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L-Carnitine improvement of cardiac function is associated with a stimulation in glucose but not fatty acid metabolism in carnitine-deficient hearts

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Abstract

Objectives: Increasing myocardial carnitine content can improve heart function in patients with carnitine deficiency. We were interested in determining the effects of L-carnitine on cardiac function and substrate metabolism in a rat model of carnitine deficiency. **Methods:** Carnitine deficiency was induced in male Sprague-Dawley rats by supplementing the drinking water with 20 mM sodium pivalate. Control animals received an equimolar concentration of sodium bicarbonate. Following treatment, cardiac function and myocardial substrate utilization were determined in isolated working hearts perfused with glucose and relevant levels of fatty acids. To increase tissue levels of carnitine, hearts were perfused with 5 mM L-carnitine for a period of 60 min. **Results:** Hearts from sodium pivalate-treated animals demonstrated a 60% reduction in total heart carnitine content, depressions in cardiac function and rates of palmitate oxidation, and elevated rates of glycolysis compared to control hearts. Treatment with L-carnitine increased total carnitine content and reversed the depression in cardiac function seen in carnitine-deficient hearts. However, this was not associated with any improvement in palmitate oxidation. Rates of glycolysis and glucose oxidation, on the other hand, were increased with L-carnitine. **Conclusions:** Our findings indicate that acute L-carnitine treatment is of benefit to cardiac function in this model of secondary carnitine deficiency by increasing overall glucose utilization rather than normalizing fatty acid metabolism.

Keywords: Myocardial function; Cardiac metabolism; L-Carnitine; Glucose transport; Fatty acids

1. Introduction

Under aerobic conditions, fatty acids are the preferred energy substrates for the heart [1]. The transport of long-chain fatty acids across the mitochondrial membranes to the site of β-oxidation is dependent on carnitine [2]. Carnitine also participates in reversible transesterification reactions, forming esters such as acetylcarnitine, via the carnitine acetyltransferase pathway [3]. This decreases the intramitochondrial acetyl CoA/CoA ratio [4], resulting in a stimulation of pyruvate dehydrogenase complex (PDC) activity [5]. For this reason, the role of carnitine as a regulator of myocardial glucose utilization should be considered [6].

The physiological importance of carnitine is evident from clinical studies demonstrating that myocardial carnitine deficiency is associated with cardiomyopathy [7–13]. In some instances, L-carnitine supplementation has been shown to be beneficial in restoring tissue levels of carnitine and improving heart function [11–13]. Cardiac depression is also evident in experimental models of carnitine deficiency, and carnitine treatment is of benefit, presumably by improving overall oxidative metabolism [14,16,17]. However, whether this is primarily due to an increase in fatty acid oxidation or glucose oxidation has not been addressed.

Carnitine deficiency can be induced in animals by oral administration of sodium pivalate, as initially described by Bianchi and Davis [18]. Administration of sodium pivalate produces a reduction in plasma and tissue carnitine levels within a few days by a mechanism of excessive formation and excretion of pivaloylcarnitine [19,20]. We have recently confirmed the work of Bianchi and Davis, and further demonstrated that, following treatment with sodium pivalate for a period of 26 weeks, the reduction in heart

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total carnitine content was associated with a depression of cardiac performance and myocardial fatty acid oxidation [21]. The magnitude of these alterations, however, was related to the level of the external work and to the concentration of fatty acids supplied to the heart [21,22].

The present study was undertaken to determine whether acute L-carnitine treatment would reverse these alterations in cardiac function and palmitate oxidation seen in carnitine-deficient hearts. Although we have demonstrated that chronic L-propionylcarnitine (LPC) treatment in combination with sodium pivalate was beneficial to tissue carnitine levels and mechanical function [22], its effects on myocardial substrate oxidation were not determined. This compound could potentially alter both glucose and fatty acid oxidation since it may enhance the tricarboxylic acid cycle [23]. For this reason, we chose to use L-carnitine in this study. We hypothesize that by acutely treating carnitine-deficient hearts with L-carnitine, this would increase tissue levels of carnitine and restore palmitate oxidation.

