# Fatigue depresses maximal in vitro skeletal muscle Na$^+$-K$^+$-ATPase activity in untrained and trained individuals

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**Fraser, Steve F., Jia L. Li, Michael F. Carey, Xiao N. Wang, Termboon Sangkabutra, Simon Sostaric, Steve E. Selig, Keld Kjeldsen, and Michael J. McKenna.** Fatigue depresses maximal in vitro skeletal muscle Na$^+$-K$^+$-ATPase activity in untrained and trained individuals. *J Appl Physiol* 93: 1650–1659, 2002. First published July 12, 2002; 10.1152/japplphysiol.01247.2001.—This study investigated whether fatiguing dynamic exercise depresses maximal in vitro Na$^+$-K$^+$-ATPase activity and whether any depression is attenuated with chronic training. Eight untrained (UT), eight resistance-trained (RT), and eight endurance-trained (ET) subjects performed a quadriceps fatigue test, comprising 50 maximal isokinetic contractions (180°/s, 0.5 Hz). Muscle biopsies (vastus lateralis) were taken before and immediately after exercise and were analyzed for maximal in vitro Na$^+$-K$^+$-ATPase (K$^+$-stimulated 3-*O*-methylfluoroscein phosphatase) activity. Resting samples were analyzed for [$^3$H]ouabain binding site content, which was 16.6 and 18.3% higher ($P < 0.05$) in ET than RT and UT, respectively (UT 311 ± 41, RT 302 ± 52, ET 357 ± 29 pmol/g wet wt). 3-*O*-methylfluoroscein phosphatase activity was depressed at fatigue by −13.8 ± 4.1% ($P < 0.05$), with no differences between groups (UT −13 ± 4, RT −9 ± 6, ET −22 ± 6%). During incremental exercise, ET had a lower ratio of rise in plasma K$^+$ concentration to work than UT ($P < 0.05$) and tended ($P = 0.09$) to be lower than RT (UT 18.5 ± 2.3, RT 16.2 ± 2.2, ET 11.8 ± 0.4 nmol·l$^{-1}$·J$^{-1}$). In conclusion, maximal in vitro Na$^+$-K$^+$-ATPase activity was depressed with fatigue, regardless of training state, suggesting that this may be an important determinant of fatigue.

3-*O*-methylfluoroscein phosphatase; exercise; Na$^+$-K$^+$ pump; potassium

RECENT STUDIES IN ISOLATED muscle preparations have demonstrated an important role for Na$^+$-K$^+$-ATPase in muscular fatigue, via prevention of a rundown in transmembrane Na$^+$ and K$^+$ gradients and thus preservation of membrane excitability (for review, see Ref. 35). Attenuation of Na$^+$-K$^+$-ATPase activity via inhibition with ouabain accelerates muscle fatigability and retards subsequent recovery (6), whereas, conversely, stimulation of Na$^+$-K$^+$-ATPase activity delays muscle fatigability and accelerates subsequent recovery in muscles paralyzed in high-K$^+$ solution (6, 7, 34). Reduced Na$^+$-K$^+$ gradients decrease rat soleus muscle M wave area and tetanic force, whereas subsequent muscle electrical stimulation of Na$^+$-K$^+$-ATPase or salbutamol-induced stimulation of Na$^+$-K$^+$-ATPase elicited a marked recovery (39, 40). These studies highlight the importance of Na$^+$-K$^+$-ATPase activity in skeletal muscle function in animal models.

Muscle excitation elicits a dramatic and rapid increase above rest levels in Na$^+$-K$^+$-ATPase activity, measured as net Na$^+$ extrusion, in isolated rat muscles (8, 33). Na$^+$-K$^+$-ATPase activity in isolated rat soleus muscle may increase up to 22-fold above rest after only 10 s of 120-Hz stimulation, thus approaching the maximal theoretical Na$^+$-K$^+$-ATPase activity (8). There are no direct measures of Na$^+$-K$^+$-ATPase activation in contracting human skeletal muscle. However, this is likely to also be dramatic, as shown by a rapid decline in femoral venous plasma K$^+$ concentration ([K$^+$]) after knee extensor exercise and by the rapid K$^+$ clearance from blood after exercise (for review, see Ref. 42). Despite this increased Na$^+$-K$^+$-ATPase activation during muscle contractions, a direct, depressive effect of fatiguing exercise on the maximal Na$^+$-K$^+$-ATPase activity can be hypothesized. There is considerable structural homology of the catalytic subunits of the Ca$^{2+}$-ATPase and Na$^+$-K$^+$-ATPase enzymes (20). It is now well known that fatiguing muscle contractions in humans induce an acute depression in the sarcoplasmic reticulum maximal Ca$^{2+}$-ATPase activity or Ca$^{2+}$-

ATPase-mediated $Ca^{2+}$ uptake rate in skeletal muscle (4, 16, 26). Hence it is conceivable that factors that adversely affect maximal $Ca^{2+}$-ATPase activity may also impair $Na^+$-$K^+$-ATPase activity, and investigation into the possible effects of fatigue on $Na^+$-$K^+$-ATPase activity is of great interest. In human skeletal muscle, it is possible to measure the maximal in vitro $Na^+$-$K^+$-ATPase activity (12). However, this assay does not measure the increase in $Na^+$-$K^+$-ATPase activation but rather reflects the theoretical maximal $Na^+$-$K^+$-ATPase activity (12, 31). A recent study has indeed demonstrated depressed maximal in vitro $Na^+$-$K^+$-ATPase activity after repeated isometric contractions (11). However, repeated isometric contractions induce marked muscle ischemia, which might be causally linked with the impaired maximal in vitro $Na^+$-$K^+$-ATPase activity and the observed postcontractile depression (11). No studies thus far have investigated whether fatiguing, dynamic contractions depress maximal $Na^+$-$K^+$-ATPase activity in human skeletal muscle, and therefore this was the first aim of the present study.

One characteristic of training is an enhanced resistance to fatigue during activity specific to the training regimen. Factors linked with $Ca^{2+}$-ATPase inactivation with exercise are affected by training, with modified muscle sarcoplasmic reticulum $Ca^{2+}$ regulation during exercise by resistance and endurance training (16, 26) and increased endogenous antioxidant enzymes also evident with training (46). If the maximal $Na^+$-$K^+$-ATPase activity is depressed with fatigue, then chronic training could conceivably confer a protective effect on $Na^+$-$K^+$-ATPase activity, thereby attenuating this decline and contributing to enhanced muscular performance. Any possible protective effect of training on $Na^+$-$K^+$-ATPase activity may also be influenced by the total muscle $Na^+$-$K^+$-ATPase content, which is increased with training (9, 15, 29, 32). Thus an increased $Na^+$-$K^+$-ATPase content with training would offset any depressive effect of fatiguing exercise on maximal $Na^+$-$K^+$-ATPase activity. There have been no studies reporting both $Na^+$-$K^+$-ATPase content and maximal activity in trained muscle. Thus the second aim of this paper was to investigate whether chronic training protects against any possible decline in the maximal $Na^+$-$K^+$-ATPase activity in human muscle. No studies have examined the effects of training on maximal $Na^+$-$K^+$-ATPase activity per se in resting muscle in humans. The 165% increase in maximal $Na^+$-$K^+$-ATPase activity reported with endurance training in canine muscle (24) far exceeds the typical 14–29% increase in [$^3$H]ouabain binding with training (31), probably because of poor and variable enzyme recovery in the isolated membrane fraction used for measurement (17). Thus the effects of chronic training on maximal $Na^+$-$K^+$-ATPase activity in skeletal muscle remain unknown and were also examined.

