

## Cardiac effects of carnitine supplementation in experimental uraemia

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### 1. ABSTRACT

Cardiovascular complications are the leading cause of death in patients with chronic kidney disease. The uraemic heart undergoes remodelling and changes in metabolic function. Experimental uraemia produces a reduction in the myocardial energy reserve phosphocreatine in parallel with left ventricular hypertrophy and depletion of serum carnitine. This study investigated the effects of chronic L-carnitine supplementation on myocardial substrate metabolism and function in the experimental uraemia. Experimental uraemia was induced surgically in male Sprague-Dawley rats via a subtotal nephrectomy. Carnitine was administered continuously via subcutaneous mini-osmotic pumps. Cardiac function and substrate oxidation were assessed *in vitro* by means of isovolumic perfusion using <sup>13</sup>C NMR, at 3 and 6 weeks. Uraemic animals exhibited anaemia, kidney dysfunction and systemic carnitine deficiency but no myocardial tissue carnitine deficiency. Myocardial hypertrophy was abolished following carnitine supplementation. This was associated with a reduction in glucose utilisation. In summary carnitine supplementation prevents cardiac hypertrophy, and this effect is amplified with the duration of treatment. This is associated with a reduction in myocardial glucose utilisation but no significant modulation of myocardial function.

### 2. INTRODUCTION

Cardiovascular complications account for 50% of all deaths among patients with chronic kidney disease (CKD) (1, 2). The majority of these deaths are from cardiac causes (3). Renal dysfunction leads to a specific uraemic cardiomyopathy in which there is an enhanced susceptibility to myocardial ischaemia and subsequent morbidity in CKD. Several metabolic derangements accompany uraemia and have been implicated but as yet the precise mechanisms remain ill-defined. Our previous studies have identified a marked reduction in the myocardial energy reserve phosphocreatine (4), together with left ventricular hypertrophy (LVH), and a depletion in serum carnitine in parallel with the degree of renal impairment in experimental uraemia (5).

Carnitine plays a pivotal role in myocardial energy metabolism, as the transporter of long chain fatty acyl intermediates across the inner mitochondrial membrane for beta oxidation (6) and as a key regulator of carbohydrate metabolism by modulation of the intramitochondrial acetyl-CoA/CoA ratio (7). Carnitine deficiency has been associated with cardiomyopathy,

cardiac hypertrophy and heart failure (8, 9). Secondary carnitine deficiency is a frequent observation in uraemic patients, particularly in those on maintenance haemodialysis therapy (10). Thus, the presence of carnitine deficiency in uraemia may exacerbate contractile dysfunction. A positive correlation has been observed between low serum carnitine concentrations and left ventricular ejection fraction (11), intradialytic hypotensive episodes (12) and anaemia (13). Carnitine supplementation in haemodialysis patients has been associated with amelioration of cardiac function (11), reduction in cardiac mass (14, 15) and reduced frequency of intradialytic hypotensive episodes (16). However, many of these studies are small and uncontrolled.

The impact of carnitine supplementation on metabolism and function of the uraemic heart has not been previously assessed. We therefore carried out a series of studies to investigate the metabolic and functional effects of carnitine supplementation in uraemic hearts.

### 3. MATERIALS AND METHODS

#### 3.1. Experimental model of uraemia

All animal experiments conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the UK Animals (Scientific Procedures) Act 1986. Uraemia was induced in male Sprague-Dawley rats (approximately 250g) (Charles River, Sussex, UK), via a one-stage 5/6<sup>th</sup> nephrectomy as described previously (5, 17). Briefly, animals were anaesthetised using a mixture of halothane in oxygen (2.5% in 1Litre), a laparotomy was performed, the left kidney exposed, and approximately two-thirds removed. Subsequently, the right kidney was completely removed. Care was taken to ensure no damage was done to the adrenal glands. Weight matched control animals underwent similar procedures except the kidneys were only decapsulated and replaced intact. Forty-eight hours later, animals were housed individually and pair-fed with the control group. Animals had free access to water and body weights were recorded weekly. Animals were divided into two groups – carnitine treated and untreated.

In the carnitine treated group, mini-osmotic pumps (Azlet, Charles River, UK) containing 10mM L-carnitine in sterile Ringers solution were inserted subcutaneously, immediately following the second surgical procedure, delivering carnitine continuously at a rate of 0.25microL/hour for a maximum period of 4 weeks. For animals treated for 6 weeks, mini-osmotic pumps were replaced after 4 weeks under anaesthesia. Weights of the pumps prior to insertion and following removal, were recorded to ensure the solution had been delivered.

#### 3.2 Blood pressure measurement

Animals were initially acclimatised to restraining cages for 2 days prior to measurement of blood pressure using a tail cuff attached to an automated non-invasive blood monitor (Harvard Apparatus) (4). Four to five measurements were made over a 30 minute period and their mean value was calculated.

