Impaired exercise performance and muscle Na\textsuperscript{+},K\textsuperscript{+}-pump activity in renal transplantation and haemodialysis patients

Aaron C. Petersen\textsuperscript{1,2}, Murray J. Leikis\textsuperscript{3,4}, Lawrence P. McMahon\textsuperscript{5}, Annette B. Kent\textsuperscript{5}, Kate T. Murphy\textsuperscript{1,6}, Xiaofei Gong\textsuperscript{1} and Michael J. McKenna\textsuperscript{1}

\textsuperscript{1}Muscle, Ions and Exercise Group, Institute of Sport, Exercise and Active Living (ISEAL), Victoria University, Melbourne, Australia, \textsuperscript{2}School of Sport and Exercise Science, Victoria University, Melbourne, Australia, \textsuperscript{3}Department of Nephrology, Royal Melbourne Hospital and Western Hospitals, Melbourne, Australia, \textsuperscript{4}Department of Renal Medicine, Wellington Hospital, Wellington, New Zealand, \textsuperscript{5}Department of Renal Medicine, Eastern Health Clinical School, Monash University, Melbourne, Australia and \textsuperscript{6}Department of Physiology, University of Melbourne, Melbourne, Victoria, Australia

Correspondence and offprint requests to: Michael J. McKenna; E-mail: michael.mckenna@vu.edu.au

Abstract

Background. We examined whether abnormal skeletal muscle Na\textsuperscript{+},K\textsuperscript{+}-pumps underlie impaired exercise performance in haemodialysis patients (HDP) and whether these are improved in renal transplant recipients (RTx).

Methods. Peak oxygen consumption (\(\text{VO}_2\text{peak}\)) and plasma [K\textsuperscript{+}] were measured during incremental exercise in 9RTx, 10 HDP and 10 healthy controls (CON). Quadriceps peak torque (PT), fatigability (decline in strength during thirty contractions), thigh muscle cross-sectional area (TMCSA) and vastus lateralis Na\textsuperscript{+},K\textsuperscript{+}-pump maximal activity, content and isoform (\(\alpha_1-\alpha_3, \beta_1-\beta_3\)) abundance were measured.

Results. \(\text{VO}_2\text{peak}\) was 32 and 35\% lower in RTx and HDP than CON, respectively (\(P<0.05\)). PT was less in RTx and HDP than CON (\(P<0.05\)) but did not differ when expressed relative to TMCSA. Fatigability (decline in strength during thirty contractions), thigh muscle cross-sectional area (TMCSA) and vastus lateralis Na\textsuperscript{+},K\textsuperscript{+}-pump maximal activity, content and isoform (\(\alpha_1-\alpha_3, \beta_1-\beta_3\)) abundance were measured.

Conclusions. \(\text{VO}_2\text{peak}\) and muscle Na\textsuperscript{+},K\textsuperscript{+}-pump activity were depressed and muscle fatigability increased in HDP, with no difference observed in RTx. These findings are consistent with the possibility that impaired exercise performance in HDP and RTx may be partially due to depressed muscle Na\textsuperscript{+},K\textsuperscript{+}-pump activity and relative TMCSA.

Keywords: extrarenal potassium regulation; fatigue; muscle mass; strength; \(\text{VO}_2\text{peak}\)

Introduction

Patients with chronic kidney disease have grossly impaired exercise tolerance [1], but differences in exercise performance between haemodialysis patients (HDP) and renal transplantation recipients (RTx) are poorly defined. Following renal transplantation, the \(\text{VO}_2\text{peak}\) of previously anaemic HDP has been reported to increase by 25–38\% [2, 3]. Post-transplantation increases in haemoglobin concentration ([Hb]) and haematocrit (Hct) may account for much of the improvement, as increases in \(\text{VO}_2\text{peak}\) of 19–33\% have been reported in HDP following treatment with erythropoietic-stimulating agents (ESA) [4, 5]. Nonetheless, no studies have investigated \(\text{VO}_2\text{peak}\) in RTx and HDP with similar [Hb] to properly compare these groups.

Muscle strength and fatigability are important determinants of exercise performance [6, 7]. Muscle strength is...
impaired in non-ESA-treated HDP [8], likely due to reduced muscle mass [9] and is unlikely to improve with ESA treatment [10]. However, RTx does not improve muscle strength [8, 11], possibly due to the muscle wasting effects of glucocorticoid therapy [12]. Muscle fatigability during repeated maximal isometric hand-grip contractions was greater and Hct lower in non-ESA-treated HDP than RTx [13]. No studies have examined fatigability during dynamic contractions in RTx or between RTx- and ESA-treated HDP with similar [Hb].

