Abnormal mitochondrial function and muscle wasting, but normal contractile efficiency, in haemodialysed patients studied non-invasively in vivo


1Department of Musculoskeletal Science, 2Magnetic Resonance and Image Analysis Research Centre and 3Department of Medical Imaging, University of Liverpool, Liverpool, 4Renal Unit, Royal Liverpool University Hospital, Liverpool and 5Countess of Chester Hospital NHS Trust, Chester, UK

Abstract

Background. Muscle dysfunction, which contributes to morbidity in patients on haemodialysis, has several manifestations and a number of possible causes. We applied the non-invasive techniques of 31P-magnetic resonance spectroscopy (31P-MRS), magnetic resonance imaging (MRI) and near-infrared spectroscopy (NIRS) to calf muscle of dialysed patients to define the abnormalities in muscle cross-sectional area (CSA), contractile efficiency, mitochondrial function and vascular O2 supply.

Methods. We performed 31P-MRS/NIRS/MRI studies on the lateral gastrocnemius during isometric plantarflexion and recovery in 23 male patients on haemodialysis (age 24–71 years; haemoglobin 9.9–14.2 g/dl; bicarbonate 17–30 mmol/l; urea reduction ratio 53–77%; parathyroid hormone 1–95 U/l) and 15 male controls (age 29–71 years). To understand the relationships between calf CSA and body mass we also performed MRI only in a further six male patients and 18 male controls.

Results. In patients, exercise duration was 30±11% lower than in controls. Muscle CSA was lower by 26±5%, but contractile efficiency (force/CSA/ATP turnover) was normal. Slowing of post-exercise phosphocreatine (PCr) recovery implied a 22±5% defect in effective 'mitochondrial capacity'. That PCr recovery was slow relative to NIRS recovery suggests that this is largely an intrinsic mitochondrial problem (not the result of impaired O2 supply), one which, furthermore, correlated with CSA. Urea reduction ratio showed a negative correlation with body mass and CSA, but none with PCr rate constant.

Conclusions. The relationships to urea reduction ratio reflect the effect of muscle mass on dialysis efficiency, rather than direct effects on muscle CSA or metabolism. The relationship between PCr recovery and calf CSA suggests a role for the mitochondrial defect, whatever its cause, in the development of muscle wasting, although a common cause (e.g. physical inactivity) for both abnormalities cannot be ruled out.

Keywords: haemodialysis; magnetic resonance spectroscopy; mitochondria; near-infrared spectroscopy

Introduction

The quality of life of patients on haemodialysis is impaired by limited exercise capacity, largely due to muscle abnormalities whose causes remain unclear. Although anaemia is a factor, erythropoietin (Epo) treatment only partly corrects aerobic exercise performance [1,2], perhaps because of adverse effects on blood flow [2]. Muscle function depends on many things and we focus here on the interactions between vascular O2 supply, muscle bulk, ATP demand and oxidative ATP supply. Whether or not these are primary or specific abnormalities, they are important in understanding how muscle function can be improved.

Simultaneous measurement of blood flow, muscle/capillary PO2, muscle O2 consumption and energy metabolism is not possible. The non-invasive technique of 31P-magnetic resonance spectroscopy (31P-MRS) has shown abnormalities in energy metabolism [3–6], but published interpretations have not always distinguished some possible implications. When muscle contracts, ATP use increases and temporary failure of (in this context, largely mitochondrial) ATP production to...
match this results in a fall in phosphocreatine (PCr) concentration [7]. An increased PCr fall during exercise is a commonly reported $^{31}$P-MRS abnormality in dialysed patients [3–6,8] and this has two possible mechanisms, which have never been separated clearly. One is increased ATP demand, which implies an increased initial rate of PCr fall [9]. This could be due to decreased contractile efficiency, perhaps resulting from altered fibre-type proportions or, in some experimental designs [5,6], simply from muscle atrophy [10]. The other cause of increased PCr depletion during exercise is defective mitochondrial ATP synthesis, which would not affect the initial rate of fall of PCr, but which would be expected to increase the (steady state) fall in PCr [9]. The importance of this distinction lies in the therapeutic implications: mitochondrial therapies, such as carnitine and coenzyme Q, may not help if normal mitochondrial function is overstressed by increased ATP demand. Evidence for a mitochondrial defect is the slow post-exercise PCr recovery seen in some [3,5,6,8], but not all [4,8], studies of dialysed patients. PCr recovery depends both on intrinsic mitochondrial function and, contrary to what is sometimes assumed [4], on vascular $O_2$ supply [9]. Both of these may be abnormal in dialysed patients; invasive methods have shown that intramuscular $O_2$ transport is impaired [2] and possible causes of intrinsic mitochondrial dysfunction include detraining, nutritional deficiencies and damage by reactive oxygen species (ROS). To distinguish ‘mitochondrial’ lesions (i.e. in $O_2$ usage) and ‘vascular’ lesions ($O_2$ supply) we use the non-invasive technique of near-infrared spectroscopy (NIRS): an $O_2$-supply defect would be favoured by observation of slow post-exercise reoxygenation, e.g. in peripheral vascular disease (PVD) [11]. The only previous study in haemodialysed patients showed no obvious abnormality [12]. However, there certainly are $O_2$-supply problems, e.g. reduced capillary $O_2$ conductance [2,8] and, of course, anaemia, which is now better treated than at the time of earlier $^{31}$P-MRS studies [3–6]. Only when these distinctions are made can we establish the relationships between muscle wasting and mitochondrial metabolic defects that are expected if, for example, both result from detraining [13].

