Effect of L-Carnitine Treatment on Lipid Metabolism and Cardiac Performance in Chronically Diabetic Rats

BRIAN RODRIGUES, HONG XIANG, AND JOHN H. McNEILL

The beneficial effects of L-carnitine administration were studied in vivo in isolated perfused working hearts from control and diabetic rats. Control and streptozocin-induced diabetic (STZ-D) rats were treated daily for 6 wk with high-dose L-carnitine (3 g · kg^{-1} · day^{-1} i.p.). STZ-D results in loss of body weight and hypoinsulinemia. These effects were not altered by L-carnitine treatment. Myocardial free-carnitine levels were decreased in the untreated diabetic rats. L-Carnitine treatment of the diabetic rats increased myocardial free-carnitine levels, which were comparable with those of control rats. Six weeks after STZ administration, hearts from untreated diabetic animals exhibited depressed left ventricular developed pressure, cardiac contractility, and ventricular relaxation rates compared with control animals. However, this depression was not seen in the L-carnitine–treated diabetic animals. L-Carnitine treatment of diabetic rats significantly reduced plasma glucose and lipid levels but had no effect on control rats. Furthermore, thyroid hormone levels were higher in the L-carnitine–treated diabetic rats than in the untreated diabetic group. The data suggest that high-dose L-carnitine treatment may reduce the severity of diabetes and result in improved cardiac performance.
***Diabetes* 37:1358–64, 1988**

Chronic diabetes mellitus is known to result in myocardial abnormalities in both clinical and experimental settings. In animal models of severe diabetes, isolated working hearts from chronically diabetic rats showed a decreased ability to respond to increased filling pressures and increasing afterloads (1,2); isolated papillary muscle from diabetic rats had a depressed velocity of shortening and a delayed onset of relaxation (3); and ventricular stiffening has been shown to be associated with shortening of left ventricular ejection time in 1-yr alloxan-induced diabetic dogs (4). The pathogenesis of these myocardial defects seen in diabetes are complex and may involve a variety of cardiovascular conditions including depressed myocardial myosin and actomyosin Ca^{2+}-ATPase (5) and sarcoplasmic reticular Ca^{2+}-Mg^{2+}-ATPase activities (6) and a defective sarcolemmal Ca^{2+} pump (7). However, the metabolic cause of these defects in cardiac performance is unclear but may relate to the inability of the diabetic heart to utilize glucose as an energy substrate (8) and hence its exclusive dependence on fatty acid for energy production (9).

Fatty acid oxidation requires the presence of both coenzyme A (CoA) and carnitine. CoA is necessary for the cytosolic activation of long-chain free fatty acids to acyl-CoA esters, which in turn serve as the substrate for β-oxidation. However, the long-chain acyl-CoA esters formed in the cytosol cannot penetrate the inner mitochondrial membrane for subsequent β-oxidation (10). The role of carnitine is to transport long-chain fatty acyl moieties across the inner mitochondrial membrane into the mitochondrial matrix (11); thus, adequate carnitine levels are required for normal fatty acid and energy metabolism in heart muscle. Lack of sufficient carnitine to transport fatty acyl groups of acyl-CoA into the mitochondria may be the factor responsible for the accumulation of free fatty acid and related intermediates, such as long-chain fatty acyl-CoA, and fatty acyl esters of carnitine and for the reduction of ATP. These metabolic abnormalities may relate to the defects in cardiac performance, because high tissue levels of these amphiphile compounds have been shown to interfer with various cellular functions by specifically inhibiting enzymes or nonspecifically altering structure of membranes by their detergent-like effects (12). For example, long-chain acyl-CoA inhibits the adenine nucleotide translocator in isolated mitochondria (13), a key factor in the regulation of normal ATP homeostasis, and long-chain acylcarnitine inhibits sarcolemmal Na^+-K^+-ATPase of the sar-

From the Division of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, British Columbia, Canada.
Address correspondence and reprint requests to John H. McNeill, PhD, Dean, Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada.
Received for publication 1 December 1987 and accepted in revised form 3 May 1988.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/10/1358/355584/37-10-1358.pdf by guest on 23 March 2023

colemma (14) and sarcoplasmic reticular Ca^{2+}-ATPase and Ca^{2+} uptake (15). Thus, adequate carnitine levels are required for normal fatty acid and energy metabolism in heart muscle, and changes in its level may affect energy production and muscle performance.

The importance of carnitine in cardiac metabolism and function is emphasized by the growing number of studies demonstrating a close association between systemic and myopathic carnitine deficiency and both hypertrophic and congestive cardiomyopathies (16), which in some cases can be reversed by carnitine treatment (17,18). Thus, the observation that diabetic hearts have a deficiency in the total carnitine pool (19,20) and the correlation between carnitine deficiency and cardiomyopathy suggested that carnitine therapy may ameliorate alterations in cardiac contractile performance seen during diabetes by decreasing accumulation of lipid intermediates (particularly long-chain acylcarnitine esters). However, previous studies in our laboratory (6) showed that diabetic rats treated with oral doses of DL-carnitine did not show any improvement in cardiac function, which remained depressed. In any pharmacological study, however, both the dose and the route of administration are important factors in establishing an effect. For example, L-carnitine administered subcutaneously to hypertriglyceridemic Zucker rats at 250–2000 $mg \cdot kg^{-1} \cdot day^{-1}$ significantly decreased plasma triglycerides in these obese rats over 8–12 wk. Oral doses at the same levels were not effective in decreasing plasma triglycerides (21). In addition, our previous study (6) employed DL-carnitine instead of L-carnitine, and studies have shown that D-carnitine is an antagonist of L-carnitine, the naturally occurring form of carnitine (22).

