L-Carnitine Attenuates Cardiac Remodelling rather than Vascular Remodelling in Deoxycorticosterone Acetate-Salt Hypertensive Rats

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Abstract: L-Carnitine is an important co-factor in fatty acid metabolism by mitochondria. This study has determined whether oral administration of L-carnitine prevents remodelling and the development of impaired cardiovascular function in deoxycorticosterone acetate (DOCA)-salt hypertensive rats (n = 6–12; #$p < 0.05$ *versus* DOCA-salt). Uninephrectomized rats administered DOCA (25 mg every 4th day s.c.) and 1% NaCl in drinking water for 28 days developed cardiovascular remodelling shown as systolic hypertension, left ventricular hypertrophy, increased thoracic aortic and left ventricular wall thickness, increased left ventricular inflammatory cell infiltration together with increased interstitial collagen and increased passive diastolic stiffness and vascular dysfunction with increased plasma malondialdehyde concentrations. Treatment with L-carnitine (1.2% in food; 0.9 mg/g/day in DOCA-salt rats) decreased blood pressure (DOCA-salt 169 ± 2; + L-carnitine 148 ± 6# mmHg), decreased left ventricular wet weights (DOCA-salt 3.02 ± 0.07; + L-carnitine 2.72 ± 0.06# mg/g body-wt), decreased inflammatory cells in the replacement fibrotic areas, reduced left ventricular interstitial collagen content (DOCA-salt 14.4 ± 0.2; + L-carnitine 8.7 ± 0.5# % area), reduced diastolic stiffness constant (DOCA-salt 26.9 ± 0.5; + L-carnitine 23.8 ± 0.5# dimensionless) and decreased plasma malondialdehyde concentrations (DOCA-salt 26.9 ± 0.8; + L-carnitine 21.2 ± 0.4# μmol/l) without preventing endothelial dysfunction. L-carnitine attenuated the cardiac remodelling and improved cardiac function in DOCA-salt hypertension but produced minimal changes in aortic wall thickness and vascular function. This study suggests that the mitochondrial respiratory chain is a significant source of reactive oxygen species in the heart but less so in the vasculature in DOCA-salt rats, underlying the relatively selective cardiac responses to L-carnitine treatment.

Mitochondrial oxidative catabolism of fatty acids is a major energy source for the adult mammalian heart although elevated serum fatty acid concentrations and enhanced fatty acid use have been implicated in the pathogenesis of heart failure [1]. The naturally occurring amino acid, L-carnitine, plays a major role, as a cofactor, in the transportation of free fatty acids from the cytosol to the mitochondria, their degradation to acyl-CoA by β-oxidation and their subsequent entry into the tricarboxylic acid cycle to produce adenosine triphosphate through oxidative phosphorylation [2]. Furthermore, L-carnitine regulates carbohydrate metabolism in the mitochondria by modulating the acetyl-CoA:CoA ratio. L-carnitine, produced primarily in the liver and kidneys from L-lysine and L-methionine, is found in high concentrations in animal skeletal and cardiac muscles with red meat and dairy products as the most common dietary sources. Decreased L-carnitine concentrations will lead to dysfunction of the mitochondrial respiratory chain, which can further cause cardiovascular disease [3]. L-Carnitine treatment restored cardiac L-carnitine concentrations and improved heart recovery after ischaemia and reperfusion through counteracting the toxic effects of high concentrations of free fatty acids, and also through improving carbohydrate metabolism [4].

Further, L-carnitine treatment improved cardiovascular parameters in patients with secondary carnitine deficiency following haemodialysis for kidney disease [5].

Damage to the heart and vascular system is associated with increased production of reactive oxygen species such as superoxide and hydrogen peroxide producing hypertension, endothelial damage, cardiac hypertrophy, inflammation, atherosclerosis, ventricular contractile dysfunction and fibrosis [6]. Rat models of mineralocorticoid hypertension such as the deoxycorticosterone acetate (DOCA)-salt or aldosterone-salt hypertensive rat [7] produced similar results, following endothelin-induced increases in vascular and cardiac superoxide production by NADPH oxidase [8–10]. Selective ET_A receptor antagonists such as A-127722 prevented and reversed the cardiovascular remodelling and improved function [11]. If the mitochondrial respiratory chain is also a major source of reactive oxygen species in this model leading to cardiovascular remodelling, then normalization of the function of the chain should be effective in preventing cardiovascular damage.

The aim of this project was to determine whether oral administration of L-carnitine to DOCA-salt hypertensive rats prevents or attenuates the development of structural and functional changes in the heart and blood vessels. Structural changes were characterized by histology while heart function was measured *ex vivo* in isolated perfused hearts. Isolated thoracic aortic rings were used to measure vascular reactivity.

