Myocardial Function, Energy Provision, and Carnitine Deficiency in Experimental Uremia

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Cardiac complications are the leading cause of mortality in patients with chronic renal failure. Secondary carnitine deficiency, which is frequently observed in hemodialysis patients, has been associated with cardiac hypertrophy and heart failure and may impair myocardial fatty acid oxidation. In chronic kidney disease, impaired carnitine homeostasis also may affect myocardial metabolism. In this study, myocardial function and substrate oxidation in conjunction with carnitine deficiency were investigated in experimental renal failure. Uremia was induced in male Sprague-Dawley rats via a two-stage five-sixths nephrectomy. Cardiac function and substrate oxidation were assessed in vitro by means of isovolumic perfusion using 13C nuclear magnetic resonance at 3 and 6 wk of uremia. Renal impairment as assessed by serum creatinine was more severe initially and was associated with a significant deficiency in serum free carnitine (43%; \( P < 0.001 \)) and elevated acyl carnitine/free carnitine ratio. Myocardial tissue carnitine concentrations, however, were unaffected. A moderate degree of cardiac hypertrophy (10 to 14%; \( P < 0.05 \)) was observed in uremia without evidence of dysfunction or changes in myocardial substrate utilization. It is concluded that renal dysfunction is associated with cardiac hypertrophy in the presence of normal myocardial carnitine levels, despite a significant depletion in serum carnitine. This may be a factor in maintaining normal cardiac function and metabolism.

Cardiovascular complications are the leading cause of mortality, accounting for 50% of all deaths among patients with end-stage renal failure in developed countries (1,2). The majority of these deaths are from cardiac causes (3). Indeed, the prevalence of heart failure among these patients is as high as 40% and is responsible for 12% of the total deaths (4). At present, the underlying mechanisms of this cardiac dysfunction remain unclear.

Our previous studies, which used an experimental model of chronic uremia, identified 40% reduction in myocardial phosphocreatine and phosphocreatine/ATP ratio (5), in parallel with left ventricular hypertrophy (LVH) (6). These observations are supported by clinical studies of patients on peritoneal dialysis, with a negative correlation between phosphocreatine/ATP ratio and dialysis duration (7). As cardiac ATP production is tightly linked to substrate metabolism, one potential mechanism for the impaired energetics may be through alterations in the profile of energy provision. The hypertrophied heart is characterized by remodeling of the left ventricle at cellular and structural levels, involving a reexpression of the fetal metabolic phenotype and a downregulation of the adult phenotype. These changes result in a switch in the profile of energy provision from fatty acid oxidation (8–10) toward a greater reliance on glucose metabolism (11,12). The extent to which this may occur in the uremic myocardium, however, is unclear.

Secondary carnitine deficiency frequently is seen in uremic patients, particularly in those who are on maintenance hemodialysis therapy (13). Carnitine plays a pivotal role in myocardial energy metabolism through (1) transport of long-chain fatty acyl intermediates, across the inner mitochondrial membrane for subsequent oxidation (9), and (2) regulation of carbohydrate metabolism by modulation of the intramitochondrial acetyl-CoA:CoA ratio (14). Thus, carnitine deficiency, a characteristic of cardiac hypertrophy and heart failure (15) may impair both fatty acid and carbohydrate oxidation. Here, we postulated that altered myocardial substrate utilization, in parallel with impaired carnitine metabolism and LVH in the setting of uremia, may contribute to cardiac dysfunction in chronic kidney disease (CKD). Currently, little is known about the role of metabolic remodeling on cardiac energy status and function in CKD. This study aimed to determine the extent of metabolic remodeling in the uremic heart, in parallel with serum and myocardial carnitine concentrations in experimental uremia, and, concurrently, assess their consequences on myocardial function.