#### 3.3. Isolated heart perfusion

Animals were studied 3 and 6 weeks post-surgery. Following anaesthesia with sodium thiopentone (100 mg/Kg body weight, intraperitoneal), hearts were rapidly excised, placed immediately in ice-cold Krebs-Henseleit (K-H) buffer and weighed. The aorta was cannulated and hearts were perfused in an isovolumic Langendorff mode using a water-jacketed oxygenator (18), with K-H buffer containing 3% bovine serum albumin (BSA) (essentially fatty acid free, (Intergen, Purchase, USA)) and the following components (in mM): NaCl 118, NaHCO<sub>3</sub> 25, KH<sub>2</sub>PO<sub>4</sub> 1.2, KCl 4.8, MgSO<sub>4</sub> 1.2, CaCl<sub>2</sub> 1.25, glutamine 0.5, sodium palmitate 0.3, glucose 5, sodium lactate 1, sodium pyruvate 0.1 and, 100microU/ml insulin (Sigma-Aldrich, Poole, UK) and equilibrated with 95%O<sub>2</sub>–5%CO<sub>2</sub> (38 degrees C, pH 7.4) (19).

Contractile function was recorded continuously via a fluid filled balloon (inserted into the left ventricle) and a physiological pressure transducer (SensoNor, Horten, Norway) connected to a bridge amplifier and linked to a two channel MacLab/2e system (AD Instruments, Hastings, England). Left ventricular end diastolic pressure (EDP) was set to 5mmHg and coronary flow rate was adjusted to maintain a perfusion pressure of 65mmHg. Following an initial 20 minute equilibration period with K-H/BSA buffer containing unlabelled substrates, the perfusion medium was switched to an identical one, containing [U-<sup>13</sup>C] palmitate and either [1-<sup>13</sup>C] glucose or [3-<sup>13</sup>C] lactate (Cambridge Isotopes) and perfused for a further 30 minutes as previous described (5). Heart rate (HR) and left ventricular developed pressure (LVDP) were recorded every 10 minutes during normoxia. Coronary effluent samples were collected at 10 minute intervals to determine myocardial oxygen consumption (MVO<sub>2</sub>). As a measure of cardiac work, rate pressure product (RPP) was calculated from the product of HR and LVDP. The ratio RPP/MVO<sub>2</sub> used as an indicator of myocardial efficiency. At the end of the perfusion period, hearts were freeze clamped using Wollenberger tongs and extracted with 6% perchloric acid for carbon<sup>13</sup> nuclear magnetic resonance (<sup>13</sup>C NMR analysis) (20).

#### 3.4. <sup>13</sup>C- NMR spectroscopy

Cardiac tissue extracts were analysed by proton decoupled <sup>13</sup>C NMR spectroscopy using a JEOL JMN-LA400 FT NMR spectrometer, interfaced with an 11.7 Tesla ultra-shielded superconducting vertical wide bore Bruker magnet and 5mm broadband probe interfaced with a Bruker spectrophotometer. <sup>13</sup>C spectra were acquired at 101MHz using a WALTZ decoupling sequence at 25 degrees C, with 24000 scans (pulse width 3.57microsec, inter-pulse delay 0.3sec, sweep width 200ppm). Relative contributions of palmitate and glucose to the total acetyl CoA pool entering the tri-carboxylic acid (TCA) cycle and substrate oxidation were determined using glutamate isotopomer analysis from TCAcalc software developed by Dr FMH Jeffrey (University of Texas, South-western Medical centre, TX) (21).

#### 3.5. Serum biochemistry and metabolite analysis

Blood samples were collected immediately from the chest cavity into heparinised syringes following

**Table 1.** Biochemical characteristics in control and uraemic animals exposed to carnitine therapy at 3 and 6 weeks

Duration	Group	Number	Serum creatinine(micromol/L)	Serum urea(mmol/L)	Haematocrit(%)
3 week	Control	Untreated (n=22)	33.6+/-1.1	5.1+/-0.2	40.1+/-0.6
		Carnitine treated (n=11)	31.0+/-1.7	6.2+/-0.4	38.5+/-1.4
	Uraemic	Untreated (n=21)	79.8+/-5.6***	15.7+/-0.5***	33.7+/-1.0***
		Carnitine treated n=11	67.5+/-4.0***	15.6+/-1.1***	34.7+/-0.9*
6 week	Control	Untreated (n=12)	37.7+/-2.3	5.8+/-0.3	36.0+/-1.3
		Carnitine treated (n=11)	32.3+/-1.6	5.9+/-0.3	36.3+/-0.8
	Uraemic	Untreated (n=12)	64.8+/-3.5***	13.4+/-1.0***	36.4+/-1.5
		Carnitine treated (n=11)	64.8+/-2.8***	14.9+/-1.1***	33.2+/-0.8*

\*p value less than 0.05/\*\*p less than 0.001 carnitine-treated uraemic or untreated uraemic groups at three or six weeks compared to their corresponding carnitine-treated or untreated control groups

excision of the hearts. Haematocrit was determined using an ABL 70 blood gas analyser (Radiometer, Sussex, UK). Serum was removed and stored at -20 degrees C for metabolite analysis. Serum urea and electrolytes were analysed using a Beckman Coulter LX20 analyser, (High Wycombe, UK) in the Department of Clinical Chemistry at Hull Royal Infirmary, Hull and East Yorkshire Hospitals National Health Service Trust. (UK). Concentrations of serum glucose, triglycerides (TG) and free fatty acids (FFA) were determined using spectrophotometric assay kits (Sigma and Boehringer Mannheim, Number 510, Number 336; Roche Number 1383175).

### 3.6. Serum and myocardial carnitine analysis

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. Cardiac tissue FC and TC concentrations were analysed by tandem mass spectrometry, at the Department of Clinical Chemistry at Newcastle Royal Victoria Hospital (UK).