If exercise performance remains impaired in RTx, an underlying muscle defect may persist, contributing to greater fatigability. A possible mechanism of fatigue is impaired muscle membrane excitability, caused by elevated interstitial [K\(^+\)] [14], which is likely exacerbated in HDP. Anaemic HDP exhibited pronounced hyperkalaemia during exercise, which was inversely correlated with VO\(_{2}\)peak [15]. A reduced muscle compound action potential in HDP [16] suggests impaired membrane excitability. Hence, abnormal K\(^+\) regulation might enhance fatigue and reduce exercise performance. We tested the hypothesis that regulation of plasma [K\(^+\)] during incremental exercise would be impaired in both HDP and RTx.

Skeletal muscle contains the largest pool of Na\(^+\), K\(^+\)-pumps [17] and is vital in extrarenal K\(^+\) regulation [18]. Reduced Na\(^+\), K\(^+\)-pump activity was found in muscle from uraemic rats [19] despite normal content and isoform abundance [19, 20], suggesting an underlying defect in existing pumps. Thus, impaired muscle Na\(^+\), K\(^+\)-pump activity could underlie the abnormal plasma K\(^+\) responses in HDP [15]. Possible abnormalities in skeletal muscle Na\(^+\), K\(^+\)-pump activity, content or isoform abundance, have not been investigated in uraemic humans. Erythrocytic Na\(^+\), K\(^+\)-pump activity was improved following renal transplantation [21], but whether skeletal muscle Na\(^+\), K\(^+\)-pump activity in RTx is impaired is not known.

This study tested the hypothesis that VO\(_{2}\)peak, muscular strength and fatigability would be worsened compared to CON but would not be different between RTx- and ESA-treated HDP with similar [Hb]. We also tested the hypotheses that muscle Na\(^+\), K\(^+\)-pump activity would be depressed in both HDP and RTx, with normal Na\(^+\), K\(^+\)-pump content and isoform abundance. Finally, we explored whether depressed muscle Na\(^+\), K\(^+\)-pump activity in HDP and RTx would be related to their poor K\(^+\) regulation and exercise performance.

**Materials and methods**

**Subjects**

Nine RTx, 10 HDP and 10 CON gave written informed consent and participated in the study. One HDP underwent all tests except the muscle biopsy. Subjects were matched for sex, age, height, body mass and body mass index (Table 1). Selection criteria for RTx were: transplanted at least 12 months prior to testing (range 16–171, 63 ± 53 months), had a stable creatinine and a calculated CCr (Cockcroft-Gault) of ≥40 mL min\(^{-1}\). Selection criteria for HDP were: stable and had been dialysing for at least 6 months (range 7–71, 38 ± 23). All HDP were anuric and urea reduction ratio was 65 ± 5%. Ultrafiltration rate was 0.4–0.7 L h\(^{-1}\) according to patient size, intra-dialytic time and pre-dialysis weight. All subjects had an [Hb] >110 g L\(^{-1}\). Subjects were excluded if they had symptomatic ischaemic heart disease, peripheral vascular disease, disabling arthritis, chronic airflow obstruction or were pregnant. Subject medications are shown in Table 2. Subject acid-base and electrolyte concentrations at rest and peak exercise are shown in Table 3. The RTx recipient received kidneys from living related donors (n = 2), living non-related donors (n = 2) and from deceased donors (n = 5). This study was approved by the Human Research Ethics Committees at Victoria University and Melbourne Health.

**Exercise tests**

For HDP, all exercise tests were performed on a non-dialysis day with a mean time of 31 ± 14 h post-dialysis.

**Peak oxygen consumption (VO\(_{2}\)peak)** Subjects cycled (≥60 r.p.m.) on an electronically braked cycle ergometer (Lode, Groningen, Holland), with increments of 15 W each minute until volitional exhaustion [22], with expired gases and ventilation continuously measured to calculate O\(_{2}\) [23].

**Quadriceps torque–velocity test.** Subjects performed three maximal isokinetic contractions at 0, 60, 120, 180, 240, 300 and 360° s\(^{-1}\), with 60 s recovery between sets, on an isokinetic dynamometer (Cybex Norm-770; Henley HealthCare, MA) [24]. The highest value of each set of three was defined as the peak torque (PT) and expressed relative to body mass (Nm kg\(^{-1}\)) and thigh muscle cross-sectional area (TMCSA, Nm cm\(^{-2}\)), to correct for differences in body size and muscle mass, respectively.

