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

Michael J. McKenna,1 Steve F. Fraser,1 Jia L. Li,1 Xiao N. Wang,2 Michael F. Carey,2 Eleonora A. Side,3,4 Judith Morton,3,4 Gregory I. Snell,3,4 Keld Kjeldsen,5 and Trevor J. Williams3,4

1Muscle, Ions and Exercise Group, School of Human Movement, Recreation and Performance, and 2School of Biomedical Science, Centre for Rehabilitation, Exercise and Sports Science, Victoria University of Technology, Melbourne 8001; 3Department of Allergy, Immunology, and Respiratory Medicine, Alfred Hospital, Melbourne 3004; and 4Department of Medicine, Monash University, Melbourne, Victoria 3800, Australia; and 5Department of Medicine B, The Heart Centre, Rigshospitalet, 2100 Copenhagen, Denmark

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LUNG TRANSPLANT RECIPIENTS (LTx) exhibit peripheral muscle O2 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\textsuperscript{+}]), sodium ([Na\textsuperscript{+}]), and calcium ([Ca\textsuperscript{2+}]) concentrations (where brackets denote concentration) are critically important.

Muscle fatigue in animal models has been associated with large transsarcolemmal Na\textsuperscript{+}/K\textsuperscript{+} shifts, leading to reduced intracellular [K\textsuperscript{+}] and two- to threefold increases in interstitial [K\textsuperscript{+}] and intracellular [Na\textsuperscript{+}], with ensuing reduced muscle excitability (for reviews see Refs. 8, 18, 41). In exercising humans, increased muscle K\textsuperscript{-} efflux, deranged muscle [K\textsuperscript{+}] and [Na\textsuperscript{+}], and depressed maximal Na\textsuperscript{+}/K\textsuperscript{-}-ATPase activity have been implicated in fatigue (13, 14, 41, 47). In LTx, there is evidence of impaired K\textsuperscript{-} regulation during exercise, with an abnormally high plasma [K\textsuperscript{+}] relative to exercise work produced, and a slower postexercise decline in [K\textsuperscript{+}] (19). These impairments might be explained by abnormalities in skeletal muscle Na\textsuperscript{+}/K\textsuperscript{-}ATPase, which could also exacerbate muscle fatigability (14). Reductions in muscle Na\textsuperscript{+}/K\textsuperscript{-}-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\textsuperscript{+}/K\textsuperscript{-} 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\textsuperscript{+}/K\textsuperscript{-}-ATPase contribute to an early muscle fatigue and consequent exercise limitation in LTx.
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 that ATP-dependent Ca\(^{2+}\) clearance might be impaired in LTx and thereby contribute to early muscle fatigue and exercise cessation. 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 that ATP-dependent Ca\(^{2+}\) release and 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 immunosuppressant drug cyclosporin A (CsA), which inhibits calcineurin-activated pathways, leading to a slow-to-fast muscle-fiber phenotype (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 isof orm 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 pathological processes underlying chronic lung disease, deconditioning due to severe illness, and chronic immunosuppressive 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 correlated 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 ± 95 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>
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<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>
</tr>
<tr>
<td>4</td>
<td>PH</td>
<td>HLTx</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>
</tr>
<tr>
<td>6</td>
<td>BO</td>
<td>DLTx</td>
<td>21</td>
<td>130/70</td>
<td>115</td>
<td>500</td>
<td>193</td>
<td>7.5</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>CF</td>
<td>DLTx</td>
<td>3</td>
<td>155/80</td>
<td>126</td>
<td>300</td>
<td>222</td>
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<tr>
<td>8</td>
<td>CF</td>
<td>DLTx</td>
<td>4</td>
<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 recipient; BP, blood pressure; [Hb], Hb concentration; PDA, patent ductus arteriosus; ES, Eisenmenger's syndrome; IPF, idiopathic pulmonary fibrosis; PLAM, pulmonary lymphangioleiomyosclerosis; 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.)