### 3.7. Isolation of cardiomyocytes

Ventricular cardiomyocytes were isolated from control and uraemic animals as previously described (5). Photomicroscopic measurements of 40 ventricular cardiomyocytes for each heart were made using a Leitz Laborlux S microscope (Leica, UK), with a x40 objective lens connected to a Cool SNAP-PRO camera, with Image-Pro Plus software (Media Cybergenetics, Maryland, USA). Only rod shaped cardiomyocytes with clear sarcomere striations were analysed.

### 3.8. Statistical analysis

Results are expressed as mean +/- standard error of the mean (SEM). Statistical significance was determined using an unpaired *t* test (for single mean comparisons) or one-way analysis of variance (ANOVA) with post hoc comparison by Bonferroni test where appropriate. Bivariate correlation analysis was performed by Pearson correlation analysis. A *p* value of less than 0.05 was considered to be statistically significant.

## 4. RESULTS

The metabolic and functional consequences carnitine supplementation was compared in both normal control and uraemic animals.

### 4.1. Characterisation of the experimental model

Characteristics of the control and uraemic untreated and carnitine treated groups are highlighted in Table 1. Biochemical markers of renal function (serum creatinine and urea) were elevated in the experimental uraemic groups, whilst haematocrit was significantly lower.

### 4.2. Effect of carnitine treatment on serum and tissue carnitine concentrations

Following 3 weeks of carnitine supplementation, serum FC increased (from 44.4+/-3.5 to 94.1+/-10.1 micromol/L, *p* value less than 0.001 and from 25.3+/-1.7 to 69.4+/-5.9 micromol/L, *p* value less than 0.001) in control and uraemic groups respectively. There was a smaller increase in control (from 51.7+/-2.2 to 88.1+/-3.9 micromol/L, *p* value less than 0.05) and uraemic groups (from 42.2+/-2.4 to 74.9+/-14.2 micromol/L, *p* value less than 0.05) (Figure 1) after 6 weeks of carnitine administration. In contrast, carnitine supplementation had little impact on myocardial tissue carnitine concentrations of free and acyl carnitine (Table 2).

### 4.3. Effect of carnitine treatment on blood pressure and cardiac hypertrophy

There was evidence of both systolic and diastolic hypertension in the uraemic groups at both stages (Figure 2). However, carnitine supplementation had no impact on blood pressure in either control or uremic groups at any stage in comparison to their respective controls (Table 3).

In untreated groups, wet heart weight (WHW), WHW: body weight (BW), and WHW: tibia length (TL) ratios were increased in uraemic versus control groups at both stages, indicating myocardial hypertrophy (Table 4). Following carnitine supplementation, this hypertrophy was abolished after 3 weeks of treatment and the effect was cumulative, with a more marked reduction in hypertrophy after 6 weeks (WHW 16%, *p* value less than 0.01; WHW: BW 9.6%, *p* value less than 0.05; WHW: TL 12.3%, *p* value less than 0.05) in the treated uraemic hearts relative to untreated.

### 4.4. Effect of carnitine treatment on surrogate markers of renal function, anaemia and serum metabolites

Carnitine supplementation had little effect on serum creatinine, urea or haematocrit in any of the groups (Table 1). In contrast, serum glucose concentration was significantly lower in the 3 week control (23%, *p* value less than 0.01) and 6 week uraemic (16%, *p* value less than 0.01) treated groups compared to corresponding untreated

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**Table 2.** Myocardial carnitine concentrations in control and uraemic animals exposed to carnitine therapy at 3 and 6 weeks

Duration	Group	Number	Free Carnitine(micromol/g wet wt)	Acyl Carnitine(micromol/g wet weight)
3 week	Control	untreated (n=6)	0.53+/-0.02	0.27+/-0.01
		Carnitine treated (n=10)	0.49+/-0.04	0.32+/-0.02
	Uraemic	untreated (n=6)	0.43+/-0.05	0.23+/-0.02
		carnitine treated (n=10)	0.40+/-0.02*	0.22+/-0.03*
6 week	Control	untreated (n=6)	0.52+/-0.07	0.24+/-0.02
		carnitine treated (n=9)	0.52+/-0.03	0.25+/-0.04
	Uraemic	untreated (n=6)	0.44+/-0.04	0.26+/-0.01
		carnitine treated (n=10)	0.40+/-0.03*	0.23+/-0.03

\*p value less than 0.05 carnitine-treated uraemic animals compared to carnitine-treated control animals

**Table 3.** Non invasive Blood pressure measurements in control and uraemic animals exposed to carnitine therapy at 3 weeks

Duration	Group	Number	Systolic pressure(mmHg)	Diastolic pressure(mmHg)	Mean arterial pressure(mmHg)
3 week	Control	Untreated (n=9)	137.8+/-4.4	89.8+/-3.2	105.7+/-3.6
		Carnitine treated (n=4)	132.7+/-6.2	92.3+/-3.3	106.8+/-4.9
	Uraemic	Untreated (n=11)	163.6+/-53**	110.2+/-4.7**	125.8+/-4.2**
		Carnitine treated (n=4)	171.1+/-9.3**	109.4+/-5.2**	130.0+/-4.1**

\*\*p value less than 0.01, uraemic versus corresponding control animal

**Table 4.** Indices of myocardial hypertrophy in control and uraemic animals exposed to carnitine therapy at 3 and 6 weeks

