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Modulation of tissue fatty acids by L-carnitine attenuates metabolic syndrome in diet-induced obese rats

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Obesity and dyslipidaemia are metabolic defects resulting from impaired lipid metabolism. These impairments are associated with the development of cardiovascular disease and non-alcoholic fatty liver disease. Correcting the defects in lipid metabolism may attenuate obesity and dyslipidaemia, and reduce cardiovascular risk and liver damage. L-Carnitine supplementation was used in this study to enhance fatty acid oxidation so as to ameliorate diet-induced disturbances in lipid metabolism. Male Wistar rats (8–9 weeks old) were fed with either corn starch or high-carbohydrate, high-fat diets for 16 weeks. Separate groups were supplemented with L-carnitine (1.2% in food) on either diet for the last 8 weeks of the protocol. High-carbohydrate, high-fat diet-fed rats showed central obesity, dyslipidaemia, hypertension, impaired glucose tolerance, hyperinsulinaemia, cardiovascular remodelling and non-alcoholic fatty liver disease. L-Carnitine supplementation attenuated these high-carbohydrate, high-fat diet-induced changes, together with modifications in lipid metabolism including the inhibition of stearoyl-CoA desaturase-1 activity, reduced storage of short-chain monounsaturated fatty acids in the tissues with decreased linoleic acid content and *trans* fatty acids stored in retroperitoneal fat. Thus, L-carnitine supplementation attenuated the signs of metabolic syndrome through inhibition of stearoyl-CoA desaturase-1 activity, preferential β-oxidation of some fatty acids and increased storage of saturated fatty acids and relatively inert oleic acid in the tissues.

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Introduction

Obesity is a chronic pathological condition characterised by excess fat deposition in adipose tissue. One of the major reasons for the increasing incidence of obesity around the world is an increased consumption of cafeteria diets with higher energy densities together with lower expenditure of this energy.[1–5] Cafeteria diet is rich in saturated and *trans* fats and simple carbohydrates, mainly fructose and sucrose. Increasing the intake of this combination can lead to disturbances in the metabolism of these macronutrients in the body.[6–8] Insulin resistance, decreased insulin sensitivity and hyperinsulinaemia associated with obesity contribute towards the imbalance in metabolism of fats and carbohydrates.[9,10] These metabolic disturbances can worsen the damage to the heart, inducing cardiovascular remodelling with decreased function, as well as damaging the liver and the skeletal muscle.[11] Decreased metabolism of fat by the liver leads to non-alcoholic fatty liver disease.[12]

L-Carnitine, a quaternary amine synthesised from lysine and methionine in the liver and kidney, is involved in the transport of fatty acids by carnitine palmitoyltransferase 1 (CPT1) across the mitochondrial membrane for β-oxidation.[13] In humans, L-carnitine is generally taken in the diet through meat products.[14] If the requirement of L-carnitine is not fulfilled from the diet, as in vegetarians, it is synthesised in the body along with an increase in the absorption from the diet.[14,15] Deficiency of L-carnitine in the body leads to organ damage including cardiomyopathy, encephalopathy and skeletal muscle myopathy.[15–17]

Our previous study with L-carnitine in DOCA-salt-induced cardiovascular remodelling in rats reported improvements in the structure and function of the heart.[18] In fructose-fed rats,

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L-carnitine supplementation reduced plasma concentrations of glucose, insulin, free fatty acids and triglycerides, improved glucose tolerance and protected the kidneys.[19–21] In high-fat diet-fed mice, a combination of acetyl-L-carnitine with lipoic acid did not change body weight, liver steatosis, oxidative stress or glucose tolerance, but reduced the plasma activities of aspartate transaminase (AST) and alanine transaminase (ALT) along with improved mitochondrial markers.[22] In another high-fat diet-fed mice study, L-carnitine reduced body weight, reduced serum triglycerides, total serum cholesterol, LDL-cholesterol and liver lipid content.[23] In aged rats, L-carnitine supplementation with physical exercise reduced body weight and abdominal fat but failed to increase CPT1 activity in liver and soleus muscles while increasing the rate of β-oxidation in soleus muscle.[24] A combination of either orlistat or sibutramine with L-carnitine improved lipid profiles, insulin resistance, body weight and inflammation in type 2 diabetic patients. The responses to the combinations were greater than with orlistat or sibutramine alone.[25,26] L-Carnitine supplementation in type 2 diabetic patients did not change body weight, body mass index, fasting plasma glucose or plasma total cholesterol, but L-carnitine reduced plasma triglycerides and total body fat.[27] In non-alcoholic steatohepatitis patients, L-carnitine reduced plasma activities of AST, ALT and γ-glutamyl transpeptidase, reduced plasma concentrations of total cholesterol, triglycerides and inflammatory markers, and attenuated non-alcoholic steatohepatitis.[28]

In this study, L-carnitine was given in a reversal protocol to rats fed with a high-carbohydrate, high-fat diet to increase the β-oxidation of stored fat. The metabolic effects of L-carnitine were characterised through measurements of body composition, glucose tolerance and plasma lipid profile. Changes in cardiovascular structure and function were evaluated with systolic blood pressure measurements, echocardiography, isolated Langendorff heart preparation, vascular responses including contraction and relaxation properties of thoracic aortic rings, and histopathology. Hepatic structure and function were determined by liver function tests and histopathology. Fatty acid contents of plasma, heart, liver, skeletal muscle and retroperitoneal fat were measured to determine the changes in metabolism and storage of fatty acids following supplementation with L-carnitine.

Experimental

Rats, diets and L-carnitine supplementation

All experimental protocols were approved by the Animal Ethics Committees of the University of Southern Queensland and The University of Queensland under the guidelines of the National Health and Medical Research Council of Australia. Male Wistar rats (8–9 weeks old, weighing 339 ± 4 g, n = 48) were supplied by The University of Queensland Biological Resources facility. Rats were randomly divided into two experimental diet groups and were fed with either corn starch diet (C; n = 24) or high-carbohydrate, high-fat diet (H; n = 24) for 16 weeks. After 8 weeks of feeding with either diet, 12 rats from each group were randomly separated and treated with 1.2% L-carnitine in food with the same diet continued for a further 8 weeks (CLC and HLC, respectively). The remaining 12 rats from both diet groups (C and H) were continued on the original diet without any additional intervention for the next 8 weeks.

C diet contained 57% corn starch, 15.5% powdered rat food (Specialty Feeds, Glen Forest, Western Australia, Australia), 2.5% Hubble, Mendel and Wakeman salt mixture and 25% water. H diet consisted of 17.5% fructose, 39.5% sweetened condensed milk, 20% beef tallow, 15.5% powdered rat food, 2.5% Hubble, Mendel and Wakeman salt mixture and 5% water. C and CLC rats were given drinking water without any additives whereas drinking water for H and HLC rats was supplemented with 25% fructose. All rats were provided *ad libitum* access to food and water and were individually housed in temperature-controlled 12 hour light-dark conditions. Energy intake was calculated from the following values in kilojoules per gram: fructose, 15.40; corn starch, 15.94; condensed milk, 13.80; beef tallow, 37.70; and powdered rat food, 13.80. The energy densities of the C and H diet were 11.23 kJ g^{-1} and 17.83 kJ g^{-1} of food, respectively, and an additional 3.85 kJ mL^{-1} in the drinking water for the H and HLC rats.[29,30]

