Fatty Acid Oxidation and Cardiac Function in the Sodium Pivalate Model of Secondary Carnitine Deficiency

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Carnitine-deficiency syndromes are often associated with alterations in lipid metabolism and cardiac function. The present study was designed to determine whether this is also seen in an experimental model of carnitine deficiency. Carnitine deficiency was induced in male Sprague-Dawley rats supplemented with sodium pivalate for 26 to 28 weeks. This treatment resulted in nearly a 60% depletion of myocardial total carnitine content as compared with control hearts. When isolated working hearts from these animals were perfused with 5.5 mmol/L glucose and 1.2 mmol/L palmitate and subjected to incremental increases in left-atrial filling pressures, cardiac function remained dramatically depressed. The effects of carnitine deficiency on glucose and palmitate utilization were also assessed in hearts perfused at increased workload conditions. At this workload, function was depressed in carnitine-deficient hearts, as were rates of 1.2-mmol/L [U-^{14}C]-palmitate oxidation, when compared with control hearts (544 ± 37 *v* 882 ± 87 nmol/g dry weight · min, $P < .05$). However, glucose oxidation rates from 5.5 mmol/L [U-^{14}C]-glucose were slightly increased in carnitine-deficient hearts. To determine whether the depressed fatty acid oxidation rates were a result of reduced mechanical function in carnitine-deficient hearts, the workload of hearts was reduced. Under these conditions, mechanical function was similar among control and carnitine-deficient hearts. Palmitate oxidation rates were also similar in these hearts (526 ± 69 *v* 404 ± 47 nmol/g dry weight · min for control and carnitine-deficient hearts, respectively). Our results show that work performed by hearts from carnitine-deficient animals is rate-limiting in the oxidation of palmitate. Despite this, our findings suggest that the sodium pivalate model of carnitine deficiency may prove to be useful for the investigation of functional and metabolic aspects of carnitine deficiency.

THE CRITICAL ROLES of carnitine in intermediary metabolism and in the pathogenesis of carnitine-deficiency syndromes are well documented.[1-5] Carnitine facilitates the transport of long-chain fatty acids into the mitochondria, where these substrates are subsequently oxidized, and is also essential in modulating the intramitochondrial acyl coenzyme A (CoA) to CoA ratio. Carnitine is also essential in the detoxification and removal of excess and potentially toxic acyl groups from the mitochondria.[1] Presumably, inadequate tissue levels of free carnitine to accomplish these roles will result in impaired long-chain fatty acid transport into the mitochondria and altered energy production. Failure of modulation of the intramitochondrial acyl CoA to CoA ratio will also impair energy production by shunting fatty acids away from oxidation and toward triglyceride synthesis. The consequences of these alterations in fatty acid metabolism, occurring in response to deficiencies in carnitine, can be manifested as lipid-storage myopathy, muscle weakness, and cardiac dysfunction.[6-13]

Since the myocardium depends largely on oxidation of fatty acids for the production of adenosine triphosphate (ATP),[14] cardiac dysfunction occurring secondary to carnitine deficiency is suspected to be associated with aberrations in myocardial substrate metabolism, particularly in fatty acid utilization. Despite the importance of total myocardial carnitine content to the outcome of cardiac function, only a few studies have provided evidence of disturbances in fatty acid metabolism in patients with carnitine deficiencies. However, to our knowledge, no studies have directly assessed the effects of carnitine deficiency on myocardial fatty acid oxidation and cardiac function in an experimental model of carnitine deficiency. In view of the problems encountered in human experimentation and the need for the study of basic pathophysiologic mechanisms associated with these deficiencies, establishment of a carnitine-deficiency model is obviously desirable.

In the present study, we induced carnitine deficiency in rats by oral administration of sodium pivalate, the pivalic acid moiety of pivampicillin. This compound is known to induce carnitine deficiency in humans by forming excessive tissue levels of the pivaloylcarnitine ester.[15-19] These esters are readily released from tissues and subsequently lost in the urine at a rate that exceeds the sum of dietary carnitine intake and synthesis. As a model of carnitine deficiency, treatment of rats with sodium pivalate is representative of the more common cases of these syndromes, the secondary carnitine deficiencies.[2-4,20,21] We demonstrate that long-term supplementation of sodium pivalate to rats produces a severe depletion in total myocardial carnitine content, along with dramatic changes in myocardial contractile function and substrate oxidation.