Duration	Group	Number	WHW (g)	WHW:BW (g/kg)	WHW:TL (g/cm)
3 week	Control	untreated (n=16)	1.8+/-0.1	4.6+/-0.2	0.43+/-0.02
		carnitine treated (n=11)	1.9+/-0.1	4.8+/-0.1	0.47+/-0.01
	Uraemic	untreated (n=16)	2.0+/-0.1*	5.3+/-0.2*	0.49+/-0.02*
		carnitine treated (n=11)	1.9+/-0.1	5.0+/-0.02	0.43+/-0.02†
6 week	Control	untreated (n=12)	2.2+/-0.06	4.6+/-0.01	0.52+/-0.01
		carnitine treated (n=12)	2.0+/-0.05†	4.5+/-0.1	0.48+/-0.01
	Uraemic	untreated (n=12)	2.5+/-0.1*	5.2+/-0.02*	0.57+/-0.02*
		carnitine treated (n=12)	2.1+/-0.1††	4.7+/-0.1 †	0.50+/-0.01†

Wet heart weight (WHW), WHW: body weight (WHW: BW) and WHW: tibia length (TL). \*p value less than 0.05 Uraemic untreated group compared to corresponding untreated control group at three or six weeks respectively. †p value less than 0.05/††p value less than 0.01 carnitine-treated groups compared to corresponding untreated groups at three or six weeks respectively

**Table 5.** Serum metabolite concentrations in control and uraemic animals exposed to carnitine therapy at 3 and 6 weeks

Duration	Group	Number	Glucose(mmol/L)	Triglyceride(mmol/L)	Free fatty acids(mmol/L)
3 week	Control	Untreated (n=6)	9.6+/-0.5	0.93+/-0.14	0.14+/-0.02
		carnitine treated (n=10)	7.4+/-0.3††	1.1+/-0.2	0.10+/-0.01
	Uraemic	Untreated (n=6)	9.2+/-0.6	0.76+/-0.11	0.11+/-0.02
		carnitine treated (n=10)	8.4+/-0.7	0.43+/-0.1*	0.08+/-0.01**
6 week	Control	Untreated (n=6)	10.7+/-0.6	1.2+/-0.1	0.11+/-0.02
		carnitine treated (n=9)	9.4+/-0.3	1.0+/-0.2	0.11+/-0.03
	Uraemic	Untreated (n=6)	10.2+/-0.3	1.0+/-0.2	0.11+/-0.02
		carnitine treated (n=10)	8.6+/-0.2*†	0.72+/-0.14†	0.10+/-0.02

\*p value less than 0.05 / \*\* p value less than 0.01 carnitine-treated uraemic group compared to corresponding carnitine-treated control group. †p value less than 0.05 / †† p value less than 0.01 carnitine-treated group compared to corresponding untreated group

groups (Table 4). Serum triglyceride (TG) concentrations were also significantly reduced in the treated uraemic group after 6 weeks (45%, p value less than 0.05). Consequently, a marked difference was evident in TG levels between carnitine-treated control and uraemic groups at 3 weeks (1.1+/-0.2 versus 0.43+/-0.1, p value less than 0.05). Although a significant difference was observed in serum free fatty acid (FFA) concentrations between treated control and uraemic groups at 3 weeks, there was no difference between these and their corresponding untreated groups (Table 5).

### 4.5. Effect of carnitine treatment on myocardial function and metabolism

Three weeks of carnitine supplementation had little impact on the haemodynamic parameters of isolated

perfused hearts (Table 6). However, in the 6 week control group, the rate pressure product (RPP) was significantly enhanced (32.6%, p value less than 0.05) following treatment. This was associated with a small increase in left ventricular developed pressure (LVDP) (12.3%, p value not significant) and heart rate (HR) (17.1%, p value not significant). In contrast, HR was unaffected in the treated uraemic group at 6 weeks. In consequence, RPP was significantly lower in the treated uraemic group relative to the treated control group at 6 weeks (16.8%, p value less than 0.05).