Paulson et al. (23) recently showed that daily intraperitoneal administration of L-carnitine for 2 wk to diabetic rats significantly increased total myocardial carnitine stores, and when exposed to varying times of ischemia followed by reperfusion, these hearts were better able to recover contractile performance than saline-treated diabetic rats. In addition, prior in vivo treatment with L-carnitine or providing L-carnitine in the perfusion medium attenuated the elevation of long-chain fatty acyl-CoA and prevented the decline in myocardial ATP levels in acutely diabetic hearts (24,25). Although the above studies suggested carnitine therapy may be beneficial to the acutely diabetic heart, no studies have been conducted to test whether long-term treatment with L-carnitine would be successful in reversing the cardiac dysfunction seen during chronic diabetes. We tested this possibility by treating streptozocin-induced diabetic (STZ-D) rats intraperitoneally with L-carnitine for 6 wk to replenish myocardial carnitine levels and to see if this intervention could prevent the depression in heart function.

MATERIALS AND METHODS

Animals. Male Wistar rats (200–220 g; Charles River Canada, Montreal, Canada) were transiently anesthetized with ether to allow injection of either STZ (Sigma, St. Louis, MO) or its vehicle into the tail vein. Diabetes was induced by a single injection of STZ (55 mg/kg) dissolved in citrate buffer (pH 4.5). Control rats were injected with citrate buffer alone. All rats injected with STZ survived and were thereafter housed two to three per cage. Food and water were provided ad libitum throughout the study. Severity of the disease was determined by measuring the extent of glycosuria with enzymatic test strips (Tes-Tape, Lilly, Toronto). The rats were then randomly divided into four groups: saline-treated control; L-carnitine–treated control; saline-treated STZ-D; and L-carnitine–treated STZ-D. The L-carnitine salt (3 $g \cdot kg^{-1} \cdot day^{-1}$) dissolved in saline was injected intraperitoneally starting 3 days after STZ administration. Varying injection sites were used because the rats tended to develop superficial lesions due to the osmotic properties of L-carnitine, which was more evident in the L-carnitine–treated control rats. At death, no gross evidence of trauma was evident within the abdominal cavity as a result of repeated injections. The injections were given at the same time of day. In these studies, a high intraperitoneal dose of carnitine (L-carnitine inner salt purchased from Sigma) was chosen because carnitine in the plasma distributes to the liver, kidney, and spleen at a faster rate than to cardiac muscle (26). In addition, a similar dose to diabetic rats has been shown to reduce glucose, free fatty acids, and triglycerides and to increase myocardial total carnitine content (23). Saline-treated animals received an equal volume of 0.9% NaCl solution. The rats were treated for 6 wk, with the last injection given 24 h before death. The length of the study was chosen because in a study conducted in our laboratory (27), alterations in cardiac performance occurred 6 wk after the onset of diabetes. Hearts were removed and perfused as described below. On death, whole-blood (arterial and venous) samples were also collected from nonfasting animals in heparinized tubes, and the plasma was separated by centrifugation (3000 $\times$ *g* for 5 min) and then assayed for glucose, insulin, T_3, T_4, and lipid profile.

Working heart perfusion. Control and diabetic animals were killed by stunning them with a blow to the head followed by decapitation. Hearts were quickly excised and placed in cold aerated (95% O_2/5% CO_2) Chenoweth-Koelle solution, and extraneous tissue was dissected free. The millimolar concentrations of solutes in the buffer were as follows: NaCl 120, KCl 5.6, $CaCl_2$ 2.18, $MgCl_2$ 2.1, $NaHCO_3$ 19, glucose 10. The aortic stump was located and tied to a 15-gauge stainless steel aortic perfusion cannula. Perfusion was according to a modification of the working heart preparation of Neely et al. (19). Perfusion was initiated in a retrograde manner through the aorta at an aortic filling pressure of 45 cmH_2O (30 mmHg). The perfusion fluid was oxygenated Chenoweth-Koelle buffer at 37 $\pm$ 1°C. A 16-gauge stainless steel cannula, connected to atrial filling reservoirs, was then inserted into and tied to the pulmonary vein. Left ventricular developed pressure (LVDP) was measured with a Statham P23 AA transducer (Statham-Gould Instruments) attached to a 3-cm piece of polyethylene (PE-90) tubing. The PE-90 was attached to a 20-gauge needle, which was inserted through the apex of the heart into the left ventricle. Cardiac work was initiated by switching the perfusion system from the retrograde mode to the working heart mode. In the working mode, the perfusate entered the left ventricle through the left atrium and was pumped out through the aortic stump. The aortic outflow was subjected to an afterload of a 75-mm column of H_2O; PE-160 tubing was used as the column. Left ventricular pressure and the first derivative of left ventricular pressure were recorded on a Grass model 79D poly-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/10/1358/355584/37-10-1358.pdf by guest on 23 March 2023