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Materials and Methods

DOCA-salt hypertensive rats. Male Wistar rats (8–10 weeks old) were obtained from the Central Animal Breeding House of The University of Queensland. All experimental protocols were approved by the Animal Experimentation Ethics Committee of The University of Queensland under the guidelines of the National Health and Medical Research Council, Australia. Rats were given *ad libitum* access to food and water and were housed in 12-hr. light:dark conditions. All treated rats were uninephrectomized. Rats were anaesthetized with an intra-peritoneal injection of Zoletil® [Virbac (Australia) Pty Ltd, Milperra, NSW, Australia] (25 mg/kg each of tiletamine and zolazepam) and Ilium Xylazil (Troy Laboratories, Smithfiled, NSW, Australia) (xylazine, 10 mg/kg). A lateral abdominal incision provided access to the left kidney, its renal vessels and ureter. The renal vessels and ureter were ligated; the left kidney was removed for weighing and the incision site was sutured. Uninephrectomized rats were either given no further treatment or were given 1% NaCl in drinking water together with subcutaneous injections of DOCA (25 mg in 0.4 ml dimethylformamide) every 4th day (DOCA-salt rats) [11–14]. ʟ-Carnitine was administered as a 1.2% mixture in powdered food for a total period of 32 days starting 4 days before surgery and ending 28 days after surgery.

Assessment of physiological parameters. Body-weight, food and water intakes were measured daily. Systolic blood pressure was measured at week 0 and at the end of weeks 2 and 4 under light sedation with i.p. injection of Zoletil® (tiletamine 15 mg/kg, zolazepam 15 mg/kg), using an MLT1010 Piezo-Electric Pulse Transducer (ADInstruments, Sydney, Australia) and inflatable tail-cuff connected to an MLT844 Physiological Pressure Transducer (ADInstruments) and PowerLab data acquisition unit (ADInstruments). Rats were killed with an injection of pentobarbitone sodium (100 mg/kg i.p.). Blood was taken from the abdominal aorta and centrifuged, and the plasma was frozen. Plasma malondialdehyde concentrations, as a measure of oxidative stress, were determined by HPLC [13].

Isolated heart preparation. The left ventricular function of the rats in all groups was assessed using the Langendorff heart preparation [11–14]. Once terminal anaesthesia was achieved with pentobarbitone sodium (100 mg/kg i.p.), heparin (1000 IU) was injected into the right femoral vein. Isovolumetric ventricular function was measured by inserting a latex balloon catheter into the left ventricle connected to a Capto SP844 MLT844 physiological pressure transducer and Chart software on a Maclab system (ADInstruments, Sydney, Australia). All left ventricular end-diastolic pressure values were measured by pacing the heart at 250 beats/min. using an electrical stimulator. End-diastolic pressures were obtained starting from 0 mmHg up to 30 mmHg. To assess contractile function, time derivatives of pressure (+dP/dt; −dP/dt) were calculated at a diastolic pressure of 10 mmHg. The right and left ventricles were separated and weighed. Diastolic stiffness constant (κ, dimensionless) was calculated as in previous studies [11–14].

Organ bath studies. Thoracic aortic rings (4 mm in length) were suspended in an organ bath chamber with a resting tension of 10 mN. Cumulative concentration-response (contraction) curves were measured for noradrenaline; concentration-response (relaxation) curves were measured for acetylcholine and sodium nitroprusside in the presence of a submaximal (70%) contraction to noradrenaline [15]. Contractile responses to noradrenaline were evaluated previously following pre-contraction and wash with isotonic KCl (100 mM) in the DOCA-salt rats [15].

Organ weights. Following euthanasia, the heart, liver, kidneys and spleen were removed and blotted dry for weighing. Organ weights were normalized relative to the body-weight at the time of their removal (in mg/g).

Histology. Tissues were initially fixed for 3 days in Telly's fixative (100 ml of 70% ethanol, 5 ml of glacial acetic acid and 10 ml of 40% formaldehyde) and then transferred into modified Bouin's fluid (85 ml of saturated picric acid, 5 ml glacial acetic acid and 10 ml of 40% formaldehyde) for 2 days. The samples were then dehydrated and embedded in paraffin wax. Thin sections (10 μm) of left ventricle, stained with haematoxylin and eosin, were used for determination of inflammatory cell infiltration while thick sections (15 μm) of left ventricle and thoracic aorta, stained with picrosirius red, were used to measure collagen distribution [13]. All sections were analysed under a laser confocal microscope as previously described [13].

Statistical analysis. All data sets were represented as mean ± standard error of mean (S.E.M.). Comparisons or findings between groups were made via statistical analysis of data sets using one-way/two-way analysis of variance followed by the Duncan test to determine differences between treatment groups. A *p*-value of <0.05 was considered as statistically significant.