MATERIALS AND METHODS

Materials

Sodium pivalate (trimethylacetic acid) was purchased from Aldrich Chemicals (Milwaukee, WI). Glucose, palmitic acid, and bovine serum albumin (fraction V) were purchased from Sigma Chemicals (St Louis, MO). D-[U-^{14}C]-glucose and D-[U-^{14}C]-palmitate were purchased from Amersham Life Sciences (Arlington Heights, IL). Hyamine hydroxide (1 mol/L methylbenzethonium hydroxide in methanol) was purchased from ICN Radiochemicals (ICN Biomedicals, Irvine, CA). Scintiverse scintil-

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lant was obtained from Fair Lawn, NJ. All other chemicals were reagent grade.

Animals

Carnitine deficiency was induced in male Sprague-Dawley rats (135 to 160 g) by treating them with 20 mmol/L sodium pivalate (pH 7.08) in their drinking water, as described previously.[22] Each animal injested, on average, 192 mg/kg body weight of sodium pivalate daily, a concentration that is threefold to 10-fold greater than what is used clinically.[17-19] Control animals received an equimolar concentration of sodium bicarbonate. Both control and sodium pivalate–treated groups were given food and water ad libitum for 26 to 28 weeks before experimentation.

Heart Perfusions

Hearts from ketamine- and xylazine-anesthetized animals were excised and cannulated as isolated working hearts as described previously.[23] Aortae were cannulated and hearts were perfused retrogradely with Krebs-Henseleit buffer (pH 7.4) gassed with 95% O_2–5% CO_2. During this initial perfusion, hearts were trimmed of excess tissue, pulmonary arteries were cut, and the openings of the left atria were cannulated. A 20-gauge needle connected to a Micron Instruments transducer (Simi Valley, CA) was then inserted through the wall of the left ventricle for measurement of cardiac contractile function. Hearts were then switched into the working mode and initially perfused at a 15-cm H_2O left-atrial filling pressure and 100-cm H_2O aortic afterload with buffer containing 2.5 mmol/L calcium, 5.5 mmol/L glucose, and 1.2 mmol/L palmitate prebound to 3% bovine serum albumin. After a 10-minute equilibration period, the left-atrial filling pressure was decreased to 5 cm water and then sequentially increased at 5-minute intervals to 25 cm water. Hearts were paced at 300 beats per minute using a Grass S88 stimulator (Grass Instruments, Quincy, MA), and mechanical function was assessed at various left-atrial filling pressures. Pressure was recorded on a PPG Simultrace AR-6 recorder (PPG Biomedical Systems, Pleasantville, NY) interfaced to a Buxco Hemodynamic analyzer (Buxco Electronics, Sharon, CT) and Epson Equity One computer (Epson, Nagano, Japan) for on-line data acquisition. Aortic output and coronary flow were measured by timed collections.

Measurement of Glucose and Palmitate Oxidation

In a separate series of control and sodium pivalate–treated hearts, the effects of work performed by the heart on substrate utilization were determined. These hearts were also perfused with Krebs-Henseleit buffer containing 2.5 mmol/L calcium, 5.5 mmol/L glucose, and 1.2 mmol/L palmitate. In a group termed "high-workload," hearts were perfused at a left-atrial filling pressure of 15 cm H_2O and an aortic afterload resistance of 100 cm H_2O. In this group, hearts were paced at a rate of 300 beats per minute using a Grass S88 electrical stimulator. In the "low-workload" group, left-atrial filling pressure was maintained at 15 cm H_2O, whereas aortic afterload resistance was reduced to 80 cm H_2O. Hearts in this group were left spontaneously beating. We chose to perfuse hearts at these various workload conditions in the presence of high levels of fatty acids to determine whether any potential differences in palmitate oxidation seen in control or pivalate-treated hearts can be explained by differences in cardiac function.

