Impaired muscle Ca\(^{2+}\) and K\(^+\) regulation contribute to poor exercise performance post-lung transplantation

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Submitted 20 December 2002; accepted in final form 10 June 2003

LUNG TRANSPLANT RECIPIENTS (LTX) exhibit peripheral rather than ventilatory limitations, resulting in grossly impaired exercise performance. Skeletal muscle abnormalities documented thus far include impaired peripheral muscle O\(_2\) extraction, mitochondrial function, and oxidative metabolism (32, 45, 48); reduced muscle ATP (48), low muscle pH during exercise (11); and a high type II fiber proportion (48). Thus peripheral muscular weakness (35) and early muscle fatigue, rather than cardiovascular and respiratory limitations, generally appear to underlie impaired exercise performance in LTX (see reviews in Refs. 2, 50 and also Refs. 1, 24, 32, 45). Whereas skeletal muscle fatigue is a multifaceted and incompletely understood phenomenon, disturbances in muscle potassium ([K\(^+\)]) and calcium ([Ca\(^{2+}\)]) concentrations (where brackets denote concentration) are critically important.

Muscle fatigue in animal models has been associated with large transsarcolemmal Na\(^+/K^+\) shifts, leading to reduced intracellular [K\(^+\)] and two- to threefold increases in interstitial [K\(^+\)] and intracellular [Na\(^+\)], with ensuing reduced muscle excitability (for reviews see Refs. 8, 18, 41). In exercising humans, increased muscle K\(^-\) efflux, deranged muscle [K\(^+\)] and [Na\(^+\)], and depressed maximal Na\(^+\)-K\(^+\)-ATPase activity have been implicated in fatigue (13, 14, 41, 47). In LTX, there is evidence of impaired K\(^+\) regulation during exercise, with an abnormally high plasma [K\(^+\)] relative to exercise work produced, and a slower postexercise decline in [K\(^+\)] (19). These impairments might be explained by abnormalities in skeletal muscle Na\(^+\)-K\(^+\)-ATPase, which could also exacerbate muscle fatigability (14). Reductions in muscle Na\(^+\)-K\(^+\)-ATPase content have been previously reported with chronic inactivity and in heart failure (8), suggesting a similar possibility in LTX. Furthermore, muscle ATP-dependent Na\(^+\)/K\(^+\) exchange might also be impaired in LTX, due to abnormal oxidative metabolism, reflected by their low muscle ATP content and elevated IMP (48). Therefore, it is possible that abnormalities in muscle Na\(^+\)-K\(^+\)-ATPase contribute to an early muscle fatigue and consequent exercise limitation in LTX.

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Disturbed intracellular Ca\(^{2+}\) regulation is strongly linked with muscular fatigue. In isolated animal muscle fibers, sarcoplasmic reticulum (SR) Ca\(^{2+}\) release declines with fatigue, resulting in depressed intracellular [Ca\(^{2+}\)] and contributing to a decline in force output; SR Ca\(^{2+}\)-ATPase activity is also impaired, depressing SR Ca\(^{2+}\) loading as well as the posttetanic decline in cytosolic [Ca\(^{2+}\)] (see reviews in Refs. 12, 23, 42, 49). In human muscle, depressed maximal in vitro rates of SR Ca\(^{2+}\) release, Ca\(^{2+}\) uptake, and Ca\(^{2+}\)-ATPase activity have each also been demonstrated with fatigue (7, 26). There are no reports of muscle SR characteristics in LTx, but abnormalities seem likely. Impaired muscle oxidative metabolism (48) suggests abnormalities in skeletal muscle, depressing Ca\(^{2+}\) release, Ca\(^{2+}\) reuptake processes might also be impaired in LTx and thereby contribute to early muscle fatigue and exercise cessation in LTx. We have shown that LTx exhibit an unusually high proportion of type II fibers (48), which are typically more fatigable (42). This abnormal fiber composition may partially result from the immunosuppres- sant drug cyclosporin A (CsA), which inhibits calcineurin-activated pathways, leading to a slow-to-fast muscle-fiber phenoconversion (5). Furthermore, there is also evidence in rats that CsA impairs Ca\(^{2+}\) regulation, disturbing myocardial SR Ca\(^{2+}\) release (4), and in skeletal muscle, depressing Ca\(^{2+}\)-ATPase activity and inducing Ca\(^{2+}\)-ATPase isofrom changes (5, 6). Whether these processes are adversely affected in LTx muscle is unknown.

Therefore, the aim of the present study was to explore muscle potassium and calcium regulatory mechanisms implicated in muscular fatigue in humans, to determine whether abnormalities might exist in LTx, in addition to impaired muscle oxidative function (48). Insights gained might have relevance to the patholog- ical processes underlying chronic lung disease, decon- ditioning due to severe illness, and chronic immuno- suppressive therapy and thus have relevance to a wide spectrum of patients.

Three hypotheses were tested in this study: 1) that muscle SR Ca\(^{2+}\) regulation (Ca\(^{2+}\) release, Ca\(^{2+}\) uptake, and Ca\(^{2+}\)-ATPase activity) and 2) muscle Na\(^{+}\)-K\(^{+}\)-ATPase function (content, maximal activity, and plasma [K\(^{+}\)]) are impaired in LTx relative to healthy controls; and 3) that these abnormalities will be corre- lated with their impaired exercise performance.

**METHODS**

**Overview**

All subjects underwent initial lung function assessment and then performed an incremental exercise test on a cycle ergometer, where arterialized venous blood samples were taken during and after exercise. On a separate occasion, but within 7 days, subjects underwent a muscle needle biopsy procedure under resting conditions. This paper complements previously published work specifying abnormalities in skeletal muscle mitochondrial ATP production rate, metabolites, metabolic enzyme activities, as well as muscle fiber-type composition for seven of these patients (48).

