Effects of Carnitine Isomers on Fatty Acid Metabolism in Ischemic Swine Hearts

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SUMMARY We studied the hemodynamic and metabolic effects of treatments with the L- and DL-isomers of carnitine in four groups (n = 42) of intact working swine hearts rendered mildly ischemic (~46% reduction in global perfusion). In three groups (n = 34), free fatty acids (FFA) in the coronary perfusate were augmented with labeled palmitate (0.72 μmol/ml). The inclusion of excess FFA per se (n = 11), as compared with nonsupplemented hearts, further and significantly depressed mechanical function (~40% and ~55% declines in left ventricular (LV)-developed pressure and work), stimulated a 2-fold increase in FFA uptake (but without a corresponding increase in 14CO2 production), raised fatty acyl-CoA levels in tissue (94 to 132 nmol/g dry), and shortened group survival. Treatments with both L- and DL-carnitine (serum values 6687 and 6398 nmol/ml) effected significant improvements in several parameters of mechanical function and survival. LV-developed pressure and work at end-ischemia were increased (+50% and +62% in L-treated hearts and +48% and +65% in DL-treated hearts). Myocardial oxygen consumption was similar to that of untreated FFA-supplemented hearts. During the middle to late portion of the perfusion trials in L-treated hearts (n = 9), FFA uptake and 14CO2 production were significantly reduced, and accumulation of tissue long-chain acyl-CoA was less (~44%, P < 0.05). Metabolic trends in DL-carnitine-treated hearts ranged between those in untreated and L-carnitine-treated hearts. These data suggest that carnitine reduces availability or incorporation of FFA intracellularly, and this benefits the heart mechanically during ischemic restrictions in coronary flow. The L-isomer appears to be the more biologically active.


GLOBAL ischemia of 30 minutes duration in intact working swine hearts causes reproducible dysfunctions in cardiac performance and metabolism (Liedtke, 1975; Liedtke et al., 1976). Additions of excess fatty acids (FFA) in this setting caused further derangements in function, together with a loss of total tissue carnitine stores and a build-up of inhibitory fatty acid intermediates, particularly long-chain acyl-CoA (Liedtke et al., 1978). Others have demonstrated that these intermediates, if sufficiently increased in concentration, can disrupt the workings of important enzyme systems in various biomembranes (Bremer and Norum, 1967; Oram et al., 1975; Wood et al., 1977).

In separate studies, pretreatment of ischemic hearts with DL-carnitine partially restored the depletions of tissue carnitine, significantly decreased the accumulation of long-chain acyl-CoA, and improved mechanical function (Liedtke and Nellis, 1979). The mechanisms for these changes are largely unknown but may relate to the reported actions of carnitine to modulate transfer of fatty acids across plasma or mitochondrial membranes (Rodis et al., 1970; Pande, 1975). The purpose of the present studies was to detail further the metabolic effects of carnitine and to evaluate the role of specific isomers on fatty acid metabolism in ischemic swine hearts. FFA were augmented to simulate levels reported in clinical settings of ischemia and infarction (Rogers et al., 1977; Mueller and Ayres, 1978) using a homogeneous solution of labeled palmitate. To prolong the exposures to global ischemia in untreated and carnitine-treated animal groups, cardiac output was regulated to prevent excessive cardiac preloading in compromised ventricles. Mechanical and metabolic functions as well as changes in tissue levels of fatty acid intermediates were compared among groups during the course of the perfusion trials.