Materials and Methods

Model of Chronic Uremia

All animal experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). Chronic uremia was induced in male Sprague-Dawley rats (Charles River, Sussex, UK) that weighed 180 to 230 g, via a two-stage five-sixths nephrectomy model, as de-
sodium pyruvate and 100 glutamine, 0.3 sodium palmitate, 5 glucose, 1 sodium lactate, and 0.1 replaced with one that contained identical substrates and concentra-

bridge amp that was linked to a two-channel MacLab/2e system (AD physiologic pressure transducer (SensoNor, Horten, Norway) and a

recorded.

age of four to five measurements per rat during a 30-min period were

conscious animals by tail plethysmography using an automated non-

weights were recorded weekly. BP was measured at 3 and 6 wk in

only decapsulated and replaced intact.

Intergen, Purchase, NY) and the following components (in mM): 118 Henseleit buffer that contained 3% BSA (essentially fatty acid–free;

isovolumic mode using a water-jacketed oxygenator (16), with Krebs-

buffer. The aorta was cannulated and hearts were perfused in an

were excised and placed immediately in ice-cold Krebs-Henseleit

right kidney was excised via a flank incision. Weight-matched control rats underwent similar procedures, except the kidneys were

only decapsulated and replaced intact.

Forty-eight hours later, rats were housed individually and pair-fed

with the control group. They had free access to water, and body weights were recorded weekly. BP was measured at 3 and 6 wk in

conscious animals by tail plethysmography using an automated non-

monitor (Harvard Apparatus, Eden Bridge, UK). An average of four to five measurements per rat during a 30-min period were

Isolated Heart Perfusions

Rats were studied 3 and 6 wk after surgery. After anesthesia with sodium pentobarbital (100 mg/kg body wt, intraperitoneally), hearts

were excised and placed immediately in ice-cold Krebs-Henseleit buffer. The aorta was cannulated and hearts were perfused in an

isovolumic mode using a water-jacketed oxygenator (16), with Krebs-

Henseleit buffer that contained 3% BSA (essentially fatty acid–free;

Intergen, Purchase, NY) and the following components (in mM): 118 NaCl, 25 NaHCO3, 1.2 KH2PO4, 1.2 MgSO4, 1.25 CaCl2, 0.5 glutamine, 0.3 sodium palmitate, 5 glucose, 1 sodium lactate, and 0.1 sodium pyruvate and 100 μU/ml insulin (Sigma-Aldrich, Poole, UK) and equilibrated with 95% O2/5% CO2 (38°C, pH 7.4) (17).

Contractile function was monitored continuously via a fluid-filled balloon that was inserted into the left ventricle (17) and connected to a

physiologic pressure transducer (SensoNor, Horten, Norway) and a

bridge amp that was linked to a two-channel MacLab/2e system (AD Instruments, Hastings, England). Left ventricular end diastolic pressure was set to 5 mmHg by adjustment of balloon volume. Coronary flow rate was adjusted to maintain a perfusion pressure of 65 mmHg.

After an initial 20-min equilibration period, perfusion medium was

switched to a circulating mode with 30 ml of the same buffer, which

contained 0.1% collagenase (type II; Worthington, Lakeland, NJ) and

0.5% BSA (fraction V, essentially fatty acid–free; Sigma, St. Louis, MO).

