procedures previously described (6). The initial biopsy was quickly plunged into liquid N\(_2\) and stored for later analysis of Na\(^+-\)K\(^+\) pump activity. The second sample was used for analysis of Na\(^+-\)K\(^+\) pump concentration. During the exercise protocol, the initial tissue sample was extracted as rapidly as possible (within 5 s) after the last 5-s contraction. In general, sample sizes of between 25 and 50 mg were obtained from each sample.

The subjects reported to the laboratory 2–4 days before the beginning of the experiment. This visit was used as an initial accommodation and testing session for measurement of MVC force and forces at different electrical stimulation frequencies. During the initial visit, the exercise task was also practiced. At Pre on the experimental day, the force-frequency measures were repeated (to account for differences in stimulating electrode placement between days). Approximately 5 min after the initial mechanical measurements, the subjects began the fatigue protocol. An oscilloscope screen was clearly marked to indicate the target force (60% MVC) that each subject was required to achieve during repetitive activity. Periodically during activity, a brief force record was used to verify that the subjects were meeting target force. When both biopsy and mechanical measurements were made at a testing time point, contractile measurements in the Ex limb followed the biopsy (~30 s), which were then followed by mechanical measurements in the Con leg.

**Muscle stimulation and force measurements.** The experimental setup for measuring muscle contractile characteristics in isometric knee extension has been described previously (22, 47). Briefly, for all force measurements, both voluntary and involuntary, the participant sat upright in a straight-backed chair with hips and leg firmly secured, the knee at 90° to the thigh, and the arms folded across the chest. Twitches (Tw) and tetani were elicited by stimulation of the quadriceps muscles using a Grass model S48 stimulator with an isolation unit. 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. The linear variable differential transducer was amplified by a Daytronic carrier preamplifier at 1 kHz, converted to a digital signal, and fed into a 12-bit analog-to-digital converter and then into an IBM computer for analysis. Calibration was performed before each test session with weights of known amounts. Two aluminum chloride electrodes (8 × 13 cm) coated with warm electrode gel were used to deliver the electrical impulse to the quadriceps muscle. The ground electrode was placed centrally on the anterior aspect of the thigh just above the patella, whereas the active electrode was toward the hip on the proximal portion of the belly of the vastus lateralis. Each electrode was secured firmly with tensor wrapped around the leg and over the top of the electrode to ensure good contact between the skin and the electrode.

Tw were evoked using a single supramaximal (~150 V) impulse of 50-μs duration, whereas tetani at low (10 and 20 Hz) and high (50 and 100 Hz) frequencies were induced using a voltage that elicited ~55% of MVC at 100 Hz with a pulse duration of 50 μs and train duration of 1 s. The voltage was...
kept constant throughout the testing for each individual subject. Tetanic force, regardless of frequency of stimulation, was taken as the peak force recorded. MVCs with Tw interpolation (IT) were obtained as the best of two trials. For each MVC, IT was used to calculate motor unit activation (MUA) by the formula of potentiated IT/potentiated Tw × 100 (4). During a typical trial, subjects were first assessed for supramaximal Tw properties, followed by tetanic stimulations from low to high frequency, followed by the MVCs.

**EMG.** The area required for muscle biopsies and positioning of stimulation electrodes precluded the use of the vastus lateralis EMG. Preliminary testing indicated that changes in vastus medialis EMG correlated with changes in vastus lateralis EMG at the specific joint angle used in this experiment (unpublished observations). EMG recordings were made with 10-mm-diameter Ag-AgCl (Medtrac 60) surface electrodes. Electrodes were placed over the belly of the vastus medialis (interelectrode distance was ~2 cm), with one ground electrode positioned on the lateral epicondyle of the tibia. The skin was shaved, abraded, and cleansed with povidone. Electrode positioning was not altered during a single testing trial.

