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Impaired exercise performance and muscle Na^+,K^+-pump activity in renal transplantation and haemodialysis patients.

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ABSTRACT

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

Methods: Peak oxygen consumption ($\dot{V}O_{2peak}$) and plasma $[K^+]$ were measured during incremental exercise in 9 RTx, 10 HDP, and 10 healthy controls (CON). Quadriceps peak torque, fatigability (decline in strength during thirty contractions), thigh muscle cross-sectional area (TMCSA) and vastus lateralis Na^+,K^+-pump maximal activity, content, and isoform (α_1-α_3, β_1-β_3) abundance were measured. Results: $\dot{V}O_{2peak}$ was 32% and 35% lower in RTx and HDP than CON, respectively ($P<0.05$). Peak torque was less in RTx and HDP than CON ($P<0.05$) but did not differ when expressed relative to TMCSA. Fatigability was ~1.6-fold higher in RTx (24±11%) and HDP (25±4%) than CON (15±5%), $P<0.05$). Na^+,K^+-pump activity was 28% and 31% lower in RTx and HDP, respectively than CON ($P<0.02$), whereas content and isoform abundance did not differ. Pooled (n=28) $\dot{V}O_{2peak}$ correlated with Na^+,K^+-pump activity ($r=0.45$, $P=0.02$).

Conclusions: $\dot{V}O_{2peak}$ and muscle Na^+,K^+-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^+,K^+-pump activity and relative TMCSA.

Keywords: extrarenal potassium regulation, fatigue, muscle mass, strength, $\dot{V}O_{2peak}$

Summary

We show that peak oxygen consumption, quadriceps strength and fatigability are not different between haemodialysis patients (HDP) and renal transplant recipients (RTx) with similar

[Hb], however they are reduced in both groups compared to matched healthy control subjects (CON). Skeletal muscle maximal Na^+,K^+-pump activity was depressed in HDP and RTx compared to CON, whereas skeletal muscle Na^+,K^+-pump content and isoform abundance did not differ. When all results were pooled, peak oxygen consumption correlated with Na^+,K^+-pump activity, suggesting that impaired exercise performance in HDP and RTx may be partially due to depressed muscle Na^+,K^+-pump activity.

INTRODUCTION

Patients with chronic kidney disease have grossly impaired exercise tolerance [1], but differences in exercise capacity between haemodialysis patients (HDP) and renal transplantation recipients (RTx) are poorly defined. Following renal transplantation, the $\dot{V}O_{2peak}$ 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 $\dot{V}O_{2peak}$ of 19-33% have been reported in HDP following treatment with erythropoietic stimulating agents (ESA) [4, 5]. Nonetheless, no studies have investigated $\dot{V}O_{2peak}$ in RTx and HDP with similar [Hb] to properly compare these groups.

Muscle strength and fatigability are important determinants of exercise capacity [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 $\dot{V}O_{2peak}$ [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 uremic 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 uremic 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 $\dot{V}O_{2peak}$, 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.

METHODS

Subjects

Nine RTx, ten HDP, and ten 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, bodymass and bodymass index (BMI) (Table 1). Selection criteria for RTx were: transplanted at least 12 mo prior to testing (range 16–171, 63±53 mo), had a stable creatinine, and a calculated CrCl (Cockcroft-Gault) of >40 $ml.min^{-1}$. Selection criteria for HDP were: stable and had been dialyzing for at least 6 mo (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, intradialytic time, and pre-dialysis weight. All subjects had [Hb] >110 $g.l^{-1}$. Subjects were excluded if they had symptomatic ischemic heart disease, peripheral vascular disease, disabling arthritis, chronic airflow obstruction, or were pregnant. Subject medications are shown in Table 3. The RTx 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 ($\dot{V}O_{2peak}$): Subjects cycled (≥60 rpm) 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 $\dot{V}O_2$ [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, Massachusetts) [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 TMCSA (Nm.cm^{-2}), to correct for differences in body size and muscle mass, respectively.

Quadriceps 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] x 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, during and after the $\dot{V}O_{2peak}$ test from an arterio-venous fistula in all HDP, and in four RTx; in all other subjects, arterialised 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 arterialised 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 (Sysmex, K-800, Kobe, Japan) and for plasma [K^+] (865pH/Blood Electrolyte and Gas Analyzer, Bayer, MA, USA). ΔPV 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. Whilst non-selective β-blockers increase plasma [K^+] during exercise [31], only β_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 Computerised 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-MFPase) 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 [^{3}H]ouabain binding site content analysis [25, 35].