Methods

Surgical Preparation

Forty-two swine, weighing 45.5-86.4 kg (average 57.5 kg), were studied following anesthesia with pentobarbital (35 mg/kg, iv) and the establishment of controlled positive pressure ventilation with 100% oxygen. Specific descriptions of the model, instrumentation, types and methods of measurements, and general format of data acquisition have been described previously (Liedtke et al., 1975; Liedtke and Nellis, 1979). Two separate extracorporeal circuits were constructed in this open-chest preparation: one to support the systemic circulation...
and a second to support the coronary circulation. The systemic circulation was regulated via a right heart bypass between both cavae and the pulmonary artery with flow maintained by a model 6002 Sarns modular pump (Liedtke et al., 1975). Cardiac output was adjusted to maintain left ventricular systolic pressures between 90 and 100 mm Hg. The bypass was primed initially with 250 ml of low molecular weight dextran. The coronary circulation was perfused with the animal’s own whole blood which was obtained from a right ventricular sump, passed through a blood oxygenator and heat exchanger, and then recirculated through the separately cannulated right and left coronary arteries. The right coronary artery was perfused from a site just distal to its origin and the left coronary artery was perfused from a Gregg cannula inserted into the ostium retrogradely from the left subclavian artery. Flow to each artery was maintained by separate, model 6050, low-flow Sarns perfusion changer, and then recirculated through the Sarns modular pump (Liedtke et al., 1975). Cardiac perfusion pressures equal to the systemic arterial pressure, after correcting for resistances of the tubing and cannulae. High-fidelity, manometer-tipped pressure devices were inserted into the left ventricle and aorta to measure pressures. Heart rate was monitored by electrocardiogram. Metabolic sampling ports were included in the coronary perfusion tubing, the right ventricular sump, and the systemic arterial and venous circulations. The hemiazygos vein draining into the coronary sinus was ligated in all animals. All hemodynamic data were displayed on an eight-channel Mark 200 Brush recorder and processed using a Digital Equipment Corporation PDP 11/10.

Data Analyses

Mechanical function was estimated from measurements of heart rate, left ventricular and aortic pressures, left ventricular max dp/dt, left ventricular work (calculated as the product of developed pressure, i.e., peak systolic minus end-diastolic pressure, × cardiac output normalized for body weight) and pressure time/min (PTM, calculated as the product of heart rate, average aortic pressure, and the time of left ventricular systolic ejection). Metabolic determinations included coronary perfusate FFA concentration, FFA:albumin molar ratios, carnitine and glucose levels, myocardial oxygen consumption, FFA uptake, and 14 CO2 production from labeled palmitate and tissue stores of high-energy phosphates, lactate, and fatty acid intermediates.

All methods of measurement, procedures, and calculations were as previously described (Liedtke, 1975; Liedtke et al., 1975; Liedtke et al., 1976; Liedtke et al., 1978; Liedtke and Nellis, 1979) with the exception of FFA uptake. Labeled and unlabeled palmitate (0.25-mmol boluses) was administered directly into the coronary perfusion circuits intermittently throughout the course of the perfusion runs (see specific details in experimental protocol section). All bolus injections of palmitate were made immediately after the preceding metabolic samplings. Palmitic acid was prepared according to the methods of Willebrands et al. (1973), dissolved in 0.1 M NaOH, warmed, and administered as an albumin-free solution. Animals received varying amounts of unlabeled palmitate (depending on the experiment group) which was prepared together with 70 µCi palmitate[14C(U)]. A subtraction routine was employed to calculate FFA uptake by following the disappearance of labeled palmitate in the arterial perfusate. Blood samples at 10-minute intervals throughout the perfusion trials were collected for analysis of radioactivity in counts/min per ml (cpm), fatty acid content in µmol FFA/ml, and the respective specific activity (SA) in cpm/µmol FFA. By determining the total blood volume (V) in the coronary circuit at the time of sampling (t), it was possible to solve for the total labeled palmitate in the perfusate (CPMp,t), as cpm t × V. By knowing the amount of labeled palmitate added to the circuit (A, in counts/min) in the interval preceding sampling (t-10), it then was possible for us to calculate FFA uptake by the heart as: FFA uptake,(µmol FFA/hr per g dry) = [(CPMp,t-10- (CPMp,t + A, t-10) + SA × 60 min/hr + dry wt of heart (g dry)]. The palmitate added (A) was corrected for the small quantity of product lost extracorporeally in the syringe, tubing, and/or oxygenator. In bench studies simulating experimental conditions but without the heart, this loss was determined to be 8–15% of the total injected palmitate and occurred either with the injection or in the first 20 minutes of circulation in the coronary bypass circuit.