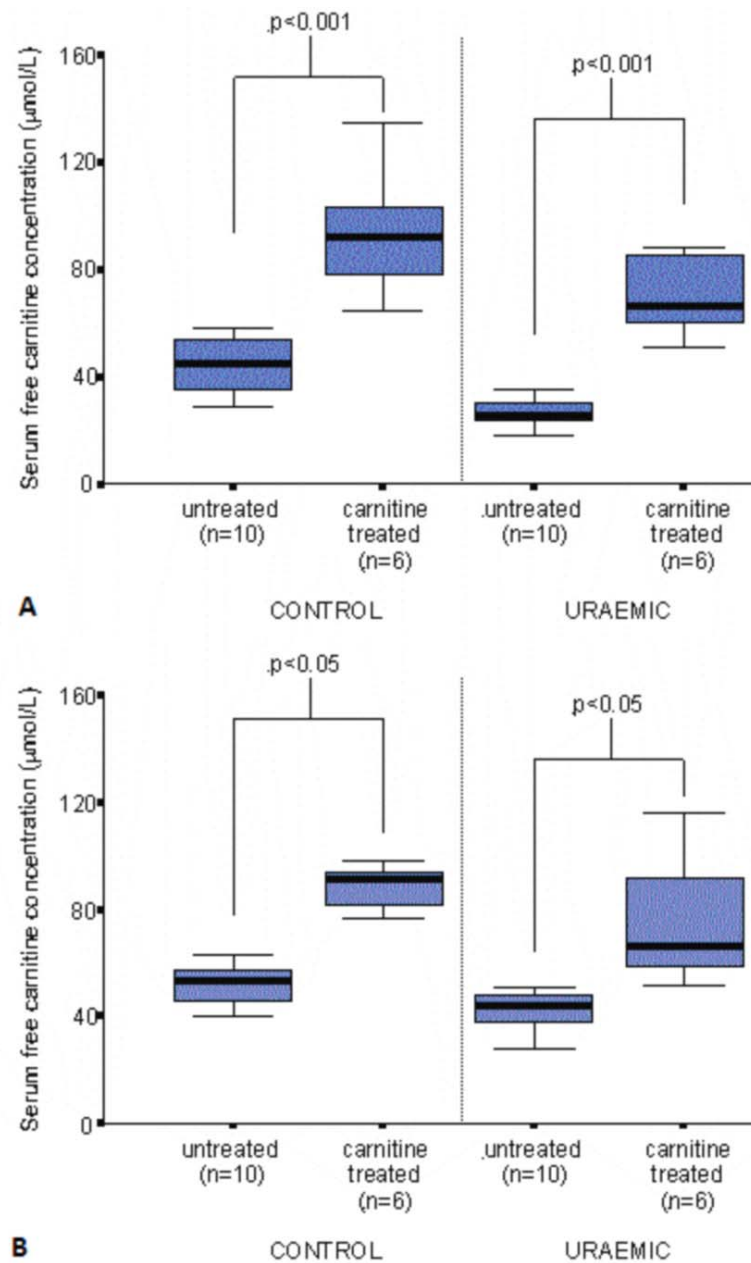
Following 3 weeks of treatment, myocardial oxygen consumption (MVO<sub>2</sub>) was unaffected in either group (23.5+/-1.6 versus 22.8+/-1.3 and 21.6+/-1.8 versus 24.8+/-2.9; untreated versus treated, control and uraemic

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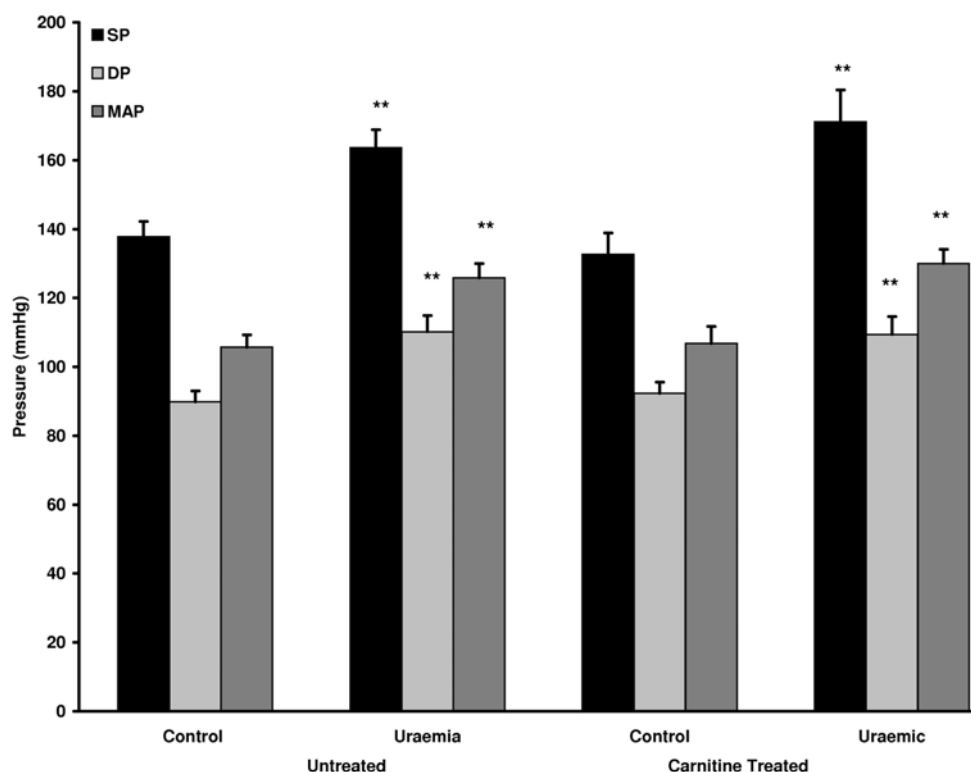
**Table 6.** Effect of carnitine on myocardial functional parameters in control and uraemic animals exposed to carnitine therapy at 3 and 6 weeks

Duration	Group	Number	LVDP (mmHg)	HR (bpm)	RPP x103 (mmHg.bpm)
3 week	Control	untreated (n=16)	110.8+/-6.7	277.7+/-9.0	31.2+/-2.6
		carnitine treated (n=11)	102.7+/-4.7	295.6+/-6.5	30.4+/-1.6
	Uraemic	untreated (n=16)	95.8+/-6.3	261.0+/-15.0	25.4+/-2.6
		carnitine treated (n=11)	93.4+/-6.6	287.0+/-13.0	26.7+/-1.9
6 week	Control	untreated (n=6)	96.4+/-8.3	252.5+/-29.1	24.2+/-3.5
		carnitine treated (n=10)	108.3+/-4.7	295.7+/-6.2	32.1+/-1.6†
	Uraemic	untreated (n=6)	88.8+/-6.1	252.1+/-34.6	21.6+/-2.4
		carnitine treated (n=12)	109.3+/-7.4	247.9+/-13.4*	26.7+/-1.9*

\*p value less than 0.05 carnitine-treated uraemia group compared to corresponding carnitine-treated control group. †p value less than 0.05 carnitine-treated group compared to corresponding untreated group



**Figure 1.** Effect of carnitine supplementation on serum carnitine concentrations at (a) 3 weeks and (b) 6 weeks post-surgery. Vertical bars represent minimum and maximum values; horizontal bar represents median value.



