Altered relative concentrations of high-energy phosphates in patients with uraemic cardiomyopathy measured by magnetic resonance spectroscopy

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Abstract

Background. Premature sudden cardiovascular death is the commonest cause of death in end-stage renal disease (ESRD) patients and is associated with uraemic cardiomyopathy [left ventricular hypertrophy (LVH), systolic dysfunction (LVSD) or LV dilation]. High-energy phosphates (HEP), quantified using phosphorus-31 magnetic resonance spectroscopy, are reduced in patients with diabetes, heart failure and uraemia. Phosphocreatine:β adenosine triphosphate (PCr:ATP) ratio is an index of metabolic activity. We compared resting HEPs in ESRD patients and hypertensive patients (with and without LVH) who had normal renal function (LVH-only or normal myocardia). We also assessed associations of HEP levels with abnormalities of uraemic cardiomyopathy.

Methods. Fifty-three ESRD and 30 hypertensive patients (18 with LVH, 12 with normal myocardia) underwent phosphorus magnetic resonance spectroscopy of their left ventricle. PCr:ATP ratios were calculated from 31P-MR spectra obtained from long-axis views of the left ventricle.

Results. There were no significant differences in age, LV mass, chamber sizes and ejection fraction between patient groups. PCr:ATP was significantly lower in ESRD patients compared to hypertensive patients, irrespective of the presence or absence of LVH (P = 0.01). In the ESRD group, PCr:ATP was significantly lower in patients with LVSD (P = 0.05) and LV dilation (P = 0.01). LVH was not associated with significant difference in PCr:ATP.

Conclusions. ESRD patients have lower HEP levels compared to hypertensive patients. Lower PCr:ATP ratio, indicating altered myocardial metabolic function in ESRD patients, is associated with features of uraemic cardiomyopathy.

Keywords: cardiovascular disease; end-stage renal disease; high-energy phosphate; uremic cardiomyopathy; 31-phosphorus magnetic resonance spectroscopy

Introduction

Premature cardiovascular (CV) disease is common in patients with end-stage renal disease (ESRD) and is the commonest cause of death in patients close to or requiring renal replacement therapy (RRT) [1]. Echocardiography and cardiovascular magnetic resonance (CMR) imaging have identified gross structural changes in left ventricular (LV) geometry that confer poorer all-cause and CV survival [2, 3]. These abnormalities include LV hypertrophy (LVH), LV systolic dysfunction (LVSD) and dilatation and are collectively termed uraemic cardiomyopathy [4]. Animal models and human studies have also demonstrated significant alteration in cardiomyocyte metabolism (e.g. intracellular calcium cycling from myofibrils to sarcoplasmic reticulum, handling of glycogen), inadequate microvascular angiogenesis leading to ischaemia and prominent cardiac interstitial fibrosis in uraemic hearts [5–8].

Localized cardiac 31-phosphorus magnetic resonance spectroscopy (31P MRS) is a non-invasive technique of measuring relative concentrations of high-energy phosphate (HEP)-containing compounds and has been used to assess cardiac metabolism in patients with ischaemic and non-ischaemic cardiomyopathy [9]. Measured phosphocreatine:β adenosine triphosphate (PCr:ATP) ratio provides an index of cardiac energetic state and HEP storage reserves. The clinical relevance of relative HEP levels has been demonstrated in heart failure patients, where reduced myocardial PCr:ATP has been significantly associated with lower LV ejection fraction (LVEF), NYHA symptoms and reduced survival. Furthermore, PCr:ATP is a better predictor of survival than LVEF or NYHA class [10].

Previously, only small studies have shown altered cardiac HEP concentrations in ESRD patients and patients with hypertensive LVH [11–13]. Similarly, abnormalities of skeletal muscle HEP concentrations have been demonstrated in haemodialysis patients [14]. In addition, the relationships between HEP and features of uraemic cardiomyopathy have
not been investigated. The aims of this study were to compare HEP levels in a cohort of ESRD patients and hypertensive patients with normal renal function to determine the additional detrimental of ureaemia and assess associations between features of uremic cardiomyopathy and altered HEP concentrations.

**Materials and methods**

**Patients**

The Western Infirmary, Glasgow, provides transplant services to a population of 2.8 million people in the west of Scotland [15]. All patients gave written informed consent and the study was approved by the local ethics committee.

