MYOCARDIAL FREE CARNITINE AND FATTY ACYLCARNITINE LEVELS IN PATIENTS WITH CHRONIC HEART FAILURE

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To study the tissue carnitine level in patients with chronic heart failure, we obtained biopsy specimens of the left ventricular papillary muscle from 8 patients with mitral valve disease undergoing valve replacement surgery. As a control group autopsy specimens from 7 patients without heart disease were obtained within 4 hours of death.

The free carnitine level in the heart was significantly lower in patients with chronic heart failure than in the control group (412 ± 142 nmol/g wet tissue vs 769 ± 267; $p < 0.01$, mean $\pm$ SD). The long-chain acylcarnitine level was significantly higher in chronic heart failure than in the control group (532 ± 169 nmol/g wet tissue vs 317 ± 72; $p < 0.01$). The total carnitine level in chronic heart failure was similar to that in the control group (1321 ± 170 nmol/g wet tissue vs 1315 ± 377).

These results show that in failing myocardium the fatty acid metabolism may be impaired, and administration of carnitine may be worth trying to treat chronic heart failure.

FREE fatty acids are major metabolic fuel for the normal heart[1-3] but at high levels they have been shown to impair myocardial cellular function in the ischemic heart[4-6] Myocardial fatty acid metabolism depends on carnitine as an essential cofactor, because carnitine is indispensable for long-chain fatty acids to penetrate the inner mitochondrial membrane and to be transported to the site of oxidation in the mitochondria[7,8]

While the tissue level of carnitine remains constant in the normal heart, it can be altered under various pathological conditions. In ischemic myocardium in experimental animals a reduction in free carnitine and an accumulation of long-chain acyl CoA and long-chain acylcarnitine have been demonstrated. This is thought to exaggerate myocardial ischemic damage[9-13]

Replacement of carnitine has been reported to alleviate the accumulation of long-chain acyl CoA and long-chain acylcarnitine, and has resulted in the improvement of energy metabolism and mechanical performance in the ischemic heart[11-13]

A reduced level of carnitine has also been reported in the hypertrophied rabbit heart[14,15] the cardiomyopathic hamster heart[16-18] and in the failing guinea pig heart[19] These have shown that myocardial usage of lipid for energy production may be impaired in the chronically failing heart. Carnitine treatment of patients with cardiomyopathy, particularly those with systemic carnitine deficiency, has been shown to improve mechanical function[20,21]

The purpose of this study was to investigate changes in human myocardial levels of free carnitine and its derivatives in patients

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TABLE I BACKGROUND OF PATIENTS

Case	Age	Sex	Name of disease	Chest X-P CTR (%)	ECG
Patients with heart failure					
1	52	F	MS	63	Atrial fibrillation
2	57	F	MS	58	Atrial fibrillation, ST-T abnormality
3	58	M	MS+TR	55	Atrial fibrillation, ST-T abnormality
4	39	F	MR	56	ST-T abnormality, LVH
5	52	F	MR	58	ST-T abnormality, LVH
6	50	F	MSR+TR	68	Atrial fibrillation, ST-T abnormality RVH
7	47	M	MSR+TR	61	Atrial fibrillation, ST-T abnormality RVH
8	60	F	MSR+AR	82	Atrial fibrillation, ST-T abnormality
Patients without heart disease					
1	42	M	Aplastic anemia	46	normal
2	70	M	Malignant lymphoma	50	normal
3	56	F	Malignant lymphoma	48	normal
4	71	F	Myelocytic leukemia	47	normal
5	54	M	Lung cancer	44	normal
6	70	M	Renal cancer	48	normal
7	52	F	Thyroid cancer	49	normal

with chronic heart failure, using biopsy specimens of left ventricular papillary muscle from patients undergoing mitral valve replacement surgery.