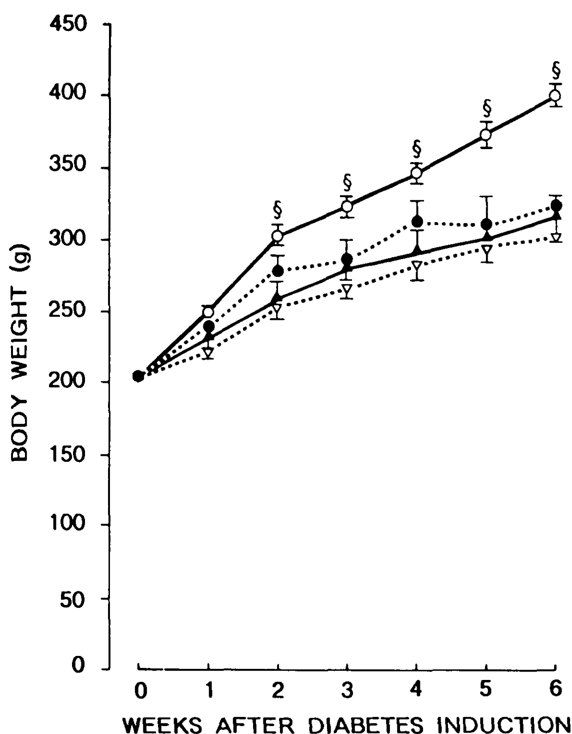

FIG. 1. Time course of body weight increases in control and diabetic rats. Body weights were measured daily before injecting L-carnitine. Values are means ± SE for *n* animals. ○, Untreated control, *n* = 8; ▲, L-carnitine–treated control, *n* = 6; ▽, untreated streptozocin-induced diabetic, *n* = 8; ●, L-carnitine–treated streptozocin-induced diabetic, *n* = 8. §Significantly different from other 3 groups ($P < .05$).

graph. Each heart was stimulated with a platinum electrode placed on the left atrium at twice the threshold voltage with square-wave pulses of 5-ms duration (from a Grass model SD9D stimulator) to give a rate of 275 beats/min. Cardiac function data were collected and analyzed with a microcomputer (Apple II with a Mountain hardware board). The pressure transducer signal from the polygraph was sampled at 667 Hz over 1.5 s at each function curve. This resulted in data being collected for six complete cardiac pulses. Three of these were analyzed with curve-fitting techniques to determine pulse height, area, start, and finish. The values from these three pulses were averaged to produce the data at that point. The hearts were equilibrated at 15 cmH_2O atrial filling pressure for 10 min before function curves were performed by estimating the left ventricular function against varying left atrial filling pressures. Different filling pressures were obtained by changing the height of the left atrial filling reservoir from 7.5 to 20.0 cm in 2.5-cm steps. The filling pressure was first reduced stepwise from 15.0 to 7.5 cmH_2O, after which it was increased stepwise to 20.0 cmH_2O, and finally decreased stepwise to 15.0 cmH_2O. At each point, pressure development was allowed to stabilize before it was recorded. In general, stable pressure development was achieved within 2 min after left atrial filling pressure was changed. A complete function curve was usually performed in ~20–30 min. The total time of perfusion of each heart was ~45 min. At the end of each experiment, hearts were frozen between aluminum tongs cooled to the temperature of liquid nitrogen with Wollenberger clamps, and stored at −70°C until assayed for free-carnitine levels.

Estimation of free carnitine. Frozen ventricular tissue was powdered in a liquid nitrogen–cooled mortar and pestle. Approximately 200 mg of frozen powder was used to estimate the dry-to-wet weight ratio. Extraction of the free carnitine was done with the method described by Idell-Wenger et al. (28). Briefly, ~500 mg of frozen powder was extracted into ice-cold perchloric acid (6%). The tissue–perchloric acid mixture was centrifuged at 4°C, and a portion (0.5 ml) of the acid-soluble supernatant was neutralized with KOH (pH < 10) and used for determination of tissue levels of free carnitine fraction (excluding short-chain acylcarnitine).

Blood analyses. Plasma was stored at −20°C until assayed. Plasma glucose levels were determined by the glucose oxidase method with a Boehringer Mannheim (Dorval, Quebec, Canada) diagnostic reagent kit. Plasma immunoreactive insulin was assayed by the radioimmunoassay method with the Amersham (Oakville, Ontario, Canada) insulin radioimmunoassay kit. The Amerlex T_4 and T_3 radioimmunoassay kit of Amersham was used for the radioimmunoassay of plasma T_4 and T_3. Plasma phospholipids, triglycerides, and total cholesterol were determined with Boehringer Mannheim diagnostic reagent kits.

Statistical analysis was performed with two-way analysis of variance followed by the Newman-Keul test. A probability of $P < .05$ was taken as the level of statistical significance.

RESULTS

General features of the experimental rats. Injecting animals with STZ resulted in a diabetic state characterized by hyperglycemia and an elevated urine glucose >2% through-

TABLE 1
General features of experimental rats

	Control		Streptozocin injected	
	Untreated (*n* = 8)	Carnitine treated (*n* = 6)	Untreated (*n* = 8)	Carnitine treated (*n* = 8)
Body wt (g)	400 ± 9	317 ± 18*	298 ± 6*	296 ± 12*
Fluid intake (ml/day)	41 ± 0.8	45 ± 0.7	123 ± 5†	56 ± 2
Wet heart wt (g)	1.64 ± 0.1	1.41 ± 0.19	1.06 ± 0.09*	1.22 ± 0.09*

Body weight was measured throughout 6-wk period, but only final values taken before death are indicated. Results are expressed as means ± SE for *n* animals.
*Significantly different from untreated control group ($P < .05$).
†Significantly different from other 3 groups ($P < .05$).