Isoform abundance: Western blotting was performed for the α_1, α_2, α_3, β_1, β_2, and β_3 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% (v/v) Nonidet P40 and 3 units N-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 α_1: monoclonal α6F (developed by D. Fambrough and obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA); α_2: polyclonal anti-HERED (kindly donated by T. Pressley, Texas Tech University); α_3: monoclonal MA3-915 (Affinity Bioreagents, Golden, Colorado); β_1: monoclonal MA3-930 (Affinity Bioreagents); β_2: monoclonal 610915 (Transduction Laboratories, Lexington, Kentucky); and β_3: monoclonal 610993 (Transduction Laboratories).

Statistics

Data are mean ± SD. A one-way 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 bodymass 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 (Fig.1). However, PT ($Nm.cm^{-2}$) did not differ between groups (Fig.1).

Quadriceps fatigue: The FI 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: $\dot{V}O_{2peak}$ 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 workrate similarly was lower by 29 and 31%, respectively in RTx (P=0.005) and HDP (P=0.003) than CON (Fig.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). RER at $\dot{V}O_{2peak}$ was not different between groups (RTx 1.17±0.08, HD 1.25±0.10, and CON 1.19±0.06. For pooled data, $\dot{V}O_{2peak}$ was correlated with TMCSA (Fig. 5), 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 FI.

Plasma [K^+] and fluid shifts

The change in plasma volume from rest (ΔPV), during incremental exercise to peak workrate was less in RTx (-12.7±2.5) than in both HDP (-15.8±2.9, P=0.032) and CON (-16.2±3.1%, P=0.014). Therefore, plasma [K^+] was corrected for the ΔPV. 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 (Fig.2). The rise in plasma [K^+] above rest (Δ[K^+]) did not differ between groups during common submaximal workrates, but was less in RTx (P=0.014) and HDP (P=0.004) at peak workrate compared to CON (Fig.2). To correct for the greater peak workrate in CON, Δ[K^+] was expressed relative to the total work done (Δ[K^+].$work^{-1}$ ratio). No difference was found between the groups in Δ[K^+].$work^{-1}$ ratio

(RTx 20.8±15.6, HDP 14.9±8.5, CON 15.6±10.4 nmol.l^{-1}.J^{-1}). When data were pooled, $\dot{V}O_{2peak}$ was inversely correlated with the $\Delta[K^+]$.$work^{-1}$ 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±0.02, HDP 0.17±0.03, CON 0.19±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 (Fig.3). In contrast, the [^{3}H]ouabain binding site content did not differ between the groups (Fig.3). Within HDP, 3-O-MFPase activity was positively correlated with $\dot{V}O_{2peak}$ (*r*=0.73, *P*<0.05, *n*=9) and isometric PT (*r*=0.84, *P*<0.0 5, *n*=9). No significant correlations were found within RTx or CON between 3-O-MFPase activity and any exercise performance variables. For pooled data, 3-O-MFPase activity correlated with $\dot{V}O_{2peak}$ (Fig.4) and [^{3}H]ouabain binding site content (*r*=0.42, *P*=0.026, *n*=28).

Isoform abundance: Each of the Na^+,K^+-pump α_1, α_2, α_3, β_1, β_2, and β_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 2).

DISCUSSION

This study identifies two possible mechanisms underlying impaired muscle function and exercise performance in renal transplantation recipients and haemodialysis patients, namely reduced skeletal muscle Na^+,K^+-pump activity and reduced TMCSA relative to bodymass. 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 ~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], muscle membrane depolarization [40], and impaired muscle membrane excitability [16]. Surprisingly, there was no difference in maximal 3-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 α_1, α_2, α_3, β_1, β_2, and β_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 mRNA 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 α_1 and α_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-O-MFPase activity and $[^3H]$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}H]ouabain binding site content [45]. We cannot compare the molecular activities with previous reports, which were calculated using different 3-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 multi-factorial. Changes in membrane lipid composition can alter Na^+,K^+-pump activity 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. Further, 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 $\dot{V}O_{2peak}$ in RTx and HDP with similar [Hb], revealing that $\dot{V}O_{2peak}$ 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 normalisation of [Hb] [5, 54] and is consistent with greater muscle fatigability. In addition, we found an apparent relationship between maximal exercise performance ($\dot{V}O_{2peak}$) and, muscle Na^+,K^+-pump maximal activity (3-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 $\dot{V}O_{2peak}$. It should be noted that Na^+,K^+-pump maximal activity explained 20% of the variance in $\dot{V}O_{2peak}$, 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].

Quadriceps strength was subnormal in RTx and HDP when corrected for bodymass 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 bodymass [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 bodymass 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, $\dot{V}O_{2peak}$ was correlated with relative TMCSA and peak torque. This is the first report of a relationship between muscle strength and $\dot{V}O_{2peak}$ in RTx. We also found an inverse relationship between $\dot{V}O_{2peak}$ and the FI. These data, together with a lack of correlation between $\dot{V}O_{2peak}$ and [Hb], support the concept that reduced muscle mass plays a vital role in limiting $\dot{V}O_{2peak}$ and strength in RTx and HDP.