Finally, the increased muscle $Na^+$-$K^+$-ATPase content with training has been suggested to be important in the improved plasma $K^+$ regulation during exercise, but no correlative evidence was found (15, 29). Here we explore whether muscle $Na^+$-$K^+$-ATPase content and maximal $Na^+$-$K^+$-ATPase activity are correlated to an index of plasma $K^+$ regulation during exercise, the rise in plasma $K^+$ concentration per unit work output (18, 29).

Three hypotheses were tested in this study: *1*) that an acute bout of fatiguing dynamic exercise would depress maximal in vitro $Na^+$-$K^+$-ATPase activity in skeletal muscle in humans; *2*) that this depression will be attenuated in chronically resistance-trained and endurance-trained athletes, and *3*) that muscle $Na^+$-$K^+$-ATPase content and maximal $Na^+$-$K^+$-ATPase activity will be correlated to the rise in plasma $K^+$ concentration per unit work output.

## METHODS

Eight healthy untrained male controls (UT), eight resistance-trained subjects (RT, 7 male, 1 female), and eight endurance-trained male subjects (ET) participated after being informed of risks associated with the study and giving written, informed consent. Our laboratory has recently reported the physical characteristics, training history, anthropometry, and muscle function test results in these subjects in a paper investigating muscle $Ca^{2+}$ regulation at rest and fatigue (26). The UT subjects were recreationally active but were not well trained and did not participate in regular sporting activities. The ET and RT athletes had been training continuously for at least 2 yr. During this period the ET athletes had performed running and/or cycling endurance training for at least 5–6 h/wk and had a peak oxygen consumption ($\dot{V}O_{2 peak}$) exceeding 60 ml·min$^{-1}$·kg$^{-1}$. The RT subjects trained with heavy weights, typically performing three sets, six to eight repetitions, for at least 1 h and at least three sessions/wk. All were able to perform a power-lifting-style squat exercise with free weights at least 1½ times their body mass. No significant differences existed between the three groups (means ± SD) for age (UT 26.4 ± 3.9, RT 26.8 ± 7.9, ET 26.4 ± 3.1 yr), body mass (UT 80.4 ± 6.8, RT 81.6 ± 3.3, ET 70.6 ± 9.9 kg), or height (UT 183.3 ± 5.7, RT 176.1 ± 4.7, ET 177.2 ± 7.1 cm). All protocols and procedures were approved by the Human Research Ethics Committee at Victoria University of Technology.

*Overview of exercise tests.* Each subject completed two tests involving invasive procedures. The first comprised an incremental cycle ergometer exercise test with arterialized-venous blood sampling conducted before, during, and after exercise. The second was a muscle fatigue test, comprising repeated maximal quadriceps contractions on an isokinetic dynamometer. A vastus lateralis muscle biopsy was taken at rest and immediately after fatiguing contractions, with arterialized venous blood sampling before, during, and after exercise. Each subject refrained from vigorous exercise, alcohol, and caffeine consumption for 24 h before each test.

*Incremental exercise test.* An incremental exercise test (25 W/min, 60 rpm except ET where 80 rpm) was performed on an electrically braked cycle ergometer (Lode N.V. Groningen, Netherlands) to determine $\dot{V}O_{2 peak}$, as detailed elsewhere (26). A catheter (20-gauge, Jelco) was inserted into a superficial dorsal hand vein of the subject before the test, and all blood samples were arterialized by heating the hand in a hot (45°C) water bath for 10 min before samples were taken (29). The catheter was kept patent by periodic infusions of heparinized isotonic saline. Arterialized venous blood was sampled at rest, in the final 10 s of each minute during graded exercise, and at 1, 2, 5, 10, 20, and 30 min in recovery, and

the samples were analyzed for Hb concentration, hematocrit, as well as $[K^+]$ and plasma hydrogen ($[H^+]$) and lactate ($[Lac^-]$) concentrations.

*Muscle fatigue test.* The muscle fatigue test was performed on an isokinetic dynamometer (Cybex II Lumex, Ronkoukowany), as previously detailed and justified (26). A muscle biopsy was taken at rest and immediately postexercise, and blood samples were taken at rest, mid- and immediately postexercise, and at 1, 2, 5, 10, 20, and 30 min in recovery and were analyzed as described for the incremental test. Subjects were strapped to the Cybex dynamometer chair by belts across the hips, chest, and legs to stabilize the upper body and thigh. Subjects performed 50 maximal knee extensions at a velocity of 180°/s and at a rate of 0.5 Hz (duration 100 s). Peak torque was measured and fatigue index (percentage decline in peak torque) calculated as described previously (26).

*Muscle biopsy sampling and analyses.* After injection of a local anesthetic into the skin and fascia (2% Xylocaine), two small incisions were made in the midportion of the vastus lateralis muscle of the right leg. The rest and fatigue biopsies were taken from separate incisions. Resting samples were analyzed for maximal in vitro $Na^+$-$K^+$-ATPase activity and $Na^+$-$K^+$-ATPase content, whereas fatigue samples were analyzed for maximal in vitro $Na^+$-$K^+$-ATPase activity. Muscle fiber-type composition, sarcoplasmic reticulum $Ca^{2+}$ regulation, and metabolite contents are reported elsewhere (26).

*Muscle [$^3$H]ouabain binding site content.* Approximately 20 mg of the frozen resting muscle was used to quantify the $Na^+$-$K^+$-ATPase content by using the [$^3$H]ouabain binding method as previously described (21, 29, 37). Samples were cut into small pieces of 2–4 mg wet wt. In all experiments, freshly made vanadate solution was used. Samples were washed at 0°C for 20 min, with a change of medium after 10 min ($2 \times 10$ min) in a buffer containing 10 mM Tris, 250 mM sucrose, 3 mM $MgSO_4$, and 1 mM vanadate, pH 7.2–7.4. This procedure was used to thaw the samples and preincubate them with vanadate and to maintain low $Na^+$ and $K^+$ concentrations so as not to interfere with vanadate-facilitated [$^3$H]ouabain binding. Incubations took place in a buffer containing 2 μCi/ml [$^3$H]ouabain and ouabain added to a final concentration of 1 μM at 37°C for 2 h, with a change of medium after 1 h. After incubations, a washout at 0°C in unlabeled buffer for 2 h with a change of medium every 30 min ($4 \times 30$ min) was performed to reduce the [$^3$H]ouabain in the extracellular space and enhance the precision of the method. After washout, samples were blotted on dry filter paper, weighed, and soaked overnight in minivials containing 0.5 ml of 5% trichloroacetic acid. The next day, 2.5 ml of scintillator (Opti-fluor) was added before liquid scintillation counting of the [$^3$H]ouabain activity was performed. The amount of [$^3$H]ouabain taken up and retained by the samples was calculated on the basis of the sample wet weight and the specific activity of the incubation medium and samples.