2. Methods

2.1. Materials

Sodium pivalate (trimethylacetic acid) was purchased from Aldrich Chemicals, Milwaukee, WI. D-[U-^{14}C]glucose, 5-[^{3}H]glucose, and D-[U-^{14}C]palmitate were obtained from Amersham Life Sciences, Arlington Heights, IL. Bovine serum albumin (fraction V) was obtained from Sigma Chemicals, St Louis, MO. Hyamine hydroxide (methylbenzethonium; 1 M in methanol solution) was purchased from ICN Radiochemicals, Irvine CA. Dowex 1-X4 anion exchange resin (200–400 mesh chloride form) was obtained from Bio-Rad Laboratories (Richmond, CA). L-Carnitine was a generous gift from Sigma Tau Pharmaceuticals, Rome, Italy. All other chemicals were of reagent grade.

2.2. Animals

Carnitine deficiency was induced in male Sprague-Dawley rats (70–80 g) by adding a 20 mM concentration of sodium pivalate in the drinking water, as described previously [18]. Control rats received an equimolar concentration of sodium bicarbonate. Sodium bicarbonate was used in control animals to balance sodium intake and because it is readily excreted in the urine when ingested in excessive amounts. All solutions were adjusted to pH 7.08–7.10. Pivalate administration was continued for a period of 26–28 weeks after which the hearts were used for experimentation. Animals used in this study were cared for according to the recommendations in *The Guide for the Care and Use of Laboratory Animals*, National Institute of Health, Publ. No. 85-23, 1986.

2.3. Heart perfusions

Following treatment, hearts from sodium-pentobarbitol-anesthetized rats were quickly excised, placed in ice-cold buffer and immediately perfused retrogradely via the aorta with Krebs-Henseleit buffer containing 11 mM glucose and 1.25 mM Ca^{2+} (pH 7.4, gassed with 95% O_2–5% CO_2). During this perfusion, the hearts were trimmed of excess tissue, the pulmonary arteries were cut, and the openings of the left atria were cannulated. Hearts were then switched to the working mode and perfused for 60 min at a 15 cmH_2O left atrial filling pressure and 80 cmH_2O hydrostatic aortic afterload in a recirculating buffer system containing 11 mM glucose and 0.4 mM palmitate prebound to 3% bovine serum albumin. L-Carnitine, when used, was added to the perfusate at the concentration of 5 mM. This condition was selected because the uptake of carnitine by the heart is a slow process, involving Na^{+}-dependent diffusion and carrier-mediated components [24]. Heart rate and aortic pressure development were recorded using a PPG Simultrace AR-6 recorder interfaced to a Buxco Hemodynamic analyzer. Aortic output was measured by timed collections. Cardiac work was expressed as the product of aortic flow and systolic pressure. Functional parameters were measured throughout the experiment, but the average obtained at 45 and 60 min was reported. At the end of the perfusion, a 5-minute Langendorff drip-out was initiated to remove any carnitine present in the extracellular space. Thereafter, hearts were rapidly frozen with clamps precooled to the temperature of liquid nitrogen.

2.4. Measurement of glycolysis, glucose oxidation, and palmitate oxidation

Myocardial substrate utilization from exogenous glucose and palmitate were measured as described previously [6]. Glycolysis and glucose oxidation rates were measured simultaneously by perfusing hearts with Krebs-Henseleit buffer containing 11 mM [5-^{3}H/U-^{14}C]glucose (0.80 μCi/ml of ^{3}H and 0.50 μCi/ml of ^{14}C) and 0.4 mM palmitate. Palmitate oxidation rates were measured in a separate series of hearts perfused with 11 mM glucose and 0.4 mM [U-^{14}C]palmitate (40 μCi/ml of ^{14}C).