Exogenous glucose oxidation was measured by perfusing hearts with buffer containing 5.5 mmol/L [U-^{14}C]-glucose (specific activity of perfusate, 0.27 μCi/μmol) and 1.2 mmol/L palmitate. To determine exogenous palmitate oxidation, hearts were perfused with 5.5 mmol/L glucose and 1.2 mmol/L [U-^{14}C]-palmitate (specific activity of perfusate, 0.23 μCi/μmol). Steady-state rates of glucose and palmitate oxidation were determined by quantitative measurement of both gaseous and perfusate $^{14}CO_2$ production by the hearts, as described previously.[24] Briefly, gaseous $^{14}CO_2$ was collected from the exhaust tube of the oxygenator and bubbled through a 1-mol/L methylbenzethonium hydroxide trap. Perfusate samples were removed directly from the system with a syringe and immediately stored under a volume of mineral oil to prevent release of $^{14}CO_2$. The $^{14}CO_2$ was extracted by injecting a volume of perfusate into sealed flasks containing 9N H_2SO_4 and methylbenzethonium hydroxide in suspended centerwells. The flasks were then gently stirred for 1 hour to release the perfusate $^{14}CO_2$ present as bicarbonate. The methylbenzethonium hydroxide traps were removed from the flasks and placed in scintillation cocktail to be counted. Glucose oxidation and palmitate oxidation were determined at 15-minute intervals throughout the 60-minute perfusion. In addition to determining exogenous rates, a standard perchloric acid–extraction procedure was performed to measure the amount of label that accumulated in heart tissue as acid-soluble metabolites. This labeled content was compared with that of $^{14}CO_2$ released from [U-^{14}C]-palmitate to determine whether the release of $^{14}CO_2$ from palmitate alone is considered an accurate indicator of palmitate oxidation or whether this underestimates the oxidation of this substrate.[25]

After the 60-minute perfusion period, hearts were rapidly frozen with serrated clamps cooled to the temperature of liquid nitrogen. The frozen ventricular tissue was then weighed and powdered in a mortar and pestle also precooled with liquid nitrogen. A portion of the powdered tissue was used to determine the dry to wet ratio of the ventricles. Frozen ventricular weight and ventricular dry to wet ratio were then used for determination of total dry weight of the heart. Total dry weight of the hearts was used to determine myocardial glucose and palmitate oxidation rates.

Measurement of Tissue Metabolites

To determine myocardial levels of carnitine and esters, powdered frozen ventricular tissue was sonicated in 12% cold perchloric acid and then centrifuged at 2,000 rpm for 5 minutes at 4°C. The supernatant was removed and neutralized to pH 7.0 to 7.4 with 6N KOH. An aliquot of this free extract was used for determination of free carnitine. Short-chain acylcarnitine was determined by using a volume of free extract adjusted to pH 11 to 12 and hydrolyzed for 1 hour at 50°C. Hydrolysis of this fraction was ended by neutralizing the pH to 7.0 to 7.4 with morpholinepropanesulfonic acid–HCl. The fraction was then centrifuged at 2,000 rpm for 5 minutes at 4°C, and the supernatant was assayed for free carnitine. The difference between these two fractions was used to estimate short-chain acylcarnitine. The level of long-chain acylcarnitine was measured from the pellet obtained following centrifugation of the initial free extract. The pellet was washed with 12% perchloric acid, sonicated with 0.5 mol/L KOH until it became dissolved, and then subjected to alkaline hydrolysis at 50°C for 1 hour. Hydrolysis was ended by decreasing the pH to 7.0 to 7.4, after which the sample was then centrifuged at 2,000 rpm for 5 minutes. The supernatant contained the free carnitine released as a result of alkaline hydrolysis of the long-chain acylcarnitine esters. Each fraction was assayed according the method described by McGarry and Foster.[26] Total carnitine content was considered the sum of carnitine found in the free, short-chain, and long-chain fractions.