**Subjects**

Eight stable LTx comprising five women and three men (age 37.8 ± 10.7 yr, height 168.3 ± 7.8 cm, body mass 64.8 ± 11.2 kg, means ± SD) and eight age- and gender-matched controls (age 37.3 ± 9.7 yr, height 169.5 ± 11.7 cm, body mass 67.1 ± 12.8 kg, means ± SD) volunteered to participate in the study. The mean duration posttransplantation was 13.5 ± 8.7 mo (means ± SD), ranging from 3 to 24 mo. The transplant recipients had stable lung function, with no evidence of recent infection or rejection, having fully rehabilitated after the lung transplant procedure. The controls were sedentary and did not exercise regularly. The diagnoses, operative procedures, and immunosuppressive medication of seven of the patients have been reported previously (48). An additional patient included here was a 24-mo post-heart and lung transplant female patient (age 43 yr, height 161 cm, body mass 48 kg) diagnosed with patent ductus arteriosus and Eisenmenger's syndrome and treated with trimethoprim and sulfamethoxazole, enalapril, pravastatin, 7.5 mg/day prednisolone, 37.5 mg/day azathioprine, and 300 mg/day CsA with a blood [CsA] trough level of 322 μg/l. Details of immunosuppressive medications are indicated in Table 1 (modified from Ref. 48). Several medications taken could affect electrolyte regulation. Electrolyte supplements included magnesium for five patients, slow-release potassium in one patient, whereas three patients received calcium. All patients were treated with the glucocorticoid prednisolone; the glucocorticoid dexamethasone increases muscle Na\(^{+}\)-K\(^{+}\)-ATPase content (9, 38). One patient was treated with the diuretic

**Table 1. Clinical characteristics of lung transplant recipients**

<table>
<thead>
<tr>
<th>LTx, subject no.</th>
<th>Diagnosis</th>
<th>Operative Type</th>
<th>Postoperative Months</th>
<th>BP, mmHg</th>
<th>[Hb], g/l</th>
<th>CsA, mg/day</th>
<th>Blood [CsA], μg/l</th>
<th>PNL, mg/day</th>
<th>AZA, mg/day</th>
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<tbody>
<tr>
<td>1</td>
<td>PDA + ES</td>
<td>HLTx</td>
<td>24</td>
<td>120/80</td>
<td>125</td>
<td>300</td>
<td>322</td>
<td>7.5</td>
<td>37.5</td>
</tr>
<tr>
<td>2</td>
<td>IPF</td>
<td>SLTx</td>
<td>15</td>
<td>150/80</td>
<td>117</td>
<td>350</td>
<td>224</td>
<td>7.5</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>PLAM</td>
<td>SLTx</td>
<td>9</td>
<td>140/80</td>
<td>106</td>
<td>300</td>
<td>336</td>
<td>15</td>
<td>NIL</td>
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<tr>
<td>4</td>
<td>PH</td>
<td>DLTx</td>
<td>24</td>
<td>140/90</td>
<td>126</td>
<td>450</td>
<td>412</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>PH</td>
<td>DLTx</td>
<td>8</td>
<td>150/90</td>
<td>116</td>
<td>400</td>
<td>251</td>
<td>12.5</td>
<td>100</td>
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<tr>
<td>6</td>
<td>BO</td>
<td>DLTx</td>
<td>21</td>
<td>130/70</td>
<td>115</td>
<td>500</td>
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<td>120/70</td>
<td>113</td>
<td>450</td>
<td>221</td>
<td>15</td>
<td>100</td>
</tr>
</tbody>
</table>

LTx, lung transplant recipients; BP, blood pressure; [Hb], Hb concentration; PDA, patent ductus arteriosus; ES, Eisenmenger's syndrome; IPF, idiopathic pulmonary fibrosis; PLAM, pulmonary lymphangioliononasitis; PH, pulmonary hypertension; BO, bronchiolitis obliterans; CF, cystic fibrosis; HLTx, heart-lung transplant; SLTx, single-lung transplant; DLTx, double-lung transplant; CsA, cyclosporine A; [CsA], predose whole blood CsA concentration; PNL, prednisolone average daily dose; AZA, azathioprine. (Modified from Ref. 48.)
frusemide; diuretics may reduce muscle Na\textsuperscript{+}-K\textsuperscript{+}-ATPase content (10). Angiotensin-converting enzyme inhibitors were receiving digitalis therapy. No patients were receiving digitalis therapy.

For electrolyte comparisons, a muscle biopsy was taken under resting conditions from an additional group of seven healthy controls (age 30.3 ± 2.8 yr, height 178.0 ± 3.4 cm, body mass 80.2 ± 5.4 kg, mean ± SD), together with a muscle biopsy from two of the above controls; this was due to procurement of insufficient tissue sample. The study was approved by both the Human Research Ethics Committee at Victoria University of Technology and The Alfred Group of Hospitals Ethics Committee. Written, informed consent was obtained from all participants.

Muscle Biopsy Sampling and Analyses

A muscle biopsy was taken from the vastus lateralis muscle by the needle biopsy technique by using a Bergstrom-Stille needle. In LTx, this procedure was performed while patients were sedated by using pethidine and midazolam at the time of a routine bronchoscopy. Brieﬂy, local anesthesia by was obtained by injection of 2% lignocaine into the skin, subcutaneous tissue, and fascia overlying the vastus lateralis muscle. A small incision was then made, and ~200 mg of tissue were sampled. One portion of muscle (~30–45 mg) was immediately weighed, diluted 1:10 (wt/vol) in a cold buffer, homogenized, and then frozen in liquid nitrogen for later measurements of SR Ca\textsuperscript{2+}-ATPase activity, Ca\textsuperscript{2+} uptake rate, and Ca\textsuperscript{2+} release rate in crude muscle homogenates, by using methods fully detailed and discussed (26). Muscle SR Ca\textsuperscript{2+}-ATPase activity was determined spectrophotometrically, whereas maximal in vitro SR Ca\textsuperscript{2+} uptake and Ag\textsuperscript{+}-induced Ca\textsuperscript{2+} release rates in SR vesicles were determined fluorimetrically by using the ratiometric dye indo-1 (26). A small portion (~10 mg) of muscle was immediately frozen in liquid nitrogen and kept separate for analysis of muscle pH and buffer capacity by using a pH microelectrode and hydrochloric acid titrations, as recently detailed elsewhere (17). Approximately 20 mg of muscle frozen in liquid nitrogen were used to quantify the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase content by using the [\textsuperscript{3}H]ouabain binding method, as previously described (14, 31). A small muscle sample (30–40 mg) was homogenized and used to determine the maximal in vitro Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity, using the K\textsuperscript{+}-stimulated 3-O-methylfluorescein phosphatase (3-O-MFPase) assay, as previously described in detail (14, 15). Muscle fiber composition was analyzed histochemically by using the myofibrillar ATPase method and was previously reported along with muscle metabolite and enzyme assays (48). Muscle Na\textsuperscript{+} and K\textsuperscript{+} contents were determined by flame photometry in five LTXs and contrasted against nine nonmatched, healthy controls.