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

Our finding of normal $\Delta[K^+]$ during exercise and $\Delta[K^+].work^{-1}$ ratio at peak workrate in RTx and HDP contrasts with our earlier reports of elevated values in HDP compared to CON [15, 58]. The difference in $\dot{V}O_{2peak}$ between the HDP and controls in our previous studies was 44 and 37%, respectively [15, 58], whereas here it was ~25%. As $\dot{V}O_{2peak}$ was related to the $\Delta[K^+].work^{-1}$ 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 workrate, the lesser $\Delta[K^+]$ in RTx and HDP compared to CON reflects their lower workrates and presumably lower muscle K^+ release during the incremental exercise test.

Changes in plasma $[K^+]$ 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 $[K^+]$ can exceed arterial or venous $[K^+]$ by 4-8 mM during exercise [61, 62]. This likely explains the unchanged rise in plasma 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 $[K^+]$ 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 $[K^+]$ 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.

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Disclosure statement

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Tables

Table 1. Physical characteristics in renal transplant recipients, hemodialysis patients and healthy controls.

	RTx	HDP	CON
n	9	10	10
Age (years)	41.3 ± 10.6	39.2 ± 8.6	39.8 ± 8.8
Sex (F:M)	3:6	3:7	3:7
Body mass (kg)	75.6 ± 15.7	76.8 ± 17.1	72.4 ± 16.0
Height (m)	1.71 ± 0.13	1.75 ± 0.11	1.75 ± 0.09
BMI (kg.m^{-2})	25.8 ± 3.3	25.2 ± 5.0	23.4 ± 3.7
[Hb] (g.l^{-1})	134 ± 9	133 ± 14	145 ± 13
Hct (%)	38.7 ± 2.5	38.3 ± 5.1	41.0 ± 3.2
Systolic BP (mmHg)	132 ± 15	127 ± 19	124 ± 10
Diastolic BP (mmHg)	86 ± 6	83 ± 13	81 ± 8
Creatinine clearance (ml.min^{-1})	75.5 ± 21.6*		108.5 ± 19.2
TMCSA (cm^2)	118 ± 22	113 ± 20	131 ± 24
Relative TMCSA (cm^2.kg^{-1})	1.58 ± 0.23*	1.53 ± 0.22*	1.82 ± 0.16

Values are mean ± SD; RTx, renal transplant recipients; HDP, hemodialysis patients; CON, healthy controls; BMI, body mass index; TMCSA, thigh muscle cross-sectional area; * less than CON, $P < 0.05$.

Table 2. Vastus lateralis Na^+,K^+-pump isoform expression in RTx, HDP and CON

	RTx	**HDP**	**CON**
α_1	2.3 ± 2.3	1.3 ± 1.1	2.1 ± 2.3
α_2	1.2 ± 0.8	1.7 ± 0.9	1.4 ± 0.6
α_3	3.2 ± 1.2	3.0 ± 1.5	4.8 ± 3.4
β_1	2.5 ± 1.7	1.6 ± 0.6	2.0 ± 1.0
β_2	1.5 ± 1.2	1.4 ± 0.5	1.8 ± 0.9
β_3	0.8 ± 0.8	0.7 ± 0.5	0.7 ± 0.3

Values are mean ± SD; RTx, renal transplant recipient, n = 9; HDP, hemodialysis patient, n = 10; CON, healthy controls, n = 10. All values are normalized to the same human muscle standard that was run in all gels.

Table 3. Patient medications

	RTx		HDP	
Medication	*n*	Dose	*n*	Dose
Darbepoetin (μg.fortnight^{-1})	1	40	6	30 ± 17
Atenolol (mg.d^{-1})	2	38 ± 18	1	25
Metoprolol (mg.d^{-1})			2	125 ± 106
Ramipril (mg.d^{-1})	3	10 ± 0		
Irbesartan (mg.d^{-1})	3	53 ± 39	1	300
Nifedipine (mg.d^{-1})	3	43 ± 15		
Cyclosporine (mg.d^{-1})	4	138 ± 66		
Blood concentration (ng.ml^{-1})		636 ± 225		
Prednisolone (mg.d^{-1})	7	5 ± 0.5	1	2.5
Azathioprine (mg.d^{-1})	3	58 ± 38		
Tacrolimus (mg.d^{-1})	5	4 ± 1		
Blood concentration (ng.ml^{-1})		10.7 ± 3.8		
Mycophenolate mofetil (mg.d^{-1})	5	1338 ± 483		

Values are mean ± SD; RTx, renal transplant recipient; HDP, haemodialysis patient.