Blood levels of glucose, free fatty acids, cholesterol, and triglycerides were measured using commercially available diagnostic kits. ATP was extracted from frozen ventricular tissue using standard perchloric acid–extraction procedures. ATP levels were measured

spectrophotometrically by a coupled-enzyme assay involving phosphoglycerate phosphokinase and glyceraldehyde phosphate dehydrogenase. Glycosylated hemoglobin levels were measured using Glyco-tek affinity columns obtained from Helena Laboratories (Beaumont, TX).

Statistical Analysis

All data are reported as the mean ± SEM. A one-way ANOVA followed by the least-significant test for post hoc analysis was used to determine statistical difference in groups containing four sample populations. The unpaired *t* test was used for determination of the statistical difference of group means. *P* less than .05 was considered significant.

RESULTS

Effects of Sodium Pivalate Treatment on Physical Characteristics

Following treatment with sodium pivalate, mean body weights were similar for both control (n = 25) and sodium pivalate–treated (n = 31) animals (740 ± 11 *v* 710 ± 18 g, respectively). Total dry heart weight was also similar between control and pivalate-treated groups (411 ± 10 *v* 419 ± 11 mg, respectively). As a result, the heart weight to body weight ratio was essentially the same between the two groups.

Effects of Sodium Pivalate Treatment on Blood Levels of Glucose and Lipids

The levels of various blood metabolites were measured in animals (n = 5 animals per group) following the 28-week treatment period with sodium pivalate. There were no significant differences in blood levels of either glucose (154 ± 9 *v* 158 ± 6 mg/dL), free fatty acids (0.28 ± 0.3 *v* 0.35 ± 0.4 mmol/L), triglycerides (114 ± 15 *v* 106 ± 11 mg/dL), cholesterol (83 ± 11 *v* 77 ± 8 mg/dL), or percent glycosylated hemoglobin (8.9 ± 0.3 *v* 9.2 ± 0.2) between control and pivalate-treated animals, respectively.

Myocardial Total Carnitine Content and Esters in Hearts From Sodium Pivalate–Treated Animals

Table 1 lists myocardial total carnitine content and the distribution of carnitine esters in hearts from control and pivalate-treated animals. Treatment with sodium pivalate resulted in a 58% depletion in myocardial total carnitine content. This reduction was seen when the data were expressed on a non–collagenase protein basis (1.7 ± 0.1 *v* 3.8 ± 0.2 nmol/mg for pivalate-treated and control hearts, respectively). Regardless of work performed by the heart, this dramatic reduction was mainly due to a decrease in both free and short-chain acylcarnitine fractions. However, under low-workload conditions, long-chain acylcarnitine content in both control and pivalate-treated hearts was higher as compared with the respective groups perfused at a higher workload.

Effects of Sodium Pivalate Treatment on Mechanical Function of Hearts

Mechanical function of isolated working hearts from control and carnitine-deficient animals is shown in Fig 1. In the control group, as expected, cardiac output was progressively increased in response to the increases in left-atrial filling pressures. Under these conditions, left-ventricular systolic pressure and positive and negative dP/dt (rate of contraction and relaxation, respectively) were also increased, but only in response to the decreased left-atrial filling pressures. In contrast, all cardiac contractile performances in hearts obtained from sodium pivalate–treated animals were dramatically depressed as compared with performances in control hearts and remained so to the various increases in preload.

Mechanical function of control and pivalate-treated hearts perfused at different workload conditions is shown in Table 2. When hearts were perfused under high-workload conditions, the heart rate–ventricular pressure product was significantly lower in pivalate-treated hearts as compared with control hearts. This was mainly due to a decrease in systolic left-ventricular pressure. However, in the low-workload group, there were no differences in the heart rate–ventricular pressure product or in the other measured cardiac parameters between the two groups of hearts.