Drugs. Deoxycorticosterone acetate, heparin, noradrenaline, acetylcholine and sodium nitroprusside were purchased from Sigma Chemical Company (St Louis, MO, USA). ʟ-Carnitine was purchased from Musashi Pty Ltd, Gold Coast, Queensland, Australia. Noradrenaline hydrochloride, acetylcholine chloride and sodium nitroprusside were dissolved in distilled water. DOCA was dissolved in dimethylformamide with mild heating.

Results

Biometrics.

DOCA-salt treated rats showed an increase in water intake and failed to gain weight compared to uninephrectomized (UNX) rats as controls (fig. 1). ʟ-Carnitine failed to alter body-weight (fig. 1) or water intake in the DOCA-salt rats. There was no change in the food intake among the groups. Thus, ʟ-carnitine intake was unchanged in the treated DOCA-salt (0.9 ± 0.1 mg/g/day) and UNX (1.1 ± 0.1 mg/g/day) rats. Systolic blood pressure was increased in DOCA-salt rats when compared with UNX rats and this increase was attenuated by ʟ-carnitine treatment (table 1). The DOCA-salt rats showed left and right ventricular hypertrophy (seen as increased left ventricle + septum and right ventricular wet weights) at 4 weeks, compared with UNX rats and this increase in wet weight of left ventricle but not the right ventricle was attenuated by ʟ-carnitine treatment (table 1). The increased wet
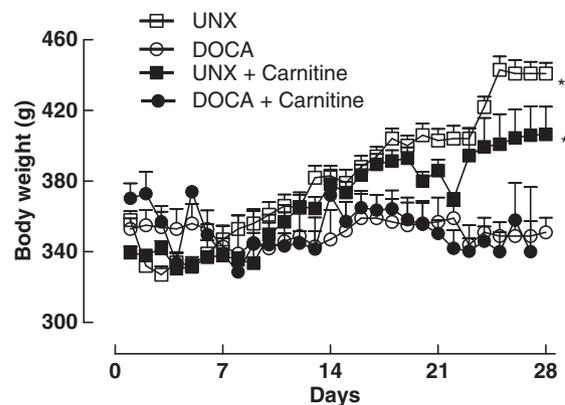
Fig. 1. Daily body-weight measurements for UNX (■), UNX + ʟ-carnitine (□), DOCA-salt (○) and DOCA-salt + ʟ-carnitine (●)-treated rats (*p* < 0.05 *versus* UNX).

Table 1.

Physiological parameters of UNX, UNX + L-carnitine, DOCA-salt and DOCA-salt + L-carnitine-treated rats.

Parameter	UNX	UNX + L-carnitine	DOCA-salt	DOCA-salt + L-carnitine
Systolic blood pressure (mmHg)	122 ± 3 (12)	125 ± 1 (12)	169 ± 2* (10)	148 ± 6# (8)
LV weight (mg/g body-wt)	1.99 ± 0.07 (8)	2.11 ± 0.04 (12)	3.02 ± 0.07* (12)	2.72 ± 0.06# (11)
RV weight (mg/g body-wt)	0.50 ± 0.03 (8)	0.53 ± 0.03 (12)	0.60 ± 0.03* (12)	0.61 ± 0.04* (11)
Remnant kidney weight (mg/g body-wt)	4.80 ± 0.16 (8)	4.81 ± 0.35 (12)	9.48 ± 0.48* (12)	8.47 ± 0.29# (11)
Thoracic aortic wall thickness (μm)	72.3 ± 5.3 (5)	71.6 ± 2.7 (5)	111.3 ± 2.8* (6)	104.2 ± 2.6 (5)
Plasma malondialdehyde concentration (μmol/l)	18.7 ± 1.0 (5)	20.3 ± 0.7 (5)	26.9 ± 0.8*(5)	21.2 ± 0.4# (5)
LV perivascular collagen fraction (% area)	23.9 ± 2.4 (4)	19.2 ± 3.9 (4)	38.9 ± 2.8* (6)	25.5 ± 4.9# (5)
LV interstitial collagen fraction (% area)	6.0 ± 0.4 (5)	6.1 ± 0.2 (5)	14.4 ± 0.2* (6)	8.7 ± 0.5# (5)
Diastolic stiffness	21.7 ± 0.5 (11)	20.7 ± 0.5 (9)	26.9 ± 0.5* (12)	23.8 ± 0.5# (6)
$+dP/dt_{max}$ (mmHg/sec.)	1890 ± 260 (9)	1460 ± 110 (6)	1710 ± 170 (12)	1350 ± 120 (6)
$-dP/dt_{max}$ (mmHg/sec.)	1260 ± 180 (9)	940 ± 90 (6)	1160 ± 130 (12)	890 ± 90 (6)
Noradrenaline ($-\log EC_{50}$)	6.9 ± 0.1 (6)	6.9 ± 0.3 (6)	7.8 ± 0.1* (6)	8.2 ± 0.1* (6)

Values are mean ± S.E.M.; number of experiments in parentheses. LV, left ventricle; RV, right ventricle. *$p < 0.05$ *versus* UNX; #$p < 0.05$ *versus* DOCA.

weight of the remnant kidney seen in DOCA-salt rats was also attenuated by L-carnitine treatment (table 1). Plasma malondialdehyde concentrations were increased after 4 weeks of DOCA-salt hypertension; this change was prevented by L-carnitine treatment (table 1).