Table 4 Plasma acid-base and electrolyte concentrations in hemodialysis patients, renal transplant recipients and healthy controls at rest and peak workrate

	RTx		HDP		CON	
	Rest	Peak	Rest	Peak	Rest	Peak
pH	7.41 ± 0.02	7.32 ± 0.06	7.44 ± 0.05	7.35 ± 0.03	7.42 ± 0.02	7.30 ± 0.03
HCO_3^- (mmol.l^{-1})	24.1 ± 2.2	16.2 ± 2.9£	27.1 ± 1.8‡	19.2 ± 2.8	26.4 ± 2.2‡	16.1 ± 2.8£
Na^+ (mmol.l^{-1})	139 ± 3	147 ± 3	138 ± 5	144 ± 6	140 ± 2	148 ± 2
Cl^- (mmol.l^{-1})	108 ± 3§	110 ± 3§	98 ± 5	100 ± 5	105 ± 2§	108 ± 2§
Ca^{2+} (mmol.l^{-1})	2.43 ± 0.16		2.57 ± 0.16			
PO_4 (mmol.l^{-1})	0.79 ± 0.16		1.62 ± 0.29‡			

Values are mean ± SD; RTx, renal transplant recipient; HDP, haemodialysis patient; ‡ greater than RTx, $P < 0.05$; § greater than HDP, $P < 0.05$;

£ less than HDP, $P < 0.05$

Titles and legend to figures

Figure 1. Quadriceps peak torque during isokinetic dynamometry, expressed relative to body mass (A) and to thigh muscle cross-sectional area (B).

Values are means ± SD. HDP (■), haemodialysis, n = 10; RTx (○), renal transplant, n = 9; CON (▲), healthy controls, n = 10. * HDP less than CON, $P < 0.05$; † RTx less than CON, $P < 0.05$.

Figure 2. Plasma [K^+] before, during, and after (A), and the rise in plasma [K^+] from rest (ΔK^+) during (B) an incremental cycle test to fatigue.

Values are means ± SD. HDP n = 10 (■), RTx n = 9 (○), CON n = 10 (▲). # 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, $P < 0.05$.

Figure 3. Muscle Na^+,K^+-pump activity and content measured by maximal *in-vitro* K^+-stimulated 3-O-methylfluorescein phosphatase activity (A) and [^{3}H]ouabain binding site content (B).

CON, healthy controls, n = 10, HDP, haemodialysis, n = 10, RTx, renal transplant, n = 9. Data expressed as mean ± SD. * less than CON, $P < 0.05$.

Figure 4. Relationship between skeletal muscle maximal Na^+,K^+-pump (3-O-MFPase) activity and $\dot{V}O_{2peak}$. HDP (■), haemodialysis, n = 10, RTx (○), renal transplant, n = 9, CON (▲), healthy controls, n = 10. Regression line is for pooled data (solid line, n =28, $r = 0.45$, $P = 0.02$, y = 4.0x + 99.1). Dotted curves indicate 95% confidence intervals.

Figure 5. Relationship between thigh muscle cross-sectional area (TMCSA) and $\dot{V}O_{2peak}$.

HDP (■), haemodialysis, n = 10, RTx (○), renal transplant, n = 9, CON (▲), healthy controls, n = 10. Regression line is for pooled data (solid line, n =28, r = 0.46, P = 0.02, y = 0.0016x + 10.8). Dotted curves indicate 95% confidence intervals.