Exercise Testing, Blood Sampling, and Analyses

All subjects completed an incremental exercise test on an electronically braked cycle ergometer, but with LTX and control tests conducted at different laboratories for ethical reasons and thus using different equipment, with oxygen uptake, ventilation, and lactate threshold as previously described (48). LTx performed a 16.3 W/min increment (Siemens cycle ergometer), whereas control tests were to determine the metabolic measurement cart (Sensormedics, Yorba Linda, CA). Controls performed a 25 W/min increment (Lode cycle ergometer, Groningen, The Netherlands) with respiratory measurements by using our laboratory’s previously described system (26). Arterialized hand dorsal venous blood samples were taken at rest, each minute during exercise, and at 1, 2, 5, and 10 min of recovery, with the hand heated in a 45°C water bath. For controls, blood samples were analyzed in duplicate for plasma acid-base status and gas tensions (H\textsuperscript{+}, Pco\textsubscript{2}, Po\textsubscript{2}), for K\textsuperscript{+} by using an automated analyzer (865 Ciba Corning, Bayer), for [Hb] spectrophotometrically (Radiometer OSM2, Copenhagen, Denmark), and for Hct in triplicate using centrifugation (Hettich Zentrifugen D-7200, Tuttinglen, Germany). For LTx, corresponding blood sample analyses were for plasma acid-base status and gas tensions (ABL500 Radiometer), for [K\textsuperscript{+}] by indirect ion-selective electrodes (Hitachi 747, Boehringer, Mannheim Germany), for [Hb] spectrophotometrically (Radiometer OSM2), and for Hct by using a hematology analyzer (Bayer H2, Leverkusen, Germany). All analytic instruments were calibrated before and during the analyses with precision standards. To ensure comparability of data collected from the two laboratories, three healthy controls (age 35.3 ± 7.6 yr, height 182 ± 9.1 cm, body mass 90.6 ± 11.4 kg, mean ± SD) performed a maximal incremental exercise test to fatigue in each laboratory, with measurement of respiratory data and arterialized venous blood sampling. Comparisons of peak incremental exercise work rate and respiratory and plasma [K\textsuperscript{+}] measurements between the two tests yielded only small differences, which include both technical and biological variability. Slightly lower values were observed in the laboratory used for testing controls with LTx for peak work rate (283 ± 8 vs. 325 ± 14 l/min; P < 0.05) and peak O\textsubscript{2} consumption (V\textsubscript{O\textsubscript{2 peak}}) [3.33 ± 0.15 vs. 3.66 ± 0.14 l/min; not significant (NS)]. The peak plasma [K\textsuperscript{+}] did not differ (6.7 ± 0.1 vs. 6.7 ± 0.2 mmol/l; NS), whereas the rise in plasma [K\textsuperscript{+}] during exercise above rest (Δ[K\textsuperscript{+}])-to-work ratio (Δ[K\textsuperscript{+}]/work) (19.3 ± 2.6 vs. 13.6 ± 2.4 mmol·l\textsuperscript{-1}·min\textsuperscript{-1}; NS) tended to be higher in the laboratory used for testing controls than for LTXs, respectively. These small differences were opposite to those observed between control and LTX (see RESULTS) and, therefore, suggest a small underestimation of the actual differences between control and LTX.

Calculations

The percent decline in plasma volume from rest (ΔPV) was calculated from changes in [Hb] and Hct from rest and the Δ[K\textsuperscript{+}] and the Δ[K\textsuperscript{+}]/work (as an index of plasma K\textsuperscript{+} regulation), as previously described (14, 31).

Statistics

All muscle and exercise data were compared by using an independent (unpaired) t-test. Blood data were analyzed by two-way ANOVA (group, sample time) with repeated measures for sample time. Post hoc analyses used the Student-Newman-Keuls test. Correlations between variables were determined by linear regression. Significance was accepted at P < 0.05. Data are expressed as means ± SE, except where otherwise indicated.

RESULTS

Muscle SR Ca\textsuperscript{2+} Regulation

The maximal rates of SR Ag\textsuperscript{+}-induced Ca\textsuperscript{2+} release, Ca\textsuperscript{2+} uptake, and Ca\textsuperscript{2+}-ATPase activity in crude muscle homogenates were, respectively, 34, 31, and 25% less in LTX compared with controls (P < 0.05), when expressed relative to muscle wet weight (μmol·min\textsuperscript{-1}·g muscle wet wt\textsuperscript{-1}; Figs. 1A, 2A, and 3A, respectively). Similarly, when expressed relative to
and controls are plotted relative to muscle total protein content (\(\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g protein}^{-1}\)), the maximal rates of SR Ag\(^+\)-induced Ca\(^{2+}\) release, Ca\(^{2+}\) uptake, and Ca\(^{2+}\)-ATPase activity were, respectively, 28% \((P < 0.05)\), 26% \((P < 0.05)\), and 16% \((P < 0.06)\) less in LTx compared with controls (Figs. 1B, 2B, and 3B, respectively).

**SR Ca\(^{2+}\) Regulation Relative to Fiber Composition**

LTx exhibited a lower proportion of type I fibers than controls (LTx, 24.9 ± 4.4 vs. controls, 56.1 ± 2.4%; \(P < 0.05)\), as previously reported (48). The SR data in LTx and controls are plotted relative to muscle fiber proportions, with individual data shown by symbols (Fig. 4). Also plotted are the SR-fiber-type relationships determined for healthy subjects using these methods in our laboratory. These were determined from previously reported data for 24 healthy controls (26) and included data from the seven controls in this study (i.e., \(n = 31)\). These figures clearly demonstrate grossly subnormal results for LTx for each of Ca\(^{2+}\) release, Ca\(^{2+}\) uptake, and Ca\(^{2+}\)-ATPase activity, relative to expected higher values, given their higher proportion of type II fibers (26).