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/10/1358/355584/37-10-1358.pdf by guest on 23 March 2023

TABLE 2
Various parameters used to assess diabetic state of experimental rats

	Control		Streptozocin injected	
	Untreated (n = 8)	Carnitine treated (n = 6)	Untreated (n = 8)	Carnitine treated (n = 8)
Plasma insulin (μU/ml)	55 ± 6	47 ± 7	20 ± 2*†	23 ± 2*†
Plasma T_3 (nM)	0.74 ± 0.05	0.68 ± 0.05	0.49 ± 0.04‡	0.66 ± 0.04
Plasma T_4 (nM)	57 ± 5	48 ± 4	30 ± 4‡	43 ± 2*
Myocardial free carnitine (nmol/g dry wt)	287 ± 37	297 ± 37	131 ± 25‡	324 ± 28

Parameters were measured in blood collected at time of death. For insulin assay, human insulin standards were used because error made in estimation of rat insulin was minimal due to very high cross-reactivity with rat insulin (~90%). Results are expressed as means ± SE for n animals.
*Significantly different from untreated control group ($P < .05$).
†Significantly different from L-carnitine–treated control group ($P < .05$).
‡Significantly different from other 3 groups ($P < .05$).

out the study period in the untreated diabetic rats. No detectable glucose was present in the urine of control animals. Surprisingly, when the extent of glycosuria was measured weekly with enzymatic test strips, seven of the eight L-carnitine–treated diabetic rats showed no detectable glucose in their urine. Weight gain during the study period was significantly less in both diabetic groups than in control animals, such that diabetic rats showed significantly lower body weights at the time of death. L-Carnitine–treated control animals also gained weight more slowly than untreated control rats, such that at 6 wk, there was a significant difference between the groups (Fig. 1). Although we did not measure food intake in these animals, this weight loss in the L-carnitine–treated control animals was a concern. In a subsequent study, animals were kept diabetic for 6 wk and then injected for 2 wk with L-carnitine (3 g · kg^{-1} · day^{-1}). The food intake (g/day) obtained from the various groups was as follows: untreated control 27 ± 0.42; L-carnitine–treated control 14 ± 0.99; untreated diabetic 46 ± 1.7; L-carnitine–treated diabetic 37 ± 2.0. The results indicate that food intake was lower in control and diabetic animals treated for 2 wk with L-carnitine, which could explain the weight loss observed in this study. Untreated diabetic rats drank more water than control rats, but this polydipsia was prevented by L-carnitine treatment (Table 1). Table 1 also shows that both diabetic groups exhibited reduced heart weights compared with untreated control animals.

Parameters used to assess diabetic state of experimental rats. Various parameters used to assess the diabetic state of the animals were recorded and are summarized in Table 2. Plasma insulin levels measured at the time of death were depressed in the untreated diabetic rats, and L-carnitine treatment of diabetic rats did not increase the insulin levels (Table 2). However, L-carnitine treatment of diabetic rats significantly decreased the plasma glucose values, which were elevated in the untreated diabetic rats (Table 3). This reduction in plasma glucose values in the treated diabetic rats is presumably due to a mechanism independent of insulin, which remained depressed in these animals. Plasma T_4 levels were significantly lower in the untreated diabetic rats, whereas the L-carnitine–treated diabetic rats did not show a reduction in T_4 levels when compared with either control group. Similarly, the T_3 levels were reduced in the untreated diabetic animals, whereas the L-carnitine–treated diabetic rats showed a definite improvement in the levels of this hormone. Diabetic hearts had lower free-carnitine levels when compared with controls. Prolonged high-dose L-carnitine treatment of diabetic rats restored the myocardial free-carnitine levels to normal. Thus, it was evident that STZ treatment resulted in a diabetic state and that L-carnitine use was an effective method of improving the depressed thyroid status in diabetic rats and reducing elevated glucose levels.

Effect of L-carnitine in control and diabetic rat hearts. Figure 2 indicates the effect of varying filling pressures on

TABLE 3
Effect of diabetes and L-carnitine treatment on plasma glucose, triglycerides, total cholesterol, and phospholipids measured 6 wk after diabetes induction with streptozocin

	Control		Streptozocin injected	
	Untreated (n = 8)	Carnitine treated (n = 6)	Untreated (n = 8)	Carnitine treated (n = 8)
Plasma glucose (mg/dl)	137 ± 3	118 ± 4	445 ± 18*	215 ± 38†‡
Plasma triglycerides (mg/dl)	144 ± 12	71 ± 8	394 ± 124*	67 ± 5
Plasma cholesterol (mg/dl)	47 ± 3	41 ± 6	72 ± 14*	48 ± 3
Plasma phospholipids (mg/dl)	110 ± 4	90 ± 7	184 ± 41*	100 ± 4

Results are expressed as means ± SE for n animals.
*Significantly different from other 3 groups ($P < .05$).
†Significantly different from untreated control group ($P < .05$).
‡Significantly different from L-carnitine–treated control group ($P < .05$).