In this study, we evaluated 53 patients with chronic kidney disease (ESRD, stage 5, including subjects within 6 months of requiring RRT (pre-dialysis)), who were being assessed for renal transplantation over a 12-month period. Thirty hypertensive patients with normal renal function (according to laboratory estimated glomerular filtration rate > 60 mL/min/1.73 m²) were recruited from medical outpatient clinics. Of these, 18 patients had evidence of LVH and 12 had normal myocardia on CMR examination. All patients provided written informed consent and the study was approved by the local ethics committee. Patients underwent CV risk factor assessment including history, clinical examination, electrocardiogram (ECG) as well as routine haematological and biochemical profile.

**Exclusion criteria**

Patients with contraindication to CMR (presence of permanent pacemaker or ferromagnetic implants, severe claustrophobia, pregnancy) were not asked to participate in the study. Furthermore, since we wished to assess the additional effect of uraemia, patients with past or current history of ischaemic heart disease (including those with previous coronary revascularization or heart failure) were excluded from the study.

**Assessment of LV chamber size and function**

Non-contrast CMR was performed using a 1.5-T magnetic resonance imaging (MRI) scanner (Sonata; Siemens, Erlangen, Germany) as previously described [16]. LVH was defined as LV mass index (LV mass/body surface area; LVMI) >84.1 (male) or >76.4 g/m² (female) and LV systolic dysfunction (LVSD) was defined as LVEF <55%. LV dilatation was defined as end diastolic volume/body surface area (BSA) >111.7 (male) or 99.3 mL/m² (female) or end systolic volume/BSA >92.8 (male) or 70.3 mL/m² (female) [17].

**Cardiac 31P MRS acquisition**

Resting 31P MRS was performed on all patients using a 1.5-T MRI scanner (Sonata; Siemens). A commercially available dual resonant 31P/1H phased array surface coil was positioned over the left ventricle on the anterior chest wall of patients allowing both proton localizer images and 31P spectra to be acquired using the same receiver coil (Figure 1). The spectroscopy acquisition was gated to the patient’s ECG. No breath holding was required. After localization, pilot scans were performed in the cardiac vertical long-axis plane (fast low-angle shot images, slice thickness 10 mm, repetition time [TR] 2/7.37 ms, echo time [TE] 7/7.37 ms, FoV 350 mm). 31P-MRS data were obtained with a two-dimensional chemical shift imaging (CSI) sequence. The acquisition matrix size was 25 × 25 mm and TR = 440 ms, TE = 2.3 ms, flip angle = 90°, no signal averaging = 60. The CSI grid was positioned over the left ventricle to ensure one voxel covered the LV apex and the remaining voxels provided coverage for the LV superior and inferior walls. Spectra were acquired from areas of uniform LV contraction. Prospective ECG gating was used with an acquisition trigger delay of 100 ms and data were acquired during diastole. An optimized radiofrequency pulse (length 2.4 ms), centred between γ-ATP and α-ATP resonance frequencies, was used to ensure uniform excitation of all spectral peaks. Spectra were obtained and areas under the curves of interest measured using spectroscopic fitting software (Siemens). The spectral resonances for β-ATP, 2,3-DPG and PCr were fitted using prior knowledge relating to peak frequencies and J-coupling patterns. PCr/ATP ratios were calculated accordingly. A correction for intraventricular blood was performed using a previously validated equation [18].

**Statistical analyses**

Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., IL). Data are described as mean ± SD for parametric data and median (interquartile range) for non parametric data. Data were compared by Chi-squared or Fisher’s test for categorical data. One-way analysis of variance combined with Tukey’s post hoc test was used to test for differences between groups.

**Results**

**Patient demographics**

Clinical, drug and blood data for ESRD (n = 53), LVH (n = 18) and normal myocardia patients (n = 12) are shown in Table 1. In the ESRD group, 16 patients were within 6 months of requiring RRT and 37 attended our centre for thrice-weekly maintenance haemodialysis. There were no significant differences in body mass index or BSA between the study groups. Despite excluding patients with symptomatic ischaemic heart disease, there was higher burden of CV disease in ESRD compared to LVH and normal myocardia patients. Furthermore, use of cardioprotective drugs was more common in ESRD patients. Comparison of blood results between ESRD and a population with normal renal function was as expected (in ESRD patients haemoglobin was lower, inflammatory markers were elevated and serum potassium was higher). In particular, serum phosphate and calcium phosphate product were significantly higher in

**Fig. 1.** Acquisition of 31P MRS spectra from apex of left ventricle.
the ESRD group compared to the hypertensive groups. Glucose was also higher in the ESRD compared to hypertensive patients due to the higher proportion of diabetic patients in this cohort. Serum cholesterol was significantly higher in the LVH group compared to patients with ESRD and normal myocardia; however, the use of statins was lower.