**Muscle Na\(^{+}\)-K\(^{+}\)-ATPase Activity and Content**

The maximal in vitro Na\(^{+}\)-K\(^{+}\)-ATPase activity (K\(^{+}\)-stimulated 3-O-MFPase activity, Table 2) was higher in LTx than in controls when expressed per gram wet weight (31%, \(P < 0.05)\) and also tended to be higher when expressed relative to muscle protein content (18%, \(P = 0.09)\). Muscle protein and Na\(^{+}\)-K\(^{+}\)-ATPase ([\(^{3}\text{H}\)]ouabain binding site, 12% higher) contents did not differ significantly between LTx and controls (Table 2).

**Muscle Electrolytes and Buffering Capacity**

Neither muscle Na\(^{+}\) (LTx, 18.5 ± 3.0 vs. controls, 22.9 ± 2.9 \(\mu\text{mol/g wet wt}\)) nor K\(^{+}\) contents differed significantly between groups (LTx, 72.5 ± 11.7 vs. controls, 78.3 ± 4.1 \(\mu\text{mol/g wet wt}\)). Muscle [H\(^{+}\)] was higher (i.e., lower pH) in LTx \((P < 0.05)\), whereas muscle buffer capacity did not differ between the groups (Table 2).

**Pulmonary Function and Incremental Exercise \(\dot{V}O_2\) peak**

Spirometry in LTx showed a mild restrictive ventilatory defect compared with the normal spirometry in controls (vital capacity: LTx, 3.50 ± 0.28 vs. controls, 4.72 ± 0.17 liters; \(P < 0.001, n = 8)\). LTx terminated exercise at substantially lower peak exercise work rates (LTx, 85 ± 8 vs. controls, 222 ± 28 W; \(P < 0.001)\), with a corresponding markedly low \(\dot{V}O_2\) peak (−47%; LTx, 18.8 ± 1.5 vs. controls, 35.6 ± 2.4 ml·kg\(^{-1}\)·min\(^{-1}\); \(P < 0.001)\).
between groups at peak exercise (Fig. 5A). Plasma [K+] was higher in LTx during submaximal exercise when expressed relative to VO2 peak (Fig. 5B). The Δ[K+] was less in LTx (LTx, 1.00 ± 0.15 vs. controls, 2.16 ± 0.19 mmol/l; *P < 0.05). However, when accounting for the lesser work performed, Δ[K+] on VO2 peak was 2.6-fold higher in LTx, indicating grossly impaired plasma K+ regulation (Fig. 6A, *P < 0.05).

The postexercise rate of decline in plasma [K+] was slower in LTx than in controls over the first 2 min (LTx, −0.44 ± 0.07 vs. controls, −0.63 ± 0.06

### Arterialization, [Hb], Hct, and Fluid Shifts During Exercise

Adequate arterialization was evidenced by high PO2 in both LTx and controls at rest, peak exercise, and recovery (Table 3). PO2 was increased above rest at 5 and 10 min of recovery (P < 0.05) and was greater in controls than in LTx (91.2 ± 2.4 vs. 76.8 ± 2.4 Torr, respectively; *P < 0.005). In controls (Table 3), [Hb] was elevated above rest at peak exercise and at 1 and 2 min (P < 0.05), and Hct was increased at 2, 5, and 10 min of recovery (P < 0.05). Plasma volume decreased by 11.2 ± 2.1% at peak exercise and remained less than rest at 30 min of recovery (P < 0.05). In contrast, in LTx, no significant changes from rest were seen in [Hb], Hct, or plasma volume (Table 3). Both [Hb] and Hct were lower in LTx than controls at rest, during exercise, and in recovery (P < 0.05); the decline in plasma volume was greater in controls than in LTx at peak exercise and until 20 min of recovery (P < 0.05, Table 3).

### Plasma [K+] During Incremental Exercise

Plasma [K+] increased with exercise intensity to peak at fatigue and declined to near rest values by 2 min of recovery in both groups (P < 0.05, Fig. 5A). A significant time × group interaction was found for plasma [K+], which was higher in LTx at rest and throughout recovery (P < 0.05), but did not differ significantly from controls until 20 min of recovery (P > 0.05, Fig. 5B). The ΔK+ increase was greater in LTx than controls throughout recovery (Table 3). PO2 was increased above rest at 5 and 10 min of recovery (P < 0.05). A linear regression line (thick dashed lines) and 95% confidence interval (thin dashed lines) is shown for SR characteristics in healthy human muscle. Data are combined from our laboratory’s previous study comprising 8 untrained, 8 endurance-trained, and 8 resistance-trained individuals (26) and pooled with data from the 7 age- and gender-matched healthy controls in this study (total, n = 31). Regression equations were as follows: Ca2+ release = (0.017 × type II%) + 1.147 (r = 0.56, P < 0.001); Ca2+ uptake = (0.011 × type II%) + 1.188 (r = 0.56, P < 0.001); Ca2+-ATPase = (0.113 × type II%) + 8.826 (r = 0.49, P < 0.01). A linear regression analysis was also shown for 7 LTx data.

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**Fig. 3.** Maximal rate of muscle homogenate SR Ca2+-ATPase activity in LTx and healthy, matched controls, expressed in μmol min−1 g muscle wet wt−1 (A) and μmol min−1 g protein−1 (B). Values are means ± SE; n = 7. *P < 0.05, *P < 0.06.