The EMG signal (20- to 500-Hz bandwidth) was passed through an alternating-current amplifier (National Instruments, AT-MIO-16H multifunction board). The gain was calibrated to optimize signal amplitude for analog-to-digital conversion and collected at 2,028 Hz. Custom-modified National Institute 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). Raw EMG signals from voluntary contractions were full-wave rectified, and the resulting signal was integrated over the duration of the contractions. Integrated EMG was divided by time (for a 1-s window) to achieve average integrated EMG (AEMG; mV) in either MVC or repetitive contractions. For electrically stimulated contractions, single-Tw compound muscle action potentials (M wave) were analyzed for peak-peak amplitude (mV), duration (ms), and area (μV·ms). Amplitude was defined as the sum of absolute values for maximum and minimum points of the biphasic (one positive and one negative deflection) M wave. Duration was defined as the time from baseline to baseline from the beginning to the end of the biphasic M wave, where the beginning is defined as a positive deflection 2 SDs above baseline harmonic mean and the end as a return to baseline. The area was calculated as the integral of the absolute value of the EMG waveform, where the beginning and end are defined by the measurement of baseline-baseline duration.

**Na⁺-K⁺-ATPase activity.** Activity of the Na⁺-K⁺-ATPase was assessed using the K⁺-stimulated 3-O-methylfluorescein phosphatase (3-O-MFPase) assay following the procedures of Fraser and McKenna (17), with minor modifications. In this assay, it is not the hydrolytic activity of the ATPase that is measured as such but the phosphatase activity. Measurement of phosphatase activity is based on the K⁺-dependence of the chormogenic substrate 3-O-methylfluorescein phosphate (3-O-MFP), which substitutes for the aspartylphosphate intermediate of the ATPase to represent the terminal step in ATP hydrolysis (25). In the measurement of 3-O-MFPase, the activity of the enzyme is based on the dephosphorylation of 3-O-MFP, which occurs during stimulation of the enzyme by K⁺. The change in fluorescence, which accompanies the hydrolysis of 3-O-methylfluorescein from 3-O-MFP and which is a measure of the 3-O-MFPase activity, is quantified by fluorescence spectrophotometry (excitation wavelength = 475 nm; emission wavelength = 515 nm, with 5-nm slit width). Briefly, tissue from the frozen muscle biopsy was homogenized (5% wt/vol) at 0°C for 2 × 20 s at 25,000 rpm (Polytron), in a buffer containing 250 mM sucrose, 2 mM EDTA, and 10 mM Tris (pH 7.40). Homogenates were freeze-thawed four times and diluted 1:4 in cold homogenate buffer. Approximately 30 μg of protein (~25-μl homogenate) were incubated for 4 min in medium containing 5 mM MgCl₂, 1.25 mM EDTA, and 100 mM Tris (pH 7.40). The K⁺-stimulated activity of the Na⁺-K⁺-ATPase was determined by the increase in activity after the addition of 10 mM KCl, at a substrate concentration of 160 μM 3-O-MFP. 3-O-MFPase activity was based on the difference in slope before and after the addition of KCl (ΔF/min). We have demonstrated that the difference in slope is completely eliminated by [³H]ouabain, which is used to inhibit Na⁺-K⁺-ATPase activity (H. Green, unpublished observations). The Na⁺-K⁺-ATPase activity, which was based on an average of four trials for each sample, is expressed in nanomoles per milligram protein per hour. This value was obtained by dividing ΔF for the sample by ΔF for a standard solution of 3-O-MFP, multiplying by the concentration of the standard, and dividing by the amount of protein in the tissue sample (μmol protein/mg tissue).

The homogenate was determined by the method of Lowry as modified by Schacterle and Pollock (42).

To test the hypothesis that M-wave area is an indicator of membrane excitability based on the electrogenic contribution of the Na⁺-K⁺-ATPase to membrane potential, we correlated M-wave area to Na⁺-K⁺-ATPase activity measured by 3-O-MFP. M-wave area is generally interpreted as a measure of the magnitude of the total current passage at the recording electrode, which is a function of the size of the individual action potentials and the number of activated fibers (39). Only time points at which a matched set of both biopsy and M-wave measurements was available (i.e., Pre-Con, PST0-Con, PST0-Ex, PST1-Ex, PST4-Ex) were used in the correlation of M-wave area with pump activity.