**Figure 2.** Non-invasive Blood pressure measurements at 3 week untreated control (n=9) and uraemic (n=11) untreated animals and carnitine treated control (n=4) and uraemic (n=4) animals. Data represent mean  $\pm$  SEM. (\*\* p value less than 0.01 uraemic versus corresponding control animal). SP= Systolic blood pressure; DP= diastolic blood pressure; MAP= mean arterial pressure.

groups respectively), but increased after 6 weeks treatment ( $17.7 \pm 0.8$  versus  $23.7 \pm 1.0$ , p value less than 0.01; control;  $16.8 \pm 2.9$  versus  $23.6 \pm 1.9$ , p value less than 0.01; uraemic). In consequence, cardiac efficiency was not altered by carnitine supplementation at any stage of the experiments (3 weeks:  $1.4 \pm 0.2$  versus  $1.4 \pm 0.1$  and  $12 \pm 0.1$  versus  $1.2 \pm 0.2$ ; 6 weeks:  $1.4 \pm 0.2$  versus  $1.4 \pm 0.1$  and  $1.4 \pm 0.2$  versus  $1.2 \pm 0.1$  in untreated versus carnitine treated control and uraemic groups respectively).

Myocardial substrate selection was comparable between treated control and uraemic groups at all stages of treatment (Figure 3). After 3 weeks, glucose contributed approximately 16% whilst palmitate contributed  $26.3 \pm 2.6$  versus  $22.3 \pm 1.9\%$  (not significant) and  $29.9 \pm 2.4$  versus  $32.8 \pm 7.1\%$  (not significant) in untreated versus treated control and uraemic groups respectively. After 6 weeks, the contribution from glucose was significantly lower in treated groups ( $19.0 \pm 1.5$  versus  $11.2 \pm 2.2$  control;  $23.4 \pm 2.8$  versus  $14.0 \pm 1.0$  uraemic) versus untreated groups, whilst palmitate contribution was constant. Unlabelled substrates (lactate, pyruvate and endogenous glycogen) made a significant contribution to acetyl CoA production in all groups.

#### 4.6. Effect of carnitine treatment on cardiomyocyte morphology

Uraemia led to an increase in cell length and width measurements in comparison to controls at both 3

and 6 weeks. At 3 weeks the increase in cell length in untreated uraemic cardiomyocytes was no longer present following carnitine supplementation. However, a significant increase in cell width (11.6%, p value less than 0.05) was still evident. After six weeks cell width was reduced in uraemic carnitine treated animals in comparison to untreated uraemic animals (Table 7).

## 5. DISCUSSION

This study has demonstrated that carnitine supplementation can limit the development of myocardial hypertrophy in experimental uraemia. This effect is amplified progressively with continued carnitine treatment. In addition, prolonged chronic carnitine therapy enhances myocardial function in control hearts, an effect that is less prominent in uraemia, but without significant impact in myocardial substrate metabolism.

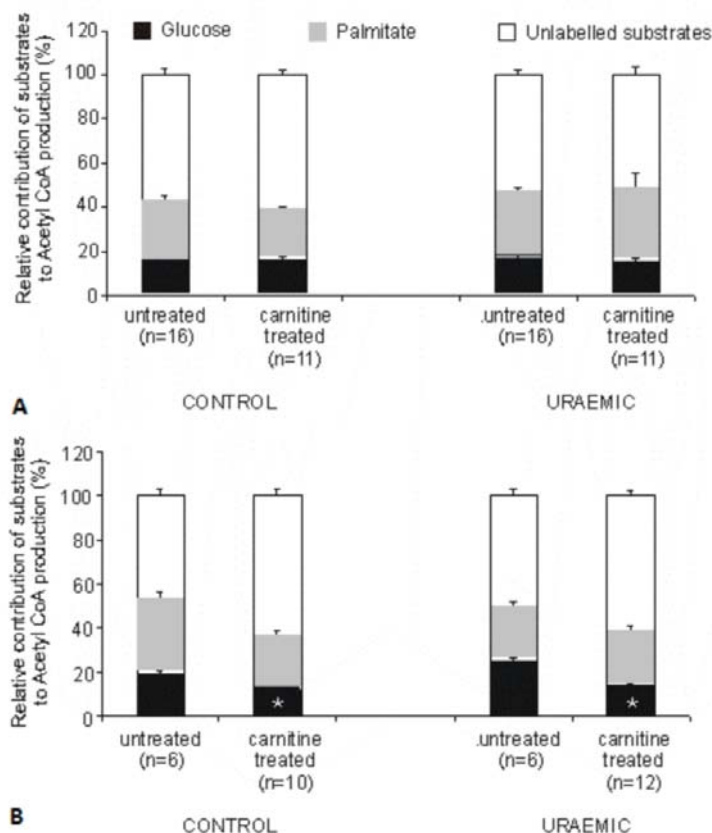
### 5.1. Serum and tissue carnitine

Increases in serum FC concentrations in all groups following carnitine supplementation indicate adequate systemic delivery of carnitine (Table 2). Despite the marked rise in serum carnitine concentration, the lack of amplification of myocardial carnitine content was intriguing. We hypothesise that this may be a consequence of limited carnitine transport into the myocardium. Carnitine is transported against a concentration gradient, via a sodium-dependent transporter (OCTN 2) (22). This