Cardiac data

Table 2 shows CMR results. There were no significant differences in LV systolic function (which was preserved) or chamber size between patient groups. As expected, LVMI was significantly lower in patients with normal myocardial structure compared to ESRD and LVH patients.

### Table 1. Clinical, drug and blood data for patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>ESRD, N = 53</th>
<th>LVH, N = 18</th>
<th>Normal myocardia, n = 12</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.7 (±12.6)</td>
<td>57.6 (±9.3)</td>
<td>51.0 (11.2)</td>
<td>0.21</td>
</tr>
<tr>
<td>Male (%)</td>
<td>33 (63.3)</td>
<td>14 (77.8)</td>
<td>10 (83.3)</td>
<td>0.23</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.69 (±0.1)</td>
<td>1.73 (±0.1)</td>
<td>1.74 (±0.1)</td>
<td>0.19</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.7 (±17.5)</td>
<td>79.2 (±13.6)</td>
<td>78.8 (16.8)</td>
<td>0.14</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2 (±5.2)</td>
<td>26.8 (±3.9)</td>
<td>27.5 (±3.3)</td>
<td>0.66</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.87 (±0.3)</td>
<td>1.97 (±0.2)</td>
<td>2.00 (±0.3)</td>
<td>0.12</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>142 (±23)</td>
<td>14 (±15)</td>
<td>136 (±19)</td>
<td>0.69</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>81 (±14)</td>
<td>86 (±9)</td>
<td>88 (±10)</td>
<td>0.09</td>
</tr>
<tr>
<td>RRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td>37 (69.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-dialysis</td>
<td>16 (30.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RRT time (years)</td>
<td>1.26 (1.9)</td>
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</table>

Primary renal disease

<table>
<thead>
<tr>
<th>Variable</th>
<th>ESRD, N = 53</th>
<th>LVH, N = 18</th>
<th>Normal myocardia, n = 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>14 (26.4)</td>
<td></td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>39 (73.6)</td>
<td>18 (100)</td>
<td></td>
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<tr>
<td>Cerebrovascular disease</td>
<td>11 (20.8)</td>
<td>1 (3.3)</td>
<td></td>
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<tr>
<td>Peripheral vascular disease</td>
<td>12 (14.5)</td>
<td>0</td>
<td></td>
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</table>

Smoking

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<th>Variable</th>
<th>ESRD, N = 53</th>
<th>LVH, N = 18</th>
<th>Normal myocardia, n = 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>22 (41.5)</td>
<td>12 (66.7)</td>
<td>7 (58.3)</td>
</tr>
<tr>
<td>Current/Ex</td>
<td>31 (58.5)</td>
<td>6 (33.3)</td>
<td>5 (41.7)</td>
</tr>
</tbody>
</table>

Haemoglobin (g/dL) 11.8 (±2.2) 14.8 (±1.4) 14.8 (±1.2) <0.01

Fibrinogen (g/L) 4.1 (±0.6) 3.3 (±0.6) 2.9 (±0.3) <0.01

ESR (mm/s) 29 (14, 56) 8.5 (4.3, 12) 5.0 (2.0, 9.0) <0.01

CRP (mg/L) 5.0 (3.3, 13.3) 1.5 (0.6, 4.8) 0.8 (0.7, 2.2) <0.01

Adjusted Ca²⁺ (mmol/L) 2.35 (±0.2) 2.43 (±0.1) 2.39 (0.1) 0.08

CaPO₄ product (mmol²/L²) 3.54 (±1.0) 2.59 (±0.4) 2.45 (±0.3) <0.01

PTH (pmol/L) 19.3 (11.6, 45.1) 5.5 (4.7, 7.2) 6.3 (4.4, 9.2) <0.01

Glucose (mmol/L) 9.1 (±1.8) 5.0 (±2.5) 5.3 (±0.9) <0.01

Albumin 37.8 (±5.6) 41.8 (±6.8) 42.1 (±7.2) 0.29

HbA1c (%) 6.9 (±2.8) 5.4 (±0.9) 5.3 (±0.5) 0.02

Potassium (mmol/L) 4.8 (±0.3) 4.1 (±0.3) 4.2 (±0.3) <0.01

Cholesterol (mmol/L) 4.3 (±1.2) 5.7 (±1.3) 5.2 (±1.0) <0.01

Triglyceride (mmol/L) 1.9 (±0.8) 2.0 (±1.0) 2.0 (±1.3) 0.85

HDL–Cholesterol (mmol/L) 1.2 (±0.6) 1.4 (±0.5) 1.4 (±0.3) 0.33

LDL–Cholesterol (mmol/L) 2.4 (±0.4) 2.78 (±0.8) 2.8 (±1.1) 0.31

Epo receptor agonist 46 (86.8) 0

β-Adrenoceptor blocker 25 (47.2) 2 (11.1) 3 (25.0)