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frusenide; diuretics may reduce muscle Na\(^+-K^+\)-ATPase content (10). Angiotensin-converting enzyme inhibitors were received by two patients (one each enalapril and lisinopril). 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, means ± 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 caudal bronchoscope, with respiratory, 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\(^{2+}\)-ATPase activity, Ca\(^{2+}\) uptake rate, and Ca\(^{2+}\) release rate in crude muscle homogenates, by using methods fully detailed and discussed (26). Muscle SR Ca\(^{2+}\)-ATPase activity was determined spectrophotometrically, whereas maximal in vitro SR Ca\(^{2+}\) uptake and Ag\(^{+}\)-induced Ca\(^{2+}\) release rates in SR vesicles were determined fluorometrically 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\(^+-K^+\)-ATPase content by using the [\(^{3}H\)]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\(^+-K^+\)-ATPase activity, using the K\(^+\)-stimulated 3-O-methyluroreoscin 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\(^+\) and K\(^+\) 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 (Siebold Lode cycle ergometer), with respiratory analyses by using a 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\(^+\), PCO\(_2\), PO\(_2\)), for [K\(^+\)] by using an automated analyzer (865 Ciba Corning, Bayer), for [Hb] spectrophotometrically (Radiometer OSM2, Copenhagen, Denmark), and for Hct in triplicate by 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\(^+\)] 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 compatibility 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\(^+\)] 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 compared with LTx for peak work rate (283 ± 8 vs. 325 ± 14 l/min; P < 0.05) and peak O\(_2\) consumption (Vo\(_2\) peak) \([3.33 ± 0.15 vs. 3.66 ± 0.14\ l/min; not significant (NS)]\).

Calculations
The percent decline in plasma volume from rest (ΔPV) was calculated from changes in [Hb] and Hct from rest and the Δ[Hb]/Δ[Hct]; for ΔPV = (Hb/[Hb] - 1) × 0.5 and when expressed relative to muscle wet weight (μmol·min\(^{-1}·g\) muscle wet wt\(^{-1}\); Figs. 1A, 2A, and 3A, respectively). Similarly, when expressed relative to

Muscle SR Ca\(^{2+}\) Regulation
The maximal rates of SR Ag\(^{+}\)-induced Ca\(^{2+}\) release, Ca\(^{2+}\) uptake, and Ca\(^{2+}\)-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\(^{-1}·g\) muscle wet wt\(^{-1}\); Figs. 1A, 2A, and 3A, respectively).
and controls are plotted relative to muscle total protein content (μmol·min⁻¹·g protein⁻¹), the maximal rates of SR Ag⁺-induced Ca²⁺ release, Ca²⁺ uptake, and Ca²⁺-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 Ca²⁺ 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 Ca²⁺ release, Ca²⁺ uptake, and Ca²⁺-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 ([³H]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 μmol/g wet wt) nor K⁺ contents differed significantly between groups (LTx, 72.5 ± 11.7 vs. controls, 78.3 ± 4.1 μmol/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 VO₂peak**

Spirometry in LTx showed a mild restrictive ventilatory defect compared with the normal spirometry in controls (vital capacity: LTx, 3.50 ± 0.28 liters; P < 0.001, n = 8). LTx terminated exercise at substantially lower peak exercise work rates (LTx, 88 ± 8 vs. controls, 222 ± 28 W; P < 0.001), with a corresponding markedly low VO₂peak (−47%; LTx, 18.8 ± 1.5 vs. controls, 35.6 ± 2.4 ml·kg⁻¹·min⁻¹; P < 0.001).

**Fig. 1.** Maximal rate of muscle homogenate sarcoplasmic reticulum (SR) Ca²⁺ release in lung transplant recipients (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.

**Fig. 2.** Maximal rate of muscle homogenate SR Ca²⁺ uptake 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.
between groups at peak exercise (Fig. 5A). Plasma $[K^+]$ was higher in LTx during submaximal exercise when expressed relative to $V_{O_2\text{peak}}$ (Fig. 5B).

The $\Delta[K^+]$ was less in LTx (LTx, $1.00 \pm 0.15$ vs. controls, $2.16 \pm 0.19$ mmol/l; $P < 0.05$). However, when accounting for the lesser work performed, $\Delta[K^+]$/work 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, $-0.44 \pm 0.07$ vs. controls, $-0.63 \pm 0.06$

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**Fig. 3.** Maximal rate of muscle homogenate SR Ca$^{2+}$-ATPase activity in LTx and healthy, matched controls, expressed in $\mu$mol min$^{-1}$ g muscle wet wt$^{-1}$ (A) and $\mu$mol min$^{-1}$ g protein$^{-1}$ (B). Values are means $\pm$ SE; $n = 7$. *$P < 0.05$; # $P < 0.06$.