Physiological and metabolic parameters

Rat body weights were measured daily. Daily food and water intakes were calculated from measurements of the weights of food bowls and water bottles, respectively. Abdominal circumference, body mass index (BMI), energy intake and feed efficiency were measured.[29] At the end of the protocol, rats were food-deprived for 12 hours and oral glucose tolerance tests were performed.[29,30] The blood glucose concentrations obtained from oral glucose tolerance tests were used to calculate the area under the curve by taking X-axis as the baseline. Body compositions of rats were measured at the end of the protocol.[29,30] During terminal experiments, abdominal fat pads (separately as retroperitoneal, epididymal and omental) were removed, weighed and normalised to tibial length at the time of terminal experiments. Plasma concentrations of albumin, total bilirubin, urea, uric acid, total cholesterol and triglycerides were determined using kits and controls supplied by Olympus using an Olympus analyser (AU 400, Tokyo, Japan).[29] Non-esterified fatty acids (NEFA) in plasma were determined using a commercial kit (Wako, Osaka, Japan).[29] Plasma insulin concentrations (ALPCO, USA) were estimated using a commercial ELISA kit according to manufacturer-provided standards and protocols.[31]

Cardiovascular structure and function

Systolic blood pressure measurements. Systolic blood pressures of rats were measured under light sedation with Zoletil (tiletamine 10 mg kg^{-1}, zolazepam 10 mg kg^{-1} intraperitoneally), using an MLT1010 Piezo-Electric Pulse Transducer (ADInstruments, Sydney, Australia) and inflatable tail-cuff connected to a MLT844 Physiological Pressure Transducer (ADInstru-

ments, Sydney, Australia) and PowerLab data acquisition unit (ADInstruments, Sydney, Australia).[29]

Echocardiography. Echocardiographic examinations (Phillips iE33, 12 MHz transducer) were performed to assess cardiovascular structure and function in rats.[29]

Isolated Langendorff heart preparation. During terminal experiments, rats were euthanased with Lethabarb (pentobarbitone sodium, 100 mg kg^{-1} intraperitoneally; Virbac, Peakhurst, NSW, Australia). After euthanasia, heparin (200 IU; Sigma-Aldrich Australia, Sydney, Australia) was injected through the right femoral vein. The abdomen was then opened and blood (~6–8 ml) was withdrawn from the abdominal aorta, collected into heparinised tubes and centrifuged at 5 000*g* for 10 minutes to obtain plasma. Plasma was stored at −20°C until further analysis. Hearts were removed and were used in isolated Langendorff heart preparation to assess left ventricular function of the rats (n = 9 from each group).[29] After performing Langendorff heart perfusion studies, hearts were separated into right ventricle and left ventricle (with septum). Both ventricles were weighed.

Vascular reactivity. Thoracic aortic rings from rats (~4 mm in length; n = 10–12 from each group) were suspended in an organ bath maintained at 35°C and filled with Tyrode physiological salt solution bubbled with 95% O_2–5% CO_2 and allowed to stabilise at a resting tension of ~10 mN. Cumulative concentration-response curves (contraction) were obtained for noradrenaline (Sigma-Aldrich Australia, Sydney, Australia) and cumulative concentration-response curves (relaxation) were obtained for acetylcholine (Sigma-Aldrich Australia) and sodium nitroprusside (Sigma-Aldrich Australia) following submaximal (~70%) contraction to noradrenaline.[29]

Histology of the heart. Three rats from each group were exclusively used for histology. Hearts were removed from these rats soon after euthanasia and were processed for histological assessments for inflammatory cells and collagen deposition.[30]

Hepatic structure and function

Livers (n = 9 from each group) were isolated and weighed. Liver portions from histology rats were isolated (n = 3 from each group) and fixed in 10% neutral buffered formalin for three days. These portions were used to determine fat deposition, infiltration of inflammatory cells and portal fibrosis.[29,30] Plasma activities of ALT, AST, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were measured to determine hepatic function.

Fatty acid analyses

Hearts (immediately after perfusion studies), liver portions (~6–8 g), retroperitoneal fat (~6–8 g), skeletal muscle (~6–8 g) and plasma (~2 mL) were isolated during terminal experiments (n = 6 from each group) and were stored at −20°C. These samples were then used for fatty acid analysis.[30–33] The concentrations of the following fatty acids were determined in these samples: capric acid (C10:0), lauric acid (C12:0), dodecenoic acid (C12:1n-1), myristic acid (C14:0), myristoleic acid (C14:1n-5), palmitic acid (C16:0), palmitoleic acid (C16:1n-7), stearic acid (C18:0), vaccenic acid (C18:1*trans*-11), oleic acid (C18:1n-9), linoleic acid (C18:2n-6), α-linolenic acid (C18:3n-3), γ-linolenic acid (C18:3n-6), eicosanoic acid (C20:0), eicosenoic acid (C20:1n-9), eicosadienoic acid (C20:2n-6), dihomo-γ-linolenic acid (C20:3n-6), eicosatrienoic acid (C20:3n-3), arachidonic acid (C20:4n-6), eicosapentaenoic acid (C20:5n-3), behenic acid (C22:0), erucic acid (C22:1n-9), docosadienoic acid (C22:2n-6), docosatetraenoic acid (C22:4n-6), docosapentaenoic acid (C22:5n-3), docosahexaenoic acid (C22:6n-3), lignoceric acid (C24:0) and nervonic acid (C24:1n-9). Margaric acid (C17:0) was used as an internal standard in this procedure. All fatty acids were expressed as % of total recovered fatty acids. The n-3 : n-6 ratio and stearoyl-CoA desaturase-1 (SCD-1) activity index were calculated from the fatty acid concentrations.[30–33]

Statistical analysis

All data are presented as mean ± SEM. Groups of rats were tested for variance using Bartlett's test and variables that were not normally distributed were transformed (using log 10 function) prior to statistical analyses. These groups were tested for effects of diet, L-carnitine and their interactions by two-way ANOVA. When interaction and/or the main effects were significant, means were compared using Newman–Keuls multiple comparison post-test. $P < 0.05$ was considered significant. All statistical analyses were performed using GraphPad Prism version 5.00 for Windows (San Diego, California, USA).

Results

Physiological and metabolic parameters

H rats had higher body weight compared to C rats at 16 weeks and HLC rats showed lower body weight than H rats at 16 weeks. Food and water intakes were lower in H rats compared to C rats. Food and water intakes were unchanged in both HLC and CLC rats. Abdominal circumference along with the weights of retroperitoneal, epididymal and omental fat pads were higher in H rats compared to C rats. These parameters were lowered in both CLC and HLC rats compared to C and H rats, respectively. HLC rats had lower total body fat mass compared to H rats with no difference in lean mass between the groups. Basal blood glucose concentrations, the area under the curve during oral glucose tolerance test and plasma insulin concentrations were normalised in HLC rats. Plasma lipid components including total cholesterol, triglycerides and non-esterified fatty acids were normalised in HLC rats (Table 1).