**Fig. 4.** Relationships between the maximal rates of SR Ca2+ release (A), Ca2+ uptake (B), and SR Ca2+-ATPase activity (C) measured in resting muscle and the proportion of type II muscle fibers. SR units are in μmol min−1 g muscle wet wt−1. Controls (n = 7); , LTx (n = 7). A linear regression line (thick dashed lines) and 95% confidence interval (thin dashed lines) is shown for SR characteristics in healthy human muscle. Data are combined from our laboratory’s previous study comprising 8 untrained, 8 endurance-trained, and 8 resistance-trained individuals (26) and pooled with data from the 7 age- and gender-matched healthy controls in this study (total, n = 31). Regression equations were as follows: Ca2+ release = (0.017 × type II%) + 1.147 (r = 0.56, P < 0.001); Ca2+ uptake = (0.011 × type II%) + 1.188 (r = 0.56, P < 0.001); Ca2+-ATPase = (0.113 × type II%) + 8.826 (r = 0.49, P < 0.01). A linear regression analysis was also shown for 7 LTx data.
Vastus lateralis muscle Na\(^+\)-K\(^+\)-ATPase activity (K\(^+\)-stimulated 3-O-methylfluorescein phosphatase activity), Na\(^+\)-K\(^+\)-ATPase content ([\(^3\)H]ouabain binding site content), protein content, fiber type, [H\(^+\)], and buffering capacity in LTx and in age- and gender-matched, healthy controls

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>LTx</th>
<th>Controls</th>
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<tr>
<td>Na(^+)-K(^+)-ATPase activity, mmol·min(^{-1})·g wet wt(^{-1})</td>
<td>8</td>
<td>220±15(^a)</td>
<td>168±9</td>
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<tr>
<td>Na(^+)-K(^+)-ATPase activity, mmol·min(^{-1})·g protein(^{-1})</td>
<td>8</td>
<td>1,441±110(\d)</td>
<td>1,219±86</td>
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<tr>
<td>Protein content, g%</td>
<td>8</td>
<td>15.5±0.8</td>
<td>14.1±1.0</td>
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<tr>
<td>Na(^+)-K(^+)-ATPase content, pmol/g wet wt</td>
<td>7</td>
<td>279±22</td>
<td>250±18</td>
</tr>
<tr>
<td>[H(^+)], mmol/l</td>
<td>7</td>
<td>88.8±2.6(\d)</td>
<td>77.4±1.2</td>
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<tr>
<td>Buffering capacity, μmol H(^+)-g wet wt(^{-1})·pH(^{-1})</td>
<td>7</td>
<td>190.8±14.8</td>
<td>196.9±10.9</td>
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</table>

Values are means ± SE. LTx, 2 single-lung transplants, 2 heart-lung transplants, 4 double-lung transplants. LTx different from controls: \(^aP<0.05\), \(^\dP<0.01\), \(\ddP<0.005\).

Correlations

Na\(^+\)-K\(^+\)-ATPase content, activity, and plasma K\(^+\) variables. The [\(^3\)H]ouabain binding site content was correlated with 3-O-MFPase activity in controls (r = 0.72, P < 0.05), but, interestingly, it was not significantly correlated in LTx alone or in the pooled data (controls and LTx). The [\(^3\)H]ouabain binding site content was not significantly correlated with any plasma K\(^+\) variables (peak [K\(^+\)], Δ[K\(^+\)], Δ[K\(^+\)]/work) in either subgroup or pooled data. Surprisingly, and contrary to findings in healthy populations (14), 3-O-MFPase activity was positively correlated with Δ[K\(^+\)]/work for pooled data (r = 0.61, P < 0.05) and inversely correlated with Δ[K\(^+\)] (r = −0.49, P < 0.05).

Na\(^+\)-K\(^+\)-ATPase, fiber type, and V\(_{O2}\)\(_{peak}\). The [\(^3\)H]ouabain binding site content was correlated with the pooled type II fiber proportion data (r = 0.52, P < 0.05), and the 3-O-MFPase activity was inversely correlated with pooled V\(_{O2}\)\(_{peak}\) data (r = −0.54, P < 0.05). As expected, the V\(_{O2}\)\(_{peak}\) was inversely correlated with the pooled type II fiber proportion data (r = −0.72, P < 0.01). In controls, Δ[K\(^+\)] was related (r = 0.79, P < 0.05) and Δ[K\(^+\)]/work was inversely related to V\(_{O2}\)\(_{peak}\) (r = −0.74, P < 0.05), with similar findings for pooled data (r = 0.87, P < 0.001 and r = −0.65, P < 0.01, respectively; Fig. 6B). The type II fiber proportion was inversely correlated to pooled Δ[K\(^+\)] data (r = −0.63, P < 0.05).

To further investigate possible fiber-type relationships with plasma K\(^+\) variables, we pooled incremental exercise data in untrained, endurance-trained, and resistance-trained individuals (14), with control data from the present study. We found no significant relationship between the proportion of type II fibers (range 23–67%) and either Δ[K\(^+\)] or Δ[K\(^+\)]/work, suggesting no apparent effect of fiber type on plasma [K\(^+\)] regulation in healthy humans. However, when the abnormally high type II data for LTx were added (type II range 23–97%), significant correlations were found. Thus the proportion of type II fibers was inversely related to Δ[K\(^+\)] (r = −0.46, P < 0.005) and positively related to the Δ[K\(^+\)]/work (r = −0.46, P < 0.005).

Na\(^+\)-K\(^+\)-ATPase and SR variables. No significant correlations were found between 3-O-MFPase activity or the [\(^3\)H]ouabain binding site content and SR Ca\(^{2+}\)-ATPase activity.

SR variables, fiber type, and V\(_{O2}\)\(_{peak}\). Several correlations between SR function and muscle performance were found. SR Ca\(^{2+}\) release tended (P < 0.06) to be significantly correlated to pooled V\(_{O2}\)\(_{peak}\) data, expressed per gram muscle (r = 0.52) or per gram protein

ArterIALIZED venous Po\(_2\), [Hb], Hct, and calculated ΔPV, during and after incremental cycle ergometer exercise continued until fatigue in LTx and in age- and gender-matched, healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Peak Exercise</th>
<th>+1 min</th>
<th>+2 min</th>
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<tr>
<td>Po(_2), Torr</td>
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<td>71.9±2.9(a)</td>
<td>72.7±4.6(a)</td>
<td>74.6±5.8(a)</td>
<td>80.1±4.3(a)</td>
<td>80.7±5.7(\d)</td>
<td>80.4±5.0(\dd)</td>
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<tr>
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<td>76.1±3.2</td>
<td>86.4±2.9</td>
<td>85.0±6.2</td>
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<td>[Hb], g/dl</td>
<td>LTx</td>
<td>11.6±0.3(a)</td>
<td>12.0±0.3(a)</td>
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<td>15.4±0.4(a)</td>
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<tr>
<td>Hct, %</td>
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<td>ΔPV, %</td>
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<td>−12.6±2.7</td>
<td>−5.5±2.4</td>
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</tbody>
</table>

Values are means ± SE; n = 8. LTx, 2 single-lung transplants, 2 heart-lung transplants, 4 double-lung transplants. ΔPV, changes in plasma volume from rest. \(^aP<0.05\), LTx different from controls; \(^\dP<0.05\) different from rest in controls only; \(\ddP<0.05\) time main effect, greater than rest, P < 0.05.