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

Adequate arterialization was evidenced by high Po$_2$ in both LTx and controls at rest, peak exercise, and recovery (Table 3). Po$_2$ was increased above rest at 5 and 10 min of recovery ($P < 0.05$) and was greater in controls than in LTx ($91.2 \pm 2.4$ vs. $76.8 \pm 2.4$ 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 $11.2 \pm 2.1\%$ 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 $\times$ group interaction was found for plasma $[K^+]$, which was higher in LTx at rest and throughout recovery ($P < 0.05$), but did not differ...
Table 2. *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>
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<tr>
<th></th>
<th>n</th>
<th>LTx</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+)-K(^+)-ATPase activity, nmol·min(^{-1})·g wet wt(^{-1})</td>
<td>8</td>
<td>220 ± 15(^a)</td>
<td>168 ± 9</td>
</tr>
<tr>
<td>Na(^+)-K(^+)-ATPase activity, nmol·min(^{-1})·g protein(^{-1})</td>
<td>8</td>
<td>1,441 ± 110(^\dagger)</td>
<td>1,219 ± 86</td>
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<td>Protein content, g(%)</td>
<td>8</td>
<td>15.5 ± 0.8</td>
<td>14.1 ± 1.0</td>
</tr>
<tr>
<td>Na(^+)-K(^+)-ATPase content, pmol/g wet wt</td>
<td>8</td>
<td>279 ± 22</td>
<td>250 ± 18</td>
</tr>
<tr>
<td>[H(^+)], nmol/l</td>
<td>7</td>
<td>88.8 ± 2.6(^\ddagger)</td>
<td>77.4 ± 1.2</td>
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<tr>
<td>Buffering capacity, (\mu)mol H(^+)-g wet wt(^{-1})·ph(^{-1})</td>
<td>7</td>
<td>190.8 ± 14.8</td>
<td>196.9 ± 10.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. LTx, 2 single-lung transplants, 2 heart-lung transplants, 4 double-lung transplants. LTx different from controls: \(^aP < 0.05, \^\daggerP < 0.01, \^\ddaggerP < 0.001\) and \(r = -0.65, P < 0.01\), respectively. The type II fiber proportion was inversely related to pooled \(\Delta[K^+]\) 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 \(\Delta[K^+]\) or \(\Delta [K^+]\) 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 \(\Delta[K^+]\) (\(r = -0.46, P < 0.005\)) and positively related to the \(\Delta[K^+]\) work (\(r = -0.46, P < 0.005\)).

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

SR variables, fiber type, and \(\dot{V}O_2\) 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 \(\dot{V}O_2\) peak data, expressed per gram muscle \((r = 0.52)\) or per gram protein.

Table 3. Arterialized venous \(P_O_2\), [\(Hb\)], Hct, and calculated \(\Delta P_V\), 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>
<th>+5 min</th>
<th>+10 min</th>
<th>+30 min</th>
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<tr>
<td>(P_O_2), Torr</td>
<td>LTx</td>
<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(^\ddagger)</td>
<td>80.4 ± 5.0(^\ddagger)</td>
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<tr>
<td>Controls</td>
<td>76.1 ± 3.2</td>
<td>86.4 ± 2.9</td>
<td>85.0 ± 6.2</td>
<td>98.8 ± 2.7</td>
<td>96.7 ± 5.3(^\ddagger)</td>
<td>98.9 ± 4.3(^\ddagger)</td>
<td>91.4 ± 3.1</td>
</tr>
<tr>
<td>[(Hb)], g/dl</td>
<td>LTx</td>
<td>11.6 ± 0.3(^a)</td>
<td>12.0 ± 0.3(^a)</td>
<td>11.8 ± 0.3(^a)</td>
<td>12.1 ± 0.3(^a)</td>
<td>11.8 ± 0.2(^a)</td>
<td>11.7 ± 0.3(^a)</td>
</tr>
<tr>
<td>Controls</td>
<td>13.8 ± 0.3</td>
<td>15.4 ± 0.4(^\dagger)</td>
<td>15.5 ± 0.3(^\dagger)</td>
<td>15.4 ± 0.4(^\dagger)</td>
<td>14.9 ± 0.6</td>
<td>15.0 ± 0.5</td>
<td>14.3 ± 0.4</td>
</tr>
<tr>
<td>Hct, %</td>
<td>LTx</td>
<td>35.0 ± 0.1(^a)</td>
<td>36.1 ± 0.1(^a)</td>
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<td>35.0 ± 0.1(^a)</td>
</tr>
<tr>
<td>Controls</td>
<td>39.8 ± 0.5</td>
<td>41.8 ± 0.1</td>
<td>42.5 ± 1.0(^\dagger)</td>
<td>42.3 ± 0.8(^\dagger)</td>
<td>43.5 ± 0.8(^\dagger)</td>
<td>43.8 ± 0.8(^\dagger)</td>
<td>41.4 ± 0.9</td>
</tr>
<tr>
<td>(\Delta P_V), %</td>
<td>LTx</td>
<td>-3.9 ± 0.8(^a)</td>
<td>-5.2 ± 1.5(^a)</td>
<td>-4.5 ± 1.2(^a)</td>
<td>-2.4 ± 1.0(^a)</td>
<td>-1.1 ± 1.1</td>
<td>0.9 ± 1.3</td>
</tr>
<tr>
<td>Controls</td>
<td>-11.2 ± 2.1(^\dagger)</td>
<td>-14.8 ± 1.8(^\dagger)</td>
<td>-15.9 ± 1.7(^\dagger)</td>
<td>-11.4 ± 3.0(^\dagger)</td>
<td>-12.6 ± 2.7(^\dagger)</td>
<td>-5.5 ± 2.4(^\dagger)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 8\). LTx, 2 single-lung transplants, 2 heart-lung transplants, 4 double-lung transplants. \(\Delta P_V\), changes in plasma volume from rest. \(^aP < 0.05, \^\daggerP < 0.005\) different from controls; \(^\daggerP < 0.05\) different from rest in controls only; \(^\ddaggertime main effect, greater than rest, P < 0.05\).