Glycolysis rates were determined by measuring tritiated water production from 5-[^{3}H]glucose which occurs at the level of the enolase reaction of glycolysis. Oxidative rates of glucose and palmitate were determined by quantitative measurement of both gaseous and perfusate $^{14}CO_2$ production by the hearts. Rates of substrate utilization were determined at 15-min intervals throughout the 60-min perfusion period, but only those measured between 30 and 60 min when rates of $^{3}H_2O$ and $^{14}CO_2$ production were found to be linear, were presented.

2.5. Measurement of tissue levels of carnitine

Myocardial levels of carnitine and esters were extracted from ventricular tissue by standard perchloric acid procedures. Briefly, powdered frozen heart tissue was sonicated in 12% cold perchloric acid and then centrifuged at 500 × g for 5 min at 4°C. The supernatant was removed and neutralized with 6N KOH. An aliquot of this free extract was used for the determination of free carnitine. Short-chain acylcarnitine was determined by using a volume of free extract adjusted to pH 11-12 and hydrolyzed for 1 h at 50°C. Hydrolysis was ended by neutralizing the pH with

MOPS-HCl. The fraction was then spun at 500 × *g* for 5 min at 4°C, and the supernatant used for short-chain acylcarnitine analysis. Long-chain acylcarnitine was measured in the pellet obtained following centrifugation of the initial free extract. The pellet was sonicated with 0.5M KOH and subjected to the same hydrolysis conditions as for short-chain acylcarnitine. Each fraction was assayed using ^{14}C-acetyl CoA and carnitine acetyltransferase, as described previously [25]. Total carnitine content was considered as the sum of carnitine found in the free, short-chain, and long-chain fractions.

2.6. Statistical analysis

Statistical significance for comparisons among groups was determined using a two-way factorial analysis of variance. When the interaction effect between carnitine deficiency and/or L-carnitine treatment was significant, the statistical significance of the differences between individual groups was further established using the Bonferroni test. When there was no significant interaction effect, the significance of the effect of carnitine deficiency and/or L-carnitine treatment was established according to the main effects observed in the two-way analysis of variance. The unpaired *t*-test was used for the determination of statistical difference of group means. A value of $P < 0.05$ was considered significant. All data are reported as mean ± s.e.m.

3. Results

3.1. Physical characteristics of animals

The heart and body weights of control and pivalate-treated animals are presented in Table 1. Body weight was

Table 1
Physical characteristics of control and pivalate-treated animals

Group	Body weight (g)	Dry heart weight (mg)	Heart-to-body weight ratio (mg/g)
Control	703 ± 20	388 ± 7	0.56 ± 0.01
Pivalate-treated	709 ± 16	438 ± 11 *	0.62 ± 0.01 *

Values are presented as mean ± s.e.m. of 24 and 27 control and pivalate-treated animals, respectively.
* $P < 0.05$ vs. control group.

Table 2
Effects of L-carnitine treatment on myocardial carnitine and ester content

Group	Free	Short-chain acyl (nmol/g dry weight)	Long-chain acyl	Total
Control	5798 ± 155	1477 ± 56	428 ± 71	7704 ± 197
(+) Carnitine	8331 ± 387	2150 ± 107	534 ± 54	11014 ± 526
Pivalate	2138 ± 217	573 ± 53	501 ± 33	3083 ± 281
(+) Carnitine	4022 ± 393	1098 ± 109	398 ± 37	5529 ± 503
		F-ratios * *		
Pivalate effect	153.86 *	118.48 *	0.32	145.49 *
Carnitine effect	47.23 *	44.40 *	0.00	47.21 *
Pivalate × carnitine	1.02	0.68	3.45	1.06

Values are presented as mean ± s.e.m. for 6–8 animals in each group. Carnitine-deficient hearts are termed pivalate.
* * *F*-ratios of a two-way factorial ANOVA to determine if carnitine deficiency and/or L-carnitine had an effect on heart carnitine and ester content.
* Significant *F*-ratios, $P < 0.05$.