Cardiovascular structure and function

Hearts from H rats showed presence of inflammatory cells (Fig. 1C) and increased ventricular collagen deposition (Fig. 1G). The infiltration of inflammatory cells and collagen deposition were inhibited in HLC rats (Fig. 1D and 1H). Systolic blood pressure was higher in H rats and normalised in HLC rats. Hearts from H rats showed higher left ventricular internal

Table 1 Effects of L-carnitine on physiological, compositional and metabolic parameters including plasma fatty acid composition

Variables	C	CLC	H	HLC	*P* Value		
					Diet	L-Carnitine	Diet × L-carnitine
Physiological, compositional and metabolic parameters (*n* = 9–12)							
Body weight at 16 week, g	432 ± 6^{c}	418 ± 6^{c}	508 ± 9^{a}	465 ± 8^{b}	<0.0001	0.0004	0.055
Water intake, mL d^{-1}	33.1 ± 1.1^{a}	31.4 ± 1.0^{a}	21.2 ± 0.8^{b}	22.2 ± 0.7^{b}	<0.0001	0.70	0.15
Food intake, g d^{-1}	30.8 ± 1.0^{a}	31.0 ± 1.1^{a}	23.5 ± 0.8^{b}	22.7 ± 0.6^{b}	<0.0001	0.74	0.58
Energy intake, kJ d^{-1}	349 ± 13^{a}	353 ± 14^{a}	498 ± 19^{b}	485 ± 18^{b}	<0.0001	0.78	0.60
Abdominal circumference, cm	20.4 ± 0.3^{b}	19.6 ± 0.3^{b}	22.7 ± 0.5^{a}	20.7 ± 0.4^{b}	<0.0001	0.0007	0.13
Abdominal fat (total), mg mm^{-1} tibial length	410 ± 31^{b}	203 ± 24^{c}	750 ± 36^{a}	421 ± 37^{b}	<0.0001	<0.0001	0.07
Retroperitoneal fat, mg mm^{-1} tibial length	191 ± 13^{b}	92 ± 10^{c}	337 ± 20^{a}	185 ± 16^{b}	<0.0001	<0.0001	0.09
Epididyml fat, mg mm^{-1} tibial length	123 ± 10^{b}	69 ± 7^{c}	247 ± 14^{a}	143 ± 13^{b}	<0.0001	<0.0001	0.33
Omental fat, mg mm^{-1} tibial length	97 ± 9^{b}	42 ± 7^{c}	169 ± 10^{a}	93 ± 8^{b}	<0.0001	<0.0001	0.23
Total body fat mass, g	81 ± 7^{c}	60 ± 9^{c}	155 ± 13^{a}	121 ± 12^{b}	<0.0001	0.014	0.54
Total body lean mass, g	310 ± 6	309 ± 8	323 ± 8	301 ± 5	0.72	0.11	0.14
Bone mineral content, g	12.2 ± 0.3	12.4 ± 0.6	12.8 ± 0.4	13.2 ± 0.7	0.19	0.57	0.85
Bone mineral density, g cm^{-2}	0.15 ± 0.00	0.16 ± 0.00	0.16 ± 0.00	0.16 ± 0.01	0.32	0.32	0.32
Basal blood glucose concentration, mmol L^{-1}	3.97 ± 0.10^{c}	3.98 ± 0.12^{c}	4.80 ± 0.09^{a}	4.30 ± 0.06^{b}	<0.0001	0.013	0.010
AUC, mmol L^{-1} min^{-1}	646 ± 10^{b}	657 ± 8^{b}	718 ± 7^{a}	666 ± 10^{b}	<0.0001	0.025	0.0009
Plasma insulin, μg L^{-1}	1.73 ± 0.16^{b}	1.48 ± 0.15^{b}	3.95 ± 0.29^{a}	2.38 ± 0.47^{b}	<0.0001	0.005	0.035
Plasma total cholesterol, mmol L^{-1}	1.3 ± 0.1^{b}	1.4 ± 0.1^{b}	2.1 ± .0.1^{a}	1.4 ± 0.1^{b}	0.0002	0.004	0.0002
Plasma triglycerides, mmol L^{-1}	0.4 ± 0.1^{b}	0.5 ± 0.1^{b}	1.0 ± 0.1^{a}	0.5 ± 0.1^{b}	0.004	0.05	0.004
Plasma non-esterified fatty acids, mmol L^{-1}	1.1 ± 0.1^{b}	1.0 ± 0.1^{b}	2.7 ± 0.2^{a}	1.4 ± 0.1^{b}	<0.0001	<0.0001	<0.0001
Plasma fatty acids, g per 100 g of total fatty acid content (*n* = 6)							
C14:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C14:1n-5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C16:0	26.60 ± 2.67^{c}	47.77 ± 2.06^{a}	23.06 ± 2.04^{c}	37.13 ± 2.71^{b}	0.008	<0.0001	0.15
C16:1n-7	30.97 ± 1.42^{a}	6.94 ± 1.74^{b}	27.66 ± 2.97^{a}	8.67 ± 3.05^{b}	0.75	<0.0001	0.31
C18:0	12.89 ± 1.51	13.26 ± 0.92	8.93 ± 1.74	12.51 ± 0.59	0.08	0.14	0.22
C18:1*trans*-11	0.00 ± 0.00^{b}	0.38 ± 0.38^{b}	14.23 ± 4.79^{a}	0.55 ± 0.36^{b}	0.007	0.012	0.009
C18:1n-9	7.59 ± 1.30^{b}	13.15 ± 1.68^{b}	10.54 ± 1.98^{b}	21.36 ± 1.97^{a}	0.005	0.0001	0.15
C18:2n-6	8.07 ± 3.13	8.36 ± 1.35	7.14 ± 1.62	9.31 ± 1.42	1.00	0.55	0.65
C18:3n-3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C18:3n-6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C20:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C20:3n-6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C20:4n-6	11.98 ± 1.21^{a}	9.04 ± 1.15^{ab}	6.41 ± 1.38^{b}	5.21 ± 0.71^{b}	0.0005	0.09	0.46
Total SFA	39.49 ± 3.11^{c}	61.89 ± 1.76^{a}	32.28 ± 2.35^{d}	54.04 ± 2.16^{b}	0.005	<0.0001	0.90
Total MUFA	40.49 ± 1.92^{b}	20.71 ± 2.63^{d}	54.17 ± 2.3^{a}	31.44 ± 2.55^{c}	<0.0001	<0.0001	0.54
Total PUFA	20.03 ± 2.37^{a}	17.40 ± 1.40^{ab}	13.55 ± 0.59^{b}	14.52 ± 1.52^{ab}	0.008	0.61	0.27
n-3 : n-6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
SCD-1 index	1.23 ± 0.13^{a}	0.14 ± 0.04^{b}	1.21 ± 0.09^{a}	0.24 ± 0.09^{b}	0.67	<0.0001	0.53

Values are mean ± SEM. Mean values within a row with unlike superscript letters are significantly different, $P < 0.05$. AUC, area under the curve; C, corn starch diet-fed rats; CLC, corn starch diet-fed rats supplemented with L-carnitine; H, high-carbohydrate, high-fat diet-fed rats; HLC, high-carbohydrate, high-fat diet-fed rats supplemented with L-carnitine; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCD-1, stearoyl Co-A desaturase-1; SFA, saturated fatty acids.

diameter during diastole (LVIDd), indicative of ventricular dilatation and increasing systolic volume. These changes decreased ventricular functional parameters shown by fractional shortening, ejection fraction, E/A ratio and left ventricular diastolic stiffness (Table 2). HLC rats showed normalised LVIDd, systolic volume, ejection fraction, fractional shortening, E/A ratio and left ventricular stiffness (Table 2). Thoracic aortic responses to noradrenaline (Fig. 2A), sodium nitroprusside (Fig. 2B) and acetylcholine (Fig. 2C) were diminished in H rats but improved in thoracic aorta from HLC rats (Fig. 2A–C).

Hepatic structure and function

H rats showed the presence of inflammatory cells (Fig. 3C), macrovesicular fat vacuoles (Fig. 3G) and mild portal fibrosis (Fig. 3K) in the liver. HLC rats showed inhibition of infiltration of inflammatory cells (Fig. 3D) and absence of both fat vacuoles (Fig. 3H) and portal fibrosis (Fig. 3L). Livers from H rats showed higher wet weight and this was unaffected by L-carnitine in HLC rats. However, the markers of hepatic function in plasma including ALT, AST, ALP and LDH, which were increased in H rats, were normalised in HLC rats. No differences were observed in the plasma concentrations of albumin and total bilirubin between the groups. Plasma uric acid concentrations were higher while plasma urea concentrations were lower in H rats than in C rats. These biochemical changes were attenuated in HLC rats (Table 3).