Experimental Protocol

The purpose of the present studies was to document the effects of excess FFA in the ischemic heart and to evaluate the possible benefits and mechanisms of treatments with carnitine. The format was to make mechanical and metabolic correlations among four groups of hearts. One group received neither excess FFA nor carnitine (LOW FFA, n = 8); a second group received excess palmitate only (HIGH FFA, n = 11); a third group was both palmitate-supplemented and treated with dl-carnitine (HIGH FFA—DL CARN, n = 14); and a final group received both excess palmitate and treatments with l-carnitine* (HIGH FFA-L CARN, n = 9). In all groups, coronary flow was maintained at normal levels for 30 minutes, after which it was reduced over 10 minutes to ischemic levels and held there for a maximum of 30 additional minutes. Lidocaine (0.6–1.8 mg/kg, iv) was administered to all animals to reduce the occurrence of ventricular dysrhythmias. As an added precaution, cardiac output was regulated via the right heart bypass so that left ventricular end-diastolic pressure did not exceed 20 mm Hg. This unloading of the left ventricle

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* Courtesy of Otsuka Pharmaceutical Factory, Naruto, Japan.
during periods of compromised performance was adopted to avoid precipitous declines in function and to prolong times of survival. Mechanical and metabolic data were sampled initially and at 10-minute intervals throughout control and ischemic perfusions. All metabolic estimates of consumption and/or production were normalized by myocardial dry weight.

Supplements of palmitate were added to the reservoir of the oxygenator of the coronary circuit in 0.25-mmol push infusions. Preliminary studies showed that these supplements were removed rapidly in the early stages of the perfusion run. To produce a sufficient excess in HIGH FFA groups, it was necessary to repeat these administrations frequently. A dose schedule was arrived at which included, for 0–10 minute perfusion, 1.25 mmol; 10–20 minutes, 1.0 mmol; 20–30 minutes, 0.5 mmol; 30–40 minutes, 0.5 mmol; 40–50 minutes, 0.5 mmol; 50–60 minutes, 0.5 mmol; and 60–70 minutes, 0.5 mmol. The palmitate dose schedule for the LOW FFA group was for 0–10 minutes, 0.30 mmol; 10–20 minutes, 0.05 mmol; 20–30 minutes, 0.05 mmol; 30–40 minutes, 0.05 mmol; 40–50 minutes, 0.05 mmol; 50–60 minutes, 0.05 mmol; and 60–70 minutes, 0.05 mmol.

In the two carnitine-treatment groups, solutions of carnitine were prepared at concentrations of 170 mg/ml for DL-carnitine and 80 mg/ml for L-carnitine. These were infused continually (0.25 ml/min) into the coronary circuit beginning at 0-minute perfusion. The doses were calculated on the basis of those used previously that were shown to have effects on the heart (Liedtke and Nellis, 1979).

At the conclusion of the 70-minute perfusion run, or with death of the animal, transmural samples of myocardium in all groups were frozen immediately and stored for subsequent analyses of several fatty acyl derivatives and other metabolites. Death was defined on the basis of mechanical deterioration. If, despite preload manipulation by the right heart bypass pump, hearts were unable to generate a developed pressure of greater than 40 mm Hg at any time during the perfusion runs, the experiments were terminated. Paired Student’s t-tests were used to test the statistical significance of intragroup comparisons. A two-component, a posteriori, statistical analysis was used for intergroup comparisons and included an F test analysis of variance followed by a Studentized Newman-Keuls test (Snedecor and Cochran, 1972). Significance was defined for probability values of less than 5%. Distribution of the data, where listed, always appears as the standard error of the mean.