J Appl Physiol • VOL 95 • OCTOBER 2003 • www.jap.org
Abnormal SR Ca\(^{2+}\) Regulation in LTx Muscle

We demonstrate, for the first time, markedly low maximal rates of Ag\(^+-\)-induced SR Ca\(^{2+}\) release, Ca\(^{2+}\) uptake, and Ca\(^{2+}\)-ATPase activity in LTx muscle, relative to controls. These were not simply due to abnormalities in muscle water content, as depressions were found whether they were expressed relative to muscle wet weight or protein content. These abnormalities in LTx muscle are even more profound, given their 31% greater type II fiber proportion (48), which, in healthy human muscle, exhibits two- to threefold higher rates (\(r = 0.53\)). SR Ca\(^{2+}\) uptake was correlated to pooled \(\dot{V}O_2\) peak, expressed per gram muscle (\(r = 0.67, P < 0.01\)) or per gram protein (\(r = 0.62, P < 0.05\)), as was Ca\(^{2+}\) uptake in controls per gram muscle wet weight (\(r = 0.74, P < 0.06\)). SR Ca\(^{2+}\)-ATPase activity per gram protein was correlated to \(\dot{V}O_2\) peak for LTx (\(r = 0.76, P < 0.05\)) and tended to correlate for pooled data (\(r = 0.52, P < 0.06\)).

DISCUSSION

We report two major novel findings of abnormal cation regulation in skeletal muscle in LTx, compared with healthy age- and gender-matched controls. Muscle SR Ca\(^{2+}\) regulation was markedly abnormal in LTx, with lower Ca\(^{2+}\) release and Ca\(^{2+}\) uptake rates, as well as Ca\(^{2+}\)-ATPase activity; these differences were even more striking when contrasted to their very high proportion of type II muscle fibers (Fig. 5). Contrary to expectations, we found that, in LTx, skeletal muscle Na\(^{+}\)-K\(^{+}\)-ATPase activity was upregulated by 31%, with a tendency also for an increased Na\(^{+}\)-K\(^{+}\)-ATPase content (12%, NS). However, LTx also demonstrated an elevated \(\Delta[K^+]\)/work during incremental exercise compared with controls, suggesting that plasma and perhaps muscle K\(^{+}\) regulation may also be abnormal during exercise, despite the increased maximal Na\(^{+}\)-K\(^{+}\)-ATPase activity. These abnormalities in muscle Ca\(^{2+}\) and K\(^{+}\) regulation in muscle and blood may lead to impaired membrane excitability and excitation-contraction coupling and thus be important contributors to impaired muscular performance in LTx, evidenced here by an approximately twofold lower \(\dot{V}O_2\) peak.

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We demonstrate, for the first time, markedly low maximal rates of Ag\(^+-\)-induced SR Ca\(^{2+}\) release, Ca\(^{2+}\) uptake, and Ca\(^{2+}\)-ATPase activity in LTx muscle, relative to controls. These were not simply due to abnormalities in muscle water content, as depressions were found whether they were expressed relative to muscle wet weight or protein content. These abnormalities in LTx muscle are even more profound, given their 31% greater type II fiber proportion (48), which, in healthy human muscle, exhibits two- to threefold higher rates.
for each of SR Ca\(^{2+}\) release, Ca\(^{2+}\) uptake, and Ca\(^{2+}\)-ATPase activity (26).

**Critique of SR methods.** Our laboratory has recently discussed these methods for investigating human skeletal muscle SR function in detail (26). Therein, we cite evidence that, whereas Ag\(^{+}\) is not the physiological mechanism for induction of Ca\(^{2+}\) release, the Ag\(^{+}\)-induced Ca\(^{2+}\) release measurement is specific for the physiological Ca\(^{2+}\) release channel, the ryanodine receptor (RyR). We also provide evidence that our SR Ca\(^{2+}\) uptake measure is mediated by SR Ca\(^{2+}\)-ATPase and that our Ca\(^{2+}\)-ATPase activity measurements are specific to the SR. One limitation of these measurements is that they are conducted by using vesicles in a crude muscle homogenate, under standardized in vitro conditions. Thus our measurements cannot predict in vivo muscle SR Ca\(^{2+}\) fluxes in an intact living fiber, where perturbations in local ATP, phosphate, temperature, pH, glycogen, Mg\(^{2+}\), and phosphocreatine may each affect function of the RyR and/or the Ca\(^{2+}\)-ATPase enzyme (23, 42). Nonetheless, it should also be appreciated that no direct method presently exists to study in vivo SR Ca\(^{2+}\) release and/or uptake in human muscle. Our data provide strong evidence of abnormalities in SR in LTx.

**Possible Mechanisms of SR Abnormalities in LTx**

The predominance of type II muscle fibers in LTx is consistent with a type I-to-II fiber transition with muscular disuse (see review by Ref. 37) and with similar findings in patients with chronic obstructive pulmonary disease (20, 44). However, the immunosuppressant drug CsA, which inhibits calcineurin-activated pathways, also leads to a slow-to-fast muscle-fiber phenocconversion (5) and probably also contributed to their profusion of type II fibers. Regardless of the underlying mechanism(s), correspondingly increased SR functional characteristics would be expected in normally functioning type II muscle fibers. Thus altered fiber-type expression without an accompanying increased SR function points to dysfunctional excitation-contraction processes in LTx.