We combined $^{31}$P-MRS, magnetic resonance imaging (MRI) and NIRS to examine this in more detail. Our aims were to define the muscle metabolic abnormalities in contemporary dialysed patients, to establish the possible contributions of reduced muscle $O_2$ supply and intrinsic mitochondrial dysfunction (i.e. ATP supply) and alterations in contractile efficiency (i.e. ATP demand), and to define the relationship to muscle wasting.

**Subjects and methods**

**Subjects**

We studied male patients on haemodialysis and sedentary male controls selected to cover a similar age range. The main aim of the study was to evaluate muscle metabolism and oxygenation kinetics and for this we studied 23 patients (age range: 24–71 years; mean age: 50 years) and 15 controls (age range: 29–71 years; mean age: 43 years) by MRI at rest and then by $^{31}$P-MRS during an exercise-recovery protocol. Interpretation of kinetic $^{31}$P-MRS data from exercise requires estimation of muscle cross-sectional area (CSA). Although we did not attempt to study body composition, we did wish to examine more closely the relationship between calf CSA and body weight. To this end we studied a further six male haemodialysed patients by MRI and also included MRI data from a further 18 male controls of appropriate ages. We recruited patients on haemodialysis who were male, current non-smokers and excluded any with evidence of diabetes, sepsis and PVD (i.e. with absent foot pulses) and those with contraindications to MRI studies. Time on dialysis ranged from 8 to 230 months (median: 26 months). Their biochemical characteristics, measured pre-dialysis within 1 week of the study, were [mean±SD (range)]: haemoglobin 11.8±1.2 (9.9–14.2) g/dl; bicarbonate 24±3 (17–30) mmol/l; urea 25±5 (18–39) mmol/l; serum creatinine 1000±220 (740–1500) μM; and serum calcium 2.48±0.19 (2.14–2.86) mmol/l. Parathyroid hormone ranged from 1 to 95 U/l (median: 24 U/l). The patients received a 4 h haemodialysis session three times a week, using a single-pump dialysate delivery system with a synthetic membrane and a glucose-free bicarbonate dialysate containing 140 mmol/l sodium, 2.0 mmol/l potassium, 1.75 mmol/l calcium, 0.5 mmol/l magnesium and 109.5 mmol/l chloride. Dialysate flow rate was 500 ml/min and blood flow rate was 300 ml/min. Three patients were dialysed using a semipermanent neck line; otherwise, all others used fistulae with two needles. Urea reduction ratio (URR) was 64±6% (53–77%). Fully informed written consent was obtained from each subject and the study was conducted with the consent of the appropriate research ethics committee.

**Methods**

These have been described elsewhere [9,11]. $^{31}$P-MRS was performed in a SIGNA Advantage 1.5 tesla whole body MR system (General Electric, Milwaukee, WI, USA) using a 10.6 cm elliptical surface coil over the lateral gastrocnemius, with simultaneous NIRS using a RunMan CWS 2000 (NIM Inc., Philadelphia, PA, USA). An axial $T_1$-weighted spin echo MR image (10 mm slices with 5 mm gaps) was obtained for measuring CSA. Muscle was studied at rest and during 3–5 min voluntary 0.5 Hz isometric plantarflexion at 50% and then at 75% maximum voluntary force (MVC), each followed by 5 min recovery. If exercise ended early because of fatigue, recovery collection started early, but the next exercise started at the normal time. The CSA of the posterior calf (soleus + gastrocnemius) was measured in the slice at which it was maximal by point-counting using ANALYZE software (Mayo Foundation, Rochester MN, USA). $^{31}$P-MRS data were analysed using Magnetic Resonance User Interface software (MRUI 97.1). Cell pH was obtained from the chemical shift of inorganic phosphate Pi relative to PCr. Changes in PCr and derived quantities were expressed relative to resting PCr concentration, which did not differ significantly between the groups.