Figure 1

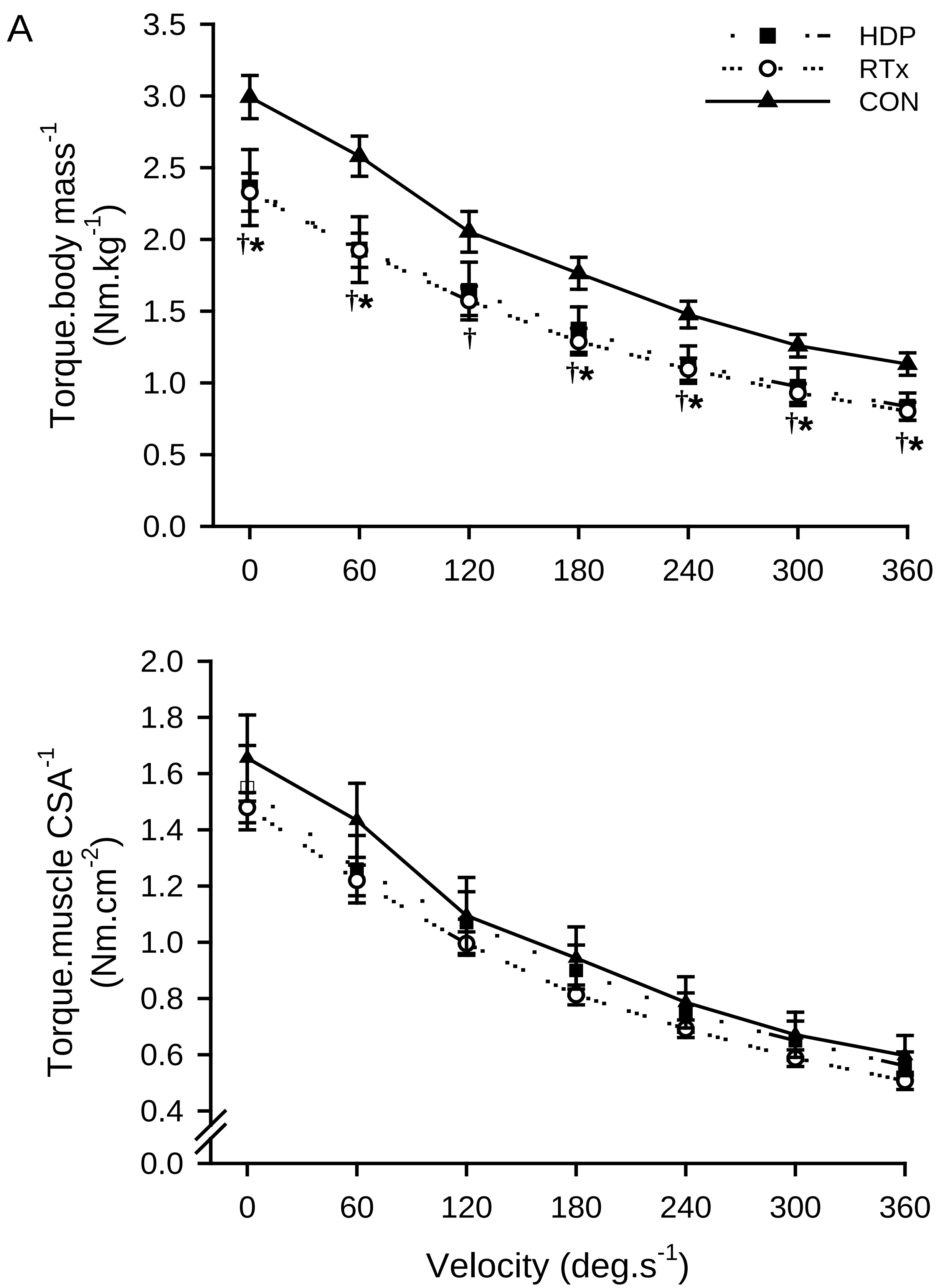

A
HDP
RTx
CON
Torque.body mass-1 (Nm.kg-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
60
120
180
240
300
360
†*
†*
†
Torque.muscle CSA-1 (Nm.cm-2)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.0
Velocity (deg.s-1)