Fatty acid content

Fatty acid contents are given in Tables 1–5. C14:0 was undetectable in plasma and the heart. It was reduced in the

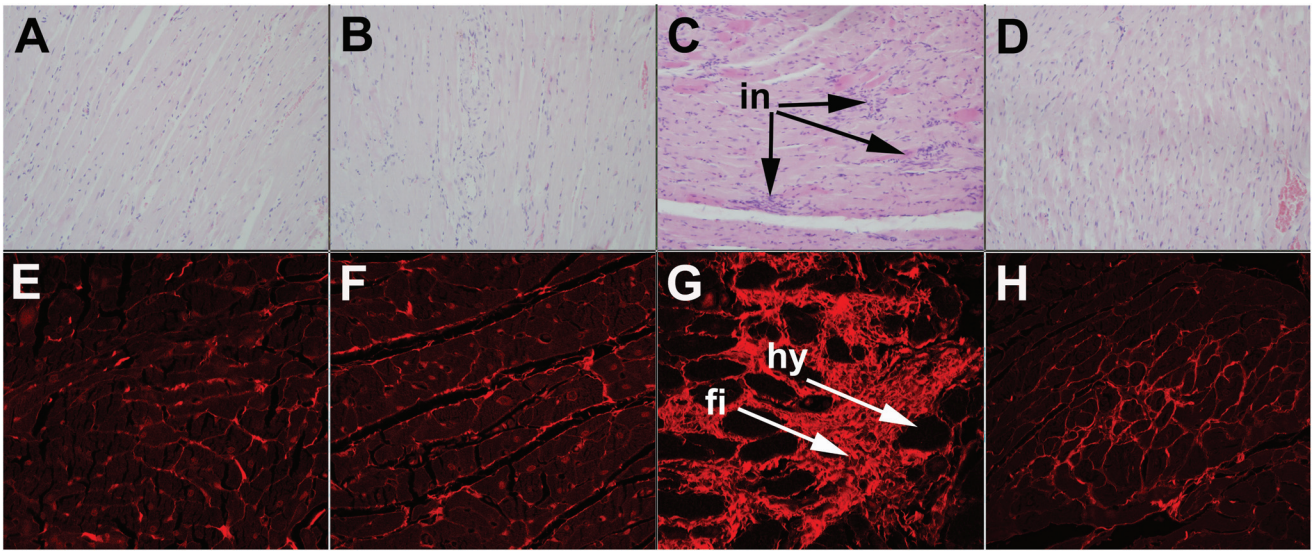

Fig. 1 Effects of L-carnitine supplementation on inflammation and fibrosis in rat heart. Top row represents haematoxylin and eosin staining of left ventricle showing infiltration of inflammatory cells (A–D, inflammatory cells marked as "in"; ×20) from C (A), CLC (B), H (C) and HLC (D) rats. Bottom row represents picrosirius red staining of left ventricle showing collagen deposition and hypertrophy (E–H, fibrosis marked as "fi" and hypertrophied cardiomyocytes as "hy"; ×40) from C (E), CLC (F), H (G) and HLC (H) rats.

liver and skeletal muscle of CLC rats compared to C rats, while it was increased in retroperitoneal fat of CLC and HLC compared to C and H rats, respectively. C14:1n-5 was undetectable in plasma and it was completely removed from heart, liver, skeletal muscle and retroperitoneal fat of CLC and HLC rats compared to C and H rats, respectively. C16:0 and C16:1n-7 were increased and decreased, respectively, by L-carnitine in plasma, heart, liver, skeletal muscle and retroperitoneal fat of both CLC and HLC rats compared to C and H rats, respectively. C18:0 was unchanged in the plasma between the groups, while increased in the heart, liver, skeletal muscle and retroperitoneal fat of both CLC and HLC rats compared to C and H rats, respectively. In plasma, C18:1*trans*-11 was decreased only in HLC rats compared to H rats. In the heart and the liver, C18:1*trans*-11 was decreased by L-carnitine in both CLC and HLC rats compared to C and H rats, respectively. In skeletal muscle, C18:1*trans*-11 was only increased in CLC compared to C rats, with HLC predominantly storing it in the retroperitoneal fat. In all the tested tissues, C18:1n-9 was increased in CLC and HLC rats compared to C and H rats, respectively. C18:2n-6 was unchanged between the groups in plasma, increased in the heart only in CLC rats compared to C rats while decreased in liver, skeletal muscle and retroperitoneal fat of CLC and HLC rats compared to C and H rats, respectively. C18:3n-3 was undetectable in plasma, heart, liver and skeletal muscle, while increased in retroperitoneal fat of CLC and HLC compared to C and H rats, respectively. C18:3n-6 was undetectable in plasma and liver while it was completely removed from heart, skeletal muscle and retroperitoneal fat in CLC and HLC rats compared to C and H rats, respectively. C20:0 was undetectable in plasma, heart and skeletal muscle. In liver and retroperitoneal fat, C20:0 was decreased in CLC compared to C rats while increased in HLC compared to H rats. While C20:3n-6 was undetectable in plasma, heart, skeletal muscle and retroperitoneal fat, it was unchanged between the groups in liver. C20:4n-6 was not changed by L-carnitine treatment in plasma and liver of CLC and HLC rats compared to their respective controls while it was undetectable in the retroperitoneal fat. In the heart, C20:4n-6 was decreased in both CLC and HLC compared to C and H rats, respectively, while it was increased in skeletal muscle of HLC rats compared to H rats.

Total saturated fatty acid (SFA) was increased in plasma, heart, liver, skeletal muscle and retroperitoneal fat of CLC and HLC rats compared to C and H rats, respectively. Total monounsaturated fatty acid (MUFA) was decreased in plasma and heart where total MUFA was increased in skeletal muscle and retroperitoneal fat of CLC and HLC rats compared to C and H rats, respectively. In liver, total MUFA was only increased in HLC rats compared to H rats. Total polyunsaturated fatty acid (PUFA) was unchanged with L-carnitine treatment in plasma of CLC and HLC rats while total PUFA was decreased in heart, liver, skeletal muscle and retroperitoneal fat. SCD-1 activity index was decreased in plasma, heart, liver, skeletal muscle and retroperitoneal fat with L-carnitine treatment.

Overall, L-carnitine increased C16:0 content in plasma through decreased SCD-1 activity. In the heart, C16:1n-7 and C18:1*trans*-11 were replaced with C16:0 and C18:0 with L-carnitine treatment. In the liver, skeletal muscle and retroperitoneal fat, C16:1n-7 was replaced with C16:0 through decreased SCD-1 activity along with increased C18:1n-9 and decreased C18:2n-6 in these tissues.