Exogenous Glucose and Palmitate Oxidation Rates in Hearts of Sodium Pivalate–Treated Animals

Steady-state rates of both glucose and palmitate oxidation were determined by fitting the slope of the remaining three 15-minute–interval time points of the perfusion (30,

Table 1. Total Carnitine Content and Esters in Hearts Perfused Under Low- and High-Workload Conditions

Group	Carnitine Esters (nmol/g dry weight)			
	Free	Short-Chain	Long-Chain	Total
High workload				
Control	4,367 ± 315	992 ± 71	810 ± 135	6,283 ± 531
Pivalate-treated	1,643 ± 159*	467 ± 45*	548 ± 68	2,695 ± 224*
Low workload				
Control	3,560 ± 202*†	1,015 ± 34†	1,592 ± 317*†	6,167 ± 104†
Pivalate-treated	1,162 ± 62*‡	398 ± 65*‡	1,093 ± 111†	2,653 ± 117*‡

NOTE. Values are presented as the mean ± SEM for 10 control and 11 pivalate-treated hearts in the high-workload group and 5 control and 5 pivalate-treated hearts in the low-workload group.

*Significantly different from control hearts under high-workload conditions.

†Significantly different from pivalate-treated hearts under high-workload conditions.

‡Significantly different from control hearts under low-workload conditions.