Results

Effects of Excess FFA on Ischemic Myocardium

Selected responses to restrictions in global coronary perfusion and to the inclusion of extra palmitate in the coronary perfusate are shown at representative perfusion times in Figures 1 and 2. In LOW FFA hearts (avg. serum FFA levels, 0.21 μmol/ml; avg. FFA:albumin molar ratios, 1.1:1), coronary flow was reduced by 46%. Heart rate remained unchanged; only a minimal adjustment in cardiac output (see Methods) was required, and no animal expired prematurely from cardiac failure or dysrhythmias. However, several derangements were observed. When we used paired Student’s t-tests and contrasted the data at 0 (initial control state) and 70 minutes (end-ischemic state) of perfusion, we noted a decline in left ventricular-developed pressure (−24%, \( P < 0.005 \)) and global work (−25%, \( P < 0.01 \)). These were determined chiefly by a decrease in left ventricular peak-systolic pressure (−17%, \( P < 0.01 \)) and a rise in end-diastolic pressure (+261%, \( P < 0.025 \)). Other mechanical changes included declines in mean aortic pressure (−17%, \( P < 0.025 \)), PTM (−14%, \( P < 0.1 \)) and left ventricular max dp/dt (−39%, \( P < 0.01 \)). Between 30 minutes (after steady state intracellular incorporation of palmitate at control flows) and 70 minutes of perfusion, these restrictions in flow were associated with reductions in myocardial oxygen consumption (−19%, \( P < 0.1 \)), FFA uptake (−42%, \( P < 0.005 \)), and \(^{14}\)CO₂ production (−28%, \( P < 0.05 \)) from labeled palmitate. Ischemic changes also were reflected in the tissue stores of high-energy phosphates. Creatine phosphate and ATP levels were 29.4 ± 2.7 and 17.6 ± 1.1 μmol/g dry, respectively.

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** Various controlled and dependent hemodynamic variables in two groups of swine hearts receiving either low (triangles) or high (squares) FFA into the coronary perfusate. Data are arrayed according to the level of coronary perfusion along the abscissa of each panel. At similar levels of ischemia, HIGH FFA hearts demonstrated significantly greater deteriorations in hemodynamic performance. Abbreviations include: \( t_\text{i} \) = initial time of perfusion trial; \( t_\text{END} \) = final time of perfusion (70 minutes for LOW FFA hearts, 63 minutes for HIGH FFA hearts); LVEDP = left ventricular end-diastolic pressure; others as described in text. * Indicates statistical significance at least \( P < 0.05 \) between groups by Studentized Newman-Keuls testings.
TIMES OF PERFUSION mm

FIGURE 2
Metabolic data for the same two groups of hearts as shown in Figure 1 arrayed along the abscissa of each panel by the time of perfusion. Symbols are as described in Figure 1. At similar levels of perfusion throughout the trials, FFA excess caused a 2-fold or greater increase in FFA uptake without a corresponding augmentation in CO₂ production.

These represented decreases of ~24% and ~18% as compared with values obtained previously from control hearts perfused at normal flows (Liedtke et al., 1978). Also, as compared with aerobic hearts (Liedtke et al., 1978), there was a 25-fold increase in long-chain acyl carnitine and a 34% increase in long-chain acyl-CoA. These values were similar to those reported previously for ischemic hearts (Liedtke et al., 1978; Liedtke and Nellis, 1979) and were again associated with a 29% loss of total tissue carnitine stores.