Figure 2

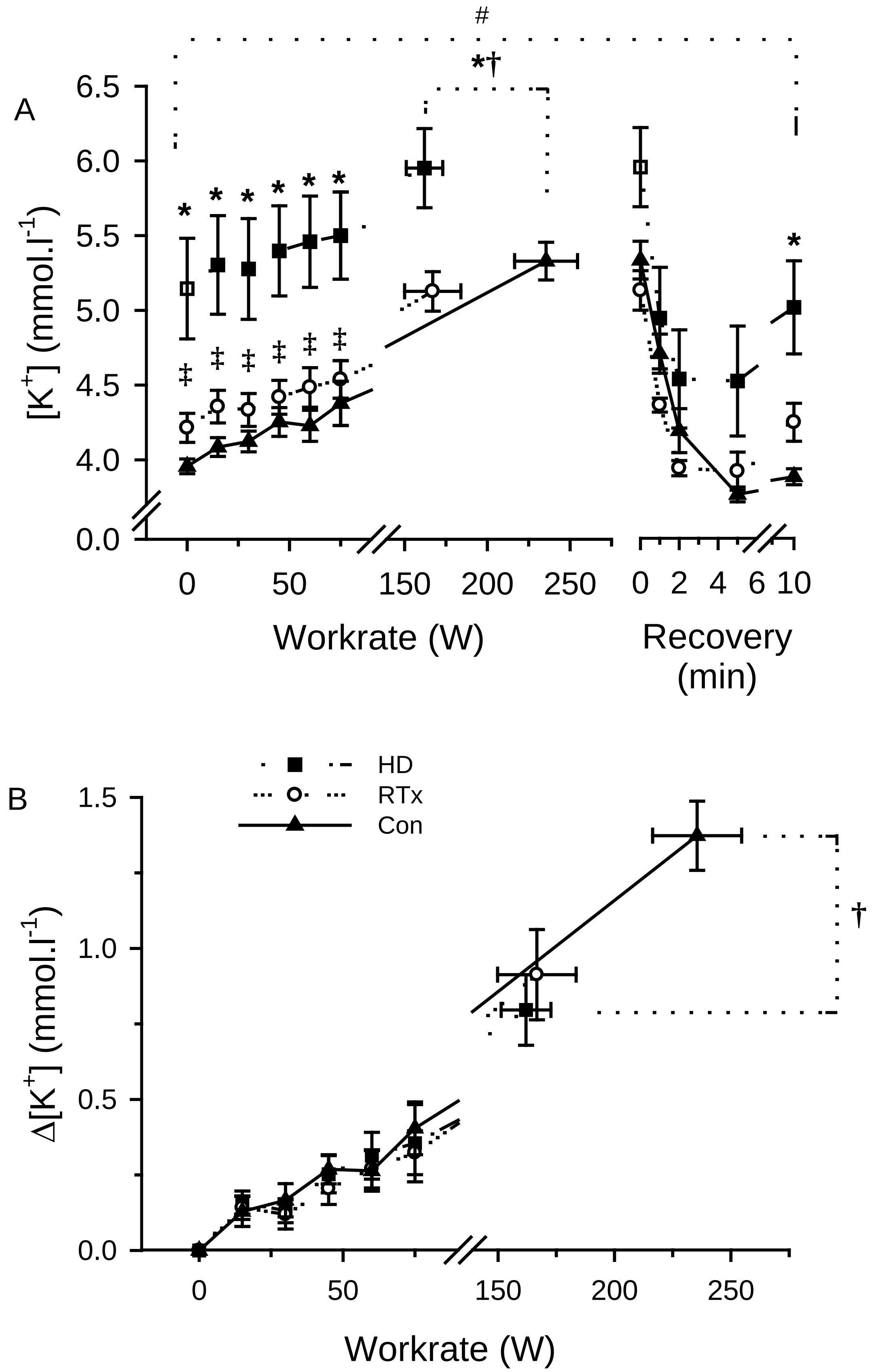

#
*†
A
6.5
6.0
5.5
5.0
4.5
4.0
0.0
[K+] (mmol.l-1)
0
50
150
200
250
0
2
4
6
10
Workrate (W)
Recovery
(min)
B
HD
RTx
Con
1.5
1.0
0.5
0.0
Δ[K+] (mmol.l-1)
†
0
50
150
200
250
Workrate (W)