Table 1. Characteristics of uremic and control rats at 3 and 6 wk after surgerya

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n = 16)</th>
<th>Uremic (n = 16)</th>
<th>Control (n = 12)</th>
<th>Uremic (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>385.5 ± 8.0</td>
<td>386.7 ± 10.6</td>
<td>483.8 ± 9.8</td>
<td>481.3 ± 11.0</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.8 ± 0.1</td>
<td>2.0 ± 0.1b</td>
<td>2.2 ± 0.04</td>
<td>2.5 ± 0.07b</td>
</tr>
<tr>
<td>Heart weight:body weight x 10^-3 (g)</td>
<td>4.6 ± 0.2</td>
<td>5.3 ± 0.2b</td>
<td>4.6 ± 0.01</td>
<td>5.2 ± 0.02b</td>
</tr>
<tr>
<td>Heart weight:tibia length (g/cm)</td>
<td>0.43 ± 0.02</td>
<td>0.49 ± 0.23b</td>
<td>0.52 ± 0.01</td>
<td>0.57 ± 0.02b</td>
</tr>
<tr>
<td>Left kidney weight (g)</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.9 ± 0.1b</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>137.8 ± 4.4</td>
<td>163.6 ± 5.3c</td>
<td>155.2 ± 5.6</td>
<td>171.3 ± 3.7b</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>89.8 ± 3.2</td>
<td>110.2 ± 4.7c</td>
<td>101.4 ± 3.8</td>
<td>111.8 ± 2.1b</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>105.7 ± 3.6</td>
<td>125.8 ± 4.2c</td>
<td>119.4 ± 4.4</td>
<td>131.6 ± 2.7b</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>33.3 ± 1.3</td>
<td>81.2 ± 7.3d</td>
<td>35.6 ± 1.3</td>
<td>64.8 ± 3.5d</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>5.0 ± 0.2</td>
<td>15.6 ± 0.6d</td>
<td>5.8 ± 0.4</td>
<td>13.4 ± 1.0d</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40.1 ± 0.6</td>
<td>33.7 ± 1.1d</td>
<td>37.1 ± 0.9</td>
<td>36.4 ± 1.5</td>
</tr>
</tbody>
</table>

aDP, diastolic BP; SBP, systolic BP.

bP < 0.05 uremic versus control.

cP < 0.01 uremic versus control.

dP < 0.001 uremic versus control.

13C-NMR Spectroscopy

Cardiac tissue extracts were analyzed by proton decoupled 13C NMR spectroscopy using a JEOL JMN-LA400 FT NMR spectrometer (JEOL, Welwyn Garden City, UK), interfaced with a 9.4 Tesla vertical bore superconducting magnet. 13C spectra were acquired at 101 MHz using a WALTZ decoupling sequence at 25°C, with 24,000 scans (pulse width 3.57 μs, interpulse delay 0.3 s, sweep width 200 ppm). The relative contributions of palmitate, glucose, and lactate to the total acetyl CoA pool that entered the TCA cycle were determined using glutamate isotope analyzer (18) from TCAcalc software developed by Dr. F.M.H. Jeffrey (University of Texas, Southwestern Medical Centre, Dallas, TX).

Isolation of Ventricular Myocytes

Ventricular cardiomyocytes were isolated from 3- and 6-wk control and uremic rats as described previously (20). In brief, after anesthesia, hearts were excised and cannulated for retrograde perfusion. After 10 min of noncirculating perfusion with buffer that contained (in mM) 60 NaCl, 16 KCl, 3.25 MgSO4, 7 H2O, 1.2 KH2PO4, 10 HEPES, 80 mannitol, 10 2,3-butanedione monoxime, 20 taurine, 11 glucose, and 5 sodium pyruvate (pH 7.2), oxygenated with 100% O2, at 37°C, perfusion was
CaCl₂ was added in 5-μl increments to achieve a final concentration of 1 mM Ca²⁺. After 45 to 60 min, ventricles were dissected and agitated gently in 10 ml modified Krebs-Ringer-HEPES buffer that contained (in mM) 120 NaCl, 2.6 KCl, 1.2 MgSO₄, 7 H₂O, 10 HEPES, 1 CaCl₂, 11 glucose, and 2 sodium pyruvate and 3% BSA (pH 7.4) to disperse the cells. The cell suspension was filtered through 25-μm nylon gauze (Miracloth; Calbiochem, La Jolla, CA) and centrifuged for 10 min at 300 rpm. The pelleted myocytes were resuspended in 5 ml of the latter buffer, incubated at 37°C, and gassed with 100% O₂. Photomicroscopic measurements of 40 ventricular cardiomyocytes for each heart were recorded using a Leitz Laborlux S microscope (Leica, Milton Keynes, UK), with a ×40 objective lens connected to a Cool SNAP-PRO camera, with Image-Pro Plus software (Media Cybergenetics, Marlow, UK). Only rod-shaped cardiomyocytes with clear sarcomere striations were analyzed.