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SR Ca\textsuperscript{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\textsuperscript{2+} uptake in controls per gram muscle wet weight \((r = 0.74, P < 0.06)\). SR Ca\textsuperscript{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\textsuperscript{2+} regulation was markedly abnormal in LTx, with lower Ca\textsuperscript{2+} release and Ca\textsuperscript{2+} uptake rates, as well as Ca\textsuperscript{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\textsuperscript{+}-K\textsuperscript{+}-ATPase activity was upregulated by 31%, with a tendency also for an increased Na\textsuperscript{+}-K\textsuperscript{+}-ATPase content (12%, NS). However, LTx also demonstrated an elevated \( \Delta[K^+]_\text{work} \) during incremental exercise compared with controls, suggesting that plasma and perhaps muscle K\textsuperscript{+} regulation may also be abnormal during exercise, despite the increased maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity. These abnormalities in muscle Ca\textsuperscript{2+} and K\textsuperscript{+} 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.

**Abnormal SR Ca\textsuperscript{2+} Regulation in LTx Muscle**

We demonstrate, for the first time, markedly low maximal rates of Ag\textsuperscript{+}-induced SR Ca\textsuperscript{2+} release, Ca\textsuperscript{2+} uptake, and Ca\textsuperscript{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.

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**Fig. 5.** A: plasma K\textsuperscript{+} concentration ([K\textsuperscript{+}]) at rest, during incremental exercise, and during 30 min of recovery for LTx (○) and for healthy, matched controls (■). Horizontal error bars for peak values indicate means ± SE of peak work rate. The zero recovery time point is the peak exercise value replotted. B: plasma [K\textsuperscript{+}] against percent peak \( \dot{V}O_2 \) consumption (\( \dot{V}O_2 \)peak\%). Horizontal error bars for submaximal values indicate means ± SE of %\( \dot{V}O_2 \)peak; \( n = 8 \). *P < 0.05, LTx different from controls. NS, not significant.

**Fig. 6.** A: rise in plasma [K\textsuperscript{+}]-to-work ratio for LTx and healthy, matched controls. Values are means ± SE; \( n = 8 \) for both groups. *LTx < controls, \( P < 0.05 \). B: relationship between \( \dot{V}O_2 \)peak (ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) and rise in plasma [K\textsuperscript{+}]-to-work ratio (nmol·l\textsuperscript{-1}·J\textsuperscript{-1}) for LTx (○) and controls (■). Regression line (solid line) and equation are shown for pooled LTx and control data; \( n = 16 \).
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 downregulation 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 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., $\sim\text{1\ \mu M}$), 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.

Present address of S. F. Fraser: Div. of Exercise Science, School of Medical Sciences, RMIT University, Bundoora, Victoria 3083, Australia.

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