*3-O-MFPase assay.* Skeletal muscle maximal in vitro $Na^+$-$K^+$-ATPase activity was determined by using the $K^+$-stimulated 3-O-methylfluoroscein phosphatase (3-O-MFPase) assay in human muscle homogenates, as previously described in detail (12). The 3-O-MFPase assay was chosen because it is highly sensitive, capable of determining extremely low levels of $Na^+$-$K^+$-ATPase activity as found in human skeletal muscle (12). This assay has two to three times higher sensitivity, therefore requiring 50–100 times less tissue than the $K^+$-stimulated *p*-nitro-phenyl-phosphatase (1, 36). Measurement of activity in whole muscle homogenates avoids the criticisms of very poor recovery inherent in techniques involving extensive enzymatic purification procedures (17). The

assay was optimized for human skeletal muscle homogenates and specifically measures $Na^+$-$K^+$-ATPase activity, as evidenced by complete ouabain inhibition and $K^+$ stimulation of activity (12). The interassay (5.3%) and intra-assay (8.1%) variation were acceptably low (12). Briefly, muscle samples (30–40 mg) were immediately blotted on filter paper, weighed, then homogenized (5% wt/vol) at 0°C for $2 \times 20$ s, 20,000 rpm (Omni 1000, Omni International) in an homogenate buffer containing 250 mM sucrose, 2 mM EDTA, and 10 mM Tris (pH 7.40). Muscle homogenates were rapidly frozen and stored in liquid nitrogen for later determination of maximal in vitro $K^+$-stimulated 3-O-MFPase activity. Before analysis, homogenates were freeze-thawed four times and then diluted one-fifth in cold homogenate buffer. The assay medium in which 3-O-MFPase activity was measured contained 5 mM $MgCl_2$, 1.25 mM EDTA, 100 mM Tris, and an 80 nM 3-O-methyl fluorescein standard (pH 7.40). The freeze-thawed, diluted homogenate (30 μl) was incubated in 2.5 ml of assay medium at 37°C for 5 min before addition of 40 μl of 10 mM 3-O-MFP to initiate the reaction. After 60 s, 10 μl of 2.58 M KCl (final concentration 10 mM) was added to stimulate $K^+$-dependent phosphatase activity, and the reaction was measured for a further 60 s. All assays were performed at 37°C, with continuous stirring, on a spectrofluorometer (Aminco Bowman AB2 SLM, Urbana, IL). Excitation wavelength was 475 nm, and emission wavelength was 515 nm, with 4-nm slit widths. The $K^+$-stimulated 3-O-MFPase activity was calculated by subtracting the initial activity (comprising unspecific-ATPase activity and any spontaneous hydrolysis of 3-O-MFP) from the activity obtained after 10 mM KCl addition (12). Maximal in vitro 3-O-MFPase activity was expressed relative to muscle wet weight (nmol·min$^{-1}$·g$^{-1}$ wet wt) and to identify possible effects due to fluid shifts, also relative to muscle protein content (pmol·min$^{-1}$·mg$^{-1}$ protein). Protein content of the homogenate was determined spectrophotometrically by using bovine serum albumin as a standard. The relationship between [$^3$H]ouabain binding site content and maximal in vitro 3-O-MFPase activity was examined in 22 of the 24 subjects, plus an additional six healthy untrained controls (age $40.7 \pm 8.7$ yr, body mass $60.8 \pm 6.2$ kg, height $163.9 \pm 5.8$ cm, means $\pm$ SD).

*Blood analyses.* The blood was mixed well, and air bubbles were removed from the syringe, which was capped tightly and placed on ice for subsequent duplicate analyses of plasma acid-base status and gas tensions ($H^+$, $P_{CO_2}$, $P_{O_2}$, and $[K^+]$) by use of an automated analyzer (865 Ciba Corning, Bayer). Hb concentration was determined in duplicate spectrophotometrically (Radiometer OSM2, Copenhagen, Denmark), whereas hematocrit was analyzed in triplicate after centrifugation (Hettich Zentrifugen D-7200, Tuttlingen, Germany). All analytical instruments were calibrated before and during the analyses with precision standards. An aliquot of whole blood was centrifuged at 4,000 rpm for 4 min, plasma was separated, a 200-μl aliquot of plasma was deproteinized in 600 μl of cold 3M perchloric acid, and the supernatant was later analyzed for plasma $[Lac^-]$ in triplicate by use of an enzymatic spectrophotometric technique.

*Calculations.* The percentage decline in plasma volume from rest ($\Delta$PV), rise in plasma $[K^+]$ during exercise above rest ($\Delta[K^+]$) and the ratio of $\Delta[K^+]$ per work done ($\Delta[K^+]$/work) were calculated as previously described (29, 30), with an example of the latter as follows. If, during incremental (25 W/min) exercise, $\Delta[K^+]$ was 2.2 mM and the subject completed 1 min at 300 W, total work equals 117 kJ and the $\Delta[K^+]$/work is 18.8 nmol·l$^{-1}$·J$^{-1}$. The decline in $Na^+$-$K^+$-ATPase activity with fatigue is calculated as rest minus fatigue in vitro 3-O-MFPase activity. Correlations involve

pooled data from all subjects in the three groups ($n$ = 22–24, as stated).

*Statistics.* Data are presented as means ± SE, except population data, for which means ± SD are shown. A two-way ANOVA (sample time, group) with repeated measures (time) was used to analyze most variables. A one-way ANOVA was used when only a single variable was compared between groups (e.g., [³H]ouabain binding). Post hoc analyses used the Newman-Keuls test. Correlations between variables were determined by least-square linear regression. Significance was accepted at $P < 0.05$.

## RESULTS

$\dot{V}O_{2\ peak}$ *and muscle fatigue test.* As a confirmation of training status, ET had a higher ($P < 0.05$) incremental exercise $\dot{V}O_{2\ peak}$ than the other groups (UT 44.4 ± 1.8, RT 43.8 ± 3.6, ET 67.6 ± 1.5 ml·kg⁻¹·min⁻¹, means ± SD), whereas RT had a higher ($P < 0.05$) quadriceps maximal peak torque during isokinetic contractions from 60–300°/s (26). Peak quadriceps muscle torque declined in all groups during the 50 contractions ($P < 0.05$), and the fatigue index was less ($P < 0.05$) in ET than in UT and RT (UT 47.4 ± 14.0, RT 43.4 ± 9.4, ET 29.9 ± 12.0%, means ± SD, Ref. 26).

*Muscle* $Na^+$-$K^+$-*ATPase content.* The resting muscle [³H]ouabain binding site content differed between groups, being 16.6 and 18.3% higher for ET than in UT and RT, respectively ($P < 0.05$, Fig. 1).

*Maximal in vitro 3-O-MFPase activity.* A significant sample time main effect was shown for maximal in vitro 3-O-MFPase activity expressed per gram wet weight, with a decline of 13.8 ± 4.1% at fatigue (Fig. 2A, $P < 0.05$). No significant group main effects or time-by-group interactions were seen, although resting 3-O-MFPase activity in ET tended to be higher (20.3%) than in UT. The decline in maximal in vitro 3-O-MFPase activity at fatigue did not differ significantly between the groups (absolute: UT −27 ± 8, RT −24 ± 13, ET −60 ± 17 nmol·min⁻¹·g wet wt⁻¹; relative: UT −13 ± 4, RT −9 ± 6, ET −22 ± 6%).
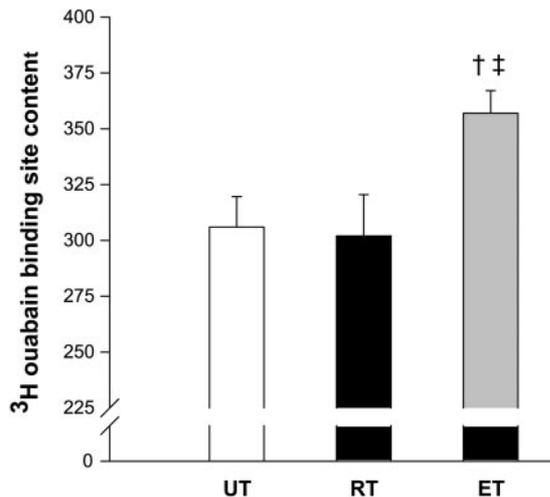