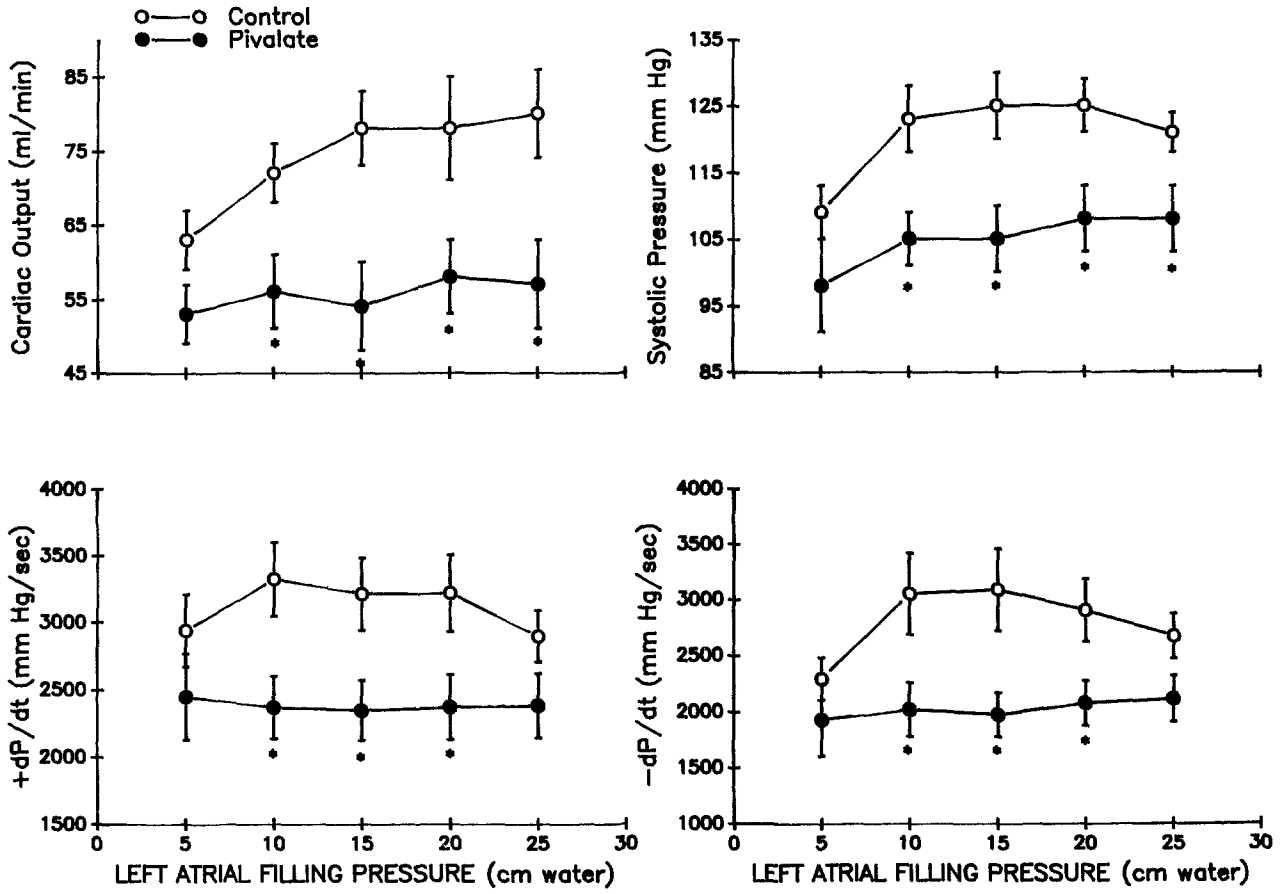

Fig 1. Effects of long-term sodium pivalate treatment on cardiac output, left-ventricular systolic pressure, +dP/dt, and −dP/dt in response to increases in left-atrial filling pressures. All values are the mean ± SEM (n = 5 to 8). *Significantly different as compared with control hearts. (○) Control; (●) pivalate-treated.

45, and 60 minutes), a time in which rates were linear in both control and pivalate-treated hearts. These rates are listed in Table 3. At a high workload, steady-state rates of palmitate oxidation were significantly lower in pivalate-treated hearts as compared with control hearts. However, under these conditions, steady-state glucose oxidation rates in these hearts tended to be higher than those seen in the control group. In contrast, under low-workload conditions, there were no differences in palmitate oxidation between the two groups.

Table 2. Mechanical Function of Control and Pivalate-Treated Hearts Perfused Under Low- and High-Workload Conditions

Group	HR (beats per min)	SLVP (mm Hg)	LVEDP (mm Hg)	HR · SLVP ($\times 10^{-3}$)
High workload				
Control	302 ± 3	109 ± 5	58 ± 3	32.8 ± 1.1
Pivalate-treated	302 ± 3	98 ± 2*	64 ± 2	29.8 ± 0.2*
Low workload				
Control	209 ± 15*†	108 ± 5	26 ± 2*†	22.3 ± 1.0*†
Pivalate-treated	223 ± 13*†	99 ± 6	26 ± 2*†	21.7 ± 0.7*†

NOTE. These mechanical data were obtained from hearts in which oxidative measurements were determined.

Abbreviations: HR, heart rate; SLVP, systolic left-ventricular pressure; LVEDP, left-ventricular end-diastolic pressure; HR · SLVP, heart rate–systolic ventricular pressure product.

*Significantly different from control hearts under high-workload conditions.

†Significantly different from pivalate-treated hearts under high-workload conditions.

The amount of label present as acid-soluble metabolites was measured and combined with the oxidative rates from the release of $^{14}CO_2$ from palmitate to obtain an overall rate of fatty acid oxidation during the 60-minute perfusion. These rates were determined only in control and pivalate-treated hearts perfused at a high workload when palmitate oxidation rates were significantly different between these two groups of hearts. Using the combined values, the difference between control and carnitine-deficient hearts remained (721 ± 48 *v* 546 ± 40 nmol/g dry weight · min, respectively). In fact these overall rates were similar to steady-state rates estimated from the release of $^{14}CO_2$ alone (Table 3). This suggests that the slope of the $^{14}CO_2$-production curve is an accurate measure and does not underestimate fatty acid oxidation due to the accumulation of label in acid-soluble intermediates.[25]

Work performed by the heart is a key determinant in overall energy substrate utilization. Therefore, glucose and palmitate oxidation rates were normalized for potential differences in mechanical function performed by the heart. When expressed in these terms, no differences in either

Table 3. Myocardial Glucose and Palmitate Oxidation Rates in Control and Pivalate-Treated Hearts Perfused Under Various Workload Conditions

Group	Absolute Rates (nmol/g dry weight · min)		Rates Normalized for Heart Function (nmol/min × HR × SLVP × 10^{-3})	
	Glucose Oxidation	Palmitate Oxidation	Glucose Oxidation	Palmitate Oxidation
High workload				
Control	315 ± 83	882 ± 87	4.3 ± 1.1	8.1 ± 0.6
Pivalate-treated	666 ± 175	544 ± 37*	6.3 ± 0.6	6.9 ± 0.3
Low workload				
Control	ND	526 ± 69*	ND	8.9 ± 0.4†
Pivalate-treated	ND	404 ± 47*	ND	7.3 ± 0.9†‡

NOTE. Values are reported as the mean ± SEM. Glucose oxidation rates were obtained from 4 control and 5 pivalate-treated hearts. Palmitate oxidation rates in the high-workload group were obtained from 6 control and 10 pivalate-treated hearts, whereas in the low-workload group, palmitate oxidation rates were obtained from 5 hearts in each group.