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/10/1358/355584/37-10-1358.pdf by guest on 23 March 2023

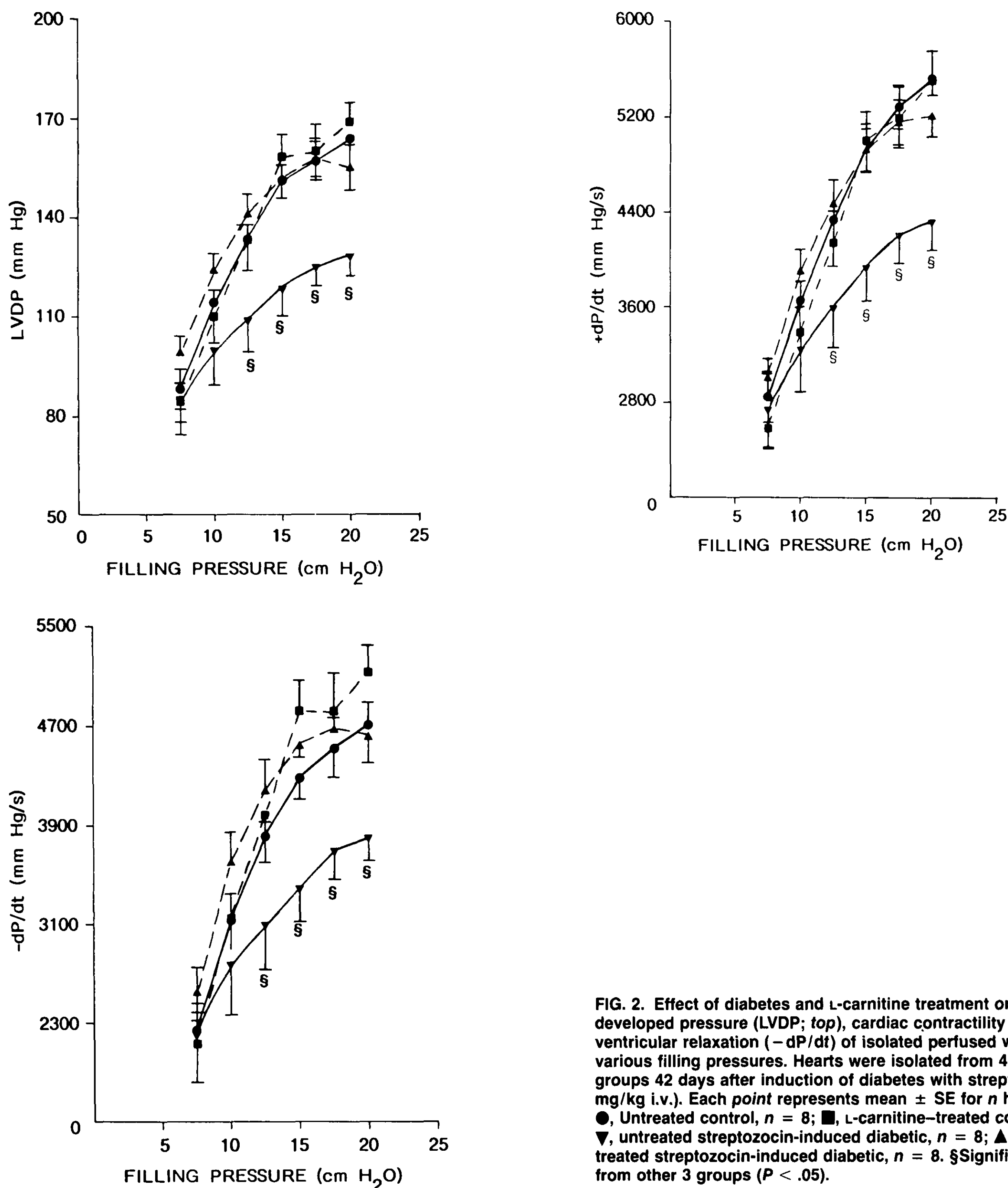

FIG. 2. Effect of diabetes and L-carnitine treatment on left ventricular developed pressure (LVDP; *top*), cardiac contractility (+dP/dt), and ventricular relaxation (−dP/dt) of isolated perfused working hearts at various filling pressures. Hearts were isolated from 4 experimental groups 42 days after induction of diabetes with streptozocin (55 mg/kg i.v.). Each *point* represents mean ± SE for *n* hearts. ●, Untreated control, *n* = 8; ■, L-carnitine–treated control, *n* = 6; ▼, untreated streptozocin-induced diabetic, *n* = 8; ▲, L-carnitine–treated streptozocin-induced diabetic, *n* = 8. §Significantly different from other 3 groups ($P < .05$).

various parameters of heart function in diabetic and control rats. Hearts from untreated diabetic rats showed lower LVDP, cardiac contractility (+dP/d*t*), and ventricular relaxation (−dP/d*t*) compared with hearts from untreated control rats at left atrial filling pressures >10 cmH_2O (Fig. 2). L-Carnitine treatment of the diabetic rats restored LVDP (Fig. 2, *top*). Similar effects of carnitine treatment occurred for +dP/d*t* (Fig. 2, *middle*) and −dP/d*t* (Fig. 2, *bottom*). The concentration of L-carnitine used in this study had no deleterious effect on the cardiac performance of control rats. In fact, although not significantly different, both L-carnitine–treated control and diabetic rats showed levels of cardiac function higher than that of control rats. These results confirm the fact that untreated diabetic rats are characterized by depressed cardiac performance. In contrast, the performance of the hearts from L-carnitine–treated diabetic rats did not differ from that of control rats. The improvement in cardiac function by L-carnitine could not be explained on the basis of its direct action on the heart. In control and diabetic left atria, L-carnitine, even at a very high dose (10^{-4} M), was found to be ineffective (data not shown).

Change in plasma lipids after L-carnitine treatment. Table 3 indicates the plasma lipid profile in the experimental rats. Plasma triglycerides, total cholesterol, and phospholipids were markedly elevated in the untreated diabetic group. In contrast, L-carnitine treatment of the diabetic rats restored these parameters to normal. The serum lipids of L-carnitine–treated control rats appeared to be normal. Thus, it seems

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/10/1358/355584/37-10-1358.pdf by guest on 23 March 2023

that intraperitoneally administered L-carnitine controlled the elevated plasma lipids of the diabetic rats, with no effect in the control rats.