**Quadriiceps fatigue test.** Subjects performed 30 maximal isokinetic contractions at 180° s\(^{-1}\), with 1 s pause between repetitions [24]. The fatigue index (FI, %) was calculated as [(starting PT–final PT)/starting PT] × 100, where starting PT is the average of the highest three of the first five repetitions; final PT is the average of the highest three of the last five repetitions.

**Blood sampling and processing**

Blood was sampled before and during and after the VO\(_{2}\)peak test from an arterio-venous fistula in all HDP and in four RTx; in all other subjects, arterialized venous blood was sampled from a heated dorsal hand vein [25]. The different blood sampling sites used are unlikely to have impacted on [K\(^+\)] since arterialized venous [K\(^+\)] did not differ from arterial [K\(^+\)] during low-to-moderate intensity exercise and was only marginally higher (4%) during high intensity exercise [26]. Blood was analysed in duplicate for [Hb] and Hct (K-800; Sysmex, Kobe, Japan) and for plasma [K\(^+\)] (865 pH/Blood Electrolyte and Gas Analyzer; Bayer, MA). APV was calculated [27] and used to correct [K\(^+\)] for fluid shifts [28]. Additional calculations were Δ[K\(^+\)] and Δ[K\(^+\)] work\(^{-1}\) ratio [28–30]. Possible medication effects on plasma [K\(^+\)] were considered. While non-selective ß-blockers increase plasma [K\(^+\)] during exercise [31], only ß\(_1\)-blockers were taken by subjects in this study, which do not affect [K\(^+\)] during exercise [32]. Prednisone increases skeletal muscle Na\(^+\), K\(^+\)-pump content [33], which could lower plasma [K\(^+\)] during exercise. However, in the present study, plasma [K\(^+\)] during exercise was not different within groups between the patients taking prednisone and those who were not (data not shown).

**Computerised tomography scan**

TMCSA of the dominant leg was measured by single-slice computerized tomography (CT) scan, taken 20 cm above the medial femoral condyle. Muscle area was calculated as the total muscle compartment area minus the femur area [24].

**Muscle biopsy**

Prior to the torque–velocity and fatigue tests, a muscle sample was collected from the vastus lateralis under local anaesthesia (1% Xylocaine) by percutaneous needle biopsy technique.

**Muscle Na\(^+\), K\(^+\)-pump analyses**

**Maximal activity and total content.** The maximal in vitro Na\(^+\), K\(^+\)-pump activity was measured in muscle homogenates using the maximal K\(^+\)-stimulated 3-O-methylfluorescein phosphatase (3-O-MFPass) assay, specific for the Na\(^+\), K\(^+\)-pump and adapted for human skeletal muscle [25, 34]. Total Na\(^+\), K\(^+\)-pump content was determined by vanadate-facilitated [18] ouabain binding site content analysis [25, 35].
Table 1. Physical characteristics in RTx, HDP and CONa

<table>
<thead>
<tr>
<th></th>
<th>RTx</th>
<th>HDP</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41.3 ± 10.6</td>
<td>39.2 ± 8.6</td>
<td>39.8 ± 8.8</td>
</tr>
<tr>
<td>Sex (female:male)</td>
<td>3:6</td>
<td>3:7</td>
<td>3:7</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>75.6 ± 15.7</td>
<td>76.8 ± 17.1</td>
<td>72.4 ± 16.0</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 ± 0.13</td>
<td>1.75 ± 0.11</td>
<td>1.75 ± 0.09</td>
</tr>
<tr>
<td>BMI (kg m−2)</td>
<td>25.8 ± 3.3</td>
<td>25.2 ± 5.0</td>
<td>23.4 ± 3.7</td>
</tr>
<tr>
<td>[Hct (%)]</td>
<td>134 ± 9</td>
<td>133 ± 14</td>
<td>145 ± 13</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>38.7 ± 2.5</td>
<td>38.3 ± 5.1</td>
<td>41.0 ± 3.2</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>132 ± 15</td>
<td>127 ± 19</td>
<td>124 ± 10</td>
</tr>
<tr>
<td>Creatinine clearance (mL min−1)</td>
<td>75.5 ± 21.6b</td>
<td>113 ± 20</td>
<td>108.5 ± 19.2</td>
</tr>
<tr>
<td>TMCSA (cm2)</td>
<td>118 ± 22</td>
<td>113 ± 20</td>
<td>131 ± 24</td>
</tr>
<tr>
<td>Relative TMCSA (cm2 kg−1)</td>
<td>1.58 ± 0.23b</td>
<td>1.53 ± 0.22b</td>
<td>1.82 ± 0.16</td>
</tr>
</tbody>
</table>

aValues are mean ± SD; BMI, body mass index.

bLess than CON, P < 0.05.