Abbreviations: HR, heart rate; SLVP, systolic left-ventricular pressure; ND, not determined.

*Significantly different from control hearts under high-workload conditions.

†Significantly different from pivalate-treated hearts under high-workload conditions.

‡Significantly different from control hearts under low-workload conditions.

glucose or palmitate oxidation were seen between the groups of hearts regardless of workload conditions (Table 3).

Estimated Rate of Myocardial ATP Production From Exogenous Substrates

Myocardial ATP production from exogenous glucose and palmitate oxidation is shown in Table 4. In control hearts, the contribution of glucose oxidation to overall ATP production was only 10% of the total exogenous ATP production, whereas palmitate oxidation contributed 90%. This pattern of ATP production from these substrates is consistent with a previous study showing that the majority of ATP production from the heart is derived from the oxidation of fatty acids.[27] In hearts from sodium pivalate–treated animals, it was interesting that the contribution of glucose oxidation to ATP production was more than twofold greater than in control hearts. The contribution of palmitate oxidation to ATP production in these hearts was reduced, but it remained the predominant substrate. Despite these shifts in substrate oxidation, ATP production from oxidation of exogenous substrates was depressed in hearts from sodium pivalate–treated animals. A similar pattern of ATP production was seen in control and pivalate-treated hearts when differences in heart function were considered (Table 4). However, it should be pointed out that the rate of ATP production from endogenous substrates such as glycogen and triglycerides was not measured in the present study.

Myocardial ATP content was assayed in both control and carnitine-deficient hearts to determine whether these changes in substrate utilization were correlated with ATP content. No differences in ATP content were observed between hearts from control and sodium pivalate–treated groups (26.1 ± 1.1 *v* 21.1 ± 3.9 μmol/g dry weight), suggesting that the rate of ATP utilization was commensurately decreased in pivalate-treated hearts.

DISCUSSION

The results of this study demonstrate that treatment of rats with sodium pivalate for a 6-month period produces a dramatic depletion in heart carnitine content. Isolated working hearts from these animals show a marked depression in cardiac function in response to increases in left-atrial filling pressures and metabolic demand imposed by pacing. Under these conditions, we also demonstrate that the severity of this myocardial carnitine deficiency is associated with a reduction in the rate at which exogenous palmitate is oxidized. When the intensity of work performed by these hearts is reduced, depressions in cardiac function and palmitate oxidation are no longer observed. This suggests that when carnitine-deficient hearts perform at increased levels of work, rates of palmitate oxidation appear to be inadequate to maintain tissue levels of acetyl CoA and high-energy phosphates. As a result, mechanical performance cannot be maintained at this increased level of external work.

Our results are in agreement with previous reports indicating that fatty acid oxidation is depressed in tissues obtained from patients with various forms of carnitine deficiency. For instance, in some cases of primary systemic carnitine deficiency, lipid accumulation observed in skeletal muscle and liver was associated with reductions in total carnitine content along with elevations in levels of long-

Table 4. Myocardial ATP Production From Exogenous Glucose and Palmitate Oxidation in Control and Pivalate-Treated Hearts Perfused Under High-Workload Conditions

Source of ATP	ATP Production (μmol/g dry weight · min)		ATP Production Normalized for Heart Function (nmol/g dry weight · min/HR × SLVP × 10^{-3})	
	Control	Pivalate-Treated	Control	Pivalate-Treated
Glucose oxidation	12 ± 3 (9.6%)	25 ± 7 (26.3%)	359 ± 77 (10.1%)	718 ± 129 (24.0%)
Palmitate oxidation	113 ± 11 (90.4%)	70 ± 5 (73.7%)*	3,191 ± 307 (89.9%)	2,271 ± 164* (76.0%)
Total ATP production	125	95	3,550	2,989
Glucose to palmitate ratio	0.11	0.36	0.11	0.32

NOTE. Values are reported as the mean ± SEM. ATP production was calculated using a value of 36 mol ATP produced per 1 mol of glucose oxidized. For palmitate oxidation, a value of 129 mol ATP produced per mol of palmitate oxidized was used.

