Myocardial Response to Pretreatment with L-Carnitine in Patients with Cardiac Valve Replacement

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Abstract

Twenty-one patients undergoing mitral valve replacement (MVR) with either isolated or combined valvular procedure were investigated to determine the effect of randomized and prospective administration of L-carnitine (car) chloride on myocardial function. Carnitine chloride (1800 mg/day, for seven consecutive days just prior to MVR) was given orally to 9 patients (car-treated group) who were compared with 12 car-untreated patients (control group) subjected to the same procedure. Valve replacement was performed using the Bjork-Shiley mechanical prosthesis in aortic and mitral position and bioprosthesis in tricuspid position in both groups under cardiopulmonary bypass (CPB) associated with cold potassium-magnesium cardioplegia and systemic hypothermia. Preoperative and intraoperative clinical profile (age, functional class, duration of aortic clamp and CPB, and level of hypothermia) was well matched in both groups. Free, short-chain acyl, long-chain acyl, and total Car were measured in the papillary muscle excised adjacent to the free wall of the left ventricle at the time of valvular surgery. Heart rate and mean arterial pressure did not differ between the two groups. Cardiac index was 3.44 ± 0.08 and 3.82 ± 0.13 L/m^2/min in the control and car-treated groups, respectively, ($p < 0.05$). Dobutamine (DOB) infusion rate was 8.55 ± 0.73 and 5.57 ± 0.95 μg/kg/min in the control and car-treated groups, respectively ($p < 0.05$). Left ventricular stroke work index

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(LVSWI)/DOB ratio was 4.50 ± 0.65 and 8.16 ± 1.72 g•m/m²/beat/unit of DOB, in the control and car-treated groups, respectively ($p < 0.05$). Long-chain acyl carnitine was 0.224 ± 0.031 and 0.460 ± 0.096 nmoL/mg noncollagenous protein in the control and the car-treated groups, respectively ($p < 0.05$). LVSWI/DOB ratio was well correlated with long-chain acyl carnitine in the tissue, $r = 0.74$, $n = 18$, $p < 0.001$.

These findings suggest that pretreatment with L-carnitine chloride appears to be effective in improving myocardial function in the early phase of reperfusion following surgery and that long-chain acyl carnitine may play a key role in the enhancement of carnitine-meditated mitochondrial metabolic process.

Introduction

Carnitine, a water-soluble, widely distributed amino acid in skeletal and cardiac muscle in the vertebrate, is the requisite carrier for transport of fatty acids from the cytosole across the mitochondrial membrane for beta oxidation.[1] Fatty acids and their intermediates are utilized to generate cardiac function by means of acyl carnitine translocase, and at the same time, free carnitine is recycled from the mitochondria to the cytosole.[2] Myocardial levels of carnitine have been determined in normal[3] and pathologic conditions.[4-7] Carnitine is depleted in association with congestive heart failure,[3,8,9] ischemia,[10] and mechanically overloaded conditions.[11,12]

Accumulation of carnitine esters of long-chain fatty acids, which occurs in ischemic myocardial cells, is attributed to the myocardial metabolic disturbances: inhibition of mitochondrial beta oxidation, increased intracellular lipolysis, increased phospholipase A2 activity, increase in circulating free fatty acid concentrations by the release of catecholamines, and accumulation of metabolites.[13,14] It is still controversial what impact long-chain acyl carnitine may have in the ischemic myocardium. It has been reported that long-chain acyl carnitine deteriorates myocardial viability because of damage to cellular membranes[15,16]; however, no correlation has been found between the recovery of cardiac function during reperfusion and the accumulation of long-chain acyl carnitine during ischemia.[17,18]

Biosynthesis of carnitine occurs in mammalian organs, the ultimate synthesis being in the liver.[19] The heart, unable to synthesize carnitine, depends on carnitine uptake from the blood. Therefore, exogenous provision of carnitine has fruitful potential. It has been clearly demonstrated that pretreatment with DL-carnitine provided better preservation of ventricular compliance and improved maintenance of contractile function without preventing adenine nucleotide depletion in a canine ischemia-reperfusion model.[20] It has been reported that L-carnitine chloride, used as a pretreatment, protected the myocardium from ischemia-reperfusion, improved cardiac metabolism, and reduced the incidence of arrhythmia in an animal model.[21-23] Furthermore, the Japanese multicenter clinical trial[24] with a protocol consisting of administration of L-carnitine chloride (1800 mg per