**Na⁺-K⁺-ATPase content.** The content of Na⁺-K⁺ pumps in muscle biopsy samples was determined using the assay developed by Nørgaard et al. (38) and employed previously in our laboratory (10). With the use of this procedure, two samples from each biopsy, weighing between 2 and 8 mg, are preserved twice within 10-min periods in a Tris-sucrose buffer (10 mM Tris·HCl, 3 mM MgSO₄, 1 mM Tris-vanadate, and 250 mM sucrose) containing sodium metavanadate (NaVO₃) at 0°C. Samples were incubated in the Tris-sucrose buffer with [³H]oubain (1.8 μCi/ml) and unlabeled ouabain (1 μM final concentration) for 2 × 60 min at 37°C. After the unbound ouabain was removed by washing four times for 30 min in ice-cold buffer, the samples were blotted, weighed, placed in 1.5-ml Eppendorf tubes, and soaked in 1 ml 5% trichloroacetic acid for 16 h at room temperature, and then 0.5 ml of sample was counted for ³H activity in a scintillation mixture. [³H]oubain binding capacity was corrected (x1.05) for loss of specifically bound [³H]oubain during washout (38). No correction was made for nonspecific uptake and retention of [³H], which was estimated at <3% (H. Green, unpublished observations). The isotopic purity of the [³H]oubain was 99% as determined by the supplier (New England Nuclear-Du Pont Canada).

**Data analysis.** Statistical analysis was performed on Statistica for Windows R.4.5 software (1993; Statsoft A, Tulsa, OK). Descriptive statistics included means and SE. One-way ANOVA with repeated measures was used to analyze Na⁺-K⁺-ATPase properties over time. Correlational analysis by linear regression was used to relate M-wave area to Na⁺-K⁺-ATPase activity and Tw force. Two-factor ANOVAs were used to compare differences in force and EMG measures between the Con and Ex condition for each time point. Dif-
ferences between Ex and Con legs were determined by paired comparison. Post hoc analysis of main effects was performed using the Tukey test. The probability level for statistical significance was accepted at \( P < 0.05 \).

RESULTS

Force measurement. The target force was maintained throughout most of the protocol, although some subjects were unable to maintain a target of \( \sim 60\% \) of MVC in the last minutes of the exercise session (data not presented). All subjects completed 30 min of exercise, despite the neuromuscular fatigue.

Only main effects were observed for the condition in which evoked force was depressed \( (P < 0.05) \) across all frequencies at PST0 in Ex (Fig. 1A). The depression ranged from \( \sim 50\% \) at low frequencies (Tw, 10 and 20 Hz) to \( \sim 33\% \) at high frequencies (50 and 100 Hz). Force remained depressed \( (P < 0.05) \) below Pre across all frequencies after 4 h of recovery. The depression ranged from \( \sim 44\% \) (10 Hz) to \( \sim 17\% \) (100 Hz). The only changes in Con-stimulated force were at PST0, at which there was an \( \sim 10\% \) increase in the last minutes of the exercise session (data not presented).

\( \text{Table 1. Force characteristics after sustained voluntary isometric exercise} \)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>PST0</th>
<th>PST1</th>
<th>PST4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tw, N</td>
<td>132</td>
<td>151</td>
<td>133</td>
<td>130</td>
</tr>
<tr>
<td>MVC, N·m</td>
<td>502</td>
<td>487</td>
<td>469</td>
<td>447</td>
</tr>
<tr>
<td>%Act</td>
<td>92.0</td>
<td>90.9</td>
<td>91.0</td>
<td>91.6</td>
</tr>
<tr>
<td>%AEMG</td>
<td>100</td>
<td>94.5</td>
<td>90.3</td>
<td>89.1</td>
</tr>
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Values are means ± SE \((n = 14 \text{ subjects})\). Pre, PST0, PST1, and PST4: before, immediately after, 1 h after, and 4 h after exercise; Tw, evoked peak twitch; MVC, maximum voluntary contraction; \%Act, percentage of motor-unit activation in an MVC (see text); \%AEMG, average integrated electromyography in an MVC as a percentage of Pre MVC AEMG; Con, control leg; Ex, exercise leg. *Significant difference from Pre \((P < 0.05)\).