Table 2 Effects of L-carnitine on structure, function and fatty acid composition of the heart

Variables	C	CLC	H	HLC	*P* Value		
					Diet	L-Carnitine	Diet × L-carnitine
Structural and functional parameters of the heart (n = 9–12)							
Systolic blood pressure, mmHg	129 ± 2^{bc}	127 ± 2^{c}	156 ± 3^{a}	135 ± 2^{b}	<0.0001	<0.0001	0.0002
Heart rate, beats per min	252 ± 6^{a}	228 ± 6^{b}	243 ± 6^{ab}	258 ± 6^{a}	0.09	0.46	0.002
LVIDd, mm	6.64 ± 0.10^{b}	6.02 ± 0.12^{c}	7.27 ± 0.09^{a}	6.39 ± 0.16^{b}	0.0002	<0.0001	0.29
LVPWd, mm	1.65 ± 0.04	1.81 ± 0.07	1.81 ± 0.06	1.87 ± 0.09	0.11	0.11	0.46
Ejection time, ms	92 ± 2	91 ± 2	88 ± 2	87 ± 2	0.05	0.62	1.00
E/A	1.88 ± 0.10^{a}	1.99 ± 0.12^{a}	1.53 ± 0.09^{b}	1.83 ± 0.09^{a}	0.015	0.048	0.35
Deceleration time, ms	53 ± 2	63 ± 3	55 ± 3	55 ± 2	0.44	0.12	0.12
Systolic volume, μL	43 ± 6^{b}	39 ± 4^{b}	101 ± 6^{a}	49 ± 7^{b}	<0.0001	<0.0001	0.0002
Relative wall thickness	0.48 ± 0.01^{b}	0.60 ± 0.03^{a}	0.48 ± 0.01^{b}	0.55 ± 0.02^{a}	0.20	<0.0001	0.20
Fractional shortening, %	53 ± 2^{a}	51 ± 2^{a}	39 ± 2^{b}	57 ± 2^{a}	0.05	0.0002	<0.0001
Ejection fraction, %	87 ± 2^{a}	88 ± 2^{a}	72 ± 2^{b}	89 ± 3^{a}	0.004	0.0003	0.001
MCMO, ms	114 ± 4^{a}	117 ± 2^{a}	114 ± 3^{a}	103 ± 2^{b}	0.019	0.17	0.019
Estimated left ventricular mass, g	0.67 ± 0.03^{b}	0.71 ± 0.05^{b}	0.91 ± 0.03^{a}	0.73 ± 0.05^{b}	0.003	0.10	0.011
Left ventricle + septum wet weight, mg mm^{-1} tibial length	19.5 ± 0.6	20.7 ± 0.5	21.0 ± 0.9	19.7 ± 0.8	0.73	0.95	0.09
Right ventricle wet weight, mg mm^{-1} tibial length	4.2 ± 0.2	4.5 ± 0.2	4.3 ± 0.2	4.5 ± 0.2	0.80	0.22	0.80
Left ventricular diastolic stiffness constant (κ)	20.2 ± 1.1^{b}	21.0 ± 1.4^{b}	28.3 ± 1.5^{a}	22.6 ± 1.3^{b}	0.0007	0.07	0.019
Fatty acids, g per 100 g of total fatty acid content (n = 6)							
C14:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C14:1n-5	1.32 ± 0.28^{a}	0.00 ± 0.00^{b}	1.49 ± 0.31^{a}	0.00 ± 0.00^{b}	0.69	<0.0001	0.69
C16:0	2.12 ± 0.12^{b}	22.39 ± 0.37^{a}	5.70 ± 4.09^{b}	22.00 ± 0.33^{a}	0.45	<0.0001	0.35
C16:1n-7	25.95 ± 1.49^{a}	1.56 ± 0.09^{b}	21.85 ± 3.59^{a}	0.37 ± 0.16^{b}	0.19	<0.0001	0.46
C18:0	0.86 ± 0.06^{c}	21.18 ± 0.28^{a}	1.51 ± 0.10^{b}	22.71 ± 0.61^{a}	0.005	<0.0001	0.21
C18:1*trans*-11	22.50 ± 0.48^{b}	4.63 ± 0.10^{c}	24.61 ± 0.43^{a}	2.64 ± 0.03^{d}	0.86	<0.0001	<0.0001
C18:1n-9	0.92 ± 0.06^{c}	10.90 ± 0.57^{b}	0.65 ± 0.14^{c}	13.99 ± 1.00^{a}	0.025	<0.0001	0.009
C18:2n-6	11.77 ± 1.12^{b}	14.89 ± 0.59^{a}	10.12 ± 0.53^{b}	10.56 ± 0.45^{b}	0.0005	0.023	0.08
C18:3n-3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C18:3n-6	12.97 ± 0.48^{a}	0.00 ± 0.00^{c}	8.91 ± 1.78^{b}	0.00 ± 0.00^{c}	0.040	<0.0001	0.040
C20:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C20:3n-6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C20:4n-6	19.64 ± 0.75^{a}	17.03 ± 0.48^{b}	19.50 ± 0.38^{a}	16.99 ± 0.72^{b}	0.88	0.0004	0.93
Total SFA	3.54 ± 0.32^{b}	43.57 ± 0.36^{a}	8.05 ± 4.35^{b}	44.71 ± 0.45^{a}	0.21	<0.0001	0.45
Total MUFA	50.70 ± 1.53^{a}	24.51 ± 0.26^{b}	48.59 ± 4.05^{a}	27.74 ± 0.91^{b}	0.80	<0.0001	0.24
Total PUFA	45.76 ± 1.41^{a}	31.92 ± 0.39^{b}	43.36 ± 0.61^{a}	27.55 ± 0.54^{c}	0.0006	<0.0001	0.25
n-3 : n-6	0.03 ± 0.00^{ab}	0.00 ± 0.00^{b}	0.10 ± 0.05^{a}	0.00 ± 0.00^{b}	0.18	0.017	0.18
SCD-1 index	12.43 ± 0.94^{a}	0.07 ± 0.00^{b}	13.47 ± 2.94^{a}	0.02 ± 0.01^{b}	0.75	<0.0001	0.73

Values are mean ± SEM. Mean values within a row with unlike superscript letters are significantly different, $P < 0.05$. C, corn starch diet-fed rats; CLC, corn starch diet-fed rats supplemented with L-carnitine; E/A, ratio of early mitral inflow velocity to late mitral inflow velocity; H, high-carbohydrate, high-fat diet-fed rats; HLC, high-carbohydrate, high-fat diet-fed rats supplemented with L-carnitine; LVIDd, left ventricular internal diameter during diastole; LVPWd, left ventricular posterior wall thickness during diastole; MCMO, time from mitral valve closure to opening; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCD-1, stearoyl Co-A desaturase-1; SFA, saturated fatty acids.

Discussion

This study has used a high-carbohydrate, high-fat diet-fed rat model to mimic human metabolic syndrome. This obesogenic diet is rich in simple sugars (such as fructose and sucrose) as well as long-chain saturated and *trans* fats.[30] Although the food intake of obese rats was lower than the food intake of lean rats, the energy content of high-carbohydrate, high-fat diet was much higher than the energy content of corn starch diet and hence the overall energy intake was higher for high-carbohydrate, high-fat diet-fed rats than corn starch rats. Intake of energy-dense diets in humans have been linked to the development of obesity and metabolic syndrome[1] which provides the validation of this model for anti-obesity interventions. The high-carbohydrate, high-fat diet-fed rats developed central obesity, impaired glucose tolerance, hypertension, cardiovascular remodelling, non-alcoholic steatohepatitis and hyperinsulinaemia.[29]

In obesity, the concentrations of L-carnitine in the body are lowered.[34] This decreased L-carnitine in obesity may explain the reduced fat oxidation and hence the increased body fat which is associated with the development of cardiovascular remodelling and non-alcoholic fatty liver disease.[35,36] L-Carnitine supplementation increased concentrations of L-carnitine in plasma as well as muscles.[24] Thus, we increased dietary L-carnitine intake to enhance L-carnitine concentration in the body so as to increase the rate of fat oxidation. Previous studies have shown that L-carnitine supplementation increased