Table 2. Patient medicationsa

<table>
<thead>
<tr>
<th>Medication</th>
<th>RTx Dose</th>
<th>HDP Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darbepoetin (µg)</td>
<td>1, 40</td>
<td>6, 30</td>
</tr>
<tr>
<td>Atenolol (mg)</td>
<td>2, 38 ± 18</td>
<td>1, 25</td>
</tr>
<tr>
<td>Metoprolol (mg)</td>
<td>2, 125 ± 106</td>
<td></td>
</tr>
<tr>
<td>Ramipril (mg)</td>
<td>3, 10 ± 0</td>
<td></td>
</tr>
<tr>
<td>Ibesartan (mg)</td>
<td>3, 53 ± 39</td>
<td>1, 300</td>
</tr>
<tr>
<td>Nifedipine (mg)</td>
<td>3, 43 ± 15</td>
<td></td>
</tr>
<tr>
<td>Cyclosporine (mg)</td>
<td>4, 138 ± 66</td>
<td></td>
</tr>
<tr>
<td>Blood concentration (ng mL−1)</td>
<td>636 ± 225</td>
<td></td>
</tr>
<tr>
<td>Prednisolone (mg)</td>
<td>7, 5 ± 0.5</td>
<td>1, 2.5</td>
</tr>
<tr>
<td>Azathioprine (mg)</td>
<td>3, 58 ± 38</td>
<td></td>
</tr>
<tr>
<td>Tacrolimus (mg)</td>
<td>5, 4 ± 1</td>
<td></td>
</tr>
<tr>
<td>Blood concentration (ng mL−1)</td>
<td>10.7 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>Mycophenolate methyl (mg)</td>
<td>5, 1338 ± 483</td>
<td></td>
</tr>
</tbody>
</table>

aValues are mean ± SD.

Quadriceps fatigue. The FL was higher in RTx (24%, P = 0.010) and HDP (25%, P = 0.003) than in CON (15%), with no difference between RTx and HDP.

Peak oxygen consumption. VO2peak was less in RTx (27.0 ± 9.6 mL kg−1 min−1, P = 0.012) and HDP (26.4 ± 6.5 mL kg−1 min−1, P = 0.006), respectively than CON (35.7 ± 4.0 mL kg−1 min−1), with no difference between RTx and HDP. Peak work rate similarly was lower by 29 and 31%, respectively, in RTx (P = 0.005) and HDP (P = 0.003) than CON (Figure 2). Total work done was also lower in RTx (60.6 ± 41, P = 0.009) and HDP (56.8 ± 23, P = 0.004) than CON (114.8 ± 54 kJ). Respiratory exchange ratio at VO2peak was not different between groups (RTx 1.17 ± 0.08, HDP 1.25 ± 0.10 and CON 1.19 ± 0.06. For pooled data, VO2peak was correlated with TMCSA (Figure 3), relative TMCSA (r = 0.72, P = 0.001, n = 28) and PT (r = 0.83, P = 0.001, n = 29) and inversely correlated with FI (r = −0.53, P = 0.004, n = 29). TMCSA was correlated with PT (r = 0.75, P = 0.001, n = 28) but not FL.

Isoform abundance. Western blotting was performed for the α1, α2, α3, β1, β2, and β3 Na+K+ pump isoforms as detailed [36], with the modification that the muscle homogenate was deglycosylated prior to electrophoresis, to enhance β isoform identification. This involved incubating the homogenate for 1 h at 37°C with 0.5% (vol/vol) Non-idet P40 and 3 U β-Glycosidase F (Boehringer Mannheim) per 0.5 mg protein. The final blot intensity was normalised to the same human muscle standard, which was run in all gels.

Antibodies. Antibodies specific to each isoform were for α1, monoclonal α6F (developed by D. Fambrough and obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health and Human Development and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA); α2, polyclonal anti-HERED (kindly donated by T. Pressley, Texas Tech University); α3, monoclonal MA3-915 (Affinity Bioreagents, Golden, CO); β1, monoclonal MA3-930 (Affinity Bioreagents); β2, monoclonal 610915 (Transduction Laboratories, Lexington, Kentucky) and β3, monoclonal 610993 (Transduction Laboratories).

Statistics

Data are mean ± SD. A one-way analysis of variance (ANOVA) was used except when repeated measures were taken (e.g. [K] data) for which a mixed-design two-way ANOVA was used. The least significant difference post-hoc test was used because of the unequal group sizes. Correlations were determined by linear regression. Significance was accepted at P < 0.05.