In the Ex leg, MVC was depressed at PST0 by 42\% \( (P < 0.05) \) and recovered by 26\% \( (P < 0.05) \) at PST4, although it still remained below Pre values \( (P < 0.05) \) (Table 1). The reduction in MUA measured during an MVC was 21\% at PST0. MUA remained below Pre by 13 and 9\% at PST1 and PST4, respectively \( (P < 0.05) \). There were no significant changes in MVC force or MUA in the Con limb.

EMG. During the exercise, AEMG during repetitive contractions increased by 20\% \( (P < 0.05) \) (results not presented). The AEMG at the beginning of the repetitive contractions was 52\% of AEMG observed with the Pre MVC. The AEMG during MVC was reduced \( (P < 0.05) \) by 32 and 20\% at PST0 and PST1, respectively \( (P < 0.05) \). There was no change in AEMG at MVC in the Con limb.

There was no difference in Pre values between Con and Ex for the M-wave parameters (amplitude, duration, and area) investigated. Pre values (Pre-Ex) for amplitude, duration, and area were 7.64 ± 0.7 mV, 25.6 ± 2.8 ms, and 463 ± 29 μV/μs, respectively. Both M-wave amplitude and area were altered \( (P < 0.05) \) in response to the exercise (Fig. 2). M-wave amplitude at PST0-Ex was 37\% less than that at PST0-Con \( (P < 0.05) \). No further differences were observed between Ex and Con in recovery. M-wave area in Ex was 25\% less than in Con at both PST0 and PST1 \( (P < 0.05) \) (Fig. 2, A and C). M-wave area and amplitude in Ex were not different from those in Con at PST4. There was no significant change in M-wave duration over time in either leg (Fig. 2B).

Na\(^+\)-K\(^+\)-ATPase function. Na\(^+\)-K\(^+\)-ATPase activity, measured by 3-O-MFPase, was 81.2 ± 12.8 nmol·mg protein\(^{-1}·h\(^{-1}\) at Pre for Con. At PST0 in Ex, 3-O-MFPase was 35\% less than at PST0 for Con \( (58.8 ± 9.8 \text{ vs. } 90.6 ± 13.2 \text{ nmol·mg protein}\(^{-1}·h\(^{-1}\) ; } P < 0.05 \) (Fig. 3A). 3-O-MFPase activity was not different from Pre at

\( P < 0.05 \) in 20-, 50-, and 100-Hz force. There were no differences from Pre at any other time in the Con condition (Fig. 1B).
any time point. [3H]Oubain binding indicated that the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase pump content quantified by this technique did not change over time (Fig. 3B). Linear regression indicated a low correlation between 3-O-MF-

\begin{align*}
\text{Fig. 2.} & \quad \text{M-wave amplitude (A), duration (B), and area (C) (see definitions in text), represented relative to Pre values in exercise (Ex) and control (Con) limbs at PST0, PST1, and PST4. M-wave properties were obtained from the vastus medialis. Values are means} \pm \text{SE. *Significantly different from Pre (} P < 0.05, n = 14).}
\end{align*}

be associated with a prolonged impairment in SR Ca\textsuperscript{2+} pump function (47). Using the 3-O-MF-

\begin{align*}
\text{DISCUSSION}
\end{align*}

In this study, we hypothesized that Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity and muscle excitability would be reduced during an isometric exercise protocol previously shown to
by depressions in M-wave amplitude and area. The fact that M-wave and Na\(^+\)-K\(^+\)-ATPase activity recovered by PST4 demonstrates the rapidly adaptable nature of the Na\(^+\)-K\(^+\) pump in skeletal muscle. These results also suggest that, at least for the isometric task studied, compromised Na\(^+\)-K\(^+\)-ATPase activity may contribute to reduced sarcolemmal excitability and contribute to muscle fatigue. It should be emphasized that, although a strong trend was observed toward a decline in Na\(^+\)-K\(^+\)-ATPase measured at PST0 compared with Pre, statistical significance was not found. Using the contralateral limb as the control would appear appropriate because the nonexercised leg would be subjected to many of the vascular influences (hormonal, ionic) as the Ex leg.