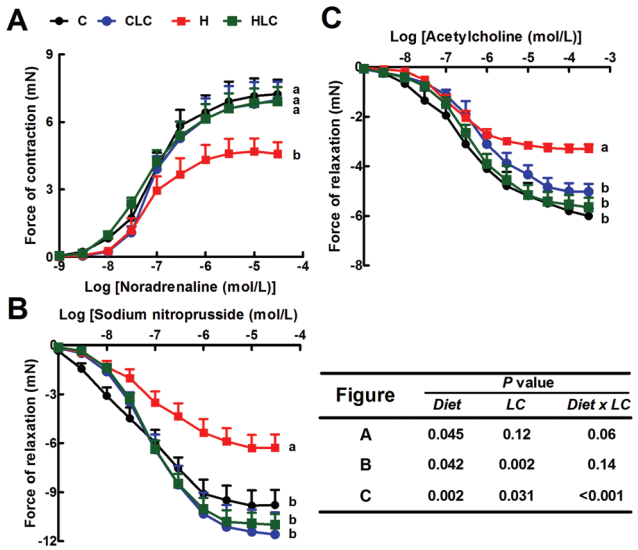

Figure	*P* value		
	Diet	*LC*	*Diet x LC*
A	0.045	0.12	0.06
B	0.042	0.002	0.14
C	0.002	0.031	<0.001

Fig. 2 Effects of L-carnitine supplementation on vascular responses of thoracic aortic preparations. Noradrenaline-induced contraction (A), sodium nitroprusside-induced relaxation (B) and acetylcholine-induced relaxation (C). Values are mean ± SEM, n = 10–12. End-point means without a common letter differ, $P < 0.05$. D, LC and D × LC represent effects of diet, effects of L-carnitine and the interaction between effects of diet and L-carnitine, respectively.

the expression of CPT1.[37] L-Carnitine-treated rats in our study showed improved metabolic status through improved glucose tolerance, improved blood lipid profile, improved body composition and reduced body fat and abdominal fat.

Removal of fat from the abdomen, reduced triglycerides and reduced NEFA in plasma support the hypothesis of increased β-oxidation with L-carnitine in obese rats. In the heart, fatty acids are the major fuel source for energy supply.[38] Supplementation of L-carnitine may further increase this energy supply in heart through increased fatty acid oxidation. In our previous study, L-carnitine supplementation in an acute model of hypertensive cardiovascular remodelling improved cardiac function.[18] Reduced β-oxidation of fatty acids coupled with increased lipogenesis in the liver leads to steatosis followed by steatohepatitis.[39,40] Normalising fatty acid β-oxidation through L-carnitine supplementation would be expected to attenuate steatohepatitis in obese rats.

L-Carnitine decreased the proportion of pro-inflammatory n-6 and *trans* fatty acids in the heart and the liver leading to the accumulation of C18:1n-9 in all tissues. However, C20:4n-6 and C18:1*trans*-11 were increased in the skeletal muscle suggesting a substrate bias in different tissues. Importantly, C18:2n-6 in the adipose tissue of L-carnitine-supplemented rats was substituted with equivalent amounts of C18:1n-9 and C18:1*trans*-11. This result strongly suggests that L-carnitine

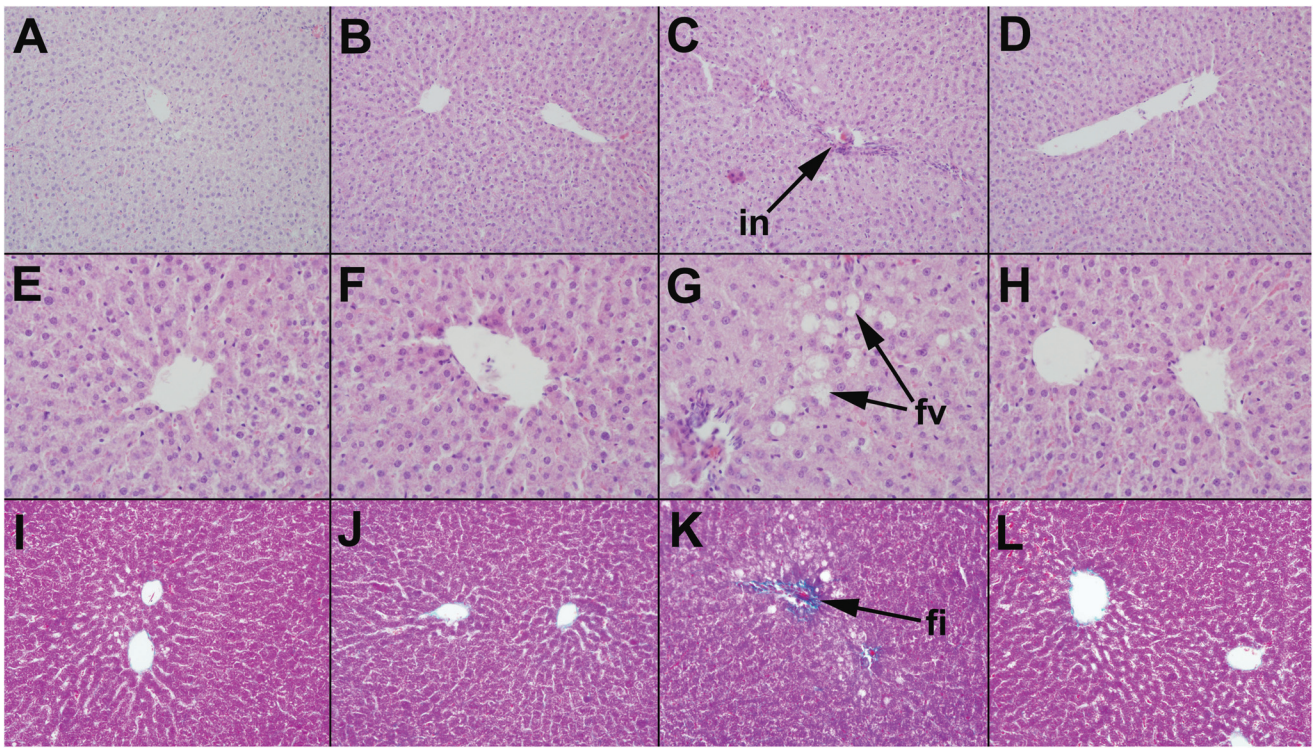

Fig. 3 Effects of L-carnitine supplementation on inflammation, macrovesicular fat deposition and fibrosis in the liver. Top and middle rows represent haematoxylin and eosin staining of the liver showing inflammatory cells (A–D, marked as "in"; ×20) and macrovesicular fat vacuoles (E–H, marked as "fv"; ×40), respectively, from C (A,E), CLC (B,F), H (C,G) and HLC (D,H) rats. Bottom row represents Milligan's trichrome staining of the liver showing fibrosis in the hepatic portal region (I–L, marked as "fi"; ×20) from C (I), CLC (J), H (K) and HLC (L) rats.