TABLE I
Clinical Profile (mean ± SEM). Preoperative and Intraoperative Factors Are Well Matched in Both Groups

	Control	L-Carnitine Treated	
n	12	9	
Age	49.1 ± 3.0	52.6 ± 3.5	NS
Duration of Cardioplegia (min)	139.0 ± 15.4	117.9 ± 13.0	NS
Duration of Cardiopulmonary bypass(min)	193.0 ± 15.6	165.3 ± 15.6	NS
Hypothermia (°C)	23.9 ± 0.5	23.6 ± 0.5	NS
NYHA	2.1 ± 0.1	2.1 ± 0.1	NS
Plasma Free Carnitine(μmoL/L)	58.4 ± 8.3	45.0 ± 5.5 (before) 59.6 ± 8.1 (after)	NS
Noncollagenous protein in tissue sample (mg/g•wet tissue)	104.3 ± 9.5	132.7 ± 12.7	NS

day for six weeks), demonstrated that L-carnitine chioride reduced the incidence of anginal attacks and improved ECG findings during exercise in patients with ischemic heart diseases.

Thus, the present study was conducted in patients undergoing cardiac valve replacement to define the pharmacokinetic effect of L-carnitine chloride (car) on cardiac function in early reperfusion following cardiac valve replacement.

Materials and Methods

Twenty-one individuals undergoing mitral valve replacement (MVR) with either isolated or combined valvular procedures were studied (7 men and 14 women). Surgical indication was rigorously conformed to symptoms, hemodynamic state evaluated by cardiac catheterization, and functional and morphological assessment of cardiac valves using ultrasonic cardiography. The following individuals were excluded from this study: those with repeated valvular surgical interventions, mitral valve disease due to infectious endocarditis, preoperative congestive heart failure, and mitral regurgitation due to ruptured chordae tendinae. The twenty-one patients were divided at random into two groups. The control group consisted of 12 patients who did not receive L-carnitine chloride, and the car-treated group consisted of 9 patients who were treated with oral car, 1800 mg per day for seven days prior to MVR. Open heart surgery was conducted in all patients under cold potassium-magnesium cardioplegia (K, 20 mEq; Mg, 16 mmoL; Ca, 1.0 mmoL; pH 7.40; osmolarity 420 mOsm; glucose 245 mmoL; and mannitol 100 mmoL per liter) in a multidose fashion associated with hypothermic cardiopulmonary bypass (CPB). Preoperative and intraoperative clinical parameters, listed in Table I, demonstrate no significant difference in any parameter. Regarding the operative procedure, the control group consisted of isolated MVR (3 patients), MVR + aortic valve replacement (AVR) (3), MVR + aortic valvular plasty (1 patient), MVR + tricuspid valve replacement (TVR) (2), and MVR tricuspid

TABLE II
Tissue Carnitine Content. Values Are Expressed as n moL/g Wet Tissue

	Control	L-Carnitine Treated	
n	12	9	
Tissue carnitine (nmoL/g Wet tissue			
• Free	414.2 ± 85.4	746.6 ± 156.7	$0.5 < p < 0.1$
• Short-chain acyl	275.5 ± 52.7	310.7 ± 35.7	NS
• Long-chain acyl	23.4 ± 3.9	59.9 ± 12.1	$p < 0.05$
• Total	762.9 ± 128.4	1117.1 ± 144.3	NS

Mean ± SEM.

valvular plaasty (TAP) (3), whereas the car-treated group consisted of isolated MVR (5), MVR + AVR + TAP (2), MVR + TAP (1), and MVR + TVR (1). The Bjork-Shiley prosthesis in the mitral and aortic position and the bioprosthetic valve in the tricuspid position were used for valve replacement.