Serum Biochemistry and Metabolite Analysis

Blood samples were collected immediately after excision of the hearts. Hematocrit was determined using an ABL 70 blood gas analyzer (Radiometer, Sussex, UK). Serum urea and electrolytes were analyzed using a Beckman Coulter LX20 analyzer (High Wycombe, UK) in the Department of Clinical Chemistry at Hull Royal Infirmary (UK). Serum free (FC) and total carnitine (TC) concentrations were determined at the Department of Clinical Chemistry at Sheffield Children’s Hospital (UK) using a Micromass Quattro LC tandem mass spectrometer. Concentrations of serum glucose, triglycerides, and free fatty acids were determined using spectrophotometric assay kits (no. 510, Sigma; no. 336, Boehringer Mannheim, Lewes, Sussex, UK; no. 1383175, Roche, Mannheim, Germany). Cardiac tissue FC and TC concentrations were analyzed using tandem mass spectrometry at the Department of Clinical Chemistry at Newcastle Royal Victoria Hospital (UK).

Statistical Analyses

Results are expressed as means ± SEM. Statistical significance was determined by unpaired t test and one-way ANOVA with post hoc comparison by Bonferroni test where appropriate. Bivariate correlation analysis was performed by Pearson correlation analysis. P < 0.05 was considered to be statistically significant.

Results

Renal Failure Model

Characteristics of the experimental model are presented in Table 1. Body weights were similar between control and uremic rats at 3 and 6 wk after surgery. However, heart weight, heart weight/body weight, and heart weight/tibia length ratios were increased in uremic rats at both stages of uremia, indicating myocardial hypertrophy. The remnant kidney weights in the uremic group were comparable to the left kidney weights of controls by 3 wk and were significantly heavier by 6 wk (18.8%; P < 0.05), suggesting progressive compensatory hypertrophy of the renal remnant. Diastolic, systolic, and mean arterial pressures were elevated in uremic rats at all times. Both serum creatinine and urea were elevated significantly (P < 0.001) in uremic rats, although the degree of renal dysfunction was more pronounced at the early stage of uremia. Hematocrit was lower in uremic rats (16%; P < 0.001) at 3 wk, although by 6 wk, it was comparable between the control and uremic groups. A significant inverse correlation was observed between serum creatinine and hematocrit in all groups (R = −0.57, P < 0.01; Figure 1).

Cardiomyocyte dimensions of control and uremic groups at 3 and 6 wk after surgery are given in Table 2. These data confirm the presence of cardiac hypertrophy suggested by the morphologic data in Table 1. Cell length increased by 6.7% (P < 0.01) and 5.4% (P < 0.05) and cell width increased by 14.3% (P < 0.01) and 10.6% (P < 0.01) at 3 and 6 wk, respectively, after induction of uremia. Cell length/width ratio remained unaltered. Induction of uremia did not modify serum glucose, triglyceride, or free fatty acid concentrations at 3 or 6 wk (Table 3).

Figure 1. Correlation between serum creatinine and hematocrit (two-tailed Pearson correlation coefficient, R = −0.566, P < 0.01).

Table 2. Cardiomyocyte dimensions of control and uremic groups at 3 and 6 wk after surgery (two hearts per group)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3 Week</th>
<th>6 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 80)</td>
<td>Uremic (n = 80)</td>
</tr>
<tr>
<td>Cell length (μm)</td>
<td>122.7 ± 2.3</td>
<td>130.9 ± 1.9</td>
</tr>
<tr>
<td>Cell width (μm)</td>
<td>19.6 ± 0.5</td>
<td>22.4 ± 0.6</td>
</tr>
<tr>
<td>Cell length: width</td>
<td>6.6 ± 0.2</td>
<td>6.3 ± 0.2</td>
</tr>
</tbody>
</table>

*P < 0.01 uremic versus control.