Results

Physical characteristics

Physical characteristics did not differ between groups (Table 1) except lower TMCSA expressed relative to body mass in RTx (P = 0.017) and HDP (P = 0.006) than in CON. Calculated CrCl was also lower in RTx (P = 0.001) than CON. When data from all groups were pooled, relative TMCSA was correlated with estimated CrCl (r = 0.44, P = 0.026, n = 28).

Exercise performance

Quadriceps torque-velocity. PT (Nm kg−1) was −25% lower in RTx and HDP than CON (P < 0.05) at all velocities except 120° s−1, where only RTx was lower (Figure 1). However, PT (Nm cm−2) did not differ between groups (Figure 1).
Plasma $[K^+]$ and fluid shifts

The change in plasma volume from rest ($\Delta PV$), during incremental exercise to peak work rate was less in RTx ($-12.7 \pm 2.5$) than in both HDP ($-15.8 \pm 2.9$, $P = 0.032$) and CON ($-16.2 \pm 3.1\%$, $P = 0.014$). Therefore, plasma $[K^+]$ was corrected for the $\Delta PV$. Corrected plasma $[K^+]$ was higher ($P < 0.05$) in HDP than in RTx and CON at rest, during exercise and at 10 min post-exercise (Figure 2). The rise in plasma $[K^+]$ above rest ($\Delta [K^+]$) did not differ between groups during common sub-maximal work rates, but was less in RTx ($P = 0.014$) and HDP ($P = 0.004$) at peak work rate compared to CON (Figure 2). To correct for the greater peak work rate in CON, $\Delta [K^+]$ was expressed relative to the total work done ($\Delta [K^+]$ work$^{-1}$ ratio). No difference was found between the groups in $\Delta [K^+]$ work$^{-1}$ ratio (RTx 20.8 $\pm$ 15.6, HDP 14.9 $\pm$ 8.5, CON 15.6 $\pm$ 10.4 nmol L$^{-1}$ J$^{-1}$).

Table 3. Plasma acid–base and electrolyte concentrations in HDP, RTx and CON at rest and peak work rate$^a$

<table>
<thead>
<tr>
<th></th>
<th>RTx Rest</th>
<th>RTx Peak</th>
<th>HDP Rest</th>
<th>HDP Peak</th>
<th>CON Rest</th>
<th>CON Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.41 ± 0.02</td>
<td>7.32 ± 0.06</td>
<td>7.44 ± 0.05</td>
<td>7.35 ± 0.03</td>
<td>7.42 ± 0.02</td>
<td>7.30 ± 0.03</td>
</tr>
<tr>
<td>$\text{HCO}_3^-$ (mmol L$^{-1}$)</td>
<td>24.1 ± 2.2</td>
<td>16.2 ± 2.9$^b$</td>
<td>27.1 ± 1.8$^c$</td>
<td>19.2 ± 2.8</td>
<td>26.4 ± 2.2$^c$</td>
<td>16.1 ± 2.8$^b$</td>
</tr>
<tr>
<td>$\text{Na}^+$ (mmol L$^{-1}$)</td>
<td>139 ± 3</td>
<td>147 ± 3</td>
<td>138 ± 5</td>
<td>144 ± 6</td>
<td>140 ± 2</td>
<td>148 ± 2</td>
</tr>
<tr>
<td>$\text{Cl}^-$ (mmol L$^{-1}$)</td>
<td>108 ± 3$^d$</td>
<td>110 ± 3$^d$</td>
<td>98 ± 5</td>
<td>100 ± 5</td>
<td>105 ± 2$^d$</td>
<td>108 ± 2$^d$</td>
</tr>
<tr>
<td>$\text{Ca}^{2+}$ (mmol L$^{-1}$)</td>
<td>2.43 ± 0.16</td>
<td>2.57 ± 0.16</td>
<td>1.62 ± 0.29$^c$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{PO}_4$ (mmol L$^{-1}$)</td>
<td>0.79 ± 0.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Values are mean ± SD.
$^b$Less than HDP, $P < 0.05$.
$^c$Greater than RTx, $P < 0.05$.
$^d$Greater than HDP, $P < 0.05$.

Fig. 1. Quadriceps PT during isokinetic dynamometry, expressed relative to body mass (A) and to TMCSA (B). Values are means ± SDs. HDP (filled squares), $n = 10$; RTx (open circles), $n = 9$; CON (filled triangles), $n = 10$. *HDP less than CON, $P < 0.05$; †RTx less than CON, $P < 0.05$.