Table 3 Effects of L-carnitine on hepatic function and fatty acid composition

Variables	C	CLC	H	HLC	*P* Value		
					Diet	L-Carnitine	Diet × L-carnitine
Hepatic functions (n = 9–12)							
Liver wet weight, mg mm^{-1} tibial length	231 ± 11^{b}	221 ± 8^{b}	291 ± 10^{a}	302 ± 9^{a}	<0.0001	0.96	0.28
Plasma ALT, U L^{-1}	37 ± 4^{b}	33 ± 3^{b}	55 ± 3^{a}	39 ± 2^{b}	0.0003	0.002	0.06
Plasma AST, U L^{-1}	81 ± 4^{b}	80 ± 3^{b}	108 ± 4^{a}	88 ± 3^{b}	<0.0001	0.005	0.010
Plasma ALP, U L^{-1}	176 ± 11^{b}	157 ± 7^{b}	240 ± 12^{a}	171 ± 10^{b}	0.0004	<0.0001	0.018
Plasma LDH, U L^{-1}	240 ± 21^{b}	230 ± 17^{b}	416 ± 28^{a}	295 ± 18^{b}	<0.0001	0.004	0.013
Plasma albumin, g L^{-1}	28.0 ± 0.4	27.3 ± 0.5	28.0 ± 0.4	27.3 ± 0.3	1.00	0.09	1.00
Plasma total bilirubin, μmol L^{-1}	2.2 ± 0.1	2.4 ± 0.2	2.5 ± 0.1	2.2 ± 0.1	0.71	0.71	0.07
Plasma urea, mmol L^{-1}	5.4 ± 0.2^{a}	5.2 ± 0.2^{a}	3.5 ± 0.2^{c}	4.6 ± 0.1^{b}	<0.0001	0.016	0.0008
Plasma uric acid, μmol L^{-1}	34 ± 2^{c}	40 ± 3^{bc}	56 ± 4^{a}	47 ± 3^{b}	<0.0001	0.63	0.019
Fatty acids, g per 100 g of total fatty acid content (n = 6)							
C14:0	7.06 ± 1.19^{a}	0.00 ± 0.00^{c}	3.11 ± 0.59^{b}	1.18 ± 0.10^{bc}	0.05	<0.0001	0.001
C14:1n-5	0.74 ± 0.13^{a}	0.00 ± 0.00^{b}	0.79 ± 0.09^{a}	0.00 ± 0.00^{b}	0.76	<0.0001	0.76
C16:0	0.03 ± 0.03^{c}	24.37 ± 0.61^{a}	0.25 ± 0.05^{c}	23.20 ± 0.31^{b}	0.18	<0.0001	0.06
C16:1n-7	26.35 ± 0.99^{a}	4.56 ± 0.64^{c}	23.33 ± 0.75^{b}	1.87 ± 0.18^{d}	0.0006	<0.0001	0.82
C18:0	3.39 ± 3.39^{bc}	13.27 ± 1.13^{a}	0.32 ± 0.07^{c}	7.89 ± 0.51^{b}	0.030	0.0001	0.53
C18:1*trans*-11	15.06 ± 2.49^{a}	4.26 ± 0.28^{b}	10.87 ± 3.37^{a}	1.81 ± 0.09^{b}	0.13	0.0001	0.68
C18:1n-9	0.00 ± 0.00^{c}	25.96 ± 2.36^{b}	1.52 ± 1.52^{c}	51.71 ± 1.57^{a}	<0.0001	<0.0001	<0.0001
C18:2n-6	27.06 ± 4.47^{b}	8.21 ± 0.58^{c}	44.31 ± 4.94^{a}	4.28 ± 0.23^{c}	0.06	<0.0001	0.005
C18:3n-3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C18:3n-6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C20:0	0.40 ± 0.12^{a}	0.00 ± 0.00^{c}	0.19 ± 0.02^{b}	0.48 ± 0.03^{a}	0.044	0.39	<0.0001
C20:3n-6	0.67 ± 0.10	0.85 ± 0.16	0.73 ± 0.11	0.52 ± 0.7	0.72	0.97	0.60
C20:4n-6	10.91 ± 2.91^{a}	14.02 ± 1.19^{a}	7.96 ± 2.31^{ab}	3.84 ± 0.30^{b}	0.003	0.80	0.08
Total SFA	11.26 ± 4.14^{b}	37.64 ± 1.00^{a}	3.92 ± 0.61^{c}	32.76 ± 0.67^{a}	0.011	<0.0001	0.58
Total MUFA	42.15 ± 2.40^{b}	39.28 ± 2.48^{b}	36.69 ± 1.85^{b}	57.93 ± 1.24^{a}	0.004	0.0002	<0.0001
Total PUFA	46.59 ± 6.43^{b}	23.07 ± 1.68^{c}	59.39 ± 2.07^{a}	9.31 ± 0.61^{d}	0.89	<0.0001	0.001
n-3 : n-6	0.20 ± 0.02^{a}	0.00 ± 0.00^{c}	0.10 ± 0.02^{b}	0.00 ± 0.00^{c}	0.002	<0.0001	0.002
SCD-1 index$^{\#}$	—	0.19 ± 0.02	78.84 ± 2.44	0.08 ± 0.01	—	—	—

Values are mean ± SEM. Mean values within a row with unlike superscript letters are significantly different, $P < 0.05$. ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; C, corn starch diet-fed rats; CLC, corn starch diet-fed rats supplemented with L-carnitine; H, high-carbohydrate, high-fat diet-fed rats; HLC, high-carbohydrate, high-fat diet-fed rats supplemented with L-carnitine; LDH, lactate dehydrogenase; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCD-1, stearoyl Co-A desaturase-1; SFA, saturated fatty acids. $^{\#}$Negligible C16:0 fatty acid detected in C diet-fed group therefore showing a very high SCD-1 desaturation index in this group.

selectively facilitated the transport and oxidation of C18:2n-6 therefore limiting the production of C20:4n-6 to exert its pro-inflammatory effects. All tissues in L-carnitine-treated rats showed extremely low indices for SCD-1 activity indicating the inhibition of conversion of SFA to MUFA. Thus, SFA was increased in the plasma and the tissues. At the same time, only plasma and the heart showed reductions in total MUFA whereas the liver and the skeletal muscle preferentially stored MUFA in the form of C18:1n-9. Retroperitoneal fat from CLC rats stored C18:1n-9 whereas HLC rats stored C18:1*trans*-11 in retroperitoneal fat. Similar results were obtained in our previous study with chia seed in obese rats.[30] Higher SCD-1 activity has been implicated in obesity and cardiovascular disease with inhibition of SCD-1 activity inducing an increase in fatty acid oxidation and decrease in body fat.[41] These results have been confirmed in this study with the decrease in the activity indices of SCD-1 in all the tissues and attenuation of obesity and cardiovascular disease. Further studies will be required to determine the mechanism of inhibition of SCD-1 by L-carnitine and also the mechanism for preferential oxidation of MUFA and C18:2n-6.

In fructose-fed rats, L-carnitine supplementation reduced plasma concentrations of glucose, insulin, triglycerides and NEFA as well as liver and muscle content of triglycerides and NEFA.[20] Also, glycogen content in the liver and the muscle were increased along with reduced activity of glycogen phosphorylase. Similarly, L-carnitine reduced glucose formation from different sources of gluconeogenesis.[20] These observations clearly suggest that L-carnitine supplementation increased glycogenesis and reduced glycogenolysis and gluconeogenesis in fructose-fed rats. These effects of L-carnitine were observed in this study through reduced blood glucose concentrations and plasma concentrations of insulin, triglyceride and NEFA, similar to the reported effects of L-carnitine on glucose metabolism.[42] These effects are strongly supported by a study in piglets supplemented with L-carnitine where 563 genes were differentially expressed.[43] Gene expression modulated by L-carnitine included genes for the proteins and enzymes controlling fatty acid uptake, fatty acid activation and fatty acid oxidation.[43] Similarly, genes for glycolysis were up-regulated and genes involved in gluconeogenesis were down-regulated.[43]