**Table 7.** Cardiomyocyte morphoogy in control and uraemic animals exposed to carnitine therapy at 3 and 6 weeks. \*

Duration	Group	Number	Cell length(um)	Cell width(um)	Cell length/width ratio
3 week	Control	untreated (n=40)	122.7 $\pm$ 2.3	19.6 $\pm$ 0.5	6.6 $\pm$ 0.2
		carnitine treated (n=40)	128.1 $\pm$ 2.6	21.5 $\pm$ 0.8	6.4 $\pm$ 0.2
	Uraemic	untreated (n=40)	130.9 $\pm$ 1.9**	22.4 $\pm$ 0.6**	6.3 $\pm$ 0.2
		carnitine treated (n=40)	124.7 $\pm$ 3.2	24.0 $\pm$ 0.7*	5.4 $\pm$ 0.2**
6 week	Control	untreated (n=40)	121.7 $\pm$ 2.0	23.6 $\pm$ 0.6	5.5 $\pm$ 0.2
		carnitine treated (n=40)	123.3 $\pm$ 1.5	22.4 $\pm$ 0.4	5.8 $\pm$ 0.1
	Uraemic	untreated (n=40)	128.3 $\pm$ 1.8*	26.1 $\pm$ 0.6**	5.3 $\pm$ 0.1
		carnitine treated (n=40)	130.1 $\pm$ 1.5**	24.1 $\pm$ 0.5**	5.7 $\pm$ 0.1*

\*p value less than 0.05. \*\*p value less than 0.01 versus corresponding control



**Figure 3.** Contribution of glucose, palmitate and unlabelled substrates to substrate oxidation in untreated and carnitine treated groups at (a) 3 weeks (n = 16 for untreated groups & n = 11 for carnitine treated groups); and (b) 6 weeks (n = 6 for untreated groups & n = 10 and 12 for carnitine treated control and uraemia groups respectively) post-surgery. \*p value less than 0.05 compared to corresponding untreated group.

transport system may become saturated with supraphysiological concentrations of carnitine. Alternatively, transporter expression and activity may be related to tissue carnitine stores and, as these were sufficient in all groups, transporter activity may remain unchanged despite the increased serum carnitine content. Recent evidence indicates a down-regulation of this carnitine transporter in the renal brush border membrane in the presence of high levels of carnitine (23). Thus a similar mechanism may explain the constancy of myocardial tissue carnitine concentration observed here. Experimental studies employing propionyl-L-carnitine treatment have resulted in enhanced myocardial tissue carnitine levels in both hypertrophied (24, 25) and diabetic (26) hearts.

However, the L-propionyl derivative is taken up more readily by myocytes than L-carnitine (27).

## 5.2. Cardiac hypertrophy

Prevention of cardiac hypertrophy in the treated uraemic groups indicates a potential contributory role for serum carnitine deficiency in the pathogenesis of LVH in uraemia. Increasing serum carnitine concentrations appears to prevent or limit the development of cardiac hypertrophy. Broderick *et al* (28) observed significant cardiac hypertrophy following induction of serum and tissue carnitine deficiency with sodium pivalate, whilst treatment with propionyl-L-carnitine resulted in regression of cardiac hypertrophy in diabetic rats (26). Clinical studies also

imply a beneficial role of carnitine in patients with LVH. A significant inverse correlation between serum FC concentration and the cardiothoracic ratio (indicating cardiac mass) has been observed in haemodialysis patients (29), whilst a reduction in cardiac mass following chronic carnitine therapy has been confirmed in a similar population by magnetic resonance imaging (14). More recently, in a randomised control study L-carnitine supplementation for 12 months resulted in significant regression of LVH in the haemodialysis population (15). These studies support the current experimental study that chronic carnitine supplementation may prevent/ limit or even reverse the development of LVH in uraemia.

The mechanism(s) underlying this beneficial effect carnitine on LVH are not clear. It is interesting that that carnitine exerted this protective effect in the absence of enhanced myocardial carnitine stores, implying an indirect mechanism of action.

Development of LVH in uraemia is mostly attributed to haemodynamic overload caused by hypertension, anaemia and extracellular volume expansion. In this study, neither blood pressure nor haematocrit were influenced by carnitine supplementation and thus could not be attributed to the underlying mechanism. Left ventricular mass can also increase from remodelling to compensate for reduced myocardial contractility. For example, when myocardial contractility becomes insufficient for general circulatory requirements, myocardial stretch enhances contractility (Frank –Starling's law) and in this process, left ventricular dilatation occurs, and the wall stress is distributed by myocardial hypertrophy. The degree of LVH in haemodialysis (HD) patients has been shown to correlate with the severity of left ventricular dilatation and with cardiac contractile failure (31). In this study, there is evidence of mild impairment of cardiac contractility in uraemia, with some improvement following carnitine treatment. This beneficial effect may at least in part explain the prevention of LVH in uraemia. Further studies are required to determine the mechanisms involved in the prevention of LVH by carnitine in uraemia.