Hemodynamic condition was evaluated in all patients during the postoperative period after open heart surgery by use of a Swan-Ganz catheter and thermodilution technique for measurement of cardiac output. Hemodynamic condition in early reperfusion, namely, at six to ten hours after surgery, was the focus in this study. All patients were treated identically by adjusting the rate of infusions of the DOB and vasodilating agents to maintain pulmonary capillary wedge pressure (PCWP) at 10-15 mmHg, total systemic resistance (TSR) at 1000-1200 dyne sec cm, and cardiac index (CI) at 3.0 ± 0.5 L/min/m^2. Hemodynamic condition was compared between the control group and the car-treated group at six to ten hours of reperfusion in terms of heart rate (HR), mean arterial pressure (mAP), cardiac index (CI), left ventricular stroke work index (LVSWI), total systemic resistance (TSR), and amount of dobutamine (DOB) administered. LVSWI and TSR were calculated by the following formulas: LVSWI = stroke index x (mAP - PCWP) x 0.0136, g•m/m^2/beat. TSR = mean arterial pressure/cardiac output x 80 dyne•sec/cm^5

Determination of Plasma and Tissue Concentrations of Carnitine

Plasma concentration of carnitine was determined by means of the method published by Marquis and Fritz,[25] with blood being withdrawn before car was started and in the morning of open heart surgery. Tissue content of carnitine was determined with a part of papillary muscle of the left ventricle excised at the time of mitral valve resection by radioimmunoassay modified from the method published by Cereblad and Linstedt.[26] The tissue sample was carefully selected from the basal portion of the papillary muscle close to the free wall of the left ventricle. The excised tissue was immediately frozen. Carnitine content was measured and expressed as content per gram of wet tissue and content per mg of noncollagenous protein to avoid contamination with tissue fibrosis. Proteins from the tissue sample yielded no significant difference between the two groups, as shown in Table I. Carnitine was measured as total carnitine and its fractions such as free, short-chain acyl, and long-chain acyl carnitine.

TABLE III
Tissue Carnitine Content. Values Are Expressed as n moL/g Noncollagenous Protein

	Control	L-Carnitine Treated	
n	12	9	
Tissue carnitine (nmoL/mg NCP)			
• Free	4.298 ± 0.528	5.474 ± 0.820	NS
• Short-chain acyl	2.546 ± 0.397	2.533 ± 0.390	NS
• Long-chain acyl	0.224 ± 0.031	0.460 ± 0.096	$p<0.05$
• Total	7.069 ± 0.886	8.467 ± 0.717	NS

Mean ± SEM, NCP = noncollagenous protein.

The relationship between either total or long-chain acyl carnitine and duration of cardioplegia was analyzed. The relationship between long-chain acyl carnitine and LVSWI/DOB was analyzed both in all 21 patients and in the 10 patients who received isolated MVR and MVR + TAP.

This clinical study was conducted after the approval by the Ethics Committee of Tokyo Medical and Dental University was given.

Data were analyzed for comparison of two groups by use of Student's t test when significance was not obtained by F test and by use of the Wilcoxon test when statistical significance was obtained by F test.

Results

In the car-treated group, no significant changes were observed in HR, mAP, and systolic and diastolic arterial pressure with the administration of car. No car-related side effects were experienced in this study.

Tissue carnitine content is shown in Table II which displays values expressed as n moL/g wet tissue. Tissue long-chain acyl carnitine in the car-treated group was significantly elevated as compared with that of the control group. Free carnitine tended to be elevated in the car-treated group over that in the control. No significant difference was noted between with groups in short-chain acyl and total carnitine. Tissue carnitine content expressed as n moL/mg noncollagenous protein (NCP) is shown in Table III. Tissue long-chain acyl carnitine in the car-treated group was again significantly higher than that in the control group.

Plasma concentration of carnitine is listed in Table I. A level of 59.6 ± 8.1 μmol/L after car treatment for seven days was not significantly different from the level of 58.4 ± 8.3 μmol/L in the control group.

Hemodynamic condition, comparable between the groups, is shown in Table IV. Cardiac index and LVSWI/DOB ratio were significantly higher in the car-treated group than in the control group. The dobutamine required for the car-treated group was significantly less than that required for the control group.

The relationship between duration of cardioplegia and long-chain acyl carnitine con-

TABLE IV

Hemodynamic Parameters at 6-10 Hours of Postoperative Period. Each Parameter is Compared Between the Two Groups

	Control	L-Carnitine Treated	
n	12	9	
Heart rate (beats/min)	109 ± 4	118 ± 8	NS
Mean arterial pressure (mmHg)	78 ± 3	86 ± 6	NS
Cardiac index (CI) (l/min/m^2)	3.44 ± 0.08	3.82 ± 0.13	$p < 0.05$
Left ventricular stroke work index (LVSWI) (g•m/m^2/beat)	32.8 ± 2.5	37.3 ± 3.0	NS
Dobutamine (DOB) (μg/kg/min)	8.6 ± 0.7	5.6 ± 1.0	$p < 0.05$
LVSWI/DOB	4.50 ± 0.65	8.16 ± 1.72	$p < 0.05$*
Total systemic resistance (dyne•sec/cm)	1132 ± 57	1075 ± 108	NS

Mean ± SEM, *Wilcoxon test.

tent in the tissue, shown in Figure 1, demonstrates no significant correlation: r = - 0.41, n = 12, NS in the control group, and no correlation in the car-treated group was found. A relationship between duration of cardioplegia and tissue total carnitine level was not demonstrated to establish any significance.