In this protocol there is little pH change and glycolytic ATP production can usually be ignored. The key $^{31}$P-MRS
measurements are of PCr kinetics, which are fitted to exponential functions. The analysis is described in detail elsewhere [7,9,11] and will be summarized briefly here. In general (see Figure 2), ATP turnover = glycolytic ATP synthesis rate + oxidative ATP synthesis rate + rate of PCr splitting. Very early in exercise the first two are negligible, so the initial rate of PCr splitting measures ATP turnover rate (F). This is proportional to force/CSA, the slope being the contractile efficiency. Subsequently, PCr splitting slows as oxidative and/or glycolytic ATP synthesis increase and a steady state may be reached. In the largely aerobic constant-power exercise used here, PCr kinetics are monoexponential. The change in PCr (Δ[PCr]) is both an overall measure of how far ATP synthesis has failed to meet ATP demand and also a correlate of the changes (in e.g. ADP) that are important in the feedback stimulation of oxidative ATP synthesis; the extrapolated steady-state PCr change (Δ[PCr]SS) is related to ATP turnover rate and mitochondrial function. The initial post-exercise PCr resynthesis rate (V) measures the end-exercise oxidative ATP synthesis rate and the rate constant of PCr recovery (= 0.693/halftime = V/Δ[PCr]SS) is proportional to the capacity for oxidative ATP synthesis (i.e. the notional rate at ‘complete’ PCr depletion). Initial post-exercise PCr resynthesis rate = end-exercise oxidative ATP synthesis rate and this declines with the same halftime as PCr. Making a small correction for end-exercise PCr splitting, total ATP turnover rate minus end-exercise oxidative ATP synthesis rate (i.e. F – V) gives end-exercise glycolytic ATP synthesis rate, which is always small here. The NIRS signal represents the difference in absorption at two wavelengths, proportional to deoxygenated haemoglobin + myoglobin content [14], normalized for instrument gain. The key measurement is the rate constant of post-exercise NIRS recovery (= 0.693/halftime), which reflects the extent to which O2 supply exceeds demand in recovering muscle [11]. To simplify the presentation, results from the two exercise intensities (50% and 75% MVC) were combined: recovery rate constants did not differ significantly and are presented as means; initial PCr depletion rates were constrained in the ratio 75:50 and a single 50% MVC value presented; exercise duration and all-exercise changes in PCr and NIRS were combined as a mean weighted 75:50 [9,11].

Results

Mean values of measured quantities in the patient and control groups are shown in Table 1, along with the