DISCUSSION

The absolute level of carnitine required to maintain normal cardiac function is unknown, and increasing evidence indicates that depletion of carnitine stores is associated with cardiomyopathy. For instance, a cardiomyopathic hamster animal model that has low cardiac carnitine concentrations has been described (29), and rats (30) and rabbits (31) subjected to surgically induced pressure overload develop cardiac hypertrophy with reduced myocardial carnitine content. In the diphtheria-infected guinea pig, Wittels and Bressler (32) showed that this toxin produced a depletion of myocardial carnitine, resulting in cardiomyopathy. Myocardial carnitine stores also significantly decrease with age (33) and diabetes (20,23) with subsequent effects on contractile function. A number of observations have shown that the cardiac defects associated with decreased carnitine stores can be alleviated by administration of L-carnitine. For example, long-term L-carnitine treatment of cardiomyopathic hamsters (34) and old rats (35) resulted in restoration of myocardial carnitine stores and significantly increased mechanical performance. Carnitine administration to acutely diabetic rats also had beneficial effects. Perfusion of diabetic rat hearts with both physiological concentrations of palmitate and concentrations simulating elevated serum fatty acid levels in the diabetic subject in vivo caused a time- and concentration-dependent reduction in myocardial ATP content and an increase in long-chain acyl-CoA (24,25). Addition of carnitine to the perfusion medium or before death in vivo attenuated or prevented the loss of ATP and the increase in long-chain acyl-CoA (24,25). These hearts were also less vulnerable to ischemia, leading to an improvement in contractile function (23).

On the basis of these reports, we decided to administer carnitine to rats from the onset of diabetes to see if this intervention could replenish total myocardial carnitine levels (which are assumed to decrease in our model) and possibly prevent the depression in heart function after chronic diabetes. In our initial studies, oral doses of DL-carnitine were employed because of the prohibitive cost of using the naturally occurring L-carnitine daily for 6 wk. The results indicated that oral DL-carnitine treatment did not prevent the onset of heart dysfunction in diabetic rats (6). Subsequently, it has been shown that the L-isomer of carnitine, not the DL-isomer, is the physiologically active form. D-Carnitine inhibits carnitine esterification and can produce a decrease in myocardial carnitine content (36), and the carnitine transport system possesses ~25-fold greater affinity for the L-isomer than the D-isomer (37). Note also that oral and injected doses of L-carnitine show varying effects. In the obese Zucker rats, Brady et al. (21) showed that the oral absorbed dose of L-carnitine apparently did not increase plasma levels to the same extent as subcutaneous doses, and York et al. (29) reported that oral L-carnitine treatment did not increase the cardiac carnitine concentration in cardiomyopathic hamsters. The authors suggested that this most likely represents poor oral absorption and subsequent metabolism of carnitine by colon bacteria. That carnitine is degraded in the gastrointestinal tract was shown by Rebouche et al. (38).

As shown in this study, the effects of L-carnitine on blood glucose are consistent with a previous report. Paulson et al. (23) reported that acute L-carnitine treatment of diabetic rats for 2 wk significantly reduced serum glucose. However, the reduction in blood glucose was more pronounced in our study and probably is a result of a longer treatment period. In this regard, Pieper and Murray (24) showed that L-carnitine injection for 2 days did not reduce blood glucose in diabetic animals. The reduction in blood glucose in our study was independent of the levels of insulin, which remained depressed in the diabetic animals. It is possible that reduction of glucose levels represents an indirect consequence of removal of lipid intermediates or reduced food intake. This reduction in blood glucose could explain the absence of glycosuria and polydipsia seen throughout the study in these diabetic animals.

Injection of the L-isomer of carnitine intraperitoneally also restored the myocardial free-carnitine fraction in the diabetic rats to levels comparable with control values. Associated with this increase in myocardial carnitine content, exogenous L-carnitine treatment prevented the onset of heart dysfunction in chronically diabetic rats. The improvement in cardiac function in the diabetic animals could be due to a number of factors. For example, the increased incidence of cardiac dysfunction in diabetic patients or drug-induced animal models could be secondary to alterations in lipid metabolism, which may modify the structure of cardiac plasma and subcellular membranes. Diabetes, which accelerates the rate of lipid metabolism in the heart, results in an elevation within the cytosol of a number of lipids and/or intermediates involved in lipid metabolism, including acyl-CoA and long-chain acylcarnitine (6,23–25). If tissue levels of these intermediates rise markedly, deleterious effects on cellular membrane structure/function can occur with a loss of sarcolemmal integrity (12) and a depression in fatty acid oxidation and ATP production (24,25). Thus, the improvement in cardiac function in our diabetic animals may be due to a lessening of the accumulation of fatty acid intermediates such as acyl-CoA and long-chain acylcarnitines, which have been shown to decrease after L-carnitine administration in the diabetic rat (23–25). Another possible mechanism for the improvement in cardiac function after L-carnitine treatment may be the ability to lower blood lipids. In this study, the effect of L-carnitine in lowering blood lipids in the diabetic rats was quite pronounced. Why serum lipids are not elevated in these rats even when insulin is lacking is unknown. However, because carnitine is a prerequisite for normal transport of fatty acids into the mitochondria to be oxidized, it may be an effective lipid-lowering agent, and administration of carnitine to hyperlipidemic patients (39) or diabetic animals (23) has been reported to have a lipid-lowering effect. Whether the lipid-lowering effect in our study was due to an increased fatty acid oxidation and uptake or, alternatively, decreased hepatic synthesis or secretion of triglycerides is also unknown. Whatever the mechanism of L-carnitine in lowering blood lipid levels, the results are certainly important, because experimental evidence indicates a possible deteriorating influence of high lipid concentrations on myocardial performance (40–43). In addition, studies from