Aspirin 35 (66.0) 3 (16.7) 2 (16.7)

Warfarin 3 (5.7) 0 0

ACEI/ARB 30 (56.6) 11 (61.1) 8 (66.7) 0.27

Diuretic 13 (24.5) 6 (33.3) 0

Nitrates 0 8 (26.7) 0

Calcium channel blocker 26 (49.1) 5 (27.7) 3 (25.0)

α Adrenoceptor bBlocker 6 (11.3) 1 (3.3) 0

Statin 30 (56.6) 5 (27.7) 3 (25.0)

Vitamin D analogue 51 (96.2) 0 0

--Clinical, drug, serum biochemistry and plasma haematology data for patients with comparisons between patients with ESRD, LVH-only and normal myocardia. Data are number with percentage in parentheses or mean ± SD. Tests of significance are one-way analysis of variance and chi-square. BMI, body mass index; HD, haemodialysis; PD, peritoneal dialysis; ADPKD, autosomal dominant adult polycystic kidney disease; Epo, Erythropoietin; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; LDL, low density lipoprotein; HDL, high density lipoprotein. --
There was no significant difference between LV mass between ESRD and LVH subjects (ESRD 87.5 versus LVH 85.2 g/m²; \( P = 0.38 \)). LVSD and dilatation were more common in the ESRD group compared to the LVH and normal myocardia groups; however, these did not reach statistical significance due to small sample size.

**Comparison of \(^{31}\text{P} MRS results between groups**

Table 2 shows comparison of \(^{31}\text{P} MRS results. There were no significant differences between PCr, \( \beta \)-ATP and 2,3-DPG values. However, mean PCr:ATP ratios (uncorrected and corrected for blood contamination) were significantly lower in ESRD patients compared to patients with LVH (corrected PCr:ATP ESRD 1.3 ± 0.5 versus LVH 1.7 ± 0.3; \( P = 0.01 \)) and normal myocardial structure (corrected PCr:ATP ESRD 1.3 ± 0.5 versus normal myocardial structure 1.9 ± 0.4; \( P = 0.01 \), Figure 2). Corrected PCr:ATP was higher in patients with normal myocardia compared to those with hypertensive LVH; however, this did not reach statistical significance (LVH 1.7 ± 0.3 versus normal myocardial structure 1.9 ± 0.4; \( P = 0.09 \)).

In the ESRD group, there were no significant differences between diabetic and non-diabetic patients (PCr:ATP diabetic 1.4 ± 0.7 versus non-diabetic 1.3 ± 0.4; \( P = 0.66 \), data not shown) and patients who were pre-dialysis or receiving RRT (pre-dialysis \( n = 16 \), PCr:ATP 1.4 ± 0.4 versus receiving RRT, \( n = 37 \), 1.3 ± 0.6; \( P = 0.73 \)). Comparing haemodialysis patients with LVH-only and normal myocardia groups, PCr:ATP remained significantly lower (\( P = 0.01 \)). Similarly, pre-dialysis patient PCr:ATP was significantly reduced compared to both hypertensive groups (\( P = 0.04 \)).

**HEP levels and uraemic cardiomyopathy**

In the ESRD group (\( n = 53 \)), PCr:ATP was not significantly correlated with measured parameters including patient haemoglobin, fibrinogen, serum potassium, bicarbonate, albumin, lipid measurements, PTH, phosphate, serum calcium and CaPO₄ product (data not shown).

PCr:ATP was significantly lower in patients with LVSD (no LVSD 1.4 ± 0.6 versus LVSD 1.0 ± 0.3; \( P = 0.01 \)) and LV dilatation (no LV dilatation 1.7 ± 0.6 versus LV dilatation 1.0 ± 0.4; \( P = 0.05 \)) compared to those with normal ventricular function or size. LVH was not associated with significant difference in PCr:ATP (Figure 3).

**Discussion**

\(^{31}\text{P} MRS has been used to in vivo to measure LV HEP of human hearts. Inherited and acquired cardiomyopathies are not only associated with abnormal cardiac structure but also reduced PCr:ATP, particularly in areas of apparently normal LV wall contraction [9, 10]. These results suggest that although echocardiography or CMR may demonstrate ‘normal’ myocardial contraction, biochemical abnormalities are present that may precede development of overt systolic dysfunction by reducing metabolic activity and/or efficiency. The clinical relevance of HEP concentrations has been demonstrated in patients with heart failure and dilated cardiomyopathy [10, 19].