Table 3
Effects of acute L-carnitine treatment on mechanical function of control and carnitine-deficient hearts

Group	HR (bpm)	Aortic SP (mm Hg)	Aortic DP (mm Hg)	Aortic flow (ml/min)	Cardiac work ($\times 10^{-3}$)
Control	254 ± 7	88 ± 2	42 ± 2	35 ± 2	3.1 ± 0.2
(+) Carnitine	257 ± 7	93 ± 2	41 ± 3	42 ± 3	3.9 ± 0.3
Pivalate	232 ± 6	93 ± 2	38 ± 3	25 ± 2	2.4 ± 0.1
(+) Carnitine	246 ± 6	93 ± 2	41 ± 3	35 ± 3	3.3 ± 0.2
			F-ratios * *		
Pivalate effect	4.31*	1.57	0.65	10.88 *	13.61 *
Carnitine effect	2.00	1.07	0.22	32.21 *	22.82 *
Pivalate × carnitine	1.60	2.50	0.38	1.22	0.05

Values are presented as mean ± s.e.m. for 13–16 animals in each group. HR = heart rate in beats per minute; SP = systolic pressure; DP = diastolic pressure; CW = cardiac work, expressed as aortic flow × systolic pressure product in mm Hg · ml/min × 10^{-3}.
Carnitine-deficient hearts are termed pivalate.
* * *F*-ratios of a two-way factorial ANOVA to determine if carnitine deficiency and/or L-carnitine had an effect on heart function. * Significant *F*-ratios, $P < 0.05$

Table 4
Effects of L-carnitine on myocardial substrate utilization in control and carnitine-deficient hearts

Group	Glycolysis (absolute rates) (nmol/g dry wt·min) *	Glucose oxidation	Palmitate oxidation
Control	1306±89	601±42	215±21
(+) Carnitine	2040±169	1081±80	186±21
Pivalate	1976±256	846±71	153±24
(+) Carnitine	2654±201	1093±83	113±12
	F-ratios **		
Pivalate effect	10.11 *	3.64	11.17 *
Carnitine effect	12.26*	28.56 *	3.11
Pivalate×carnitine	0.02	2.74	0.07

Values are presented as mean±s.e.m. of 6–8 hearts for each group. Carnitine-deficient hearts are termed pivalate.
* Rates are eitehr [^{3}H] glucose utilized, [^{14}C] glucose–oxidized, or [^{14}C] palmitate oxidized for glycolysis, glucose oxidation, and palmitate oxidation, respectively.
** *F*-ratios of a two-way factorial ANOVA to determine if carnitine deficiency and/or L-carnitine had an effect on myocardial substrate use. * Significant *F*-ratios, $P < 0.05$.

similar between control and pivalate-treated animals. However, heart weight was higher in those following treatment with sodium pivalate. As a result, the heart-to-body weight ratio was higher in these animals.

3.2. Effects of L-carnitine treatment on myocardial total carnitine content and esters

As shown in Table 2, carnitine deficiency had a significant main effect on myocardial total carnitine content, resulting in a 60% reduction. This was a result of a main effect on both free and short-chain fractions. Treatment with L-carnitine for a period of 60 min had a significant main effect of increasing the levels of these fractions, resulting in a major increase in total heart carnitine content. Perfusion of control hearts with L-carnitine also had a significant effect of increasing intracellular myocardial carnitine content. No significant interactions were observed between carnitine deficiency and carnitine treatment on total carnitine and ester content.

3.3. Effects of L-carnitine on mechanical function of control and carnitine-deficient hearts

Table 3 shows that mechanical function is depressed in carnitine-deficient hearts. In fact, the significant main effect of carnitine deficiency was reflected as depressions in heart rate, aortic flow, and cardiac work. The effects of L-carnitine treatment were significant, reversing the depressions in aortic flow and cardiac work. Interestingly, L-carnitine treatment also had a significant main effect of enhancing the cardiac work of control hearts. The aortic pressures were not altered by carnitine deficiency or L-carnitine treatment. No significant interactions were seen between carnitine deficiency and carnitine treatment on these functional parameters.