*P < 0.05 uremic versus control.
surgery are shown in Figure 2. Serum FC concentration (Figure 2a) was reduced significantly in the uremic group at 3 wk (43%; \( P < 0.001 \)) with a concomitant decrease in TC concentration (40%; \( P < 0.01 \)). However, at 6 wk, serum FC concentration remained significantly reduced (18%; \( P < 0.01 \)), whereas the reduction in serum TC did not reach significance (13%; NS). Overall, these changes resulted in an increase in AC/FC ratio in uremia (0.35 versus 0.26 \( P < 0.04 \) and 0.30 versus 0.21 \( P < 0.02 \)) at 3 and 6 wk, respectively. A significant inverse correlation was observed between serum creatinine and serum FC and TC concentrations in all groups, suggesting that the degree of FC and TC depletion may parallel the severity of renal dysfunction (Figure 3). Despite significant serum carnitine deficiency, myocardial FC and TC levels were unaltered in uremia (Figure 2B).

### Table 3. Serum metabolite concentrations in control and uremic groups at 3 and 6 wk after surgery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 16)</th>
<th>Uremic (n = 16)</th>
<th>Control (n = 12)</th>
<th>Uremic (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose (mmol/L)</td>
<td>9.6 ± 0.5</td>
<td>9.2 ± 0.6</td>
<td>10.7 ± 0.6</td>
<td>10.2 ± 0.3</td>
</tr>
<tr>
<td>Serum triglyceride (mmol/L)</td>
<td>0.93 ± 0.14</td>
<td>0.76 ± 0.11</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Serum free fatty acids (mmol/L)</td>
<td>0.14 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>0.09 ± 0.03</td>
</tr>
</tbody>
</table>

Function of Isolated Isovolumic Hearts

Mean heart rate, LVDP, and RPP of hearts from control and uremic rats are shown in Figure 4. Heart rates were similar in all groups. At 3 and 6 wk, LVDP and, consequently, RPP were lower in uremia, although not significantly different. MVO\(_2\) was unchanged at 3 \((23.5 ± 1.7 \text{ mol/min per g dry heart wt})\) and 6 wk \((17.7 ± 0.8 \text{ versus } 16.9 ± 2.9)\) in control versus uremic hearts, respectively. Similarly, cardiac efficiency was comparable between the two groups at both time points \((1.4 ± 0.01 \text{ versus } 1.2 ± 0.01 × 10^3, 3 \text{ wk}; 1.4 ± 0.2 \text{ versus } 1.4 ± 0.2 × 10^3, 6 \text{ wk})\). Therefore, there was no evidence of cardiac dysfunction in this model of uremia.

13C NMR Analysis

Three weeks after induction of uremia, glucose contributed \(16.4 ± 1.5 \text{ versus } 16.7 ± 1.5\%\) (NS) to substrate oxidation, whereas palmitate contributed \(26.3 ± 2.6 \text{ versus } 29.9 ± 2.4\%\) (NS) and lactate contributed \(59.1 ± 1.2 \text{ versus } 52.7 ± 3.3\%\) in control and uremic hearts, respectively (Figure 5a). Unlabeled substrates (exogenous pyruvate, endogenous triglycerides, and glycogen) made up the remaining contribution.

At 6 wk, a small reduction in palmitate use and increase in glucose utilization was observed in the uremic hearts, although these changes were NS (Figure 5b). Unlabeled substrates including lactate contributed \(47.9 ± 4.8 \text{ and } 50.8 ± 1.5\%\) (NS) in control and uremic hearts, respectively. Thus, no change in the profile of substrate utilization was observed at either 3 or 6 wk.

Discussion

This study has demonstrated that renal dysfunction is associated with cardiac hypertrophy, without any alterations in cardiac function or myocardial energy metabolism, in contrast to our original hypothesis. Although there is a significant depletion in serum FC and TC concentration, cardiac tissue carnitine content remains unchanged, which may be sufficient to maintain fatty acid oxidation and thereby prevent early metabolic changes in the hypertrophied uremic myocardium. This is the first study to characterize myocardial energy provision over time in an experimental uremic model.
Model of Uremia

The greater severity of uremia 3 wk after surgery is most probably due to acute tubular necrosis in the renal remnant, resulting from temporary occlusion of renal vasculature during the surgical procedure. Partial or complete recovery from acute tubular necrosis and hypertrophy of the remnant kidney may account for the improvement in renal function by 6 wk. Similar findings of enhanced renal function over time have been made in an in vivo study using the same uremic model (21). Hypertension was evident from the early phase of uremia and increased with progressive exposure to uremia (Table 1). Diastolic and mild systolic hypertension in the 6-wk control group may be due to a sympathetic response from restraining the rats, although any such effect should be consistent between the two groups. BP assessment under anesthesia might resolve this issue but could result in underestimation of the true values.