Left ventricular structure and function.
In comparison to UNX rats, collagen content was increased in the left ventricles of DOCA-salt rats (table 1, figs 2A and B). L-Carnitine treatment did not alter collagen content in UNX rats (table 1, fig. 2C) but attenuated the increased collagen deposition in DOCA-salt rats (table 1, fig. 2D). Hearts from DOCA-salt rats showed significantly increased diastolic stiffness that was attenuated by L-carnitine treatment (table 1). The functional parameters, +dP/dt (ventricular contraction) and −dP/dt (ventricular relaxation), were unchanged compared to UNX rats; L-carnitine treatment did not alter these parameters (table 1).

Left ventricular monocyte/macrophage infiltration.
Infiltrating inflammatory cells occurred in low numbers and always as single cells in the left ventricles of UNX rats (fig. 3A). Many more inflammatory cells were observed in the left ventricle sections of DOCA-salt rats, and these cells were usually found in clusters of cells located throughout the interstitium and especially at scar sites and areas of fibrosis (fig. 3B). Treatment with L-carnitine did not change the den-

sity of inflammatory cells in UNX rats (fig. 3C) but markedly reduced inflammatory cell infiltration and the overall area of scar tissue in DOCA-salt hypertensive rats (fig. 3D). However, some inflammatory cells were still found within the interstitial component of the left ventricle.

Vascular structure and function.
Thoracic aortic rings showed an increased wall thickness in DOCA-salt rats that was not reduced by L-carnitine treatment (table 1; fig. 4). Thoracic aortic rings from DOCA-salt hypertensive rats showed an increased potency with unaltered maximal responses to noradrenaline across all treatment groups. In previous studies, we showed no differences between the maximal contractile responses to KCl (100 mM) (9.1 ± 1.4 mN; n = 15) and to noradrenaline (1×10^{-5} M) (7.7 ± 2.2 mN; n = 7) in thoracic aortic rings from the DOCA-salt rats (Brown L, unpublished data). Treatment with L-carnitine did not modify responses to noradrenaline (fig. 5A). Furthermore, relaxant responses to sodium nitroprusside were unchanged among the groups (fig. 5B). Relaxant responses to acetylcholine were reduced in DOCA-salt rats; treatment with L-carnitine did not prevent this damage (fig. 5C).

Discussion

Cardiovascular remodelling includes hypertension, endothelial damage, cardiac hypertrophy, inflammation, ventricular
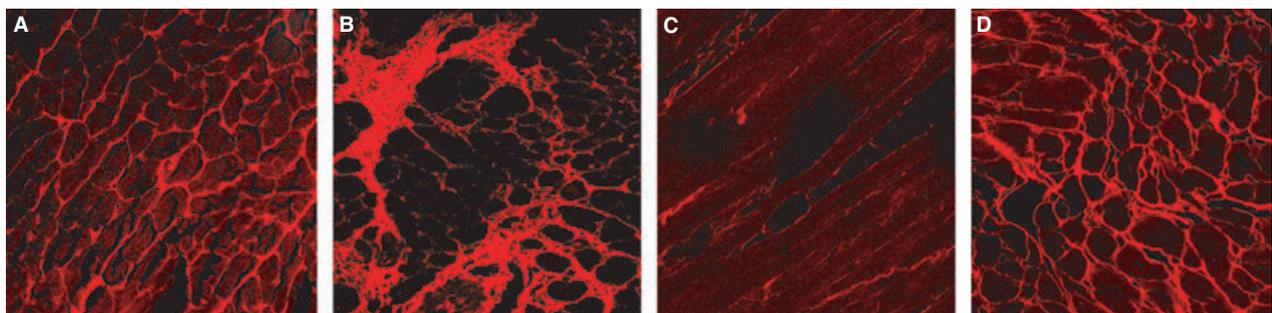
Fig. 2. Picrosirius red staining of left ventricular interstitial collagen deposition (magnification, ×40) in UNX (A), DOCA-salt (B), UNX + L-carnitine (C), DOCA-salt + L-carnitine (D)-treated rats; collagen is stained light red.
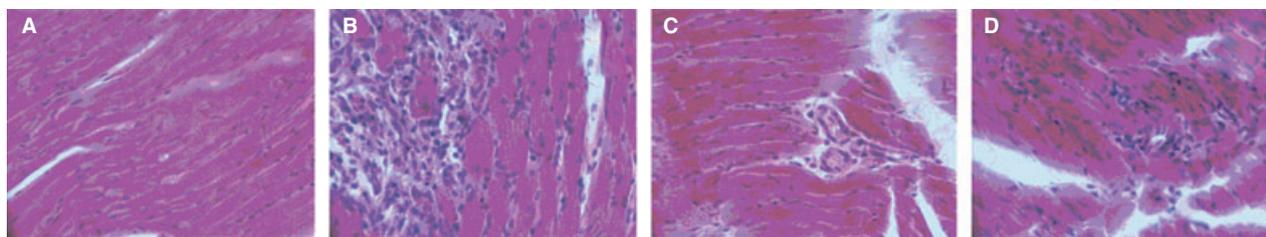
Fig. 3. Haematoxylin and eosin staining showing infiltrating inflammatory cells in the left ventricular interstitial region (magnification, ×40) in UNX (A), DOCA-salt (B), UNX + L-carnitine (C), DOCA-salt + L-carnitine (D)-treated rats.