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/10/1358/355584/37-10-1358.pdf by guest on 23 March 2023

our laboratory have shown that diabetic rats pretreated with hydralazine (44), choline, and methionine (45) and diabetic WKY rats (46) that did not show elevated plasma lipid levels exhibited an improved cardiac performance compared with untreated Wistar rats. Thus, the lack of cardiac dysfunction in L-carnitine–treated diabetic rats may be explained partly by the fact that these animals have a normal lipid metabolism.

In conclusion, L-carnitine treatment of rats with experimentally induced diabetes improved cardiac function. It is possible that carnitine deficiency contributes to muscle dysfunction because of reduced energy production, which promotes lipid accumulation in the cytoplasm with subcellular membrane alterations. Pretreatment with L-carnitine could possibly prevent both the depletion of free carnitine and tissue ATP levels in the myocardium and the accumulation of lipid intermediates, resulting in improved use of other substrates like glucose. Because carnitine deficiency may be involved in the cardiac complications of diabetes, it will be of interest to study the mechanism of action of L-carnitine in future studies.

ACKNOWLEDGMENTS

This study was supported by a grant from the British Columbia Heart Foundation.

H.X. and B.R. are Predoctoral Trainees of the British Columbia Heart Foundation and the Canadian Heart Foundation, respectively.