MATERIALS AND METHODS

1. *Postmortem changes in levels of carnitine and its derivatives*

Autopsy specimens were used as a control group, so postmortem changes in the tissue levels of carnitine and its derivatives were studied. Seven mongrel dogs weighing 10—20 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg), and left thoracotomized. To measure carnitine and its derivatives, specimens of the left ventricular muscle were taken every 2 hours for 10 hours after death from animals left at room temperature.

2. *Levels of carnitine and its derivatives in patients*

Eight biopsy specimens of left ventricular papillary muscle were obtained from patients (average age 51.9±6.9 years) undergoing valve replacement surgery because of chronic heart failure due to mitral valve disease. As a control group, 7 autopsy specimens were obtained from age-matched patients (average age 59.3±11.2 years) without heart disease within 4 hours of their death (Table I).

3. *Measurement of tissue carnitine and its derivatives*

Tissue samples were immediately frozen in liquid nitrogen and stored at −80℃.

The assay methods for free carnitine and its acyl derivatives are: One gram of the frozen tissue was homogenized in 5 ml of cold 600 mM perchloric acid with a polytron homogenizer, then centrifuged at 4000 rpm for 20 min at 4℃. The supernatant, 3.5 ml, was adjusted to pH 6.5 to 7.0 with 1N KOH and kept in ice water for 1 hour. After additional centrifugation at 4000 rpm for 20 min at 4℃, the supernatant was used to determine free carnitine. Short-chain (C 3-C 10) and long-chain (C 12 and upward) acylcarnitine were assayed as free carnitine after alkaline hydrolysis at pH 13 for 1 hour at 40℃ and 2 hours at 55℃[22] Free carnitine was determined enzymatically using carnitine acetyl transferase by the method of Marquis and Fritz[23] The basic reaction mixture con-

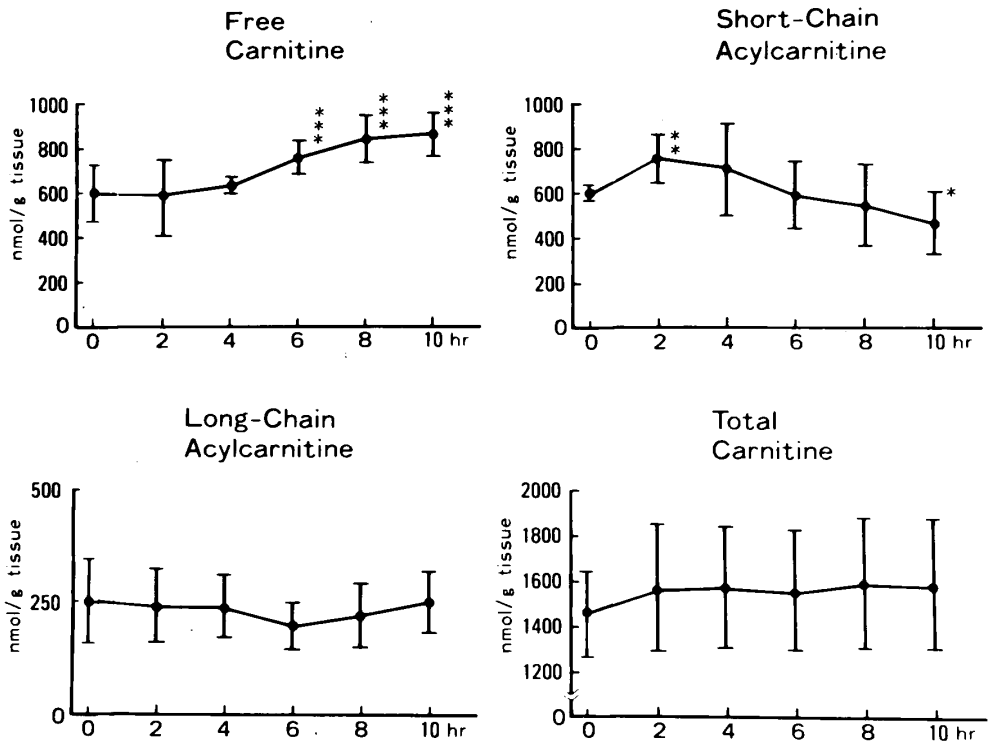

Fig.1. Postmortem changes in carnitine and its derivatives of dog hearts (n=7). Values are expressed as per gram wet tissue weight and presented as the mean±SD. The significance of the changes is as follows: at death vs every two hours after death; *** $p<0.001$, ** $p<0.01$, * $p<0.05$