contractile dysfunction and fibrosis [16]. In this study, we have shown that treatment with L-carnitine markedly attenuated cardiac remodelling including hypertrophy and fibrosis as well as hypertension in DOCA-salt hypertensive rats. Furthermore, L-carnitine prevented the increase in plasma malondialdehyde concentrations indicating decreased oxidative stress in DOCA-salt rats. However, the pronounced endothelial dysfunction and the increased thoracic wall thickness, characteristic of DOCA-salt hypertensive rats, were minimally changed with L-carnitine treatment.

L-Carnitine plays a major role, as a cofactor, in the transportation of free fatty acids from the cytosol to the mitochondria for adenosine triphosphate synthesis. An altered metabolic substrate use in the failing heart also contributes to the dysfunction of the mitochondrial electron transport chain, resulting in enhanced production of superoxide [17]. Mitochondrial dysfunction and increased mitochondrial superoxide production, preceding endothelial dysfunction, might favour the development of hypertension [18]. Free radicals also potentiate mitochondrial dysfunction by further damaging mitochondrial DNA, with resultant impairment in the synthesis of some components of the respiratory chain and further increases in superoxide production [18–21].

The substrate usage pattern for cardiac energy shifts to decreased fatty acid oxidation and increased glucose oxidation within the myocardial mitochondria during the development of pathophysiological conditions such as ischaemia, hypertrophy and heart failure [22–24]. L-Carnitine, by allowing efficient fatty acid transport, changes this energy shift back to increased fatty acid oxidation so that the elevated energy demands of the hypertensive heart can be met. This shift with L-carnitine prevents the increase in production of mitochondrial superoxide [25]. This potential of L-carnitine to increase fatty acid oxidation in the myocytes while reducing oxidative stress provides a likely explanation for the attenuation of cardiac remodelling observed in the current study. L-Carnitine treatment improved heart function after ischaemia and reperfusion injury [4], and also improved heart rate regulation and ventricular size in streptozotocin-diabetic rats [26]. In spontaneously hypertensive rats, oxidative stress in heart, liver and plasma was decreased following L-carnitine supplementation, although blood pressure was unchanged and contractile responses of isolated blood vessels were not measured [27]. In the current study, L-carnitine attenuated cardiac hypertrophy, possibly due to antioxidant and anti-inflammatory effects, but did not reverse vascular hypertrophy. Myocardial fibrosis was also attenuated resulting in a reduced diastolic stiffness in the isolated heart studies. However, the functional performance of the DOCA-salt rat hearts was not significantly different to that of UNX controls; heart failure is unlikely in this 4-week model, compared with 8-week DOCA-salt rats [15].