In HIGH FFA (palmitate-supplemented) hearts, coronary perfusate FFA and FFA:albumin ratios (avg. values, 0.75 µmol/ml and 3.8:1, respectively) were increased to levels reported clinically during acute events in ischemic heart disease (Rogers et al., 1977; Mueller and Ayres, 1978). At comparable control and ischemic flows, several additional derangements were observed as compared with LOW FFA hearts (Figs. 1 and 2). There was progressive mechanical failure in palmitate-treated hearts (despite a significant decrease in cardiac output), and group survival time (63 ± 2 min) was reduced (P at least < 0.05 by Studentized Newman-Keuls tests). Other disturbances at end-ischemia included further significant (P at least < 0.05) declines in left ventricular-developed pressure (~40%) and work (~55%). These were determined by at least a 2-fold increase in end-diastolic pressure (P < 0.05) despite a forced reduction in cardiac output (~29%, P < 0.05) and a decrease in left ventricular peak-systolic pressure (59 ± 5 vs. 79 ± 5 mm Hg, P < 0.05). Left ventricular max dp/dt was attenuated further (~50%, P < 0.05), as was mean aortic pressure (49 ± 5 vs. 70 ± 6 mm Hg, P < 0.05) and PTM (1640 ± 203 vs. 2130 ± 150 mm Hg-sec/min, P < 0.05). These changes resembled those reported previously for ischemic hearts exposed to excess FFA effected by treatments of an emulsion of several FFA (Intralipid) given with heparin (Liedtke et al., 1978; Liedtke and Nellis, 1979).

Metabolically in HIGH FFA hearts and in concert with the declines in mechanical function, myocardial oxygen consumption was decreased further at end-ischemia (~36%, P at least < 0.05 as compared with LOW FFA hearts). Conversely, palmitate treatments induced a 2-fold increase in FFA uptake (P < 0.05, Fig. 2) at both control and ischemic coronary flows. However, this increase was not reflected in any corresponding increase in ¹⁴CO₂ production from mitochondrial oxidation. Such a dissociation suggests that the excess incorporated FFA alternatively entered extramitochondrial lipid pools in the cytosol or biomembranes. For the level of coronary flow restriction selected in these studies, there were no significant differences in tissue stores of high energy phosphates in HIGH as compared with LOW FFA hearts nor was there further loss of total tissue carnitine. However, long-chain acyl carnitine increased from 527 to 851 nmol/g dry (+20%) and fatty acyl-CoA increased from 94 to 132 nmol/g dry (+40%, P < 0.05).

**Effects of Carnitine Treatments in the Presence of Excess FFA in Ischemic Hearts**

In two additional palmitate-supplemented groups of hearts, (avg. serum FFA, 0.71 µmol/ml; avg. FFA:albumin molar ratios, 3.3:1), DL- and L-isomers of carnitine were infused into the coronary perfusate (terminal serum carnitine values 6398 nmol/ml in DL-treated hearts; 6687 nmol/ml in L-treated hearts, Fig. 3). Selected hemodynamic and metabolic responses as compared with HIGH FFA...
High FFA-dL-carnitine-treated hearts (half filled circles); and 69.1 minutes for High FFA hearts (filled circles). Treatments with carnitine, and particularly L-carnitine, appeared to decrease FFA uptake during the middle portion of the perfusion trials. A decrease in CO₂ production following L-carnitine treatment was also demonstrated.

Metabolic trends with dL-carnitine were between those observed in untreated and L-carnitine-treated hearts (Fig. 5). As compared with High FFA hearts, myocardial oxygen consumption in dL-carnitine-treated hearts shifted relatively little over the course of the perfusion, whereas FFA uptake decreased by 15% during the middle portion (30–50 min) of the trials. Slight reductions in ¹⁴CO₂ production over this same period were also noted. As compared with L-carnitine-treated hearts, the decrease in FFA uptake was not as great and was statistically no different from that of either untreated or L-carnitine-treated values (except at 50-min perfusion). Tissue concentrations of high-energy phosphates were similar to those in untreated hearts, whereas long-chain acyl-carnitine was increased by 9% (924 ± 149 nmol/g dry) and long-chain acyl-CoA decreased by 6% (123.8 ± 5.9 nmol/g dry) over those in High FFA hearts.