Table 4 Effects of L-carnitine on fatty acid composition of skeletal muscle

					P Value		
Variables	C	CLC	H	HLC	Diet	L-Carnitine	Diet × L-carnitine
Fatty acids, g per 100 g of total fatty acid content (n = 6)							
C14:0	14.35 ± 3.23[a]	1.59 ± 0.10[b]	6.26 ± 1.58[b]	2.35 ± 0.10[b]	0.06	0.0002	0.023
C14:1n-5	1.76 ± 0.10[a]	0.00 ± 0.00[b]	2.50 ± 0.23[a]	0.00 ± 0.00[b]	0.008	<0.0001	0.008
C16:0	0.59 ± 0.15[c]	28.82 ± 0.44[a]	0.43 ± 0.03[c]	26.26 ± 0.67[b]	0.003	<0.0001	0.008
C16:1n-7	28.41 ± 0.89[a]	7.92 ± 0.70[c]	20.94 ± 1.28[b]	2.37 ± 0.13[d]	<0.0001	<0.0001	0.28
C18:0	0.33 ± 0.07[c]	7.30 ± 0.82[b]	0.58 ± 0.03[c]	10.46 ± 0.54[a]	0.003	<0.0001	0.008
C18:1*trans*-11	0.67 ± 0.67[b]	4.06 ± 0.08[a]	1.07 ± 1.07[b]	2.15 ± 0.06[b]	0.25	0.002	0.08
C18:1n-9	4.24 ± 1.02[c]	36.24 ± 1.95[b]	5.27 ± 1.06[c]	44.91 ± 2.12[a]	0.007	<0.0001	0.028
C18:2n-6	36.64 ± 3.98[b]	7.57 ± 0.37[c]	50.53 ± 3.51[a]	6.44 ± 0.36[c]	0.027	<0.0001	0.011
C18:3n-3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C18:3n-6	7.41 ± 1.52[a]	0.00 ± 0.00[b]	10.79 ± 2.18[a]	0.00 ± 0.00[b]	0.22	<0.0001	0.22
C20:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C20:3n-6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C20:4n-6	2.94 ± 0.73[a]	3.69 ± 0.74[a]	0.84 ± 0.18[b]	2.67 ± 0.47[a]	0.014	0.037	0.36
Total SFA	15.58 ± 3.45[b]	37.71 ± 0.97[a]	7.56 ± 1.55[c]	39.06 ± 1.01[a]	0.11	<0.0001	0.031
Total MUFA	35.35 ± 1.18[b]	51.03 ± 1.97[a]	30.08 ± 1.56[c]	51.83 ± 1.75[a]	0.19	<0.0001	0.08
Total PUFA	49.07 ± 3.42[b]	11.26 ± 1.05[c]	62.36 ± 2.44[a]	9.11 ± 0.79[c]	0.020	<0.0001	0.002
n-3 : n-6	0.04 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.20	0.20	0.20
SCD-1 index	63.09 ± 13.71[a]	0.28 ± 0.02[b]	49.07 ± 1.18[a]	0.09 ± 0.01[b]	0.31	<0.0001	0.33

Values are mean ± SEM. Mean values within a row with unlike superscript letters are significantly different, $P < 0.05$. C, corn starch diet-fed rats; CLC, corn starch diet-fed rats supplemented with L-carnitine; H, high-carbohydrate, high-fat diet-fed rats; HLC, high-carbohydrate, high-fat diet-fed rats supplemented with L-carnitine; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCD-1, stearoyl Co-A desaturase-1; SFA, saturated fatty acids.

Table 5 Effects of L-carnitine on fatty acid composition of retroperitoneal fat

					P Value		
Variables	C	CLC	H	HLC	Diet	L-Carnitine	Diet × L-carnitine
Fatty acids, g per 100 g of total fatty acid content (n = 6)							
C14:0	0.00 ± 0.00[c]	1.71 ± 0.02[b]	0.00 ± 0.00[c]	2.55 ± 0.09[a]	<0.0001	<0.0001	<0.0001
C14:1n-5	2.17 ± 0.04[b]	0.00 ± 0.00[c]	2.81 ± 0.10[a]	0.00 ± 0.00[c]	<0.0001	<0.0001	<0.0001
C16:0	0.22 ± 0.05[c]	28.40 ± 0.27[a]	0.42 ± 0.08[c]	20.84 ± 0.12[b]	<0.0001	<0.0001	<0.0001
C16:1n-7	31.51 ± 1.06[a]	9.41 ± 0.59[c]	22.40 ± 0.21[b]	2.55 ± 0.08[d]	<0.0001	<0.0001	0.08
C18:0	0.22 ± 0.03[d]	3.66 ± 0.18[b]	0.62 ± 0.02[c]	6.92 ± 0.08[a]	<0.0001	<0.0001	<0.0001
C18:1*trans*-11	3.46 ± 0.23[c]	3.91 ± 0.08[c]	6.97 ± 0.45[b]	60.96 ± 0.58[a]	<0.0001	<0.0001	<0.0001
C18:1n-9	0.00 ± 0.00[c]	46.30 ± 0.43[a]	0.00 ± 0.00[c]	1.37 ± 0.32[b]	<0.0001	<0.0001	<0.0001
C18:2n-6	51.23 ± 0.96[b]	6.24 ± 0.19[c]	60.76 ± 0.47[a]	3.87 ± 0.12[d]	<0.0001	<0.0001	<0.0001
C18:3n-3	0.00 ± 0.00[b]	0.35 ± 0.01[a]	0.07 ± 0.07[b]	0.31 ± 0.02[a]	0.69	<0.0001	0.15
C18:3n-6	10.12 ± 0.32[a]	0.00 ± 0.00[c]	5.18 ± 0.08[b]	0.00 ± 0.00[c]	<0.0001	<0.0001	<0.0001
C20:0	0.66 ± 0.13[a]	0.00 ± 0.00[c]	0.35 ± 0.02[b]	0.63 ± 0.02[a]	0.026	0.010	<0.0001
C20:3n-6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
C20:4n-6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	1.00	1.00
Total SFA	1.10 ± 0.14[c]	33.78 ± 0.37[a]	1.48 ± 0.13[c]	30.94 ± 0.16[b]	<0.0001	<0.0001	<0.0001
Total MUFA	37.24 ± 0.86[c]	59.63 ± 0.48[b]	32.40 ± 0.43[d]	64.88 ± 0.23[a]	0.71	<0.0001	<0.0001
Total PUFA	61.66 ± 0.92[b]	6.59 ± 0.19[c]	66.12 ± 0.48[a]	4.18 ± 0.13[d]	0.07	<0.0001	<0.0001
n-3 : n-6	0.00 ± 0.0[c]	0.06 ± 0.00[b]	0.00 ± 0.00[c]	0.08 ± 0.00[a]	<0.0001	<0.0001	<0.0001
SCD-1 index	129.55 ± 16.75[a]	0.32 ± 0.03[c]	44.38 ± 0.51[b]	0.13 ± 0.00[c]	<0.0001	<0.0001	<0.0001

Values are mean ± SEM. Mean values within a row with unlike superscript letters are significantly different, $P < 0.05$. C, corn starch diet-fed rats; CLC, corn starch diet-fed rats supplemented with L-carnitine; H, high-carbohydrate, high-fat diet-fed rats; HLC, high-carbohydrate, high-fat diet-fed rats supplemented with L-carnitine; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCD-1, stearoyl Co-A desaturase-1; SFA, saturated fatty acids.

This study along with earlier studies supports that L-carnitine supplementation rectifies the changes in carbohydrate and lipid metabolism produced by an obesogenic diet.[15,44,45] The metabolic disturbances and diminished endothelial responses were associated with the presence of oxidative stress.[46–48] L-Carnitine supplementation attenuated the metabolic complications and improved cardiovascular responses suggesting decreased oxidative stress. This hypothesis is

supported by the anti-oxidative responses shown by L-carnitine in L-*N*-nitroarginine methyl ester-induced hypertensive rats.[49]

In conclusion, high-carbohydrate, high-fat diet-fed rats serve as a suitable animal model for the metabolic, cardiovascular and hepatic complications seen in human metabolic syndrome. L-Carnitine supplementation in these rats attenuated the symptoms of metabolic syndrome, cardiovascular remodelling and non-alcoholic fatty liver disease. Also, SCD-1 activity was inhibited by L-carnitine supplementation. These changes were accompanied by the differential metabolism of fatty acids and differential storage of fatty acids in various tissues including retroperitoneal fat mainly storing *trans* fats in the rats given L-carnitine with high-carbohydrate, high-fat diet.

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