REFERENCES

1. Penpargkul S, Schaible T, Yipinstoi T, Scheuer J: The effect of diabetes on performance and metabolism of rat hearts. *Circ Res* 47:911–21, 1980
2. Vadlamudi RVSV, Rodgers RL, McNeill JH: The effect of chronic alloxan and streptozotocin-induced diabetes on isolated rat heart performance. *Can J Physiol Pharmacol* 60:902–11, 1982
3. Fein FS, Kornstein LB, Strobeck JE, Capasso JM, Sonnenblick EH: Altered myocardial mechanisms in diabetic rats. *Circ Res* 47:922–33, 1980
4. Regan TJ, Ettinger PO, Khan MI, Jesran MV, Lyons MM, Oldewurtel HA, Weber M: Altered myocardial function and metabolism in chronic diabetes mellitus without ischemia in dogs. *Circ Res* 35:222–37, 1974
5. Malhotra A, Penpargkul S, Fein FS, Sonnenblick EH, Scheuer J: The effect of streptozotocin induced diabetes in rats on cardiac contractile proteins. *Circ Res* 49:1243–50, 1981
6. Lopaschuk GD, Tahiliani RVSV, Vadlamudi S, Katz S, McNeill JH: Cardiac sarcoplasmic reticulum function in insulin- or carnitine-treated diabetic rats. *Am J Physiol* 245:H969–76, 1983
7. Heyliger CE, Prakash A, McNeill JH: Alterations in cardiac sarcolemmal Ca^{2+} pump activity during diabetes mellitus. *Am J Physiol* 252:H540–44, 1987
8. Miller TB: Cardiac performance of isolated perfused hearts from alloxan diabetic rats. *Am J Physiol* 236:H808–12, 1979
9. Garland PB, Randle PJ: Regulation of glucose uptake by muscle. 10. Effects of alloxan-diabetes, starvation, hypophysectomy and adrenalectomy and of fatty acids, ketone bodies and pyruvate on the glycerol output and concentration of free fatty acids, long-chain fatty acyl coenzyme A, glycerol phosphate and citrate cycle intermediates in rat heart and diaphragm muscles. *Biochem J* 93:678–87, 1964
10. Haddock BH, Yates DW, Garland PB: The localization of some coenzyme A-dependent enzymes in rat liver mitochrondia. *Biochem J* 119:565–73, 1970
11. Pande SV, Parvin R: Characterization of carnitine acyl carnitine translocase system in heart mitochondria. *J Biol Chem* 25:6683–91, 1976
12. Katz AM, Messineo FC: Lipid membrane interactions and the pathogenesis of ischemic damge in the myocardium. *Circ Res* 48:1–24, 1981
13. Vaartjes WJ, Kemp A Jr, Souverijon JHM, Van Den Gergh SG: Inhibition by fatty acyl esters of adenine nucleotide translocator in rat-liver mitochondria. *FEBS Lett* 23:303–308, 1972
14. Wood JM, Bush B, Pitts BJR, Schwartz A: Inhibition of bovine heart Na^+, K^+-ATPase by palmitylcarnitine and palmityl-CoA. *Biochem Biophys Res Commun* 74:677–84, 1977
15. Lopaschuk GD, Katz S, McNeill JH: The effect of alloxan and streptozotocin-induced diabetes on calcium transport in rat cardiac sarcoplasmic reticulum. The possible involvement of long chain acyl carnitines. *Can J Physiol Pharmacol* 61:439–48, 1983
16. Rebouche CJ, Engel AG: Carnitine metabolism and deficiency syndromes. *Mayo Clin Proc* 58:533–40, 1983
17. Cederbaun SD, Auestad N, Bernar J: Four year treatment of systemic carnitine deficiency: *N Engl J Med* 310:1395–96, 1984
18. Tripp ME, Shug AL: Plasma carnitine concentrations in cardiomyopathy patients. *Biochem Med* 32:199–206, 1984
19. Feuvray D, Idell-Wenger JA, Neely JR: Effects of ischemia on rat myocardial function and metabolism in diabetes. *Circ Res* 44:322–29, 1979
20. Vary TC, Neely JR: A mechanism for reduced myocardial carnitine levels in diabetic animals. *Am J Physiol* 243:H154–58, 1982
21. Brady LJ, Knoeber CM, Hoppel CL, Leathers CW, McFarland D, Brady PS: Pharmacologic action of L-carnitine on hypertriglyceridemia in obese Zucker rats. *Metabolism* 35:555–62, 1986
22. Leichter J, Ottem A, Hahn P: Does carnitine have a role in fat absorption? *Life Sci* 41:941–44, 1987
23. Paulson DJ, Schmidt MJ, Traxler JS, Romacci MT, Shug AL: Improvement of myocardial function in diabetic rats after treatment with L-carnitine. *Metabolism* 33:358–63, 1984
24. Pieper GM, Murray WJ: In vivo and in vitro intervention with L-carnitine prevents abnormal energy metabolism in isolated diabetic rat heart: chemical and phosphorous-31 NMR evidence. *Biochem Med Metabol Biol* 38:111–20, 1987
25. Pieper GM, Murray WJ, Salhany JM, Wu ST, Eliot RS: Salient effects of L-carnitine on adenine-nucleotide loss and coenzyme A acylation in the diabetic heart perfused with excess palmitic acid. A phosphorous-31 NMR and chemical entract study. *Biochim Biophys Acta* 803:229–40, 1984
26. Brooks DE, McIntosh JEA: Turnover of carnitine by rat tissues. *Biochem J* 148:439–45, 1975
27. Tahiliani AG, Vadlamudi RVSV, McNeill JH: Prevention and reversal of altered myocardial function in diabetic rats by insulin treatment. *Can J Physiol Pharmacol* 61:516–23, 1983
28. Idell-Wenger JA, Gratyohann LW, Neely JR: Coenzyme A and carnitine distribution in normal and ischemic hearts. *J Biol Chem* 253:4310–18, 1978
29. York CM, Cantrell CR, Borum PR: Cardiac carnitine deficiency and altered carnitine transport in cardiomyopathic hamsters. *Arch Biochem Biophys* 221:526–33, 1983
30. Reibel DK, Uboh CE, Kent RL: Altered coenzyme A and carnitine metabolism in pressure overload hypertrophied hearts. *Am J Physiol* 244:H839–43, 1983
31. Reves NW, Cameron AJV: Metabolism of lipids in experimental hypertrophic hearts of rabbits. *Metabolism* 28:601–13, 1979
32. Wittels B, Bressler R: Biochemical lesion of diptheria toxin in the heart. *J Clin Invest* 43:630–37, 1964
33. Abu-Erreish GM, Neely JR, Whitmer JT, Whitman V, Sanadi DR: Fatty acid oxidation by isolated perfused working hearts of aged rats. *Am J Physiol* 232:E258–62, 1977
34. Whitmer JT: L-Carnitine treatment improves cardiac performance and restores high-energy phosphate pools in cardiomyopathic Syrian hamster. *Circ Res* 61:396–408, 1987
35. Lucreziotti R, Gaetoni F, Pacifici L, Ramacci MT: L-Carnitine long term administration improves the myocardial function in the aged rat (Abstract). *J Mol Cell Cardiol* 15 (Suppl. 3):10, 1983
36. Paulson DJ, Shug AL: Tissue specific depletion of L-carnitine in rat heart and muscle by D-carnitine. *Life Sci* 28:2931–38, 1981
37. Vary TC, Neely JR: Characterization of carnitine transport in isolated perfused adult rat hearts. *Am J Physiol* 242:H585–92, 1982
38. Rebouche CJ, Mack DL, Edmonson PF: L-Carnitine dissimilation in the gastrointestinal tract of the rat. *Biochemistry* 23:6422–26, 1984
39. Pola P, Savi L, Grilli M, Flore R, Serricchio M: Carnitine in the therapy of dyslipidemic patients. *Curr Ther Res* 27:208–16, 1980
40. Kannel WB, McGee DL: Diabetes and cardiovascular risk factors: the Framingham Study. *Circulation* 59:8–13, 1979
41. Opie LH: Effect of fatty acid on contractility and rhythm of the heart. *Nature* (Lond) 227:1055–56, 1970
42. Willebrands AF, Terivelle HF, Tarseron SJA: The effect of a high molar FFA/albumin ratio in the perfusion medium on rhythm and contractility of the isolated rat heart. *J Mol Cell Cardiol* 5:259–73, 1973
43. Oram JF, Bennetch SL, Nelly JR: Regulation of fatty acid utilization in isolated rat hearts. *J Biol Chem* 248:5299–309, 1973
44. Rodrigues B, Goyal RK, McNeill JH: Effects of hydralazine on streptozotocin-induced diabetic rats: prevention of hyperlipidemia and improvement in cardiac function. *J Pharmacol Exp Ther* 237:292–99, 1986
45. Heyliger CE, Rodrigues B, McNeill JH: Effect of choline and methionine treatment on cardiac dysfunction of diabetic rats. *Diabetes* 35:1152–57, 1986
46. Rodrigues B, McNeill JH: Cardiac function in spontaneously hypertensive diabetic rats. *Am J Physiol* 251:H571–80, 1986

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/10/1358/355584/37-10-1358.pdf by guest on 23 March 2023