**Exercise protocol and fatigue.** PCD is a type of fatigue characterized by a depression in force across low and high frequencies of stimulation after exercise (45, 47). The repetitive isometric exercise protocol employed in this experiment depressed force output across all muscle stimulation frequencies and in MVCs. Although some recovery was evident, fatigue persisted up to PST4. This protocol was previously observed to cause acute alterations of the Ca\(^{2+}\) pump, reducing maximal activity of the Ca\(^{2+}\)-ATPase for at least PST1 (47). The authors suggested that Ca\(^{2+}\) cycling could be one of several sites of fatigue with this protocol. Previous investigations on the cause(s) of PCD identified a failure of neuromuscular transmission (8), although it was later demonstrated that muscle fibers retained the ability to generate normal action potentials during PCD (33). The results of this experiment indicate that, given the high level of activation required to sustain the voluntary isometric exercise schedule, impaired excitability of the sarcolemma could contribute to neuromuscular fatigue that was observed. However, definitive evidence that impaired sarcolemmal excitability is mechanistically linked to fatigue would depend on demonstrating impaired t-tubule excitability and coupling between the t-tubule and the SR Ca\(^{2+}\)-release channel. During recovery, excitability was restored at a point at which force was still depressed. This observation supports other findings that indicate that other peripheral sites of fatigue are responsible for more prolonged reductions in force during the period after exercise [as reviewed by Fitts (16)]. The possibility that central fatigue, because of an impairment in neural drive, also occurred must be acknowledged. The reductions that were observed in MUA during the MVC and the reductions in AEMG observed both after exercise and in recovery are consistent with this notion.

**Inactivation of the Na\(^+\)-K\(^+\)-ATPase.** The capacity of the Na\(^+\)-K\(^+\) pump to restore ionic gradients can be challenged by heavy contractile demands and resultant changes in the local environment (i.e., ionic and metabolite buildup, substrate depletion), and, therefore, appropriate regulation of Na\(^+\)-K\(^+\)-ATPase activity is essential for maintaining transport capacity and muscle excitability (37). Complex regulatory controls exist to ensure that Na\(^+\)-K\(^+\) pump capacity is “activated” during exercise to maintain excitability. Intrinsic activity of the pump is increased over a 20-fold range by mechanisms associated with the action potential (14) and acute hormonal control (7). Pump capacity may also be increased by translocation of a specific pool of Na\(^+\)-K\(^+\) pump subunits from intracellular sites to the muscle membrane (28). Despite a wealth of information published on the role of the Na\(^+\)-K\(^+\)-ATPase in muscle excitability and fatigue, the actual enzymatic capacity of the pump in skeletal muscle to meet the demands of exercise remains relatively unknown. Most experiments rely on indirect measures of excitability and pump function in response to a short-term fatiguing event. However, there has recently been evidence that Na\(^+\)-K\(^+\) pump inactivation can occur during sustained exercise in humans. Verburg et al. (48) determined that, after a period of sustained submaximal exercise, increased loss of K\(^+\) from muscle occurred, which persisted in brief recovery. This observation suggests that a reduction in Na\(^+\)-K\(^+\)-ATPase activity occurred as the exercise progressed. This conclusion is supported by a study reported in abstract form that found direct reductions in Na\(^+\)-K\(^+\)-ATPase activity as measured by 3-O-MFPase during prolonged exercise (36). Our results offer further evidence that inactivation of the Na\(^+\)-K\(^+\)-ATPase can occur, at least with sustained isometric exercise.

There are a number of possible mechanisms to explain the intrinsic reduction in pump activity that was observed in this experiment when measured in vitro under supposedly optimal conditions. Changes in the metabolic environment were observed previously with this protocol (47); however, these effects are most likely to exact their greatest impact on Na\(^+\)-K\(^+\)-ATPase activity in vivo. Reduction in Na\(^+\)-K\(^+\)-ATPase activity measured under “optimal” conditions in vitro likely reflects structural alterations to the enzyme. The most notable causes for acute inactivation include temperature denaturation (15), Ca\(^{2+}\)-activated proteolysis (5), and free radical damage (31), all of which can increase with exercise (44). Studies on mouse diaphragm indicate that excessive intracellular Ca\(^{2+}\) can inhibit Na\(^+\) pump activity (46). Reports also indicate that free radical damage can reduce Na\(^+\)-K\(^+\)-ATPase activity in cardiac muscle (29, 30). Free radical damage could also affect the Na\(^+\)-K\(^+\)-ATPase, because the \(\alpha\)-subunit of the pump has a number of disulphide bonds that are susceptible to oxidation (31). Interestingly, the SR Ca\(^{2+}\)-ATPase activity is also inhibited by repetitive contractions, and structural modifications to the nucleotide binding domain appear to be responsible for the impairment (13). Regardless of the mechanisms, the fact that Na\(^+\)-K\(^+\)-ATPase activity had returned to Pre values within 4 h of recovery indicates that the alterations that occur as a result of exercise are rapidly reversible.