A significant correlation was found between tissue concentration of long-chain acyl carnitine and LVSWI/DOB ratio in all patients of both groups: r = 0.74, n = 20, $p < 0.001$ (Fig. 2). Significant correlation was again found between long-chain acyl carnitine and LVSWI/DOB ratio in patients who underwent MVR (n = 6) or MVR-TAP (n = 4) in both groups: r = 0.87, n = 10, $p < 0.001$ (Fig. 3).

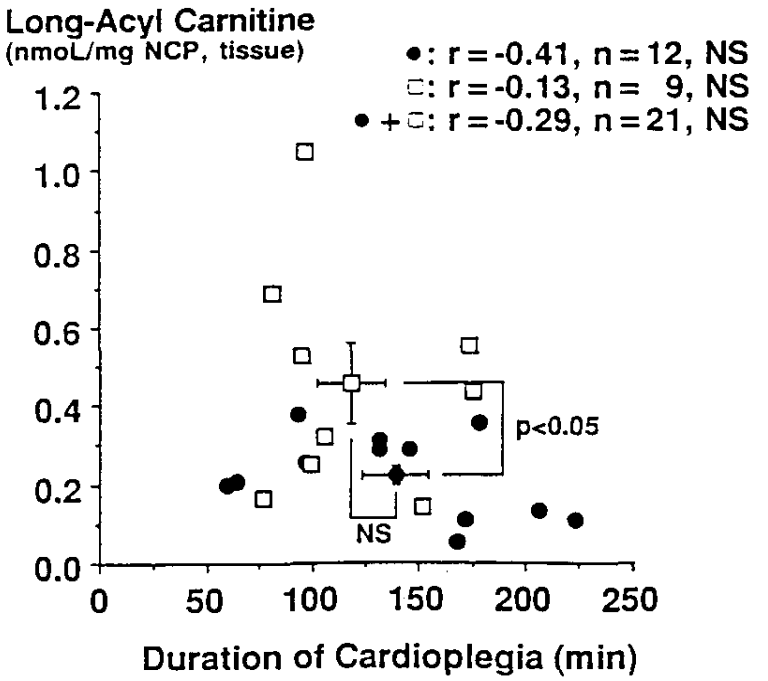

FIG. 1. Relationship between duration of cardioplegia and tissue long-chain acyl carnitine content is shown. No significant correlation is observed in any group.

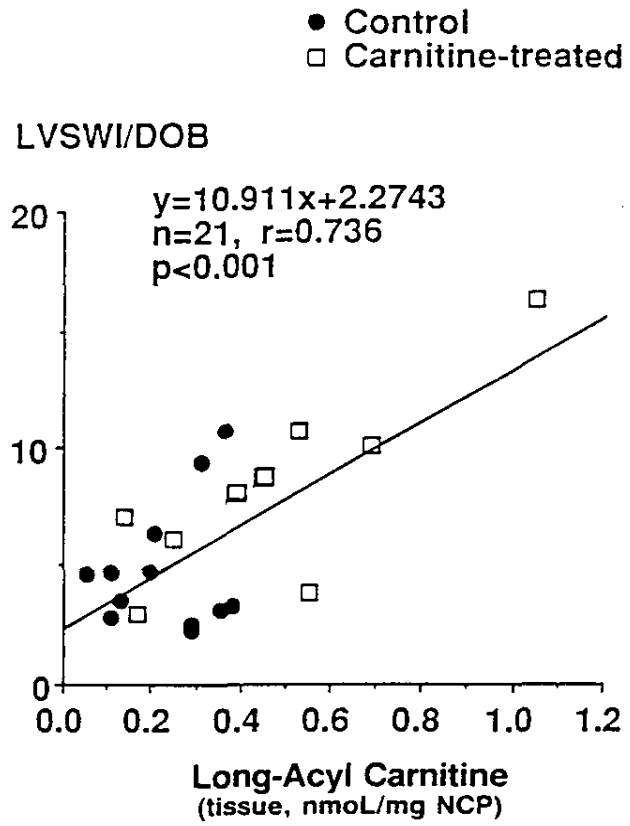

FIG. 2. Relationship between tissue long-chain acyl carnitine content and LVSWI/DOB ratio. Significant correlation is found in patients of both groups.