Treatments with L-carnitine brought significant improvements in mechanical function similar to those observed with dL-carnitine (Fig. 4). As compared with High FFA hearts, group survival was prolonged (69.1 ± 0.9 min, P < 0.05), and hemodynamic function at end-ischemia was preserved (left ventricular-developed pressure increased by 50% and work by 62% over values in untreated hearts at nearly identical cardiac outputs). Both changes were significant (P < 0.05). Other improvements included increases in left ventricular peak-systolic pressure (+27%, P < 0.05), max dp/dt (+49%), mean aortic pressure (64.4 ± 5.2 mm Hg, P < 0.05) and PTM (2092 ± 161 mm Hg-sec/min), and a decrease in left ventricular end-diastolic pressure (−31%). Metabolic changes were more evident than in High FFA-dL-CARN hearts. At oxygen consumptions similar to those of High FFA hearts (Fig. 5), FA uptake in High FFA-L-CARN hearts was decreased below levels noted in both untreated and dL-carnitine-treated hearts. Between 30 and 50 minutes of perfusion, FA uptake in L-carnitine-treated hearts was reduced by 33% below untreated values (P < 0.05) and was accompanied by a significant decline in ¹⁴CO₂ production at 90 minutes of perfusion. Tissue accumulation of long-chain acylcarnitine was not increased above untreated values (795 ± 148 nmol/g dry), whereas long-chain acyl-CoA was significantly reduced (−44%, P < 0.05). High energy phosphate stores were similar to those in untreated and dL-carnitine-treated hearts.

**Figure 4** Hemodynamic responses before and after restrictions in coronary perfusion in palmitate-supplemented hearts receiving either no treatment or constant infusions of carnitine. The format of data display is identical to that in Figure 1. Abbreviations and symbols include: t₀ = initial time of perfusion trial; tₚ = final time of perfusion (63 minutes for HIGH FFA hearts (squares, same group as in Figure 1); 70 minutes for HIGH FFA-dL-CARNITINE-treated hearts (half circles); and 69.1 minutes for HIGH FFA-L-CARNITINE-treated hearts (filled circles)]. Treatments with both isomers of carnitine significantly improved mechanical function and survival during ischemia.

**Figure 5** Metabolic responses in the same three heart groups as shown in Figure 3 and 4. Treatments with carnitine, and particularly L-carnitine, appeared to decrease FFA uptake during the middle portion of the perfusion trials. A decrease in CO₂ production following L-carnitine treatment was also demonstrated.
Discussion

The present data support previous observations that excess FFA can promote further mechanical failure and shorten group survival in ischemic swine hearts. These changes were associated with increased intracellular incorporation of fatty acids (but without increased mitochondrial oxidation) and accumulation of inhibitory intermediates of acyl-CoA and carnitine. Treatments with DL- and L-carnitine partially restored mechanical function in this setting and appeared to modulate fatty acid uptake, particularly for the L-isomer. Before proceeding with a discussion of these findings, a critique of the methods and protocol is presented.

The conditions of the study were selected in part to evaluate the effects of only modest elevations of a long-chain fatty acid at less than severe restrictions in coronary flow. The levels of flow restriction were chosen to prolong the studies since it had been shown previously in this preparation that greater restrictions (>60% Δ) in global perfusion led to precipitous declines in function and early death (Liedtke et al., 1975). As an adjunct to prolonging survival, cardiac output, as regulated by the right ventricular bypass pump, was reduced during ischemia to prevent excessive engorgement of the left ventricle during mechanical failure. These maneuvers were used to provide more time for separation of potential differences in mechanical and metabolic responses among groups, lessen the excessive accumulation of inhibitory intermediates which might be masking other processes, and better limit the variability in times for sampling of tissue metabolites obtained at the conclusion of the studies. Fatty acid supplements were not augmented to levels above those observed clinically (Mueller and Ayres, 1978). This not only satisfied recent objections criticizing the use of overly large amounts of FFA in most previous studies on animals (Rogers et al., 1977) but also established a previously unrecognized threshold for mechanical dysfunction which can result from clinical levels of FFA excess in mildly ischemic hearts. The combination of FFA excess and ischemia chosen in these studies (LOW and HIGH FFA hearts) may explain why losses of total tissue carnitine stores were not as great as previously reported in ischemic swine hearts (Liedtke et al., 1978; Liedtke and Nellis, 1979). We elected not to calculate the total tissue carnitine stores in carnitine-treated hearts because of the high levels of carnitine in the aqueous phase of the tissue. Although the wet weight:dry weight ratios and distributions of water between intra- and extracellular spaces were known, the error of subtracting the total measured acid-soluble fraction of carnitine from the estimated extracellular levels was deemed too large for accuracy in determining the derived difference.