Reduced hematocrit at 3 wk also may be related to the severity of renal dysfunction. This assumption is supported by the inverse correlation that was observed between serum creatinine and hematocrit (Figure 1). With acute deterioration in renal function, a marked decrease in erythropoietin production can cause anemia. Alternatively, blood loss during surgery may cause anemia at this stage, which is then replaced by erythropoiesis, resulting in an almost complete recovery of anemia by 6 wk.

Cardiac Hypertrophy

LVH is the most frequent cardiac adaptation in CKD, developing early and progressing in prevalence and severity with deterioration in renal function (22). It is evident in >75% of patients at the time of initiation of renal replacement therapy. In our study, a mild (10 to 14%) yet significant hypertrophy was
observed from the early stages of uremia, consistent with previous observations using the same model (6). The myocyte data (Table 2) demonstrate an increase in both cell length and cell width, consistent with eccentric hypertrophy (23). Hypertension per se leads to concentric enlargement of the ventricle, characterized by an increase in cell width (wall thickness) during developing and compensated phases, whereas volume overload leads to a proportional growth in cell length and width (24). Therefore, our findings indicate a developing adaptive hypertrophy secondary to volume overload (Table 2). Comparable body weights between control and uremic groups suggest that volume overload was not overt, but we hypothesize that a degree of volume overload could be present at the cellular level, through an osmotic effect of uremic toxins. Assessment of tissue tonicity would assist in deciphering this.

Although the pathogenesis of LVH in renal disease remains unclear, anemia, together with volume overload and hypertension, is a potential contributory factor (25). Intervention studies have found that correction of anemia with recombinant erythropoietin can reduce left ventricular dimensions (26,27), with partial regression of LVH and left ventricular dilation. In our study, recovery of anemia to near-normal levels by 6 wk was not associated with regression of hypertrophy, highlighting the involvement of other contributory factors in the development of cardiac hypertrophy. Therefore, development of cardiac hypertrophy that was observed in this study arises from a combination of factors, including hypertension, volume overload, and, in the initial phase, anemia.

Uremia and Carnitine

Secondary carnitine deficiency in patients with end-stage renal failure results from reduced dietary intake, impaired synthesis and handling by the kidney, and, most common, from chronic dialytic loss (28). The marked deficiency in serum FC at 3 and 6 wk (Figures 2 and 3) most probably is related to the degree of renal dysfunction, because dietary and dialytic losses of carnitine have not played a role here. Under normal conditions, more than >90% of the filtered carnitine is reabsorbed (29), with FC being preferentially reabsorbed, resulting in four to eight times higher urinary excretion of carnitine esters (30). Thus, a deterioration in renal function is associated with decreased carnitine clearance and impaired excretion of AC. Studies of predialysis uremic patients have found increased levels of FC and TC, with markedly elevated concentrations of AC (31), resulting in elevated AC:FC ratios. However, carnitine metabolism in the early stages of renal failure has not been assessed to date. Our study highlights that in early uremia, both serum FC and TC concentrations decrease in parallel with renal dysfunction (Figure 3), whereas AC:FC ratio increases significantly. Reduced renal biosynthesis may account for the carnitine deficiency in early uremia.

It is of interest that although there is pronounced serum FC and TC depletion in uremia, myocardial carnitine concentrations were unchanged. Carnitine distribution in skeletal and cardiac muscles accounts for 97% of total body carnitine, with 2% in the liver and kidneys and the remainder in extracellular fluid (32). AC:FC ratio is considered normal when it is ≤0.40 (13), as is observed in this study. Thus, prolonged and progressive serum carnitine deficiency may be required before any alterations in myocardial carnitine content occur.