Discussion

This study clearly demonstrates that pretreatment with oral car for seven days did not increase both plasma concentration of free carnitine and total and free carnitine in the left ventricular papillary muscle, although long-chain acyl carnitine tissue content was significantly increased as compared with the nontreated hearts. In the car-treated patients, CI was significantly better, being attained with significantly lower doses of dobutamine in early reperfusion following valvular surgery. Likewise, LVSWI/DOB was significantly higher in the patients treated with car.

Cardiac performance in early reperfusion is determined by various perioperative factors: circulating blood volume; preload and afterload; status of preoperative ventricular function; status of valvular function in which the involved valve is repaired, replaced, or left untouched; neurohumoral condition; and status of myocardial contractility. The latter is closely related to preexisting myocarditis and quality of intraoperative myocardial protection. This study included patients with multivalvular lesions; therefore, it might be difficult to interpret our hemodynamic result in early reperfusion in relation to the complex valvular effect on myocardial function and pretreatment with carnitine. However, preoperative functional class and intraoperative factors such as duration of cardioplegia and cardiopulmonary bypass were comparable between the two groups.

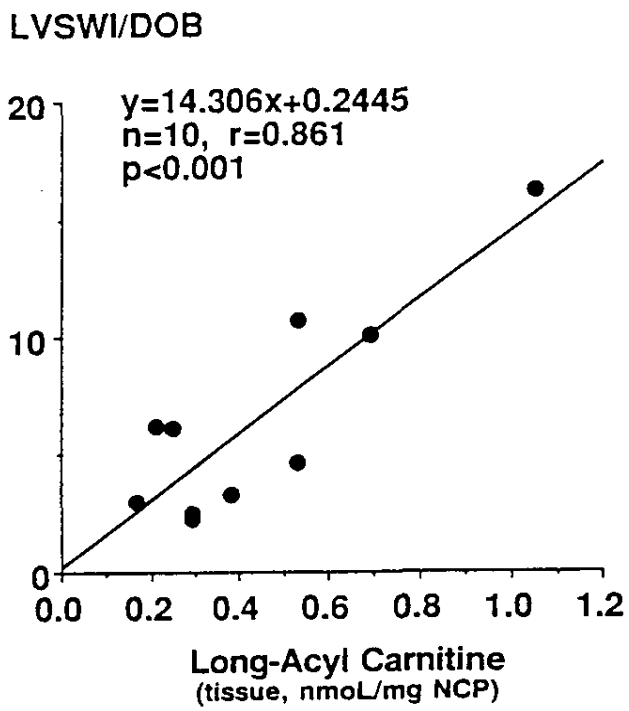

FIG. 3. Relationship between tissue long-chain acyl carnitine content and LVSWI/DOB ratio in early reperfusion in patients who underwent MVR or MVR-TAP. Significant correlation is found between the two variables.

With respect to plasma concentration or tissue content of carnitine, the duration of oral administration of car in this study was not prolonged enough to saturate plasma and tissues, for previous reports have suggested that it would take three to four weeks following oral administration to reach a plateau of carnitine content.[27] Also, we should take into account that selection criteria of patients were extremely important in this study, since carnitine content is influenced by cardiac conditions associated with congestive heart failure,[3,8,9] ischemia,[10] and mechanically overloaded settings,[11,12] in which tissue carnitine content becomes depleted. We have, therefore, carefully excluded such patients from the present study. However, differences in hemodynamic stresses or mechanically overloaded conditions that might have existed preoperatively and been attributed to a variety of valvular lesions appear to be incriminated in relation to tissue carnitine content. Pretreatment with car in our study provided the myocardium with significant increase in long-chain acyl carnitine as opposed to controls. These results suggest that Car was incorporated into the tissue unquestionably by some metabolic process.