Fig. 2. Plasma $[K^+]$ before, during and after (A) and the rise in plasma $[K^+]$ from rest ($\Delta [K^+]$) during (B) an incremental cycle test to fatigue. Values are means ± SDs. HDP $n = 10$ (filled squares), RTx $n = 9$ (open circles), CON $n = 10$ (filled triangles). #main effect exercise > rest ($P < 0.0001$); *HDP greater than CON, $P < 0.05$; †HDP greater than RTx, $P < 0.05$; †RTx less than CON, $P < 0.05$. 

Plasma $[K^+]$ and fluid shifts
When data were pooled, $\dot{V}O_2$peak was inversely correlated with the $A[K^+]$ work$^{-1}$ ratio ($r = -0.42, P = 0.030, n = 29$).

**Muscle Na$^+$,K$^+$-pump activity, content and isoforms**

**Total muscle protein content.** Muscle protein content did not differ between groups [RTx $0.18 \pm 0.02$, HDP $0.17 \pm 0.03$, CON $0.19 \pm 0.03$ mg protein mg muscle (wet weight)$^{-1}$].

**Maximal activity and content.** Muscle maximal 3-O-MFPase activity was 28 and 31% lower in RTx ($P = 0.020$) and HDP ($P = 0.010$) than CON, respectively (Figure 4). In contrast, the $[^3H]$-ouabain binding site content did not differ between the groups (Figure 4). Within HDP, 3-O-MFPase activity was positively correlated with $\dot{V}O_2$peak ($r = 0.73, P < 0.05, n = 9$) and isometric PT ($r = 0.84, P < 0.05, n = 9$). No significant correlations were found within RTx or CON between 3-O-MFPase activity and any exercise performance variables.

**Isoform abundance.** Each of the Na$^+$,K$^+$-pump $\alpha_1$, $\alpha_2$, $\alpha_3$, $\beta_1$, $\beta_2$ and $\beta_3$ isoforms were expressed in muscle from all HDP and RTx patients and CON. There was no significant difference between groups in protein abundance for any Na$^+$,K$^+$-pump isoform (Table 4).

**Discussion**

This study identifies two possible mechanisms underlying impaired muscle function and exercise performance in RTx and HDP, namely reduced skeletal muscle Na$^+$,K$^+$-pump activity and reduced TMCSA relative to body mass. Furthermore, both Na$^+$,K$^+$-pump maximal activity and relative TMCSA as well as muscle fatigability and exercise performance did not differ between RTx and HDP with normal [Hb]. Significant inter-relationships further strengthen the possibility that the impaired exercise performance in RTx and HDP may be linked to reduced Na$^+$,K$^+$-pump maximal activity and relative TMCSA.

**Reduced maximal Na$^+$,K$^+$-pump activity but not abundance**

This is the first study to measure skeletal muscle Na$^+$,K$^+$-pump activity, content or isoforms in uraemic patients. Muscle maximal Na$^+$,K$^+$-pump activity was reduced by $\sim 30\%$ in RTx and HDP. This is consistent with reports in uraemic patients of reduced Na$^+$,K$^+$-pump activity in other tissues [37, 38], reduced muscle intracellular [K$^+$] [39],
membrane excitability [16]. Surprisingly, there was no difference in maximal $3^\text{H}$DP. We hypothesized that Na

Exercise and Na$^+$,K$^+$-pumps in HDP and RTx

Table 4. Vastus lateralis Na$^+$,K$^+$-pump isoform expression in RTx, HDP and CON

<table>
<thead>
<tr>
<th></th>
<th>RTx</th>
<th>HDP</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_1$</td>
<td>2.3 ± 2.3</td>
<td>1.3 ± 1.1</td>
<td>2.1 ± 2.3</td>
</tr>
<tr>
<td>$\alpha_2$</td>
<td>1.2 ± 0.8</td>
<td>1.7 ± 0.9</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>$\alpha_3$</td>
<td>3.2 ± 1.2</td>
<td>3.0 ± 1.5</td>
<td>4.8 ± 3.4</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>2.5 ± 1.7</td>
<td>1.6 ± 0.6</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>1.5 ± 1.2</td>
<td>1.4 ± 0.5</td>
<td>1.8 ± 0.9</td>
</tr>
<tr>
<td>$\beta_3$</td>
<td>0.8 ± 0.8</td>
<td>0.7 ± 0.5</td>
<td>0.7 ± 0.3</td>
</tr>
</tbody>
</table>

$^a$Values are mean ± SD; RTx, n = 9; HDP, n = 10; CON, n = 10. All values are normalized to the same human muscle standard that was run in all gels.

muscle membrane depolarization [40] and impaired muscle membrane excitability [16]. Surprisingly, there was no difference in maximal 3-$\text{O}$-MFPase activity between RTx and HDP. We hypothesized that Na$^+$,K$^+$-pump activity would be normal in RTx, based on findings in erythrocytes of normal or elevated Na$^+$,K$^+$-pump activity after RTx in many [21, 41, 42] although not all studies [43].