The abnormal SR-fiber-type relationship evident in LTx suggests dissociation between coexpression of myosin ATPase and SR proteins normally observed in fast-twitch muscle, as reported during fast-to-slow-fiber transition with chronic electrical stimulation (39). It is possible that increased SR protein expression that normally occurs in type II fibers is inhibited in LTx muscle.

Hindlimb unweighting in rats increased SR Ca\(^{2+}\) uptake and Ca\(^{2+}\) release rates (43), Ca\(^{2+}\)-ATPase SERCA1 mRNA and protein expression (40), and RyR protein expression in soleus muscle (21). Similarly, immobilization or short-term hindlimb suspension in rats increased SR Ca\(^{2+}\) uptake and Ca\(^{2+}\)-ATPase activity in oxidative muscles (22). These changes are consistent with the typical slow-to-fast-fiber phenocconversion and suggest that disuse per se cannot account for the abnormally low SR function in LTx. Furthermore, training does not increase SR Ca\(^{2+}\)-ATPase content in human muscle (29). In contrast, there is also some evidence of downregulated SR function with inactivity (22, 46). Thus the effects of chronic disuse on SR function remain unresolved.

The effects of the immunosuppressant CsA on SR function are unclear. Chronic CsA increased myosin heavy chain type IIa and SERCA1 protein expression in rat soleus muscle, proportional to blood [CsA] (5). Others found that CsA reduced SR Ca\(^{2+}\)-ATPase activity in rabbit fast-twitch muscle (6), whereas SR Ca\(^{2+}\) uptake in rat white muscle was unchanged (36). The RyR density in rat white muscle was not affected by CsA (36), suggesting that depressed Ca\(^{2+}\) release in LTx likely results from RyR inhibition, as found in rat myocardium (4). We have found that acute CsA addition to rat muscle homogenates depressed SR Ca\(^{2+}\) release, Ca\(^{2+}\) uptake, and Ca\(^{2+}\)-ATPase rates in soleus and extensor digitorum longus muscles, by similar magnitudes to those observed in LTx muscle (J. L. Li et al., unpublished data). It is possible that Mg\(^{2+}\) deficiency due to CsA adversely affected SR Ca\(^{2+}\) regulation (3). Further investigations into CsA effects on skeletal muscle SR Ca\(^{2+}\) regulation in humans are clearly warranted.

The gross depression in SR function in LTx must reflect underlying differences in protein content, structural alterations, and/or changes in SR membrane properties that exert regulatory effects, but our results do not distinguish between these possibilities. Depressed SR function does not necessarily imply down-regulation of SR protein contents in LTx, as seen with altered muscle activity patterns (25, 34). Measurement of muscle SR protein contents and isoform expression in LTx is clearly indicated.

**Increased Muscle Na\(^{+}\)-K\(^{+}\)-ATPase Activity, but Impaired Plasma K\(^{+}\) Regulation in LTx**

An important finding was that skeletal muscle maximal in vitro Na\(^{+}\)-K\(^{-}\)-ATPase activity was elevated in LTx, yet, paradoxically, an abnormal plasma K\(^{+}\) response to exercise was also evident. Thus LTx exhibited a dissociation between muscle Na\(^{+}\)-K\(^{-}\)-ATPase and plasma K\(^{+}\) regulation during exercise. The nonsignificant difference in Na\(^{+}\)-K\(^{-}\)-ATPase content (12% higher in LTx) suggests a possible type II error. However, the significant correlation between Na\(^{+}\)-K\(^{-}\)-ATPase content and activity in controls, but not in LTx, suggests that additional factors affected activity. Na\(^{+}\)-K\(^{-}\)-ATPase upregulation in LTx is interesting, because reduced Na\(^{+}\)-K\(^{-}\)-ATPase content in skeletal muscle could be expected as a consequence of chronic muscular disuse and as also seen in heart failure (for references, see Ref. 8). Upregulated Na\(^{+}\)-K\(^{-}\)-ATPase in LTx can, in part, be explained by glucocorticoid treatment, which increased muscle Na\(^{+}\)-K\(^{-}\)-ATPase content in patients with chronic obstructive lung disease (38) and in rats (9). CsA might also be influential, as CsA inhibited Na\(^{+}\)-K\(^{-}\)-ATPase activity in rabbit cardiac myocytes (30). It is unlikely that the increased
Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in LTx muscle is due to their higher type II muscle fiber proportions, because fiber-type dependence for Na\textsuperscript{+}-K\textsuperscript{+}-ATPase content and/or activity has not been detected in human skeletal muscle (14). Patients demonstrated elevated resting plasma [K\textsuperscript{+}], increased \(\Delta[K\textsuperscript{+}]/\text{work} \) during exercise, and a reduced postexercise decline in plasma [K\textsuperscript{+}], confirming previous results for heart and lung transplant patients (19). Higher resting plasma [K\textsuperscript{+}] in LTx (19) is probably due to CsA, acting via either aldosterone deficiency or distal renal tubular dysfunction (33). The abnormal plasma K\textsuperscript{+} regulation during exercise was independent of both Na\textsuperscript{+}-K\textsuperscript{+}-ATPase content and maximal activity in skeletal muscle. Indeed, the relationship between Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity and plasma K\textsuperscript{+} variables in LTx was opposite to that seen in controls (14). The high proportion of type II fibers might contribute to abnormal plasma K\textsuperscript{+} regulation in LTx. In healthy humans, we found no significant relationships between type II fiber proportion and markers of K\textsuperscript{+} regulation. However, with inclusion of LTx, extending the type II fiber maximum to 97%, a significant inverse relationship was found between the type II fiber proportion and the \(\Delta[K\textsuperscript{+}]/\text{work} \). Hence, the type II fiber dominance in these patients might be linked with their poor K\textsuperscript{+} regulation during exercise. This could occur via enhanced K\textsuperscript{+} release from contracting muscles, because K\textsuperscript{+} efflux is greater in glycolytic than in oxidative muscles (8, 27). Whereas the muscle maximal in vitro Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity is upregulated in LTx, it is possible that the normal exercise-induced in vivo increase in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity is compromised. This would be consistent with their exaggerated \(\Delta[K\textsuperscript{+}]/\text{work} \) and reduced postexercise decline in plasma [K\textsuperscript{+}]. Furthermore, mitochondrial ATP production is abnormal in resting muscle in LTx (48) and likely also during exercise. This could, consequently, impair muscle in vivo Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity, whereas our measures of in vitro maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity, where energy supply is not limiting, would be normal. CsA-induced Mg\textsuperscript{2+} deficiency might also adversely affect muscle Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity. A further possibility is that muscle cellular K\textsuperscript{+} efflux in LTx is enhanced via alterations in muscle K\textsuperscript{+} channels, which might include voltage-sensitive ATP-sensitive K\textsuperscript{+} or Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels (41). Muscle K\textsuperscript{+} loss during exercise would then be augmented in LTx in each of these scenarios. These possibilities all require further investigation.