With regard to tissue content of carnitine, the left ventricular free wall seems to provide the best tissue for sampling purposes. We chose the base of the papillary muscle of the left ventricle excised at the time of MVR without any increased clinical risk; in addition, the specimen was suitable for evaluating pathologic conditions of the free wall of the left ventricle.[28] Furthermore, there was no significant difference in tissue protein levels between the two groups; our data showing carnitine content ranging between 2 and 14 n moL/mg NCP were consistent with a previous report.[3] The papillary muscle was excised and frozen approximately ten minutes after global ischemia. Our data values in connection with tissue carnitine may, however, be lower than those in noncompromised

conditions, for Shug and colleagues[10] and Liedtke and Nellis[29] have documented that cardiac tissue content of free carnitine thirty minutes after ischemia is reduced by 50%. Neither total nor long-chain acyl carnitine tissue content in our study correlated with duration of cardioplegia. This result was expected because tissue was sampled at the almost identical phase of early cardioplegia rather than taken at the end of cardioplegia.

Ischemic depletion of carnitine results in the toxic accumulation of esterified fatty acids intermediates. These metabolites inhibit crucial intracellular enzymes such as Na, K-ATP ase, K phosphatase,[15,30-32] and adenine nucleotide transferase.[33,34] High levels of carnitine esters of long-chain fatty acids accumulated in myocardial tissue during ischemia are also attributed to myocardial metabolic disturbances.[13,14] There is evidence that long-chain acyl carnitine is responsible for reducing viability of myocardium and cellular membranes.[15,16] However, no correlation was found between the recovery of cardiac function during reperfusion and the accumulation of long-chain acyl carnitine during ischemia.[17,18] Furthermore, Lamers et al[35] have reported that intracellular increase in long-chain acyl carnitine is not critical to sarcolemmal sodium and calcium permeability or to sacoplasmic reticulum calcium pumping activity. This study aimed to define the effect of pretreatment with carnitine on pharmacokinetic activity in relation to hemodynamic myocardial response in cardiac valvular surgery. Further investigation needs to be done in coronary revascularization in order to elucidate the efficacy of pretreatment with carnitine.

Correlation between tissue long-chain acyl carnitine and LVSWI/DOB in early reperfusion was evaluated in patients who had various degrees of valvular lesion. The complexity of valvular lesion and multivalvular surgery may reflect differences in hemodynamic stress. Therefore, we examined a rather small number of patients who had mitral valve disease treated with MVR or MVR-TAP. Significant correlation was found between tissue long-chain acyl carnitine and LVSWI/DOB in early reperfusion in patients following both complex and almost isolated MVR. Our results show that tissue long-chain acyl carnitine correlates positively with LVSWI/DOB and suggest that long-chain acyl carnitine is at least significant for maintaining better cardiac function. Since carnitine has no inotropic action on myocardium,[36] this consequence may suggest that a carnitine-mediated metabolic process enhances energy production in the mitochondria.

Since cardiac tissue cannot synthesize carnitine,[19] though it can take up carnitine from the blood pool, several reports[21-23] have supported the argument that exogenous provision of carnitine is efficacious in ameliorating the manifestations of myocardial ischemia. Shug et al[33] reported that carnitine, added to mitochondrial preparations, prevented long-chain acyl-CoA from suppressing adenine nucleotide translocase. Folts et al[37] reported that carnitine was effective in preventing adenine nucleotide depletion resulting from ischemia. On the other hand, Silverman et al[20] reported that carnitine has a dissociative and dose-dependent beneficial effect on myocardial ischemia by promoting preservation of ventricular compliance and improved maintenance of contractile function without preventing adenine nucleotide depletion.

Clinical benefits reported with use of carnitine are that it can increase the tolerance to stress[24] and reduce the ischemic changes observed on ECG.[27,38]

Conclusion

Oral administration of L-carnitine chloride, 1800 mg/day for seven days, is not sufficient to increase plasma free carnitine and tissue free and total carnitine contents, although long-chain acyl carnitine increased significantly with this method. The carnitine-treated patients exhibited a significantly better cardiac index with significantly less dobutamine requirements in the early reperfusion following valvular surgery. These results suggest that pretreatment with L-carnitine chloride is efficacious in maintaining a better hemodynamic state in patients subjected to valvular surgery and that long-chain acyl carnitine appears to have significance in the function of carnitine-mitochondrial biochemical reactions for energy production and utilization.

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