We confirmed the expression of the Na$^+$,K$^+$-pump $\alpha_1$, $\alpha_2$, $\alpha_3$, $\beta_1$, $\beta_2$ and $\beta_3$ isoforms [36] in muscle from RTx and HDP and demonstrate an unchanged relative isoform abundance compared to CON. These results support the findings of unchanged Na$^+$,K$^+$-pump isoform messenger RNA expression or protein abundance in skeletal muscle of nephrectomized and sham-operated rats [20]. There was a non-significant $-2$-fold difference between HDP and CON in the relative abundance of the $\alpha_1$ and $\alpha_3$ isoforms. Thus, we cannot exclude the possibility that some differences in isoform expression exist but that these were not detectable due to the intrinsic variability of western blotting. However, failure to detect significant differences between groups for isoform abundance was unlikely due to the small sample size as effect sizes were very small (range 0.03–0.15).

Possible mechanisms of impaired maximal Na$^+$,K$^+$-pump activity

As muscle Na$^+$,K$^+$-pump content and isoform expression were not reduced in RTx and HDP, their depressed Na$^+$,K$^+$-pump activity appears to be due to defective Na$^+$,K$^+$-pump function. We show that Na$^+$,K$^+$-pump content is normal in uraemic human skeletal muscle, consistent with findings in uraemic rats [19]. The lower activity in HDP and RTx did not abolish the previously described positive association between maximal 3-$\text{O}$-MFPase activity and $[^3\text{H}]$-ouabain binding site content [30]. As the HDP were hyperkalaemic, which increases Na$^+$,K$^+$-pump content in rat muscle [44], possible hyperkalaemic effects on Na$^+$,K$^+$-pump content and activity cannot be excluded.

Normal muscle Na$^+$,K$^+$-pump content in these patients suggests that their depressed maximal Na$^+$,K$^+$-pump activity is due to reduced molecular activity per pump or grossly impaired activity in some pumps. This is consistent with a previous finding in erythrocytes from HDP of reduced ouabain-sensitive $^{86}$Rb$^+$ uptake but normal $[^3\text{H}]$-ouabain binding site content [45]. We cannot compare the molecular activities with previous reports, which were calculated using different 3-$\text{O}$-MFPase assay conditions [46]. With numerous myopathies identified in RTx and HDP, the cause of reduced Na$^+$,K$^+$-pump activity is likely to be multifactorial. Changes in membrane lipid composition can alter Na$^+$,K$^+$-pump function without altering Na$^+$,K$^+$-pump content [47] and abnormal membrane lipid composition has been found in erythrocytes from HDP [37] and RTx [48] as well as in kidney, liver and testis microsomal membranes from rats following renal transplantation [49]. In uraemia, endogenous digitalis-like factors [37] and uraemic toxins [50] in plasma can depress Na$^+$,K$^+$-pump activity. In RTx, calcineurin inhibitors reduce Na$^+$,K$^+$-pump activity in heart [51] and kidney [52]. Conversely, prednisolone increases muscle Na$^+$,K$^+$-pump content [33] and possibly also activity [53]. Further research is required to determine the mechanisms inhibiting Na$^+$,K$^+$-pump activity in skeletal muscle in RTx and HDP.

Reduced relative TMCSA and impaired exercise performance in HDP and RTx with normal [Hb]

This is the first report demonstrating exacerbated muscle fatigability during repeated dynamic contractions in RTx compared to CON. Furthermore, we show similar fatigability between RTx and HDP. This contrasts findings of greater fatigability in HDP than RTx during repeated isometric hand-grip contractions [13], possibly due to a lower Hct in HDP in that study.

This is the first study comparing VO$_{2\text{peak}}$ in RTx and HDP with similar [Hb], revealing that VO$_{2\text{peak}}$ did not differ between RTx and HDP, but was $-25\%$ less than CON. This confirms the poor exercise performance in RTx and HDP, which persists despite normalization of [Hb] [5, 54] and is consistent with greater muscle fatigability. In addition, we found an apparent relationship between maximal exercise performance (VO$_{2\text{peak}}$) and muscle Na$^+$,K$^+$-pump maximal activity (3-$\text{O}$-MFPase activity). This is consistent with the possibility that impaired exercise performance in RTx and HDP may be related to reduced muscle function associated with lower Na$^+$,K$^+$-pump activity. Since the Na$^+$,K$^+$-pump protects muscle membrane excitability and delays fatigue [14, 18], depressed maximal Na$^+$,K$^+$-pump activity in HDP and RTx may impair exercise performance and VO$_{2\text{peak}}$. It should be noted that Na$^+$, K$^+$-pump maximal activity explained 20% of the variance in VO$_{2\text{peak}}$, and thus other factors must also contribute to their poor exercise performance, including limb muscle atrophy [9], altered muscle fibre type [55], impaired blood flow [56], reduced muscle capillarity [56] and mitochondrial function [9].