Reduced K\textsuperscript{+} clearance by inactive muscles might also conceivably contribute to abnormal plasma K\textsuperscript{+} regulation during exercise in LTx. This might result from depressed Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activation in noncontracting, as well as in contracting muscles. Diminished skeletal muscle mass in LTx, if present, would further reduce the capacity for extracellular K\textsuperscript{+} clearance and could also, in part, explain these discordant findings. The elevated resting muscle [H\textsuperscript{+}] in LTx (11) was not due to reduced muscle buffer capacity but is compatible with elevated lactate, impaired mitochondrial ATP production in resting muscle, and early plasma lactate threshold during exercise in LTx (48).

**Functional Consequences of Impaired Muscle Cation Regulation in LTx**

Muscle Na\textsuperscript{+}-K\textsuperscript{+}-ATPase plays a crucial role in maintaining membrane excitability and force (8). The upregulated muscle in vitro Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in LTx might then suggest an enhanced functional capacity, but the opposite was found. The \(\Delta[K\textsuperscript{+}] \) was abnormal, and the \(\Delta[K\textsuperscript{+}]/\text{work} \) was inversely related to \(V_{O_2\text{peak}} \), which was abnormally low. These indicate that LTx were unable to adequately regulate K\textsuperscript{+} in vivo during exercise and suggest that disturbances in muscle [K\textsuperscript{+}] may be important in the early muscle fatigue in LTx. This scenario is compounded by the striking abnormalities in SR Ca\textsuperscript{2+} regulation, because SR Ca\textsuperscript{2+} release and Ca\textsuperscript{2+} uptake processes directly regulate cytosolic [Ca\textsuperscript{2+}] and thus muscle force development and relaxation (23). Impaired SR Ca\textsuperscript{2+} release in LTx would probably reduce cytosolic [Ca\textsuperscript{2+}] and force output during contractions (49). Using force-pCa relationships in healthy human vastus lateralis muscle fibers (28), assuming proportionate reductions in SR Ca\textsuperscript{2+} release and tetanic cytosolic [Ca\textsuperscript{2+}] and physiological tetanic cytosolic [Ca\textsuperscript{2+}] (i.e.,~1 \(\mu\text{M} \)), we estimate that a 34% lower Ca\textsuperscript{2+} release in LTx would reduce muscle force in type IIA and type IIB fibers by as much as 30 and 37%, respectively. The lower SR Ca\textsuperscript{2+} uptake rate would lessen the decline in tetanic cytosolic [Ca\textsuperscript{2+}] with reduced Ca\textsuperscript{2+} release. However, even if this decline were reduced by one-third, muscle force in type IIA and type IIB fibers in LTx would still be reduced by 18 and 23%, respectively. Thus impaired SR Ca\textsuperscript{2+} release would have important adverse functional consequences in LTx muscle. Furthermore, these SR functional deficiencies identified in resting muscle in LTx would probably be exacerbated in vivo during exercise. Maximal SR Ca\textsuperscript{2+} release, Ca\textsuperscript{2+} ATPase activity, and Ca\textsuperscript{2+} uptake rates in healthy human muscle were depressed with fatigue (26), which probably also occurs in LTx. Furthermore, impaired mitochondrial function and reduced ATP (11, 48) suggest further inhibition of SR RyR opening and Ca\textsuperscript{2+} release, leading to muscle fatigue during exercise. The reduced SR Ca\textsuperscript{2+} uptake in LTx, together with phosphate accumulation, could also limit the Ca\textsuperscript{2+} available for release (16). Thus the lower skeletal muscle SR Ca\textsuperscript{2+} release and Ca\textsuperscript{2+}-ATPase activity could conceivably make a major contribution to the marked exercise limitation in these patients. Consistent with this, correlations were found between each of the SR function variables and \(V_{O_2\text{peak}} \). These impairments in SR function are thus consistent with, and may even be primarily responsible for, the muscle weakness that persists after lung transplantation (1, 24, 35).

In conclusion, we demonstrate here a range of abnormalities in Ca\textsuperscript{2+}, Na\textsuperscript{+}/K\textsuperscript{+}, and H\textsuperscript{+} regulation in peripheral skeletal muscle in LTx. Muscle SR Ca\textsuperscript{2+} release, Ca\textsuperscript{2+} uptake, and Ca\textsuperscript{2+}-ATPase activity were...
reduced in LTx compared with controls and were considerably subnormal compared with muscle SR function-fiber-type relationships found in healthy controls. The underlying mechanisms are not known, but they may result from chronic disuse, CsA, and disease effects. A paradox was evident between muscle in vitro Na\(^+/\)K\(^{-}\)-ATPase measures, content and activity, which were respectively maintained or increased, and the in vivo measure, plasma [K\(^+\)] regulation with exercise, which was impaired. The maintenance of Na\(^+/\)K\(^{-}\)-ATPase activity and content occurred, despite chronic inactivity, possibly due to medication and disease effects. We speculate that impaired muscle Ca\(^{2+}\) and Na\(^+/\)K\(^{-}\) regulation may be important contributory factors in the early muscle fatigue and grossly restricted exercise capacity evident in LTx.

We thank all participants in this study. This project arose from work initiated by our esteemed former colleague, the late Dr. Michael Hall.

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REFERENCES