Finally, we employed a new method for calculating FFA uptake in these studies based on the rate of disappearance of labeled palmitate from the arterial perfusate in the closed coronary perfusion circuit. This approach was adopted to avoid errors introduced by mixing problems using the bolus method of palmitate administration and the standard way of calculating FFA uptake from the measured arteriovenous differences of labeled palmitate across the myocardium (Liedtke et al., 1975). Separate studies using just the coronary-oxygenator loop without the heart revealed that the extracardiac loss of labeled palmitate was small (less than 10% of myocardial consumption). This loss was corrected for and included in the calculations. Estimates of FFA uptake in LOW FFA hearts agreed closely with prior results in similar studies (Liedtke et al., 1975).

The observation has long been held that fatty acids in excess may be detrimental to cardiac function. Hoak et al. (1968) artificially elevated long-chain saturated fatty acids in plasma of dogs, ducks, and geese and produced acute heart failure, dysrythmias and other electrocardiographic abnormalities, and death. Light and electron microscopy revealed myocardial necrosis and degeneration, suggesting a direct toxic effect upon myocardial cells by FFA. Oliver et al. (1968) added clinical relevance to these findings when they documented a strong correlation in patients with acute myocardial infarction between elevated serum levels of FFA (>1200 μEq/liter) and an increased prevalence of serious ectopic dysrhythmias and disorders of conduction. Since then, several investigations have been reported supporting these early claims (Kjekshus and Mjos, 1972; Willebrands et al., 1973; DeLeris et al., 1975) although agreement has not been universal (Opie et al., 1971).

Several mechanisms have been proposed to account for these mechanical and electrical derangements. FFA have been shown in mitochondria to uncouple electron transport from oxidative phosphorylation and impair oxidation (Pressman and Lardy, 1956; Hulsman et al., 1960). In excess concentrations within the cell, FFA also may act as nonspecific detergents locally on biomembranes to promote swelling and leakage of intracellular contents. DeLeris et al. (1975) in working rat hearts showed that adding FFA to the coronary perfusate, and in particular palmitate, led to a 5- to 10-fold increase in the release of LDH enzyme. More recently, attention has shifted to examine the role of fatty acid intermediates, in particular the acyl derivatives of CoA and carnitine, on membrane function. Long-chain acyl-CoA which may increase dramatically in ischemia, has been reported in a variety of subcellular preparations to inhibit adenine nucleotide transferase (Ho and Pande, 1974; Shug et al., 1978), carnitine-palmitoyl CoA transferase (Bremer and Norum, 1967), and phosphate and potassium exchange (Wojtczak, 1974; Flatmark and Pederson, 1975) in the membrane of mitochondria as well as Na⁺,K⁺-ATPase (Lamers and Hulsman, 1974).
1977; Wood et al., 1977) in sarcolemma. Long-chain acyl carnitine also has been shown to interfere with Na+,K+-ATPase and K+ phosphatase in sarcolemma (Wood et al., 1977) and Ca2+ binding in sarcoplasmic reticulum (Adams et al., 1979).