Fig. 1. Vastus lateralis muscle [³H]ouabain binding site content (Na⁺-K⁺-ATPase content) for untrained (UT), resistance-trained (RT), and endurance-trained (ET) subjects; $n$ = 8 subjects/group (means ± SE). †ET > UT; ‡ET > RT ($P < 0.05$).
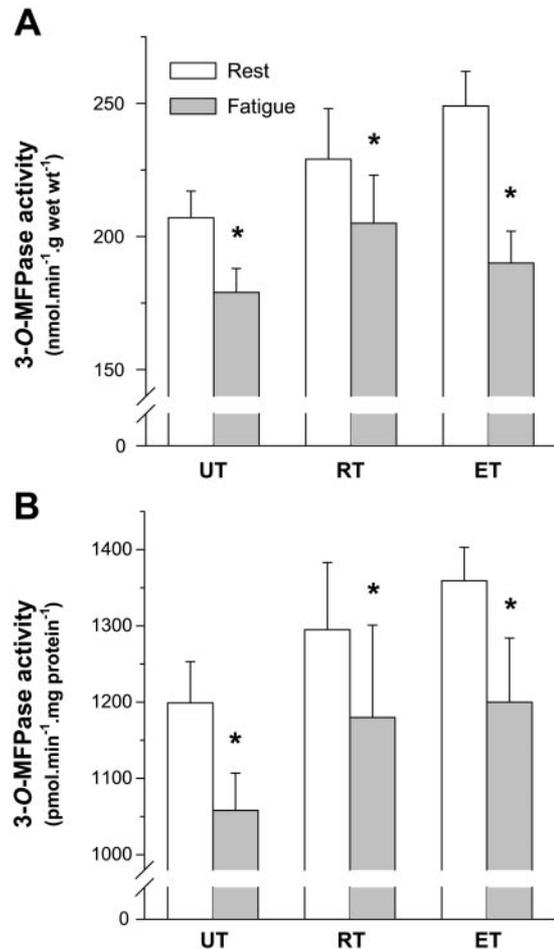


Fig. 2. Maximal in vitro K⁺-stimulated 3-O-methylfluoroscein phosphatase (3-O-MFPase) activity (Na⁺-K⁺-ATPase activity) in skeletal muscle obtained at rest and fatigue for UT, RT, and ET subjects. *A*: activity expressed per gram weight wet. *B*: activity expressed per milligram protein. Data are means ± SE; $n$ = 7 subjects for UT and ET and $n$ = 8 subjects for RT. *Main effect of exercise, fatigue < rest ($P < 0.05$).

To determine whether the decline in activity with exercise may be due to muscle water accumulation, 3-O-MFPase activity was also expressed relative to muscle protein content, which did not differ between rest and fatigue samples or between groups (data not shown). A significant time main effect for maximal in vitro 3-O-MFPase activity expressed per milligram protein was again evident, with a −10.5 ± 3.4% reduction at fatigue (Fig. 2B, $P < 0.05$). No significant group main effects or time-by-group interactions were seen for maximal in vitro 3-O-MFPase activity per milligram of protein. There were no differences between groups in the absolute or percentage decline from resting values in maximal in vitro 3-O-MFPase activity (absolute: UT −141 ± 26, RT −115 ± 93, ET −159 ± 88 pmol·min⁻¹·mg protein⁻¹; relative: UT −12 ± 2, RT −8 ± 9, ET −11 ± 6%).

*Plasma electrolytes and plasma volume changes during the muscle fatigue test.* A significant time main effect was found for arterialized-venous plasma [K⁺] ($P < 0.05$), which increased midexercise ($P < 0.05$),

peaked at fatigue, and returned to rest values by 2 min recovery (Fig. 3*A*). No significant group main effect or time-by-group interactions were found for plasma [K⁺]. No between-group differences were found during the fatigue test for peak plasma [K⁺] (UT 4.81 ± 0.17, RT 4.57 ± 0.17, ET 4.60 ± 0.09 mmol/l), Δ[K⁺] (UT 0.92 ± 0.13, RT 0.60 ± 0.12, ET 0.85 ± 0.09 mmol/l), or Δ[K⁺]/work (UT 85.7 ± 13.0, RT 59.7 ± 11.6, ET 77.6 ± 8.5 nmol·l⁻¹·J⁻¹).

A significant time main effect was found for ΔPV ($P < 0.05$), whereby PV fell at fatigue until 2 min of recovery ($P < 0.05$) and returned to rest by 10 min recovery (Table 1). No significant group main effect or time-by-group interactions for ΔPV were found; thus electrolytes were not corrected for ΔPV. A significant time main effect was found for arterialized-venous plasma [Lac⁻] ($P < 0.05$), which peaked at 1–5 min recovery. A significant time-by-group interaction was
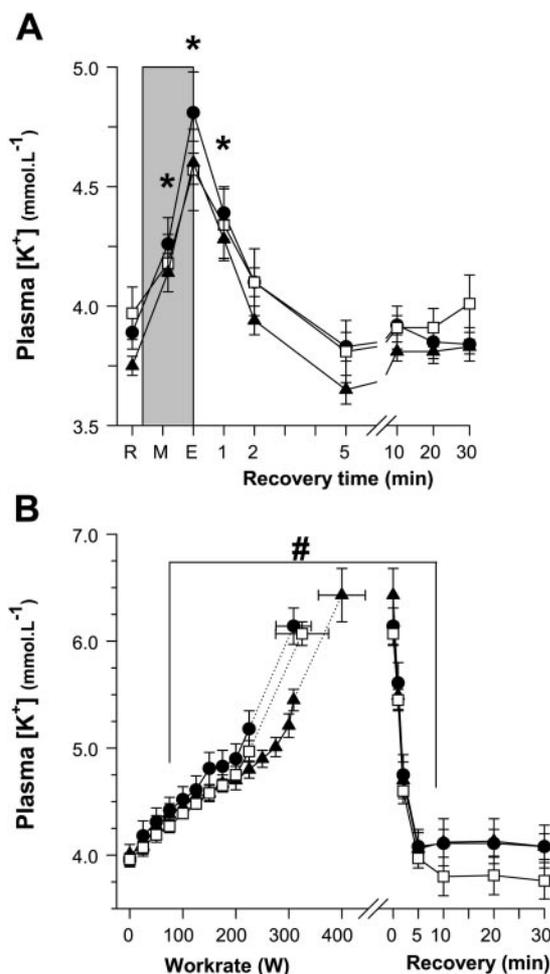
found for plasma [Lac⁻], which was less in ET than in UT and RT from 2 until 10 min recovery ($P < 0.05$, Table 1). A significant time main effect was found for plasma [H⁺] ($P < 0.05$), which increased at fatigue, peaked at 5 min postexercise, and returned to rest levels by 20 min recovery. A significant time-by-group interaction was found for plasma [H⁺], which was lower at 5 min recovery in ET than UT and RT ($P < 0.05$, Table 1).