*Significantly different from control hearts.

chain acylcarnitine.[6-12] Cultured fibroblasts and muscle mitochondrial preparations obtained from these patients exhibit reductions in both the activity of long-chain acyl CoA dehydrogenase and the oxidation of palmitate.[9,11,12,28] Interestingly, the activity of pyruvate dehydrogenase complex and the oxidation of pyruvate in fibroblasts were normal in these cases of carnitine deficiency.[11] Lipid droplets and fragmented and distorted cristae have also been observed in mitochondria from skeletal muscle of patients with severe carnitine deficiency, clearly suggesting impaired fatty acid metabolism. Although heart was the only tissue in which palmitate oxidation was measured in the present study, we cannot rule out that a chronic carnitine deficiency induced by treatment with sodium pivalate could also alter fatty acid metabolism in other tissues.

It has been previously assumed that carnitine levels in tissues are not found in excess, but rather at set levels appropriate for optimal substrate metabolism. In fact, the amount of carnitine required for maximal stimulation of fatty acid oxidation paralleled the endogenous content of this compound in tissues.[29] However, the observation that the K_m for carnitine palmitoyltransferase is much lower than the in vivo carnitine concentration in the heart suggests that this may not be the case.[1] Maximal stimulation of fatty acid oxidation by carnitine in isolated skeletal muscle mitochondria can be obtained with 0.4 mmol/L, a concentration that not only corresponds to a cytosolic carnitine content of 1.2 μmol/g tissue but also is much lower than levels found in skeletal muscle.[30] In the chronic–volume-overload rat heart, a reduction in total carnitine content was associated with a decrease in palmitate oxidation.[31] When mitochondrial function was measured in these hearts, respiration rates in the presence of palmitate and carnitine were also depressed. Taken together, these findings suggest that the lack of carnitine itself in the carnitine acyltransferase-translocase transfer system may not be the primary defect, but rather that alterations in mitochondrial long-chain acyl CoA dehydrogenase may be involved (unpublished observations, August 1993). Defective mitochondrial β-oxidation has also been demonstrated in tissues of patients with severe carnitine deficiency.[9,11,12]

An interesting observation from this study is that exogenous glucose oxidation rates in carnitine-deficient hearts were higher than those seen in control hearts perfused at increased-workload conditions. This was expected, since the contribution of fatty acid–derived acetyl units from palmitate was markedly depressed in carnitine-deficient hearts. If fatty acid flux into the mitochondria for oxidation is limited because of low tissue levels of carnitine and increased work demand, the myocardium must rely on alternate sources for a supply of acetyl residues to maintain tricarboxylic acid cycle activity.[32,33] Under these conditions, the uptake and subsequent overall utilization of glucose through the glycolytic pathway can be rate-limiting for the production of acetyl units. Increased myocardial pyruvate dehydrogenase complex activity then becomes of critical importance and can provide an additional and readily available source of acetyl units to the heart.[34] This is supported by the observation that $^{14}CO_2$ production from [U-^{14}C]-glucose is enhanced in carnitine-deficient hearts (Table 3), suggesting a greater shift of pyruvate-derived acetyl CoA production. Despite this, the contribution of glucose to overall myocardial ATP production in carnitine-deficient hearts was not sufficient to maintain an ATP production rate comparable to that seen in control hearts.

In summary, we show that long-term oral sodium pivalate treatment of rats produces a marked deficiency in myocardial carnitine content. When hearts from these animals are perfused at increased workloads, glucose oxidation is enhanced, whereas that of palmitate is dramatically reduced. These alterations in myocardial substrate utilization are also accompanied by a depression in cardiac function. Our findings indicate that the secondary carnitine deficiency model induced by sodium pivalate may be useful for the study and understanding of basic metabolic mechanisms associated with secondary carnitine deficiency.

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