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Controls</th>
<th>Patients</th>
<th>P-value</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>83±4</td>
<td>76±3</td>
<td>0.2 (0.01)</td>
<td>(0.89±0.04)</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>178±2</td>
<td>172±2</td>
<td>0.03 (0.008)</td>
<td>0.97±0.01</td>
</tr>
<tr>
<td>Body mass index (kg.m⁻²)</td>
<td>26±1</td>
<td>26±1</td>
<td>0.6 (0.2)</td>
<td>(0.97±0.01)</td>
</tr>
<tr>
<td>Calf muscle dimensions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior calf CSA (cm²)</td>
<td>52±4</td>
<td>38±2</td>
<td>0.002 (0.0001)</td>
<td>0.73±0.06</td>
</tr>
<tr>
<td>CSA/height¹ (x 10⁴)</td>
<td>16±1</td>
<td>13±1</td>
<td>0.001 (0.01)</td>
<td>0.78±0.07</td>
</tr>
<tr>
<td><strong>Resting muscle biochemistry</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>pH</td>
<td>7.02±0.01</td>
<td>7.02±0.01</td>
<td>0.9</td>
<td>n/a</td>
</tr>
<tr>
<td>PCr/γ-ATP</td>
<td>3.07±0.10</td>
<td>3.06±0.09</td>
<td>0.9</td>
<td>n/a</td>
</tr>
<tr>
<td>Pi/γ-ATP</td>
<td>0.40±0.02</td>
<td>0.47±0.03</td>
<td>0.1</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Exercising muscle function, metabolism and deoxygenation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ATP turnover rate (min⁻¹)</td>
<td>0.7±0.1</td>
<td>0.9±0.1</td>
<td>0.1</td>
<td>n/a</td>
</tr>
<tr>
<td>Exercise duration (min)</td>
<td>2.8±0.1</td>
<td>1.9±0.1</td>
<td>0.0001</td>
<td>0.69±0.06</td>
</tr>
<tr>
<td>Observed end-exercise Δ[PCr]</td>
<td>0.35±0.03</td>
<td>0.44±0.04</td>
<td>0.1</td>
<td>n/a</td>
</tr>
<tr>
<td>Fitted steady state Δ[PCr]SS</td>
<td>0.37±0.04</td>
<td>0.31±0.04</td>
<td>0.01</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>Observed end-exercise ΔNIRS (au)</td>
<td>76±12</td>
<td>83±12</td>
<td>0.7</td>
<td>n/a</td>
</tr>
<tr>
<td>Force (au)</td>
<td>1.04±0.06</td>
<td>0.87±0.05</td>
<td>0.04</td>
<td>0.84±0.07</td>
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<tr>
<td>Force/CSA (au.cm⁻²)</td>
<td>21±2</td>
<td>24±2</td>
<td>0.2</td>
<td>n/a</td>
</tr>
<tr>
<td>Contractile efficiency (au.cm².min⁻¹)</td>
<td>32±3</td>
<td>31±2</td>
<td>0.8</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Recovering muscle metabolism</strong></td>
<td></td>
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</tr>
<tr>
<td>PCr recovery rate constant (min⁻¹)</td>
<td>2.1±0.1</td>
<td>1.5±0.1</td>
<td>0.0001</td>
<td>0.72±0.05</td>
</tr>
<tr>
<td>NIRS recovery rate constant (min⁻¹)</td>
<td>1.0±0.1</td>
<td>0.9±0.1</td>
<td>0.4</td>
<td>n/a</td>
</tr>
<tr>
<td>‘Excess’ PCr rate constant (min⁻¹)</td>
<td>0.00±0.08</td>
<td>-0.51±0.06</td>
<td>0.0001</td>
<td>n/a</td>
</tr>
<tr>
<td>End-exercise oxidative fraction</td>
<td>0.73±0.02</td>
<td>0.56±0.02</td>
<td>0.0001</td>
<td>0.77±0.04</td>
</tr>
</tbody>
</table>

All values are means±SEM. Data are shown for 31P-MRS studies at rest and during exercise/recovery. P = difference by unpaired t-test. If this is <0.05, then ‘ratio’ = patient/control (except for ‘excess PCr rate constant’ where control mean = zero); otherwise, ‘n/a’ = not applicable. Values in parentheses show ratios and P-values using the expanded set of MRI studies at rest; means and SEMs are essentially identical for this larger group, so are omitted. PCr changes, ATP turnover and contractile efficiency are given relative to resting [PCr]. Resting muscle ratios are not corrected for saturation. See Figure 3.
Relative abnormality (i.e. patient/control value) when the difference is significant.

Muscle cross-sectional area

The CSA of posterior calf muscles was significantly smaller in the patients. For proper comparison we must express this as a function of height; on allometric grounds we used height squared. This correlated with calf CSA (Figure 1A), with no significant different between the slopes, but a significant vertical separation between the regression lines. Thus, measured calf CSA is lower in patients, both absolutely and for a given height. Average MVC force was reduced in patients compared with controls, congruent with the reduction in calf CSA, so that MVC force per unit CSA was normal (Table 1).

Muscle metabolism: concentrations and concentration changes

There were no significant differences between controls and patients with respect to resting pH and the metabolite ratios PCr/ATP and Pi/ATP and, therefore, also absolute PCr and Pi concentration. Tolerated exercise duration was reduced in patients compared with controls. Figure 2A shows the time course of PCr concentration and the calculated fluxes in 50% MVC exercise and recovery. PCr falls during exercise and rises during recovery, as expected. PCr depletion at the end of exercise (ΔPCr) did not differ significantly, although the extrapolated steady-state fall in PCr was increased in patients compared with controls (Figure 2A).