Figure 3

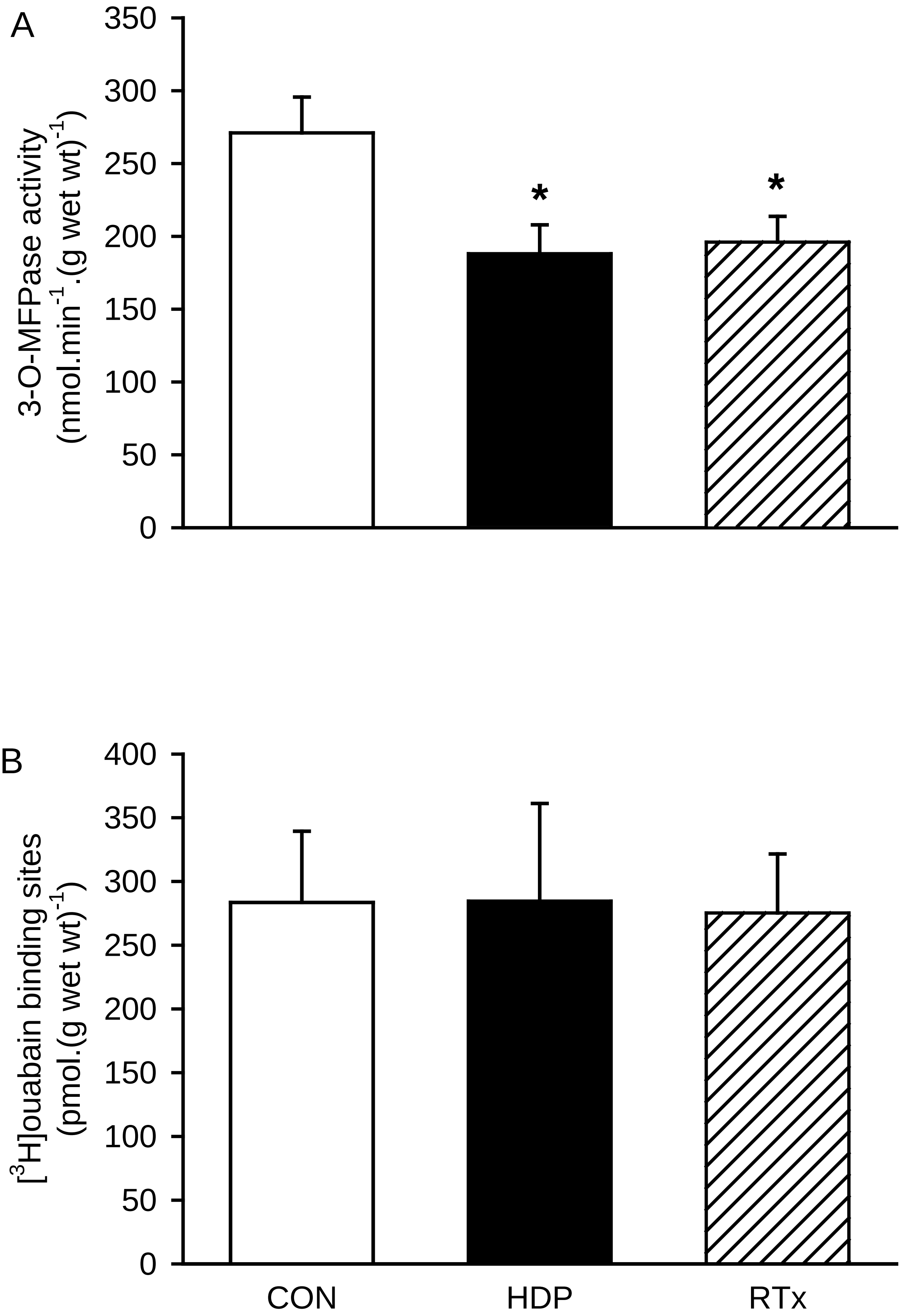

A
3-O-MFPase activity
(nmol.min-1.(g wet wt)-1)
350
300
250
200
150
100
50
0
*
*
B
[3H]ouabain binding sites
(pmol.(g wet wt)-1)
400
350
300
250
200
150
100
50
0
CON
HDP
RTx