In this current study, HEPs were measured in a cohort of ESRD patients. Patients with known ischaemic heart disease...
were excluded to assess the effect of uraemia and small vessel ischaemia. Acquisition voxels were placed on visibly functioning myocardium to ensure that PCr:ATP was measured from viable cardiac tissue as opposed to scar/fibrotic tissue. As control groups, hypertensive patients with LVH or normal myocardial structure were assessed to allow the additional effect of myocardial abnormalities (LVSD and LV dilatation) and uraemia to be determined.

The values obtained for patients with ESRD, LVH and normal myocardial structure are comparable to previous studies [11, 12]. These results show that despite similar LV mass, function and chamber size, PCr:ATP ratios were significantly lower in ESRD patients compared to hypertensive patients with LVH (Figure 2). Similarly, PCr:ATP was significantly lower in ESRD patients compared to individuals with hypertension and normal myocardial structure. This study also demonstrates for the first time significant associations between features of uraemic cardiomyopathy (LVSD and LV dilatation) and reduced PCr:ATP ratio (Figure 3). Consistent with a previous smaller study, which compared HEP levels between diabetic and non-diabetic uremic patients [11], there was no significant difference in PCr:ATP between diabetic and non-diabetic ESRD patients.

The biochemical changes that cause reduction of PCr:ATP are not completely understood and derived mostly from animal studies. Lowered PCr:ATP, which is closely related to free intracellular adenosine diphosphate (ADP) concentration, indicates alteration of myocardial energy supply in the resting state [20]. In animal models for cardiac hypertrophy and failure, a reduction in PCr:ATP has been demonstrated [21, 22] and has been associated with reduced activity of the creatine kinase/phosphocreatine energy shuttle. Impaired ATP synthesis and/or utilization may reduce PCr due to the buffering effect of the creatine kinase system. Contractile dysfunction may be further exacerbated by subsequent elevation of free cytoplasmic ADP, which inhibits myosin ATPase activity [23]. To this end, abnormal HEP levels have also been detected by $^{31}$P MRS in skeletal muscle of ESRD patients [14].

The differences between PCr:ATP of ESRD and LVH patients are most likely due to alteration of myocardial interstitial tissue composition and cardiomyocyte metabolic function. In patients with systemic hypertension and ESRD, LVH is associated with sarcomere volume and density expansion and myocardial fibrosis [8, 24]. Post-mortem, endomyocardial biopsy and gadolinium-enhanced MRI studies of hypertensive and ESRD patients have demonstrated more fibrosis in uraemic hearts [3, 24–26]. Furthermore, in patients with dilated cardiomyopathy, dialysed patients have been shown to have more severe and disorganised myocardial fibrosis on endomyocardial biopsies compared to their non-dialysed counterparts [27]. Reduction of measured HEP levels may be a consequence of impeded nutrient/metabolic substrate (e.g. glucose, oxygen) transfer to cardiac myocytes as a result of intervening collagen and reduced cardiomyocyte volume per acquisition voxel.

Mismatch of energy demand (from hypertrophied myocytes) and supply (due to inadequate angiogenesis) has been demonstrated in animal models and patients with uraemic cardiomyopathy [7, 28] and may be an important cause of reduced PCr:ATP ratio in uraemic hearts. Although care was taken to avoid spectra acquisition from areas of obvious wall motion abnormality, it is likely that presence of microvascular ischaemia in areas of apparently normal myocardial contraction may contribute to altered HEP concentrations in ESRD patients.

Several animal models and clinical studies have also shown abnormalities of cardiomyocyte metabolism in
uræmia. Altered intracellular Ca\(^{2+}\) cycling has been demonstrated in isolated cardiac perfusion and single cardiomyocyte models of uræmia that may significantly affect excitation coupling and relaxation of cardiac tissue during the cardiac cycle [29–31].

As in similar human investigations using the MRS technique, this study was limited by noisy signal during MRS acquisition, which was minimized by ensuring the magnetic field was homogenized completely. In addition, it is difficult to assess the effect of other metabolic and haemodynamic differences between uræmic and non-uræmic patients from these data.

In conclusion, this study shows that despite similar myocardial mass, ESRD patients have significantly lower HEP levels compared to hypertensive patients with LVH. This may be due to greater myocardial fibrosis or altered myocyte metabolic function in ESRD patients. Lower PCr:ATP ratio is associated with features of uræmic cardiomypathy.

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Conflict of interest statement. None declared.

References

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