Quadriiceps strength was sub-normal in RTx and HDP when corrected for body mass but not when expressed relative to TMCSA. This suggests that muscle contractile function is normal in RTx and HDP with normal [Hb] and that their strength deficit may be entirely due to reduced lean body mass [57]. This is an important finding as it indicates that strategies to improve physical functioning in HDP and RTx should focus on increasing muscle mass. It is also consistent with our finding of reduced TMCSA relative to body mass in RTx and HDP. We also report no difference in quadriceps strength in RTx compared to HDP, which supports previous studies [8, 11]. Not surprisingly, VO$_{2\text{peak}}$ was correlated with relative TMCSA and PT. This
is the first report of a relationship between muscle strength and $\text{VO}_2\text{peak}$ in RTx. We also found an inverse relationship between $\text{VO}_2\text{peak}$ and the FI. These data, together with a lack of correlation between $\text{VO}_2\text{peak}$ and [Hb], support the concept that reduced muscle mass plays a vital role in limiting $\text{VO}_2\text{peak}$ and strength in RTx and HDP.

**Normal plasma $K^+$ regulation during exercise in HDP and RTx**

Our finding of normal $\Delta[\text{K}^+]$ during exercise and $\Delta[\text{K}^+]$ work$^{-1}$ ratio at peak work rate in RTx and HDP contrasts with our earlier reports of elevated values in HDP compared to CON [15, 58]. The difference in $\text{VO}_2\text{peak}$ between the HDP and controls in our previous studies was 44 and 37%, respectively [15, 58], whereas here, it was ~25%. As $\text{VO}_2\text{peak}$ was related to the $\Delta[\text{K}^+]$ work$^{-1}$ ratio in each study, this may explain the contrasting $K^+$ regulation data. The small sample size is unlikely to have limited our ability to detect a difference between HDP, RTx and CON as the effect size was very small (effect size $= 0.03$). At peak work rate, the lesser $\Delta[\text{K}^+]$ in RTx and HDP compared to CON reflects their lower work rates and presumably lower muscle $K^+$ release during the incremental exercise test.

Changes in plasma $[\text{K}^+]$ may not accurately reflect changes in net muscle $K^+$ efflux [59] due to $K^+$ uptake by inactive tissues and incomplete $K^+$ equilibration between the muscle cell, interstitium and blood [60]. For example, muscle interstitial $[\text{K}^+]$ can exceed arterial or venous $[\text{K}^+]$ by 4–8 mM during exercise [61, 62]. This likely explains the unchanged rise in plasma $\text{K}^+$ in RTx and HDP during exercise despite their substantially reduced muscle Na$^+$.K$^+$-pump maximal activity and also the lack of correlation between plasma $[\text{K}^+]$ variables and Na$^+$.K$^+$-pump maximal activity (data not shown). Furthermore, the possibly confounding effects of hyperkalaemia at rest in HDP may have contributed to the apparent disconnect between plasma $[\text{K}^+]$ and Na$^+$.K$^+$-pump maximal activity in these patients.

In conclusion, maximal exercise performance was similarly reduced in RTx and HDP with near normal [Hb], as was muscle maximal Na$^+$.K$^+$-pump activity and relative TMCSA. The depressed Na$^+$.K$^+$-pump activity is likely due to direct inhibition of Na$^+$.K$^+$-pumps since muscle Na$^+$.K$^+$-pump content and isoform abundance did not differ between RTx and HDP. Finally, impaired exercise performance in HDP and RTx may be partially due to depressed muscle Na$^+$.K$^+$-pump activity and relative TMCSA.

**Acknowledgements.** We sincerely thank our participants for their time and effort. This research was supported by funding from Janssen-Cilag, Australia.

**Conflict of interest statement.** None declared.

**References**


Exercise and Na⁺,K⁺-pumps in HDP and RTx


44. Fervenza FC, Hendry BM, Ellory JC. Effects of dialysis and transplantation on red cell Na pump function in renal failure. Nephron 1989; 53: 121–128


Received for publication: 26.11.11; Accepted in revised form: 4.9.11