Figure 4

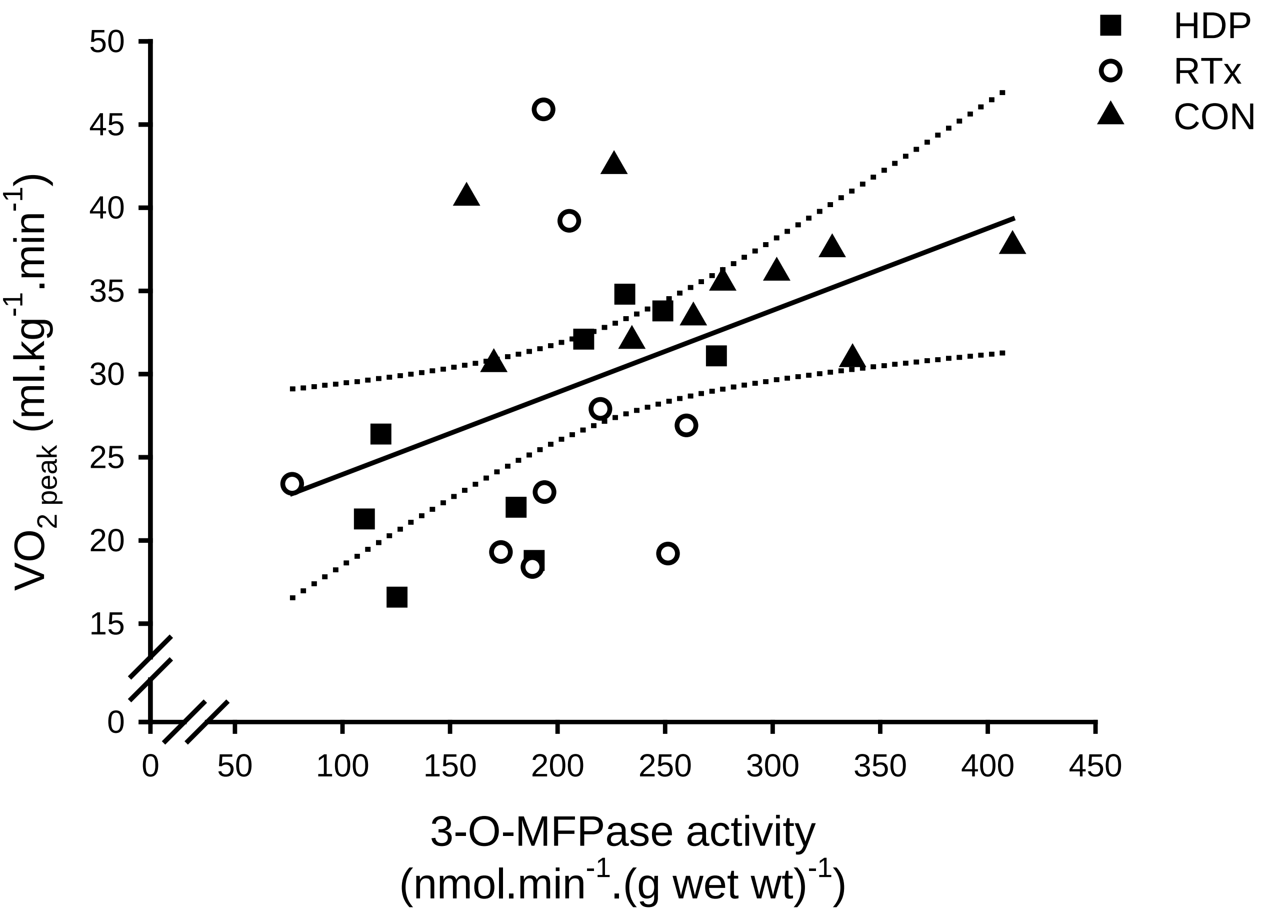
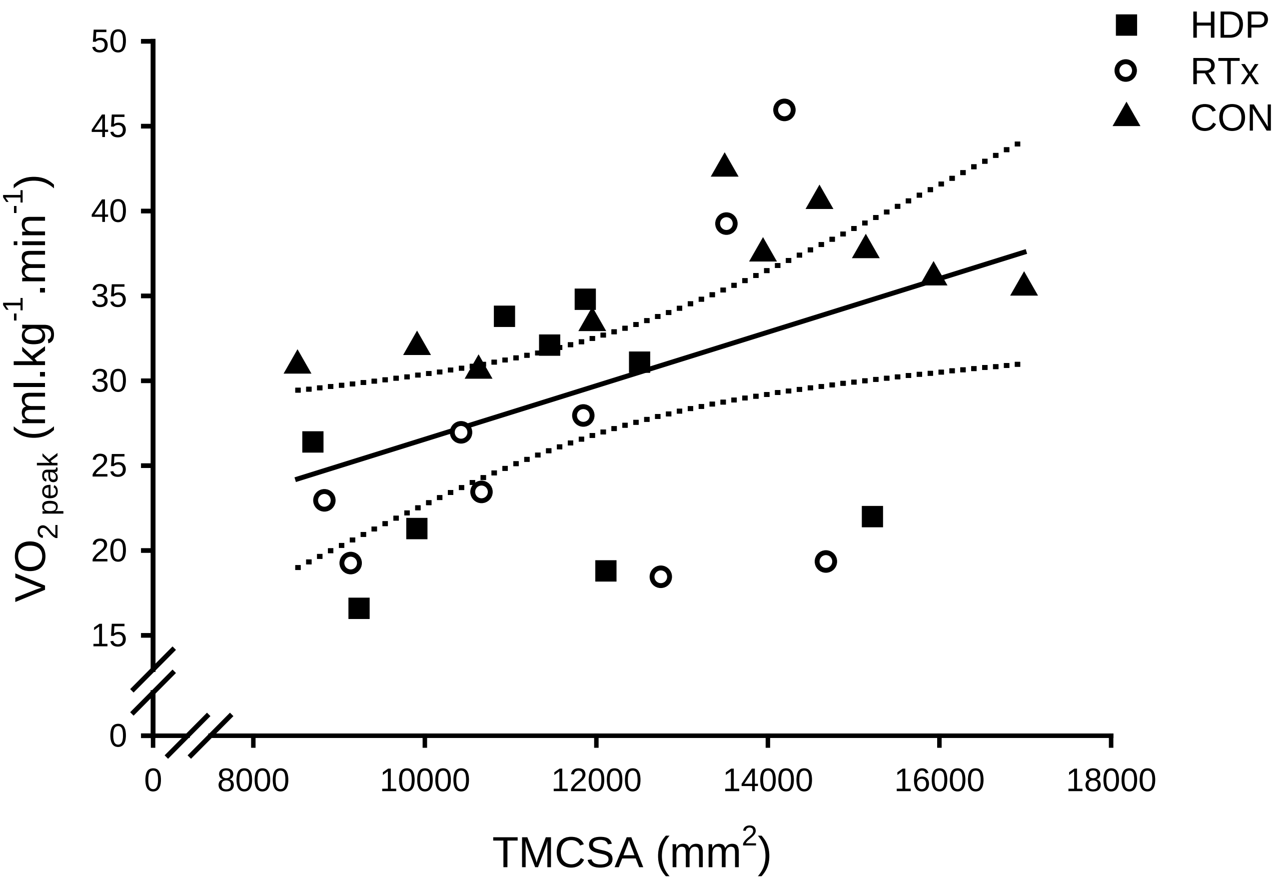

HDP
RTx
CON
$VO_{2\,peak}$ (ml.kg^{-1}.min^{-1})
50
45
40
35
30
25
20
15
0
0
50
100
150
200
250
300
350
400
450
3-O-MFPase activity
(nmol.min^{-1}.(g wet wt)$^{-1}$)

Figure 5


HDP
RTx
CON
50
45
40
35
30
25
20
15
0
VO2 peak (ml.kg-1.min-1)
0
8000
10000
12000
14000
16000
18000
TMCSA (mm2)