Muscle metabolism: fluxes

Neither ATP turnover rate nor the contractile cost was significantly abnormal in patients (Figure 1B). The end-exercise oxidative fraction at 50% and at 75% MVC was not significantly different from unity in controls, implying that glycolytic ATP production was negligible. In patients it was significantly less than unity at both 50% MVC and 75% MVC (Table 1 gives a mean). Figure 2B shows how estimated oxidative ATP synthesis rate was reduced in patients throughout exercise at 50% MVC, the shortfall being made up by extra PCr depletion and glycolytic ATP production. Glycolysis, however, was not enough to produce significant acidification: pH changes during exercise were <0.15 units in controls and not significantly abnormal in patients (data not shown). PCr recovery was slowed in patients, the rate constant being reduced (Table 1). The end-exercise oxidative fraction correlated (P = 0.007) with the PCr recovery rate constant, as expected if the reduced oxidative contribution during exercise is mainly due to the defect in mitochondrial function, which is measurable during recovery, rather than to increased ATP demand.

Muscle metabolism and muscle deoxygenation

NIRS showed the expected pattern of deoxygenation during exercise followed by recovery: the exercise changes were not significantly abnormal in patients. The NIRS recovery rate constant was not significantly abnormal overall. However, there was a correlation between the PCr and NIRS rate constants in both groups (Figure 3A): the slopes not being significantly different, the significant vertical difference between the two lines measures the amount by which PCr recovery was slower than expected for given a NIRS recovery rate constant. Figure 3B shows a relationship in patients (not control subjects) between the PCr rate constant and calf CSA expressed relative to height squared. A similar correlation is seen with a number of other measures of CSA, as well as CSA itself (P = 0.008).
Fig. 2. MRS changes during exercise and recovery and principles of analysis. This figure combines (A) observed changes in PCr concentration and (B) inferred rates of ATP turnover during exercise at 50% MVC and subsequent recovery, together with the principles of analysis. Results are means ± SEM for dialysed patients (solid circles) and control subjects (open circles), shown as composite presentations of mean values from studies in which, especially for patients, exercise duration was often reduced; this is indicated by breaks in the graphs. (A) PCr kinetics, expressed relative to resting [PCr]. Changes in free [ADP] (data not shown) have roughly the opposite form to those in PCr. (B) ATP turnover rates, scaled to initial ATP turnover and expressed additively so that ATP turnover rate (broken line) is one during exercise (assuming constant work rate and contractile cost) and zero during recovery. Oxidative ATP synthesis is shown as circles (glycolytic + oxidative ATP synthesis is essentially the same in controls and patients).

Fig. 3. Relationships between PCr and NIRS recovery kinetics and muscle mass. (A) The PCr rate constant as a function of the NIRS rate constant, in patients and controls (see key for identification), and lines fitted by grouped linear regression analysis. The slopes are significantly non-zero but not significantly different, while the vertical separation is significantly non-zero ($P < 0.0001$). Mean PCr rate constant was significantly lower in patients, while there was no overall difference in NIRS rate constant (Table 1). (B) A function of calf CSA plotted against a function of PCr recovery rate constant. To correct for difference in height, muscle CSA is expressed relative to the square of height (see Figure 1A): patients have a significantly lower value than controls (Table 1). To isolate the ‘metabolic’ abnormality in the PCr rate constant, the x-axis here is the amount by which the individual PCr rate constant exceeds the value expected for the measured NIRS rate constant, i.e. in (A) the vertical distance from the observed point to the control regression line. For the patients the average value of this ‘excess PCr rate constant’ is significantly less than zero.
**Relationships to plasma biochemistry and haematology**

URR correlated inversely with body mass (Figure 4A) and with weight/height (both $P < 0.007$), but not with either PCr rate constant or ‘excess’ rate constant (Figure 4B). Furthermore, when the patient group is divided into two on the basis of URR <65% and ≥65%, the abnormality in the PCr rate constant is not significantly different between these two groups; the reduction compared with control being $24 \pm 6\%$ and $33 \pm 7\%$, respectively (both significant, $P < 0.002$). URR showed a negative correlation with both calf CSA and body mass (Figure 4A) and with CSA/height$^2$ ($P = 0.02$; data not shown), but not with CSA/weight$^{2/3}$ ($P = 0.2$; data not shown). Plasma Pi correlated positively with body mass ($P = 0.04$) and calf CSA ($P = 0.03$) and negatively with URR ($P = 0.03$). There were no other significant correlations with biochemical plasma measurements or with haemoglobin.