In the current study, L-carnitine treatment attenuated the increases in the blood pressure of DOCA-salt hypertensive rats. Several other studies have also proved the efficiency of L-carnitine in reducing blood pressure in patients with pulmonary hypertension [28], in rats with L-NAME-induced hypertension [29] and in fructose-fed hypertensive rats [30]. The mechanisms by which L-carnitine can decrease blood pressure include its role in enhancing fatty acid oxidation [28] and the consequent role to reduce the production of superoxide [25], and further increasing the availability of nitric oxide [30]. In addition, the anti-hypertensive effects of L-carnitine in this study may result from inhibition of
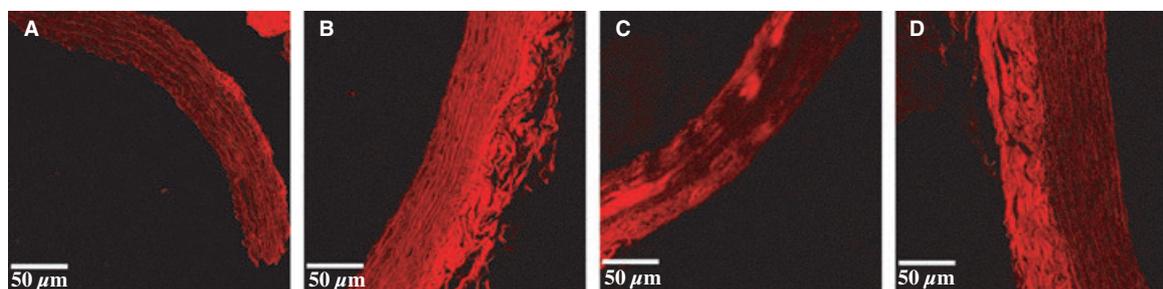
Fig. 4. Picrosirius red staining of thoracic aortic rings to define vascular smooth muscle (magnification, ×20) in UNX (A), DOCA-salt (B), DOCA-salt + L-carnitine (C)-treated rats.
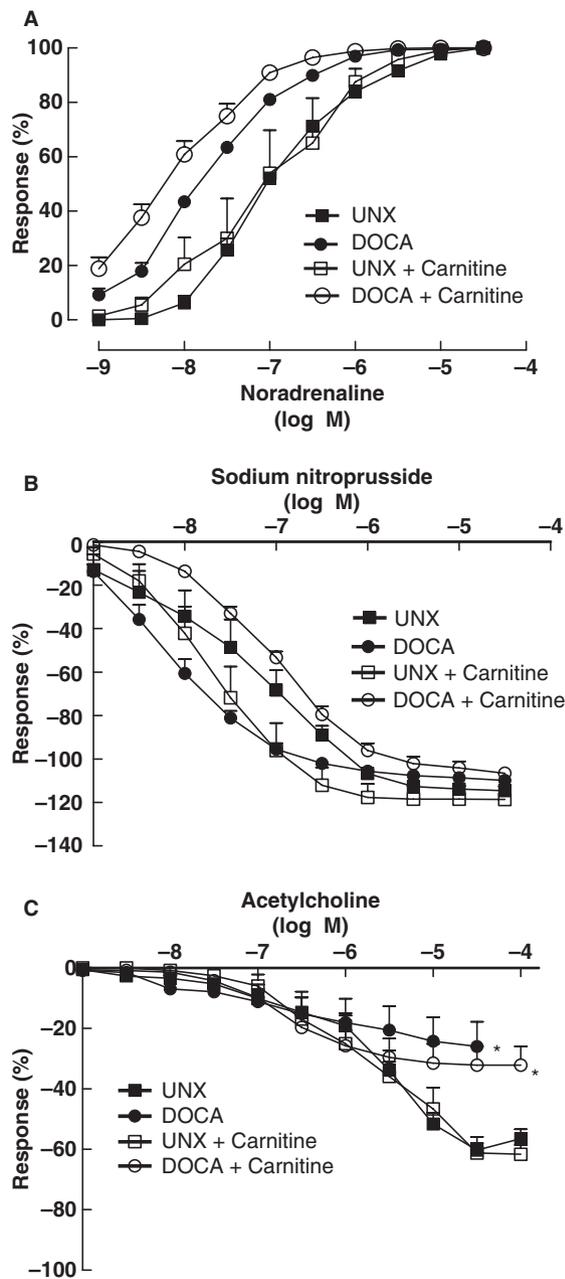
Fig. 5. Cumulative concentration-response curves for noradrenaline (A), sodium nitroprusside (B) and acetylcholine (C) in thoracic aortic rings from UNX (■), UNX + L-carnitine (□), DOCA-salt (○) and DOCA-salt + L-carnitine (●)-treated rats (*$p < 0.05$ *versus* UNX).

inflammation, as inflammation is an integral part of the cardiovascular remodelling observed in DOCA-salt hypertensive rats [13,16]. Furthermore, plasma concentrations and cardiac expression of inflammatory markers such as IL-6 and TNF-α were reduced after L-carnitine treatment in L-NAME-induced hypertensive rats [29]. Both the anti-inflammatory and antioxidant properties of L-carnitine can explain the reduced weight of the remnant kidney in DOCA-salt rats. Renal hypertrophy was reduced and function improved

through the scavenging of free radicals and reducing tissue inflammation in DOCA-salt hypertensive rats treated with hemin, an up-regulator of haemoxygenase [31].