3.4. Effects of L-carnitine on myocardial glucose utilization and palmitate oxidation

Myocardial rates of glycolysis, glucose oxidation, and palmitate oxidation are shown in Table 4. As shown by the *F*-ratios, carnitine deficiency had a significant main effect on myocardial glycolysis only. L-Carnitine treatment had a significant main effect on glycolysis and glucose oxidation, which were both elevated. Although this effect was significant in both carnitine-deficient and control hearts, based on the absolute rates, L-carnitine had more of an effect on glucose oxidation in control hearts. No significant interaction was observed between carnitine deficiency and carnitine treatment on overall glucose metabolism.

Table 4 also shows that carnitine deficiency had a

Table 5
Effects of L-carnitine on myocardial substrate utilization rates normalized for work performed by control and carnitine-deficient hearts

Group	Glycolysis (rates normalized for cardiac work) (nmol substrate metabolized/min·AF×SP×10^{-3}) *	Glucose oxidation	Palmitate oxidation
Control	200±15	92±7	24±2
(+) Carnitine	241±50	121±11	18±3
Pivalate	346±70	145±22	31±5
(+) Carnitine	341±26	141±12	16±2
		F-ratios **	
Pivalate effect	5.85 *	5.70 *	0.36
Carnitine effect	0.13	0.70	8.37 *
Pivalate×carnitine	0.20	1.08	1.90

Values are presented as mean±s.e.m. of 6–8 hearts for each group. Carnitine-deficient hearts are termed pivalate.
* Substrate metabolized considered as either [^{3}H] glucose utilized, [^{14}C] glucose oxidized, or [^{14}C] palmitate oxidized for glycolysis, glucose oxidation, and palmitate oxidation, respectively.
** *F*-ratios of a two-way factorial ANOVA to determine if carnitine deficiency and/or L-carnitine had an effect on myocardial substrate use. * Significant *F*-ratios, $P < 0.05$.

significant main effect of decreasing palmitate oxidation. Interestingly, treatment with L-carnitine had no significant effect on palmitate oxidation in either carnitine-deficient or control hearts. In fact, palmitate oxidation rates tended to be further decreased following treatment with L-carnitine. As with glucose metabolism, there was no significant interaction between carnitine deficiency and L-carnitine treatment on palmitate oxidation.

As shown in Table 5, there is a significant effect of carnitine deficiency when rates of glycolysis and glucose oxidation are normalized for cardiac work, whereas this effect is not observed for palmitate oxidation. In contrast, there was no effect of L-carnitine treatment on glucose utilization, whereas the effect of L-carnitine was significant on decreasing palmitate oxidation. This suggests that the stimulation of glucose utilization is commensurate with the enhancement of mechanical function, but not for normalized rates of fatty acid oxidation. There was no interaction between carnitine deficiency and treatment with L-carnitine on all myocardial substrate utilization rates normalized for work.

L-Carnitine, by stimulating PDC, could potentially lower pyruvate levels and hence lactate release. Lactate production by hearts was estimated by subtracting the rate of glucose oxidation from the rate of glycolysis and multiplying by 2. In control hearts, lactate production was estimated to be at 1.4 ± 0.1 μmol/g dry wt $\cdot$ min^{-1}, and increased to 1.9 ± 0.5 μmol/g dry wt $\cdot$ min^{-1} in hearts perfused in the presence of L-carnitine. In carnitine-deficient hearts, lactate production was significantly higher at 2.2 ± 0.4 μmol/g dry wt $\cdot$ min^{-1} compared to control hearts, and increased to 3.1 ± 0.4 μmol/g dry wt $\cdot$ min^{-1} with L-carnitine. However, the estimated increases in lactate production seen in control and carnitine-deficient hearts perfused with L-carnitine were not significant.