### 5.3. Renal function, anaemia and serum metabolites

Consistent with previous reports (31) carnitine had no detrimental effect on surrogate markers of renal function over the 6 week period (Table 1). Although serum creatinine is not a precise measure of renal function, in this setting it provides a reasonable means of comparison between groups. One clinical study has shown a reduction in pre-dialysis serum urea and creatinine concentrations within two months of treatment with intravenous L-carnitine (16). The authors speculated that this effect may be due to decreased protein catabolism and/ or enhanced protein synthesis.

Carnitine treatment had little impact on anaemia of uraemia at 3 or 6 weeks and this is consistent with the findings by Nilsson-Ehle *et al* (32) who observed no effect of carnitine on anaemia in haemodialysis patients following 6 weeks of treatment. In contrast Travato *et al* (33) reported

a significant increase in haematocrit (26% to 37%) after 12 months treatment with L-carnitine, whilst more recent studies report a reduction in the dose of erythropoietin required to maintain adequate haemoglobin levels (34), an inverse correlation between erythropoietin dose and serum carnitine concentration (35), and a direct correlation between haemoglobin and serum carnitine levels (13) in patients supplemented with carnitine for 4 to 6 months. It is perhaps likely that prolonged treatment with carnitine is necessary for its beneficial effects on anaemia.

Carnitine therapy here was associated with a significant reduction in serum glucose and TG concentrations, whilst serum FFA levels were unaffected (Table 5). Previous clinical studies show conflicting effects of carnitine on serum TG concentrations in dialysis patients (36, 37), whilst, in experimental uraemia, TG levels were reduced following 4 weeks of carnitine therapy (38). Carnitine may lower TG concentrations by increasing uptake and utilisation of FFA, thereby promoting conversion of TG to FFA. Although myocardial fatty acid utilisation was not enhanced by carnitine in this study, uptake and utilisation of FFA by other tissues such as skeletal muscle may be increased, and consequently lower serum TGs. Insulin resistance is increasingly seen in chronic uraemia (39, 40), which can also contribute to hypertriglyceridaemia (41). It is feasible that carnitine may modify insulin sensitivity, which may account for the decrease in glucose and triglyceride concentrations. Further studies to assess insulin levels in the presence and absence of carnitine supplementation would assist with this.

### 5.4. Myocardial function and metabolism

Prolonged carnitine therapy (6 weeks) augmented myocardial function in control hearts (Table 6). Although the impact is less marked in uraemia, it may be further improved by extending the period of carnitine supplementation. In support of this, clinical studies have highlighted enhancement of cardiac function with L-carnitine treatment for 6 to 8 months in haemodialysis patients (14, 42), whilst shorter treatment times (1 to 2 months) have had no effect (43).

The mechanisms underlying the positive inotropic effect of carnitine are poorly understood. LV abnormalities are highly predictive of future cardiac failure and regression of LV mass is strongly associated with subsequent reduced likelihood of cardiac failure in haemodialysis patients (44). Thus, prevention of cardiac hypertrophy in the 6 week treated uraemic group and reduction in cardiac mass in the 6 week control group may contribute to the positive inotropic effects observed in these groups. Carnitine deficiency and subsequent lipid peroxidation can be considered as contributory factors to cardiac dysfunction in CKD (45). Lipid peroxidation, mediated by free oxygen radicals is an important cause of damage to cell membranes, with consequent disruption of membrane integrity. Chronic carnitine supplementation in experimental uraemia can ameliorate oxidative organ injury, potentially via its free radical scavenging and antioxidant properties (46). This in turn may enhance cardiac function.



The most salient effect of carnitine supplementation on myocardial metabolism was a reduction in glucose utilisation at 6 weeks in both groups. Determinants of glucose uptake include substrate supply and hormonal milieu (47), which were comparable between the treated and untreated perfused hearts. Thus, the observed decrease in glucose utilisation with carnitine treatment is likely to be related to glucose metabolism rather than its uptake. In contrast to these observations, an increase in fatty acid metabolism has been reported in healthy adults (48) and haemodialysis patients (43) following L-carnitine supplementation. The contrasting findings between clinical and experimental studies suggest that the metabolic effects of chronic carnitine treatment may vary depending on the model and length of treatment.

## 6. CONCLUSIONS

This study has demonstrated that chronic carnitine supplementation prevents cardiac hypertrophy in experimental uraemia, and this effect is more marked with the duration of treatment. This is associated with a significant reduction in myocardial glucose utilisation and a moderate enhancement of cardiac function. The mechanisms underlying the prevention of myocardial hypertrophy remain unclear. With prolonged treatment, this beneficial effect may relate to improvement of anaemia, although this does not play a role in the early stages of uraemia.

## 7. LIMITATIONS

Firstly vehicle pumps were not utilised in the control groups. Secondly, we administered a relatively low dose of carnitine, and perhaps dose-dependent studies may be useful in further assessing the effects of carnitine on many of the studied parameters. Finally, further studies with assessment of insulin levels would be useful to corroborate our theory that carnitine may modify insulin sensitivity and therefore account for the reduced plasma glucose concentrations following carnitine treatment.

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