**EMG.** M-wave amplitude and area assessed in the Ex limb were significantly lower than in the unexercised Con limb at PST0. Although the decrease in M-wave amplitude in the Ex leg of 12–14% was not significant on its own, similar but significant decreases
have been shown with a long-duration voluntary fatigue protocol using the ankle plantarflexors and leg extensors (3) or first dorsal interosseus muscle (18). It has been proposed that the rundown of gradients for Na\(^+\) and K\(^+\) during intense exercise reduces the transmembrane ion potential and thereby reduce M-wave amplitude and force (37). A reduction in ion gradients with exercise can result from Na\(^+\) and K\(^+\) fluxes during successive action potentials, exceeding the ability for active transport mechanisms to maintain the gradients. Although complex regulatory controls exist, including the concentration of Na\(^+\) and specific ion channels in the sarcolemma, the Na\(^+\)-K\(^+\)-ATPase is the principal mechanism for maintaining excitability (43). The present results suggest that reduced M-wave amplitude after exercise may be due, at least in part, to intrinsic modifications of the Na\(^+\)-K\(^+\)-ATPase. According to this reasoning, modifications to the Na\(^+\)-K\(^+\)-ATPase should result in transmembrane disturbances in Na\(^+\) and K\(^+\). Impaired pump function would then indicate a compromised capability of the enzyme to maintain membrane excitability through its electrochemical contribution to membrane potential, resulting in depolarization, and reduced M wave.

It should be noted that the reduced M wave observed in this experiment at PST0 was relative to the M wave in an unperturbed Con limb. The trend toward higher M-wave amplitude and area in Con after exercise was significant. This effect has been observed previously (32), when noncontracting muscle fibers exhibit hyperpolarization, likely the result of catecholamine spillover associated with exercise of a single limb (41). The fact that a trend toward increased M-wave amplitude was observed in the nonexercised limb would suggest that the exercised-induced effects on M-wave amplitude are best appreciated by comparing the Ex limb with the nonexercised limb.

**Relationship of M wave and Na\(^+\)-K\(^+\)-ATPase.** The depression of M wave after activity and subsequent return to Pre levels within 4 h could be explained, at least in part, by alteration of structure and subsequent repair, or activation of, Na\(^+\)-K\(^+\)-ATPase subunits in recovery. M-wave area is interpreted as a measure of the magnitude of the total current passage at the recording electrode, which is a function of the size of the individual action potentials and the number of activated fibers (39). Either factor may be affected by fatigue; however, the integrity of the Na\(^+\)-K\(^+\)-ATPase determines the ion gradients that these variables represent in a measure of M-wave area, making it suitable for correlation to Na\(^+\)-K\(^+\)-ATPase measured in vitro. A positive and significant correlation was observed between Na\(^+\)-K\(^+\)-ATPase activity and content \((r = 0.59, P < 0.01, n = 16)\), which supports the hypothesis that the measure of activity is related to the Na\(^+\)-K\(^+\)-ATPase content. The capacity of these pumps is indicated by the measure of activity.