*Plasma electrolytes and plasma volume changes during the incremental exercise test.* A significant time main effect was found for arterialized-venous plasma [K⁺] ($P < 0.05$), which increased above rest from 75 W until the peak incremental exercise work rate and had returned to rest by 5 min recovery (Fig. 3*B*). No significant group main effect or time-by-group interactions were found for plasma [K⁺]. No between-group differences were found for peak plasma [K⁺] (UT 6.14 ± 0.17, RT 6.07 ± 0.11, ET 6.43 ± 0.25 mmol/l) or Δ[K⁺] (UT 2.20 ± 0.16, RT 2.11 ± 0.15, ET 2.42 ± 0.19 mmol/l), but the Δ[K⁺]/work was 36% lower in ET (11.8 ± 0.4 nmol·l⁻¹·J⁻¹) compared with UT (18.5 ± 2.3 nmol·l⁻¹·J⁻¹, $P < 0.05$) and also tended to be lower than in RT (16.2 ± 2.2 nmol·l⁻¹·J⁻¹, $P = 0.09$).

A significant time main effect was found for ΔPV ($P < 0.05$), whereby PV fell below rest from 125 W until 1 min of recovery ($P < 0.05$) and returned to rest by 30 min recovery (Table 2). No significant group main effect or time-by-group interactions for ΔPV were found, and electrolytes were not corrected for ΔPV. The ΔPV at the peak incremental exercise work rate did not differ between groups (Table 2). Significant time main effects were found for arterialized-venous plasma [Lac⁻] and [H⁺] ($P < 0.05$), which rose above rest from 175 W, peaked at 1 and at 5 min recovery, respectively, and remained above rest at 30 min recovery (Table 2).

*Functional correlates of muscle 3-O-MFPase activity and Na⁺-K⁺-ATPase content.* In resting muscle samples, a significant correlation was found between [³H]ouabain binding site content and the maximal in vitro 3-*O*-MFPase activity (Fig. 4*A*, $r = 0.61$, $n = 28$, $P < 0.05$). The [³H]ouabain binding site content was inversely correlated with the fatigue index (Fig. 4*B*, $r = -0.42$, $n = 24$, $P < 0.05$) and correlated with V̇O₂ peak (Fig. 5*A*, $r = 0.64$, $n = 23$, $P < 0.05$). The maximal in vitro 3-*O*-MFPase activity was also correlated with V̇O₂ peak (Fig. 5*B*, $r = 0.46$, $n = 22$, $P < 0.05$) but not with the fatigue index ($r = 0.24$, NS).

For the incremental test, significant inverse relationships were found between the incremental Δ[K⁺]/ work and both the maximal in vitro 3-*O*-MFPase activity ($r = -0.53$, $n = 22$, $P < 0.05$, Fig. 6*A*) and the Na⁺-K⁺-ATPase content ($r = -0.49$, $n = 24$, $P < 0.05$, Fig. 6*B*). However, for the fatigue test, which involved only a small contracting muscle mass, no significant relationships were found between either maximal in vitro 3-*O*-MFPase activity or Na⁺-K⁺-ATPase content in resting muscle samples, against Δ[K⁺] or Δ[K⁺]/ work ($n = 22$).



Fig. 3. Arterialized-venous plasma K⁺ concentration ([K⁺]) for UT (●), RT (□), and ET (▲) subjects during muscle fatigue test (*A*) and during recovery incremental test (*B*). In *A*, plasma K⁺ concentration ([K⁺]) is shown at rest (R), midexercise (M), and end exercise (E). In *B*, plasma [K⁺] is shown at rest, each minute during exercise, and during 30 min of recovery. In *B*, horizontal error bars indicate mean ± SE peak work rate. The peak incremental exercise plasma [K⁺] is replotted as the zero-recovery time point. *Main effect of exercise time (*A*) and/or work rate (*B*) [K⁺] > rest ($P < 0.05$). Data are means ± SE; $n = 8$ for UT and ET, $n = 7$ for RT.

Table 1. $\Delta PV$, plasma [lactate], and [$H^+$] during the quadriceps muscle fatigue test in UT, RT, and ET subjects

| | $\Delta PV$, % | | | Plasma [lactate], mmol/l | | | Plasma [$H^+$], nmol/l | | |
|---|---|---|---|---|---|---|---|---|---|
| | UT | RT | ET | UT | RT | ET | UT | RT | ET |
| Rest | | | | $1.07 \pm 0.12$ | $1.59 \pm 0.18$ | $1.61 \pm 0.15$ | $37.5 \pm 0.5$ | $37.5 \pm 0.4$ | $37.7 \pm 0.4$ |
| Fatigue | $-3.9 \pm 1.9$ | $-5.4 \pm 1.3$ | $-4.2 \pm 0.4$ | $4.21 \pm 0.39$ | $3.89 \pm 0.41$ | $3.45 \pm 0.29$ | $39.4 \pm 0.6$ | $38.3 \pm 1.2$ | $38.3 \pm 0.4$ |
| +1 min | $-6.9 \pm 1.1$ | $-5.3 \pm 1.7$ | $-7.0 \pm 0.9$ | $6.29 \pm 0.66$ | $5.56 \pm 0.96$ | $4.36 \pm 0.56$ | $41.4 \pm 0.7$ | $40.7 \pm 0.9$ | $40.1 \pm 0.6$ |
| +2 min | $-7.5 \pm 1.2$ | $-5.8 \pm 1.8$ | $-6.2 \pm 1.0$ | $6.83 \pm 0.64$ | $6.30 \pm 1.09$ | $4.36 \pm 0.57$*† | $41.6 \pm 0.8$ | $41.9 \pm 1.1$ | $40.8 \pm 0.7$ |
| +5 min | $-5.9 \pm 1.5$ | $-2.6 \pm 2.0$ | $-1.2 \pm 1.5$ | $6.38 \pm 0.93$ | $5.96 \pm 0.58$ | $3.91 \pm 0.44$*† | $42.2 \pm 1.0$ | $42.4 \pm 1.1$ | $40.2 \pm 0.8$*† |
| +10 min | $-2.9 \pm 1.3$ | $-0.7 \pm 1.8$ | $0.2 \pm 1.5$ | $5.21 \pm 1.05$ | $5.64 \pm 0.60$ | $3.21 \pm 0.38$*† | $40.7 \pm 0.9$ | $40.5 \pm 1.0$ | $39.0 \pm 0.4$ |
| +30 min | $2.0 \pm 0.9$ | $1.9 \pm 1.7$ | $0.7 \pm 1.4$ | $2.08 \pm 0.32$ | $2.19 \pm 0.28$ | $1.84 \pm 0.15$ | $38.4 \pm 0.5$ | $36.7 \pm 0.8$ | $37.1 \pm 0.3$ |

Values are means $\pm$ SE; $n = 8$ subjects per group. $\Delta PV$, plasma volume changes from rest; [lactate], lactate concentration; UT, untrained; RT, resistance trained; ET, endurance trained. *†ET < UT and RT ($P < 0.05$).

## DISCUSSION

*Fatigue depresses muscle maximal Na+-K+-ATPase activity.* The most important and novel finding of this study was that an acute bout of fatiguing dynamic exercise depressed the skeletal muscle maximal in vitro 3-*O*-MFPase activity, which is a measure of Na+-K+-ATPase activity. The similar decline at fatigue in 3-*O*-MFPase activity expressed relative to muscle wet weight or protein content argues strongly against the possibility of an artifactual effect due to a contraction-induced vascular fluid shift into muscle. Although we did not measure [³H]ouabain binding site content in the fatigued sample, it seems improbable that a loss of Na+-K+-ATPase pump units could occur in this time frame. Rather, an inactivation of these pump units is the more likely explanation. This is the first time such a depression has been demonstrated with dynamic exercise and implicates Na+-K+-ATPase as an additional potential site for muscle fatigue during intense muscle contractions.