4. Discussion

In the present study, we show that carnitine content is reduced in hearts following treatment with sodium pivalate, exerting significant depressions on palmitate oxidation and cardiac function. In an attempt to reverse these changes, we show that acute treatment with L-carnitine is of benefit in restoring heart levels of carnitine and improving cardiac function. To our surprise, however, fatty acid oxidation rates are not increased if carnitine levels are elevated in carnitine-deficient hearts. In fact, the beneficial effects of L-carnitine on heart function are better accounted for by its effects in stimulating overall myocardial glucose utilization.

There is now mounting evidence that carnitine can exert a key role in the regulation of glucose metabolism in the heart. By binding acetyl groups, carnitine can decrease the intramitochondrial acetyl CoA/CoA ratio [4], resulting in a stimulation of PDC activity [5]. Stimulation of PDC activity is also supported by the observation that extramitochondrial carnitine can buffer matrix acetyl CoA in heart mitochondria, thereby enhancing pyruvate utilization [26]. In the intact fatty acid-perfused heart, stimulation of glucose oxidation also occurs with L-carnitine [6]. Ironically in severe carnitine-deficient states, an increase in glucose oxidation is also seen [21]. This could occur as a result of a decrease in the intramitochondrial acetyl CoA/CoA ratio, secondary to a decrease in fatty acid oxidation.

An interesting observation from this study is that rates of glucose utilization are higher in carnitine-deficient hearts than those seen in control hearts. The reason for this increased reliance on glucose metabolism could relate to the limitation in fatty acid oxidation, since the heart must rely on alternate substrates for its provision of ATP [27]. Under these conditions, the uptake and subsequent utilization of glucose is critical and provides immediate energetic support [28]. In carnitine-deficient hearts treated with L-carnitine, it is interesting to observe that glucose metabolism is further increased, although to a lesser extent than seen in control hearts. The reasons for these differences are not certain, but may relate to the fact that in carnitine-deficient hearts, glycolysis and glucose oxidation rates are already high and hearts may be utilizing glucose at maximal rates.

It is possible that the improvement in mechanical function that we observed in control and carnitine-deficient hearts treated with L-carnitine is occurring in response to a positive inotropic effect. In dog and pig heart, L-carnitine has been shown to have a direct effect on contractility and left ventricular pressure, without affecting myocardial oxygen consumption [29,30]. Interestingly in humans, the derivative of L-carnitine, L-propionylcarnitine, also exerts an acute positive inotropic effect of function without altering oxygen consumption [31]. In our study, the possibility that the improvement in cardiac function by L-carnitine, such as aortic flow, is mediated through inotropy cannot be discounted.

The demonstration that fatty acid oxidation rates were not stimulated with L-carnitine appears to be consistent with the recently proposed mechanism involving malonyl CoA inhibition of fatty acid oxidation in the heart. As reported by Saddik et al. [32], increasing malonyl CoA production by direct activation of PDC can actually reduce mitochondrial oxidation of fatty acids. Although conjectural, it is possible that L-carnitine can down-regulate fatty acid oxidation by increasing the levels of malonyl CoA by a mechanism of increased cytosolic supply of acetyl CoA [4]. In support of this, Schonekess et al. [33] have shown that increasing carnitine levels in hypertrophied hearts will result in a dramatic increase in malonyl CoA levels.

In summary, we have demonstrated that acute L-carnitine treatment is of benefit in restoring heart levels of carnitine and preventing the depression in cardiac function from occurring in the pivalate-induced model of carnitine deficiency. In addition, the anticipated increase in palmitate oxidation with L-carnitine was not observed in carnitine-deficient hearts. Rather, our data suggested that acute carnitine treatment is clearly beneficial to heart function by increasing overall glucose utilization.

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