Cardiac Metabolism and Function

The profile of myocardial substrate oxidation was unaltered in uremia despite the presence of cardiac hypertrophy and significant serum carnitine deficiency (Figure 5). At 3 wk, lactate was the predominant substrate for oxidation in all hearts, whereas palmitate and glucose contributed approximately 30 and 16% respectively. Contributions from unlabeled substrates

Figure 4. Mean cardiac functional parameters in control and uremic groups at 3 and 6 wk. (a) Heart rate. (b) Left ventricular developed pressure (LVDP). (c) Rate pressure product (RPP).
(exogenous pyruvate, endogenous triglycerides, or glycogen), were minimal in both groups. At 6 wk, contributions from palmitate and glucose were similar to those at 3 wk in both groups. The remaining contribution (approximately 50%) was from unlabeled substrates. Our previous studies on control animals of the same age demonstrated lactate as the predominant substrate of energy provision, accounting for approximately 60 (33) and 49% (34). Because no alteration in substrate metabolism is observed between control and uremic groups, it is most likely that the majority of the unlabeled substrate that is oxidized by control and uremic hearts at 6 wk is lactate.

Previous studies of the hypertrophied heart have demonstrated a reexpression of the fetal metabolic phenotype, with a downregulation of fatty acid oxidation (8,35) and increase in glucose metabolism (11,12). However, it is unclear whether these metabolic adaptations precede the development and contribution to the severity of hypertrophy. This study indicates that myocardial hypertrophy in uremia can occur without any metabolic alterations. In support of this, recent clinical and experimental studies have demonstrated that the switch in substrate selection occurs primarily in severe end-stage heart failure while remaining normal in mild to moderate hypertrophy (36–38). In addition, maintenance of the metabolic profile that was observed here may result from sufficient tissue concentrations to sustain fatty acid oxidation. It is possible that the extent of metabolic remodeling in uremia may be determined by the combination of the degree of cardiac hypertrophy, carnitine deficiency, and cardiac workload (39). Here, cardiac hypertrophy in uremia was moderate (10 to 14%), serum carnitine deficiency was insufficient to deplete myocardial carnitine concentrations (Figure 2), and cardiac workload was low; therefore, substrate selection remained unaltered. These data suggest that in early uremia, mild cardiac hypertrophy develops without any alteration in cardiac function or metabolic profile.

The absence of significant cardiac contractile dysfunction in our study (Figure 4) was a surprising finding in the face of many clinical studies that have demonstrated prevalent myocardial dysfunction in early and later stages of uremia (40,41). However, many patients with CKD, in addition to having risk factors for heart failure such as LVH, hypertension, and anemia, have a number of comorbidities, including diabetes and ischemic heart disease, which may cause further deleterious effects on the myocardium. Our study used relatively moderate workloads, under which cardiac dysfunction was not apparent (Figure 4). Increasing cardiac workload, via adrenergic stimulation, or increasing perfusion pressure may unmask or exacerbate any underlying cardiac dysfunction. Indeed, Raine et al.
(5) observed impaired cardiac contractility in isolated working hearts only under conditions of increased workload. Similarly, in cardiomyocytes that were isolated from uremic hearts, a depression in contractility and rates of contraction and relaxation were observed only after stimulation with calcium and isoproterenol (42). Extending the period of uremia within the experimental setting may generate a more progressive decline in cardiac function, which would help us to identify the underlying mechanisms of cardiac dysfunction in ureemia, without the presence of confounding variables that are present in the clinical setting.

Conclusion
This study has demonstrated that in early stages of chronic uremia, cardiac hypertrophy develops with no impact on cardiac function or alteration in myocardial metabolism. Maintenance of normal tissue carnitine stores in the setting of significant serum carnitine depletion may sustain myocardial fatty acid oxidation, thereby preventing metabolic remodeling in the early stages of cardiac hypertrophy in uremia.

Acknowledgments
We are grateful to Jenny Foster for invaluable technical support and Dr. Ian Hanning of Hull Royal Infirmary for analyses of serum urea and electrolytes.

Disclosures
None.

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