This finding appears somewhat paradoxical, because it is well established that Na+-K+-ATPase activity is increased during muscle contractions, both in isolated animal muscles (8, 33) and in humans (42, 45). This finding of depressed maximal in vitro Na+-K+-ATPase activity, consistent with a recent report after repeated isometric contractions (11), does not argue against an increase above rest in Na+-K+-ATPase activity in contracting muscle. Indeed, the rapid postexercise decline in plasma [K+] provides some evidence that Na+-K+-ATPase activity is raised well above resting levels.

Rather, these findings indicate a reduction in the maximal attainable Na+-K+-ATPase activity with fatigue. In isolated rat soleus muscle stimulated at high frequency (e.g., 120 Hz), Na+-K+-ATPase activity, measured by intracellular Na+ extrusion, increased to maximal theoretical levels (8). In human muscles, however, in which excitation frequencies are much lower, it is likely that activation is less than maximal theoretical levels (see Ref. 31). Thus a decline in the maximal in vitro Na+-K+-ATPase activity suggests a reduced safety factor in the attainable Na+-K+-ATPase activation, which may then be important in fatigue. Marked disturbances in muscle intracellular Na+ and both intracellular and extracellular K+ concentrations, reductions in muscle membrane potential, and excitability have been shown with fatigue in human muscles (Ref. 11 and references in Ref. 42). We speculate that the depressed maximal Na+-K+-ATPase activity with fatigue might exacerbate these perturbations and thus accelerate muscular fatigue. It is important to note that ouabain-induced inhibition of Na+-K+-ATPase rat soleus muscle markedly enhanced the rate of fatigue (6). Whether the smaller fraction of Na+-K+-ATPase inhibited in this study has similar effects on fatigue has not yet been tested. Interestingly, this depression in Na+-K+-ATPase activity also appears to be reversible, at least after isometric contractions (11), further suggesting a link with fatigue rather than muscle damage.

One possible criticism of this finding is that the 3-*O*-MFPase activity represents steps performing only

Table 2. *Plasma volume changes from rest ($\Delta PV$), plasma [lactate] and [$H^+$] during an incremental cycle ergometer exercise test in UT, RT, and ET subjects*

| | $\Delta PV$, % | | | Plasma [lactate], mmol/l | | | Plasma [$H^+$], nmol/l | | |
|---|---|---|---|---|---|---|---|---|---|
| | UT | RT | ET | UT | RT | ET | UT | RT | ET |
| Rest | | | | $0.86 \pm 0.10$ | $0.98 \pm 0.14$ | $1.26 \pm 0.14$ | $37.7 \pm 0.4$ | $36.2 \pm 0.6$ | $36.8 \pm 0.9$ |
| Fatigue | $-13.1 \pm 1.7$ | $-12.1 \pm 1.3$ | $-12.3 \pm 2.3$ | $12.45 \pm 1.05$ | $14.91 \pm 1.24$ | $15.63 \pm 1.18$ | $49.8 \pm 1.7$ | $46.7 \pm 2.5$ | $51.4 \pm 2.6$ |
| +1 min | $-13.7 \pm 2.1$ | $-16.3 \pm 1.5$ | $-15.5 \pm 2.8$ | $15.24 \pm 1.04$ | $17.29 \pm 1.24$ | $16.90 \pm 0.67$ | $58.1 \pm 2.0$ | $55.4 \pm 2.7$ | $63.5 \pm 2.2$ |
| +2 min | $-13.1 \pm 2.7$ | $-14.2 \pm 1.9$ | $-15.9 \pm 2.0$ | $15.86 \pm 1.07$ | $16.43 \pm 1.23$ | $16.40 \pm 0.79$ | $60.7 \pm 2.2$ | $58.6 \pm 3.0$ | $64.0 \pm 0.9$ |
| +5 min | $-12.7 \pm 1.9$ | $-13.7 \pm 1.2$ | $-11.4 \pm 1.2$ | $16.15 \pm 1.34$ | $16.13 \pm 0.60$ | $15.68 \pm 0.80$ | $64.6 \pm 2.7$ | $61.1 \pm 3.0$ | $66.2 \pm 1.7$ |
| +10 min | $-10.6 \pm 2.9$ | $-8.8 \pm 1.4$ | $-8.3 \pm 2.3$ | $13.65 \pm 1.38$ | $14.05 \pm 1.48$ | $13.85 \pm 0.95$ | $62.3 \pm 2.5$ | $58.2 \pm 2.9$ | $59.6 \pm 2.4$ |
| +30 min | $-2.8 \pm 2.8$ | $2.0 \pm 1.9$ | $-1.1 \pm 0.8$ | $6.63 \pm 1.17$ | $6.38 \pm 0.68$ | $6.28 \pm 0.31$ | $44.4 \pm 1.3$ | $41.9 \pm 0.8$ | $41.8 \pm 0.8$ |

Values are means $\pm$ SE; $n = 8$ subjects per group.
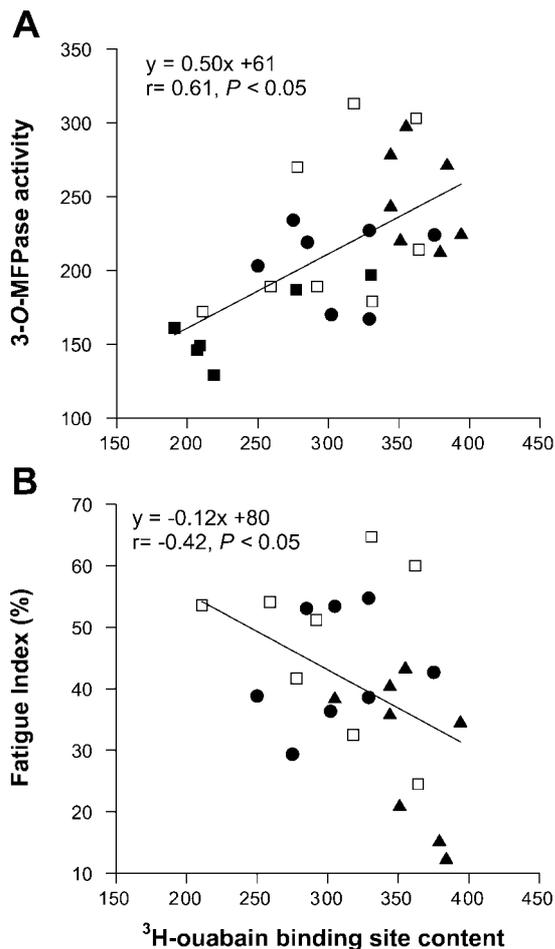
Fig. 4. Relationship between [$^3$H]ouabain binding site content (Na$^+$-K$^+$-ATPase content; pmol/g wet wt) and 3-$O$-MFPase activity (Na$^+$-K$^+$-ATPase activity; nmol·min$^{-1}$·g$^{-1}$ wet wt, $n = 28$) (A) and fatigue index during the muscle fatigue test (%, $n = 24$) (B) in UT (●), RT (□), ET (▲), and age-matched controls (■).

binding site content value of 333 pmol/g, in excellent agreement with our measured value of 311 pmol/g. Although we cannot be certain that 3-$O$-MFPase activity inhibition will reflect inhibition of total Na$^+$-K$^+$-ATPase activity, this seems highly probable.