However, the pronounced endothelial dysfunction seen in the DOCA-salt hypertensive rats in this study was not altered by L-carnitine supplementation. Similar to the heart, increased superoxide production is an important cause of oxidative stress in the vasculature of the DOCA-salt hypertensive rats. However, the major source of superoxide is the mitochondria in the heart compared to the activation of NADPH oxidase in the vasculature [32,33], although vascular xanthine oxidase and mitochondrial oxidation phosphorylation complexes can contribute to vascular superoxide production in DOCA-salt rats, especially in mesenteric resistance arteries [34]. Since the primary role of L-carnitine is to mediate the transport of fatty acids into the mitochondria of cardiac muscle [35], L-carnitine controls oxidative stress by improving mitochondrial function [25,35]. In the vasculature of DOCA-salt rats, endothelin activation of NADPH oxidase, acting via ET_A receptors, increases superoxide production [8,33]; L-carnitine does not appear to inhibit this pathway but NADPH oxidase-independent pathways may also be involved [36]. However, in the spontaneously hypertensive rats, L-carnitine reduced endothelial dysfunction, especially through its antioxidant properties [37,38]. In streptozotocin-diabetic rats, L-carnitine partially restored endothelium-dependent relaxation to acetylcholine with no changes in responses to sodium nitroprusside [39]. In conclusion, this study suggests that the mitochondrial respiratory chain is a significant source of reactive oxygen species in the heart but a relatively minor source in the vasculature. As a consequence, L-carnitine attenuates cardiac remodelling more than vascular remodelling because of its actions on the mitochondria.

References

1 Tominaga H, Katoh H, Odagiri K, Takeuchi Y, Kawashima H, Saotome M *et al.* Different effects of palmitoyl-L-carnitine and palmitoyl-CoA on mitochondrial function in rat ventricular myocytes. Am J Physiol Heart Circ Physiol 2008;**295**:H105–12.

2 Bremer J. Carnitine – metabolism and functions. Physiol Rev 1983;**63**:1420–80.

3 Carvajal K, Moreno-Sánchez R. Heart metabolic disturbances in cardiovascular diseases. Arch Med Res 2003;**34**:89–99.

4 Ferrari R, Merli E, Cicchitelli G, Mele D, Fucili A, Ceconi C. Therapeutic effects of L-carnitine and propionyl-L-carnitine on cardiovascular diseases: a review. Ann NY Acad Sci 2003;**1033**:79–91.

5 Pauly DF, Pepine CJ. The role of carnitine in myocardial dysfunction. Am J Kid Dis 2003;**41**:S35–43.

6 Takimoto E, Kass DA. Role of oxidative stress in cardiac hypertrophy and remodeling. Hypertension 2007;**49**:241–8.

7 Doggrell SA, Brown L. Rat models of hypertension, cardiac hypertrophy and failure. Cardiovasc Res 1998;**39**:89–105.

8 Ammarguellat F, Larouche I, Schiffrin EL. Myocardial fibrosis in DOCA-salt hypertensive rats. Effects of endothelin ETA receptor antagonism. Circulation 2001;**103**:319–24.

9 Callera GE, Touyz RM, Teixeira SA, Muscara MN, Carvalho MH, Fortes ZB *et al.* ETA receptor blockade decreases vascular

superoxide generation in DOCA-salt hypertension. Hypertension 2003;**42**:811–7.

10 Pu Q, Neves MF, Virdis A, Touyz RM, Schiffrin EL. Endothelin antagonism on aldosterone-induced oxidative stress and vascular remodelling. Hypertension 2003;**42**:49–55.

11 Allan A, Fenning A, Levick S, Hoey A, Brown L. Reversal of cardiac dysfunction by selective ET-A receptor antagonism. Br J Pharmacol 2005;**146**:846–53.

12 Mirkovic S, Seymour A-ML, Fenning A, Strachan A, Margolin SB, Taylor SM *et al.* Attenuation of cardiac fibrosis by pirfenidone and amiloride in DOCA-salt hypertensive rats. Br J Pharmacol 2002;**135**:961–8.

13 Fenning A, Harrison G, Rose'meyer R, Hoey A, Brown L. L-Arginine attenuates cardiovascular impairment in DOCA-salt hypertensive rats. Am J Physiol 2005;**289**:1408–16.

14 Brown L, Duce B, Miric G, Sernia C. Reversal of cardiac fibrosis in deoxycorticosterone acetate-salt hypertensive rats by inhibition of the renin-angiotensin system. J Am Soc Nephrol 1999;**10**:167–73.

15 Brown L, Ooi S-Y, Lau K, Sernia C. Cardiac and vascular responses in DOCA-salt hypertensive rats. Clin Exp Pharmacol Physiol 2000;**27**:263–9.

16 Weber KT, Anversa P, Armstrong PW, Brilla CG, Burnett JC Jr, Cruickshank JM *et al.* Remodeling and reparation of the cardiovascular system. J Am Coll Cardiol 2001;**20**:3–16.

17 Rosca MG, Vazquez EJ, Kerner J, Parland W, Chandler MP, Stanley W *et al.* Cardiac mitochondria in heart failure: decrease in respirasomes and oxidative phosphorylation. Cardiovasc Res 2008;**80**:30–9.

18 Puddu P, Puddu GM, Cravero E, Rosati M, Muscari A. The molecular sources of reactive oxygen species in hypertension. Blood Press 2008;**17**:70–7.