**Discussion**

Clinical research on muscle metabolism is difficult using invasive methods, although some information can be obtained in other ways. We have combined three non-invasive methods – $^{31}$P-MRS and NIRS, which look at two aspects of the O$_2$ delivery/usage system, and MRI – to measure muscle CSA. We had three aims, which we discuss in turn.

**Abnormalities in muscle**

First, we wished to establish the muscle abnormalities in our patients. We found, as expected, slow PCr recovery, which suggests a functional defect of mitochondrial ATP synthesis and, consistent with this [7], a significant increase in the steady-state PCr change.

Unlike in previous studies [3–6,8], this analysis is not complicated by a significant glycolytic contribution and the consequent pH change [8]. ATP demand was normal and, accordingly, the reduced oxidative contribution during exercise is due to the mitochondrial defect measurable during recovery. We also found evidence of muscle wasting, assuming the controls represent the patients’ pre-morbid state.

It is of interest to compare our results with those of invasive studies of maximal muscle O$_2$ consumption $V_{O_{2max}}$ [2,15]. To a first approximation, the PCr recovery rate constant is proportional to maximum O$_2$ consumption [7,16]. Direct arteriovenous-differences measurements of muscle $V_{O_{2max}}$ are usually expressed per leg, or exercising muscle, and so the appropriate comparison is with the product of PCr recovery rate constant and muscle CSA: this is significantly reduced, by $\sim 50\%$, a similar abnormality to that seen in $V_{O_{2max}}$ in quadriceps in young patients in [2] and larger than in [15].

**Contributions to mitochondrial dysfunction**

Second, we wished to estimate the contributions to abnormalities in exercising muscle of reduced muscle O$_2$ supply, intrinsic mitochondrial dysfunction (i.e. ATP supply) and alterations in contractile efficiency (i.e. ATP demand). Compared with controls, the patients exert less force because they have less muscle; contractile cost is normal and so is the ATP demand. There is a relative failure of oxidative ATP production during exercise, due to the reduced capacity for oxidative synthesis inferred from the slow PCr recovery [7]. This depends on actual mitochondrial content, intrinsic mitochondrial function and vascular O$_2$ delivery, any of which might be abnormal.

A candidate cause for impaired O$_2$ delivery is anaemia. The results of a comparison with the
literature are suggestive: the mitochondrial abnormality of ~30% seen in the present study (Table 1) and others where mean haemoglobin was 10–12 g/dl [12,15] is smaller than abnormalities of 40–50% seen in earlier studies [3,5,6] where mean haemoglobin was 7–8 g/dl (calculations based [7,9] on published data). Against this, pre- and post-Epo studies conducted over periods of a few months showed little improvement in mitochondrial function measured by $^{31}$P-MRS [6] or invasively [8], while haemoglobin increased from 7–8 to 12 g/dl. Also, Epo treatment lowers peak blood flow and does not normalize the reduced $O_2$ conductance [2]. Recent work has shown that elevating haemoglobin from 10 to 14 g/dl does improve various measures of exercise performance [1]. Nevertheless, in the present work there was no correlation between mitochondrial function and haemoglobin. It is, therefore, likely that there is no direct influence on muscle mitochondrial function over this range.

Another possible cause of impaired $O_2$ delivery is the reduced muscle $O_2$ conductance in patients on haemodialysis [2]. We tested this using NIRS to report cellular/capillary deoxygenation. Unlike in PVD, where the lesion is straightforwardly in blood supply [11], the absolute change in NIRS was normal. This cannot be interpreted in any more detail because of the current debate about the source of the NIRS signal: intracellular deoxymyoglobin [17] or intracapillary deoxyhaemoglobin [14]. However, the recovery kinetics of NIRS should be largely independent of this. The overall normal NIRS recovery shows that, unlike in PVD [11], there is no gross defect of $O_2$ supply. NIRS recovers because vascular $O_2$ supply, which we do not measure non-invasively, exceeds $O_2$ use, which is declining during recovery (Figure 2B). Given that PCr resynthesis can be limited by $O_2$ supply [16], theory and simulation predict the observed correlation between the rate constants of PCr and NIRS recovery (Figure 3A), which allows us to control for the $O_2$ supply by means of a joint linear regression. That PCr recovery is slow for a given NIRS recovery suggests an oxidative defect independent of $O_2$ supply.