The relatively low correlation observed between Na\(^+\)-K\(^+\)-ATPase activity and M wave highlights the more complicated nature of human experimentation compared with the isolated nature of animal research, where studies support a direct relationship between recovery of membrane potential with activity of the Na\(^+\)-K\(^+\)-ATPase (24, 39, 40). A number of factors could affect the correlation coefficient. Because the M wave represents a composite of individual action potentials obtained during supramaximal stimulation, synchronization, conduction velocity, temperature, and transmembrane fluid shifts, in addition to changes in the action potentials themselves, could alter the individual M-wave properties (23). Measurements of Na\(^+\)-K\(^+\)-ATPase activity are not based on the hydrolytic activity of the Na\(^+\)-K\(^+\)-ATPase itself but on the phosphatase activity (25). This factor, in combination with the large, nonspecific activity, may compromise the measurement of the true catalytic activity of the enzyme. Finally, it must be recognized that the M-wave measurements were obtained from the vastus medialis, whereas the Na\(^+\)-K\(^+\)-ATPase was measured on tissue extracted from the vastus lateralis. Although we found a similar change in EMG activity between the two muscles during this contraction schedule, it is possible that M-wave characteristics and/or the Na\(^+\)-K\(^+\)-ATPase activity may vary between muscles. Finally, the relatively low correlation that we have observed may have resulted because of the data used to determine the relationship. We have used all data points, both during exercise and in recovery. It is possible that the relationship might have changed if we could have examined the exercise response only or multiple time points in recovery. Unfortunately, the limitations on the number of biopsies that could be performed necessitated the use of the full data set to examine the relationship between Na\(^+\)-K\(^+\)-ATPase activity and M-wave characteristics. A similar relation in Na\(^+\)-K\(^+\)-ATPase activity with exercise has not been previously published for human muscle because of difficulties in measuring the Na\(^+\)-K\(^+\)-ATPase activity. We used a newly modified assay for Na\(^+\)-K\(^+\)-ATPase activity (17) to show that the combined effects of increased M wave in Con and decreased M wave in Ex were related to the measured activity of the Na\(^+\)-K\(^+\)-ATPase. There are a number of assumptions and limitations associated with the use of M wave as an indication of excitability and force in skeletal muscle (12). A positive correlation of M-wave amplitude with force (Tw) was also identified in this experiment (unpublished observations), demonstrating a link among Na\(^+\)-K\(^+\)-ATPase, M wave, and force in human muscle, as has been demonstrated previously for animal muscle (40).

**Conclusions.** The results of this experiment indicate that the Na\(^+\)-K\(^+\)-ATPase activity can be altered by a single bout of heavy isometric exercise in humans. The association observed between Na\(^+\)-K\(^+\)-ATPase activity and measures of membrane excitability as obtained by the M wave support the contention that indirect measures of neuromuscular activity can reflect, at least in part, cellular processes measured through biochemical procedures in human muscle. The alteration in activity and the short time course for recovery emphasize the rapidly adaptive nature of the Na\(^+\)-K\(^+\)-ATPase and imply that reduced sarcolemmal excitability may con-
tribute to neuromuscular fatigue in humans during the exercise. It is likely that other cellular processes contribute more to fatigue during the prolonged period of recovery. No doubt the role of Na\(^+\)-K\(^+\)-ATPase in a multifactorial fatigue process is dependent on the intensity, duration, and type of exercise performed.

**Perspectives.** Investigation of the skeletal muscle Na\(^+\)-K\(^+\)-ATPase indicates that it is highly adaptable to a wide variety of training protocols (21, 37). The rapid content changes observed for the Na\(^+\)-K\(^+\)-ATPase in response to training imply that acute events likely lead to the chronic adaptation. There are a number of intracellular signals that may predispose the enzyme to rapid adaptations, with one of them possibly being inactivation of the enzyme and the need to stimulate turnover and remodeling. It is reasonable to postulate that the stress of exercise induces structural modifications that reduce the activity of the enzyme and result in a need for compensation and adaptation. Heavy contractile activity that induces both high- and low-frequency fatigue challenges the capacity of the Na\(^+\)-K\(^+\)-ATPase to maintain transmembrane Na\(^+\) and K\(^+\) gradients and excitability. By mechanisms that are as yet uncertain, structural modifications appear to occur to the Na\(^+\)-K\(^+\)-ATPase, resulting in reduced activity. The fact that the Na\(^+\)-K\(^+\)-ATPase activity and EMG were restored within 4 h indicates that this modification has a rapid time course for recovery, as is observed in response to repeated training bouts for this key sarcolemmal protein. It must be emphasized, however, that the specific effect of alterations in Na\(^+\)-K\(^+\)-ATPase in modifying contractile activity needs to be established. Its role as a mechanism of fatigue may well depend on the characteristics of the task.

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