19 Esposito LA, Melov S, Panov A, Cottrell BA, Wallace DC. Mitochondrial disease in mouse results in increased oxidative stress. Proc Natl Acad Sci U S A 1999;**96**:4820–5.

20 Shibutani S, Takeshita M, Grollman AP. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. Nature 1991;**349**:431–4.

21 Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial ROS-induced ROS release: an update and review. Biochim Biophys Acta 2006;**1757**:509–17.

22 Massie BM, Schaefer S, Garcia J, McKirnan MD, Schwartz GG, Wisneski JA *et al.* Myocardial high-energy phosphate and substrate metabolism in swine with moderate left ventricular hypertrophy. Circulation 1995;**91**:1814–23.

23 Sack MN, Rader TA, Park S, Bastin J, McCune SA, Kelly DP. Fatty acid oxidation enzyme gene expression is downregulated in the failing heart. Circulation 1996;**94**:2837–42.

24 Yamashita H, Bharadwaj KG, Ikeda S, Park TS, Goldberg IJ. Cardiac metabolic compensation to hypertension requires lipoprotein lipase. Am J Physiol Endocrinol Metab 2008;**295**:E705–13.

25 Gülçin I. Antioxidant and antiradical activities of L-carnitine. Life Sci 2006;**78**:803–11.

26 Malone JI, Cuthbertson DD, Malone MA, Schocken DD. Cardio-protective effects of carnitine in streptozotocin-induced diabetic rats. Cardiovasc Diabetol 2006;**5**:2, doi:10.1186/1475-2840-5-2.

27 Gómez-Amores L, Mate A, Miguel-Carrasco JL, Jiménez L, Jos A, Cameán AM *et al.* L-carnitine attenuates oxidative stress in hypertensive rats. J Nutr Biochem 2007;**18**:533–40.

28 El-Beshlawy A, Youssry I, El-Saidi S, El-Accaoui R, Mansi Y, Maklouf A *et al.* Pulmonary hypertension in beta-thalassemia major and the role of L-carnitine therapy. Pediatr Hematol Oncol 2008;**25**:734–43.

29 Miguel-Carrasco JL, Mate A, Monserrat MT, Arias JL, Aramburu O, Vázquez CM. The role of inflammatory markers in the cardioprotective effect of L-carnitine in L-NAME-induced hypertension. Am J Hypertens 2008;**21**:1231–7.

30 Rajasekar P, Palanisamy N, Anuradha CV. Increase in nitric oxide and reductions in blood pressure, protein kinase C beta II and oxidative stress by L-carnitine: a study in the fructose-fed hypertensive rat. Clin Exp Hypertens 2007;**29**:517–30.

31 Jadhav A, Torlakovic E, Ndisang JF. Hemin therapy attenuates kidney injury in deoxycorticosterone acetate-salt hypertensive rats. Am J Physiol Renal Physiol 2009;**296**:F521–34.

32 Li JM, Shah AM. Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiology. Am J Physiol Regul Integr Comp Physiol 2004;**287**:R1014–30.

33 Li L, Fink GD, Watts SW, Northcott CA, Galligan JJ, Pagano PJ *et al.* Endothelin-1 increases vascular superoxide via endothelinA-NADPH oxidase pathway in low-renin hypertension. Circulation 2003;**107**:1053–8.

34 Viel EC, Benkirane K, Javeshghani D, Touyz RM, Schiffrin EL. Xanthine oxidase and mitochondria contribute to vascular superoxide anion generation in DOCA-salt hypertensive rats. Am J Physiol Heart Circ Physiol 2008;**295**:H281–8.

35 Calvani M, Reda E, Arrigoni-Martelli E. Regulation by carnitine of myocardial fatty acid and carbohydrate metabolism under normal and pathological conditions. Basic Res Cardiol 2000;**95**:75–83.

36 Callera GE, Tostes RC, Yogi A, Montezano AC, Touyz RM. Endothelin-1-induced oxidative stress in DOCA-salt hypertensive hypertension involves NADPH-oxidase-independent mechanisms. Clin Sci (Lond.) 2006;**110**:243–53.

37 de Sotomayor MA, Mingorance C, Rodriguez-Rodriguez R, Marhuenda E, Herrera MD. L-Carnitine and its propionate: improvement of endothelial function in SHR through superoxide dismutase-dependent mechanisms. Free Radic Res 2007;**41**:884–91.

38 Alvarez de Sotomayor M, Bueno R, Pérez-Guerrero C, Herrera MD. Effect of L-carnitine and propionyl-L-carnitine on endothelial function of small mesenteric arteries from SHR. J Vasc Res 2007;**44**:354–64.

39 Irat AM, Aktan F, Ozansoy G. Effects of L-carnitine treatment on oxidant/antioxidant state and vascular reactivity of streptozotocin-diabetic rat aorta. J Pharm Pharmacol 2003;**55**:1389–95.