The implication of the recent finding that $O_2$ supplementation normalizes $VO_{2\text{max}}$ [15] is that the entire mitochondrial defect is secondary to impaired $O_2$ supply. However, there must be a difference between the patients in our studies and those patients, because $O_2$ supplementation could normalize whole-leg $O_2$ consumption in the presence of reduced muscle mass. Comparison is made difficult by the differences in the muscle studied and because that protocol gives highly pH-dependent PCr recovery rate constants [8] (see above).

The older literature on muscle content and activity of mitochondria in dialysed patients is confused [18,19]. A recent finding of normal mitochondrial content and activity [20] has been taken as evidence that the oxidative defect is all in muscle $O_2$ supply, although it might also be related to a difference in the studied populations, or a defect in mitochondrial control in vivo. A mitochondrial defect could be related to fibre-type change; however, the relative type 1 (fast) fibre atrophy sometimes reported [10] would tend to have the opposite effect. Furthermore, in quadriceps muscle of 10 patients on haemodialysis and 10 control subjects we have found no difference in the proportion of type 1 vs type 2 fibres, but there is a significant decrease relative to control in the mean diameter of both fibre types (A.V.Crowe, unpublished data).

Muscle metabolism and muscle atrophy

Thirdly, we wished to establish the relationship of metabolic abnormalities to muscle atrophy. Muscle wasting is a known feature of chronic renal failure [10] and many mechanisms have been proposed. As muscle makes substantial contribution to body weight, an appropriate comparison is not entirely straightforward, but several methods agree in finding a reduced calf muscle CSA in these patients, while the ability to generate force was unchanged. Figure 3B shows that the 'metabolic' component of the PCr recovery defect (i.e., as we argue, the intrinsic mitochondrial defect) correlates with the abnormality in CSA. This could, of course, mean that both are consequences of some other factor. Age can be provisionally ruled out, as neither quantity shows a significant age-dependence in this group. Detraining is a possible cause, as experimental disuse decreases both muscle CSA and mitochondrial content [13] and it is known that haemodialysed patients are less active than even sedentary controls [21]. Another possible link is damage by ROS: mitochondria [22] are damaged by ROS in dialysed patients and are themselves a source of ROS, and this might be important in disuse atrophy.

Even though these may be, at least in part, rather general features of chronic disease, defining their contributions may be useful. For example, the persistence of mitochondrial dysfunction might suggest that it is still worth testing 'mitochondrial therapies', such as carnitine and coenzyme Q. One would expect this to be one of the benefits of endurance-training.

Muscle metabolism, muscle atrophy and plasma biochemistry

Some other findings are of pathophysiological relevance. The range of URR seen here is fairly wide, with about half of the patients falling below the currently recommended minimum of 65% [23]. Although URR is not an entirely satisfactory measure of dialysis dose, in the present study it shows some interesting relationships. First, URR correlated inversely with body mass (Figure 4A) and with weight/height, as in large patient series [24], presumably because, for a given dialysis dose, the smaller the body mass the greater the reduction in urea [24]. We cannot be sure that more complete dialysis would not have resulted in smaller muscle metabolic abnormalities, although the lack of correlation between URR and mitochondrial function (Figure 4B) argues against this. Second, the negative
correlation between URR and muscle CSA (Figure 4A) no doubt reflects the negative correlation between URR and body mass (Figure 4A) and remains even after correcting CSA for height\(^2\). However, the lack of correlation with CSA/weight\(^{2/3}\), which should control for the contribution of CSA to body mass, suggests that URR has no causal relationship to muscle wasting. URR also correlates negatively with plasma Pi, as one might expect [25], and this no doubt explains the negative correlations between plasma Pi and both calf CSA and body mass: all result from the influence of mitochondrial defect rather than a result of impaired O\(_2\) supply. We noted a relationship between PCr recovery and muscle mass on dialysis effectiveness [24].

In summary, we observed slow PCr recovery relative to NIRS recovery, suggesting an intrinsic mitochondrial defect rather than a result of impaired O\(_2\) supply. We also observed relationships to URR that reflect the effect of muscle mass on dialysis efficiency, but not direct effects of URR on muscle CSA or metabolism. We noted a relationship between PCr recovery and calf CSA, which suggests a role for the mitochondrial defect in the development of muscle wasting, although a common cause (e.g. physical inactivity) for both abnormalities cannot be ruled out.

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References


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