It is not possible from this study to ascertain the exact mechanisms underlying this depression with fatigue in maximal in vitro 3-$O$-MFPase activity. However, given identical and controlled assay conditions for the rest and fatigue muscle samples, this most likely reflects a structural alteration in the Na$^+$-K$^+$-ATPase enzyme and/or altered characteristics of the membrane in which it is embedded. Fowles et al. (11) similarly reported a depression in the maximal in vitro 3-$O$-MFPase activity after repeated isometric contractions. Their depression was larger than in the present study, possibly reflecting the greater disturbances occurring with ischemia than with dynamic contractions. Possible underlying mechanisms include elevated intracellular Na$^+$ and Ca$^{2+}$ concentrations ([Na$^+$] and [Ca$^{2+}$], respectively) and reactive oxygen species. Intracellular [Na$^+$] is increased twofold with exercise in human muscle (42) and causes reduced Na$^+$-K$^+$-
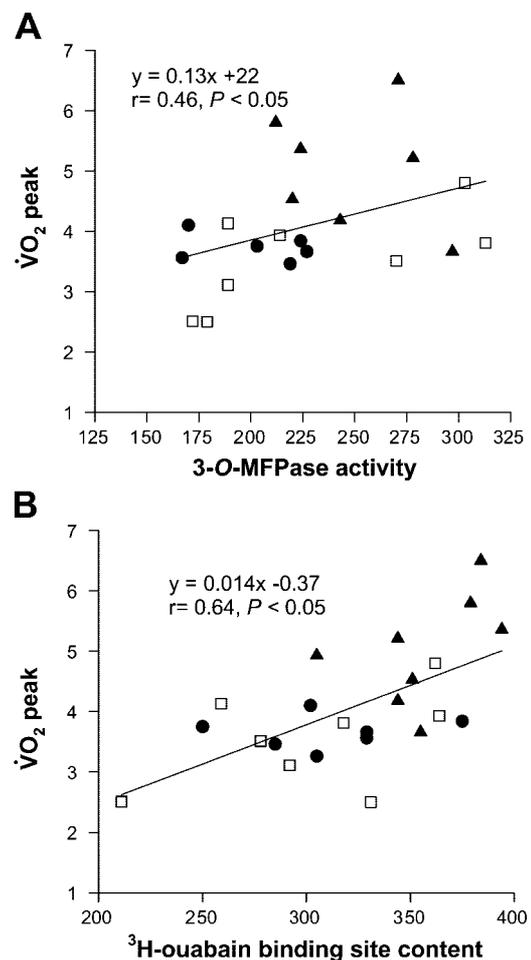
part of the overall Na$^+$-K$^+$-ATPase cycle (2), and thus reduced phosphatase activity may not reflect reduced maximal Na$^+$-K$^+$-ATPase activity. It is important to appreciate, however, that methodological considerations govern our using this assay rather than utilizing the traditional direct measures of activity via rates of P$_i$ accumulation. In human muscle, it is not possible to detect Na$^+$-K$^+$-ATPase activity by P$_i$ liberation because of the small sample yield of the biopsy technique, together with the overwhelmingly high total ATPase activity relative to Na$^+$-K$^+$-ATPase activity (3). Hence, the 3-$O$-MFPase activity assay was utilized here as the best available method (3, 12). Furthermore, through abolition of 3-$O$-MFPase activity by ouabain, we have demonstrated that this assay is specific for Na$^+$-K$^+$-ATPase (12). In addition, we report a significant correlation between 3-$O$-MFPase activity and ouabain binding site content in human muscle, as has been previously reported in rat (38) and human muscle (11). Assuming a molecular activity of 620 cycles/min, the 3-$O$-MFPase activity in UT muscle of 207 nmol·g wet wt$^{-1}$·min$^{-1}$ corresponds to an estimated [$^3$H]ouabain



Fig. 5. Relationship between peak O$_2$ consumption (V̇o$_{2peak}$; l/min) and 3-$O$-MFPase activity (Na$^+$-K$^+$-ATPase activity, nmol min$^{-1}$·g$^{-1}$ wet wt, $n = 22$) (A) and Na$^+$-K$^+$-ATPase content (pmol/g wet wt, $n = 23$) (B) for pooled UT (●), RT (□), and ET (▲) subjects.

## A



y = -0.07x +31
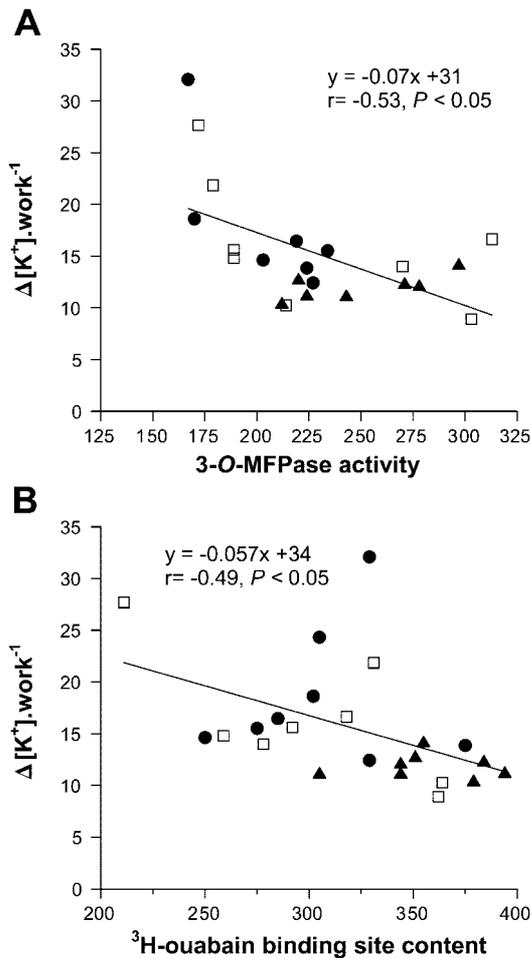r= -0.53, *P* < 0.05

**3-*O*-MFPase activity**

$\Delta$[K$^+$]·work$^{-1}$

## B



y = -0.057x +34
r= -0.49, *P* < 0.05

**$^3$H-ouabain binding site content**

$\Delta$[K$^+$]·work$^{-1}$

Fig. 6. Relationships between the incremental exercise ($\Delta$[K$^+$]/work; nmol·l$^{-1}$·J$^{-1}$) and maximal in vitro 3-*O*-MFPase activity (Na$^+$-K$^+$-ATPase activity; nmol·min$^{-1}$·g$^{-1}$ wet wt, *n* = 22) (*A*) and [$^3$H]ouabain binding site content (Na$^+$-K$^+$-ATPase content; pmol/g wet wt, *n* = 24) (*B*) for UT (●), RT (□), and ET (▲) subjects.