Thus one approach to therapy in ischemic hearts would be to reduce intracellular accumulations of FFA and their intermediates and/or to redistribute these products away from important membrane systems in myocytes. To these ends, carnitine was employed in the present studies. Carnitine, a naturally occurring and actively synthesized quaternary ammonium compound, is an essential cofactor in the transfer of activated fatty acids from the cytosol to mitochondrial matrix. This process is both temperature dependent (Ramsey and Tubbs, 1976) and isomer specific (Pande and Parvin, 1976); i.e., the L-isomer stimulates transfer and the D form inhibits it. Carnitine, particularly the L-isomer, is actively taken up by the heart and is concentrated against a plasma-cytosol gradient (Böhmer et al., 1977). The uptake of carnitine by sarcolemma appears to result from active selective transport which is saturable and energy dependent (Bressler et al., 1979).

In experimentally induced heart failure resulting from hypertension (Wittels and Spann, 1968) and coronary ischemia (Schwartz et al., 1973; Shug et al., 1978), carnitine is lost from the myocyte as the acid-soluble fraction. Early attempts at replacing carnitine in ischemic hearts have been encouraging. Thomsen et al. (1979) demonstrated positive benefits with carnitine in patients with coronary artery disease whose hearts were paced until angina occurred. In this group, carnitine significantly increased the heart rate-blood pressure product, the pacing duration to angina, and lactate extraction while decreasing left ventricular end-diastolic pressure and ST-T wave abnormalities. Fols et al. (1978) in ischemic dog hearts documented that carnitine decreased electrocardiographic changes, increased tissue stores of high energy phosphates, and in harvested mitochondria restored adenine nucleotide translocase activity. They also concluded that carnitine possessed antidysrhythmic actions which prevented the development of ventricular fibrillation.

In ischemic swine hearts supplemented with FFA we also noted a variety of improvements with DL-carnitine (Liedtke and Nellis, 1979). As compared with untreated hearts, carnitine induced significant increases in left ventricular and aortic pressures, left ventricular mass dp/dt and global work, and regional left ventricular shortening. These increased work expenditures did not further increase oxygen consumption or further deplete tissue stores of high energy phosphates during ischemia and thus suggested an increased mechanical/metabolic efficiency. Tissue levels of long-chain acyl-CoA also were reduced in the treated group. The present studies were designed to follow up these initial observations, with particular attention focused on documenting important metabolic events and determining the influence of different carnitine isomers.

The present data supported initial findings that treatments with both DL- and L-isomers preserved mechanical function at no further cost to oxygen consumption during ischemia. Clear metabolic differences in fatty acid metabolism were best seen in carnitine-treated hearts. The decrease in fatty acid uptake, CO2 production, and tissue stores of long-chain acyl-CoA, taken together, suggest a reduced availability of FFA intracellularly. Such effects on fatty acid uptake were reported previously in isolated aerobic hearts (Rodis et al., 1970). The mechanism responsible for these changes is as yet unknown, but the present data suggest the possibility of a stereospecific action with the L-isomer being biologically more active. Uptake in DL-carnitine-treated hearts was between that in L-isomer-treated and untreated hearts and not significantly different from either (except at 50 minutes, at which time a statistical separation was noted between the L- and DL-isomer-treated groups). This suggested the D-isomer was either biologically inert or opposite in action from the L-isomer, both of which possibilities support an isomer-specific effect. Another question still to be resolved is how carnitine modifies fatty acid uptake. Possibilities include a direct interference with the passive uptake of FFA at the plasma membrane or perhaps a yet-to-be-identified translocase system in the sarcolemma to discharge activated fatty acids in the cytosol back into plasma, possibly in the form of acyl carnitine. The background level of long-chain acyl carnitine in the perfusate of FFA- and carnitine-treated hearts was too high to test for this latter possibility in the present study.

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doi: 10.1161/01.RES.48.6.859

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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