ATPase activity in rat cerebellum slices, probably because of increased intracellular [Ca$^{2+}$] (28). Intense muscle contractions induce Ca$^{2+}$ entry via Na$^+$ channels and Ca$^{2+}$ accumulation (14), increased resting [Ca$^{2+}$], and delayed posttetanic Ca$^{2+}$ transients (for references, see Ref. 26). Furthermore, increased [Ca$^{2+}$] can decrease the Na$^+$-K$^+$-ATPase hydrolytic and transport activities (19, 43, 44, 48), even at nanomolar [Ca$^{2+}$] (44). Reactive oxygen species are produced during intense muscle contractions (41) and inhibit Na$^+$-K$^+$-ATPase activity in a variety of tissues (25). Finally, these mechanisms may also be linked with increased [Na$^+$] and [Ca$^{2+}$] culminating in nitric oxide and peroxynitrite formation (47). Na$^+$-K$^+$-ATPase inactivation may also occur as a result of phosphorylation of the α subunit (5). It is unlikely that muscle metabolic perturbations can account for the present findings, because no significant correlations were found between muscle metabolites (26) and the maximal in vitro 3-*O*-MFPase activity (data not shown). It is unclear whether the depression in activity was due to a small depression in activity in all Na$^+$-K$^+$-ATPase enzymes

in all muscle fibers or represented larger depressions in activity in Na$^+$-K$^+$-ATPase enzymes in selected fibers. However, we found no relationship between the decline in maximal in vitro 3-*O*-MFPase with fatigue and fiber composition (*r* = 0.19, NS), arguing against a fiber-specific effect. In the present and a previous paper (26), our laboratory has demonstrated for the first time an impairment with fatigue in both of the major cation active transport regulatory proteins, Na$^+$-K$^+$-ATPase and Ca$^{2+}$-ATPase. With a similar structural homology (20), this leads to the intriguing question as to whether common mechanisms underlie these reductions. Surprisingly, however, we found no significant correlations between the percentage reduction with fatigue in the maximal in vitro 3-*O*-MFPase and Ca$^{2+}$-ATPase activities (*n* = 22, *r* = −0.03). Thus different mechanisms appear to be involved in these processes. Further work is clearly required to determine the mechanisms involved in depression of Na$^+$-K$^+$-ATPase activity.

The second major and unique finding from this study was that chronic training did not attenuate the decline in maximal in vitro Na$^+$-K$^+$-ATPase activity with fatigue. However, this observation further strengthens the validity of the depression in Na$^+$-K$^+$-ATPase activity, suggesting that this is an obligatory acute response to fatiguing muscular contractions. The ET athletes had higher $\dot{V}$o$_{2 \text{ peak}}$ and muscular fatigue resistance, suggesting that underlying enhanced muscle oxidative capacity typical of ET does not protect against the depression in Na$^+$-K$^+$-ATPase activity with fatigue. The lack of training status effect on the depression in maximal Na$^+$-K$^+$-ATPase activity does not invalidate our argument that this is intimately involved in fatigue. One possibility is that the upregulation in Na$^+$-K$^+$-ATPase content with training (9, 15, 29) is an adaptive process to offset the functional consequences of a decline in maximal activity. Thus ET would demonstrate enhanced muscle performance despite an unchanged depression in maximal Na$^+$-K$^+$-ATPase activity. We acknowledge that one limitation in this study was the cross-sectional design, and thus our data cannot exclude a possible protective adaptation as may be ascertained with a longitudinal training program.

We report for the first time skeletal muscle maximal Na$^+$-K$^+$-ATPase activity in athletes. The maximal in vitro 3-*O*-MFPase activity in resting muscle tended to be 20% higher in the ET group compared with UT, consistent with their 17% higher Na$^+$-K$^+$-ATPase content and with similar findings from longitudinal endurance training studies (9, 15, 16, 27). The lack of significance may reflect a type II error due to the higher variability seen in the ET group. Surprisingly, no difference was found in either the 3-*O*-MFPase activity or [$^3$H]ouabain binding site content between the RT and UT groups. This contrasts the 16% increase in [$^3$H]ouabain binding content reported with resistance training (16), the 15% higher [$^3$H]ouabain binding site with intensified resistance training (32), and the 45% higher [$^3$H]ouabain binding site content in resistance-trained older men (23). The reason for this discrepancy

is unclear but might reflect a lesser training level or shorter training duration in our subjects compared with previous studies (16, 23).

*Functional implications for Na$^+$-K$^+$-ATPase: muscle performance and plasma [K$^+$].* We demonstrate an important functional role of Na$^+$-K$^+$-ATPase for muscle contractile performance in humans via relationships between the maximal in vitro 3-*O*-MFPase activity, [$^3$H]ouabain binding site content, and two indexes of dynamic muscular performance: fatigability during repeated quadriceps contractions and the peak incremental exercise O$_2$ uptake. Both the maximal in vitro 3-*O*-MFPase activity and the [$^3$H]ouabain binding site content were significantly correlated with $\dot{V}o_{2\ peak}$. A novel finding in human muscle was the significant inverse relationship between the [$^3$H]ouabain binding site content and fatigability. This is consistent with studies in rat muscle in which Na$^+$-K$^+$-ATPase activation correlated with contractile performance (7, 33), although other studies in humans have failed to find a relationship between Na$^+$-K$^+$-ATPase content and muscle endurance during fatiguing isometric contractions (23) or repeated sprints (29). Others found depressions in each of muscle isometric force, M-wave area, and maximal in vitro 3-*O*-MFPase activity after isometric contractions, although these were not reported as being directly linked (11). Surprisingly, we did not find a positive relationship between either the maximal in vitro 3-*O*-MFPase activity or the fatigue-induced decline in 3-*O*-MFPase activity with the fatigue index. This may reflect variability in the 3-*O*-MFPase activity measures but is also consistent with multiple additional factors contributing to muscle fatigue, including impaired sarcoplasmic reticulum Ca$^{2+}$ release and uptake (4, 26), K$^+$ loss (42), metabolic perturbations such as increased intracellular P$_i$ (10), and fatigue of the central nervous system (13).

Finally, we tested whether the maximal in vitro 3-*O*-MFPase activity and the [$^3$H]ouabain binding site content in muscle were linked with plasma K$^+$ regulation during exercise. We have previously used $\Delta$[K$^+$]/work as a marker of adaptive training effects on plasma K$^+$ regulation during exercise (18, 29). Here we show a lesser $\Delta$[K$^+$]/work during the incremental test in ET (and a tendency in RT) compared with UT subjects, in support of similar findings after sprint training (18, 29) and reduced hyperkalemia reported after endurance training (15, 22). The reduced $\Delta$[K$^+$]/work seen in the incremental cycling test was not evident during the muscle fatigue test, but this is not unexpected because of the smaller contracting muscle mass and consequent lower plasma [K$^+$] during one-leg maximal exercise. An important functional role for muscle Na$^+$-K$^+$-ATPase in plasma K$^+$ regulation during exercise in humans was shown by the significant inverse relationship between the incremental exercise $\Delta$[K$^+$]/work and both the maximal in vitro 3-*O*-MFPase activity and the [$^3$H]ouabain binding site content. The reduced $\Delta$[K$^+$]/work with chronically trained subjects may be due to reduced K$^+$ release from contracting muscles, as well as enhanced K$^+$ clearance by noncon-tracting muscle and/or other tissues, but the relative importance of these with training remains to be verified. Reduced Na$^+$-K$^+$-ATPase activity with fatigue in the endurance-trained subjects could be offset by their increased Na$^+$-K$^+$-ATPase content, thereby reducing the rise in plasma [K$^+$] and helping explain their lower $\Delta$[K$^+$]/work. If an inhibition of Na$^+$-K$^+$-ATPase activity also occurred in noncontracting muscle, this would exacerbate the rise in plasma [K$^+$]; however, this seems unlikely and was not tested in this study.

In conclusion, acute exercise depressed maximal in vitro 3-*O*-MFPase activity in untrained, resistance-trained, and endurance-trained individuals. This finding, together with the related paper (26), points to a generalized downregulation of muscle cation regulatory active transport processes during intense contractions and suggests that these are intimately involved in fatigue. Important functional roles for muscle Na$^+$-K$^+$-ATPase were shown for both muscle performance and plasma K$^+$ regulation during exercise.

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