TABLE II TISSUE LEVELS OF CARNITINE AND ITS DERIVATIVES IN PATIENTS

Patients	*Free carnitine (nmol/g)*	*Short-chain acycarnitine (nmol/g)*	*Long-chain aclycarnitine (nmol/g)*	*Total carnitine (nmol/g)*
With heart failure (n=8)	*412±142***	*377±163*	*532±169***	*1321±170*
Without heart failure (n=7)	*769±267*	*228±144*	*317±72*	*1315±377*

Values are expressed as per gram wet tissue weight and presented as the mean±SD.
*The significance of the changes is presented as follows: with heart failure vs without heart failure, ** $p<0.01$*

tained, in a volume of 1.0 ml, 200 μmol of Tris-HCI buffer at pH 7.8, 0.2 μmol of 5,5-dithiobis-2-nitrobenzoic acid, 0.3 μmol of acetyl CoA and 2.5 μmol of EDTA. L-carnitine standards (10 to 80 nM) were included with each assay. Reactions were initiated by adding 0.1 ml of the basic reaction mixture to a test tube containing 1.0 ml of the sample. Before and 5 min after adding 10 μl carnitine acetyl transferase solution (1 mg protein/ml, pH 7.5), the absorption of 5,5-dithiobis-2-nitrobenzoic acid with sulfhydryl was measured at 412 nm and the carnitine level was calculated from the absorbency changes before and after adding the carnitine acetyl transferase.

4. *Statistical analysis*

The levels of the metabolites were expressed for wet tissue weight as the

mean ± SD. Statistical analysis was made using the paired or non-paired Student's t test. P values of less than 0.05 were considered statistically significant.

RESULTS

1. *Postmortem changes in carnitine and its derivatives of dog hearts*

Figure 1 shows the postmortem changes in the levels of free carnitine and its derivatives of dog hearts during 10 hours after death. The free carnitine levels showed no significant changes from death to 4 hours later. However, at 6, 8 and 10 hours after death the levels were significantly higher than at death. The short-chain acylcarnitine levels were significantly higher 2 hours after death, and significantly lower 10 hours after death. Long-chain acylcarnitine and total carnitine levels showed no significant changes at any time during the 10 hours. From this we decided that human autopsy specimens from the control group had to be obtained within 4 hours of death.

2. *Levels of tissue carnitine and its derivatives in patients*

Table II shows the myocardial levels of free carnitine and its derivatives in patients with and without chronic heart failure. The free carnitine level was significantly lower in patients with chronic heart failure than in patients without heart desease (412 ± 142 nmol/g wet tissue vs 769 ± 267, $p < 0.01$). The short-chain acylcarnitine level was higher in patients with chronic heart failure than in patients without heart disease, but the difference was not significant (377 ± 163 nmol/g wet tissue vs 228 ± 144). The long-chain acylcarnitine level was significantly higher in patients with chronic heart failure than in patients without heart disease (532 ± 169 nmol/g wet tissue vs 317 ± 72, $p < 0.01$). But there was no significant difference in the total carnitine levels between patients with and without heart failure (1321 ± 170 nmol/g wet tissue vs 1315 ± 377).

DISCUSSION

Evaluation of the method and results

As a control we used postmortem specimens from autopsy patients without heart disease, therefore the postmortem changes in the tissue levels of carnitine and its derivatives had to be studied. The free carnitine, long-chain acylcarnitine and total carnitine levels did not change significantly up to 4 hours after death. So the results of the control group using autopsy specimens within 4 hours of death (average 3.4 h) can be admitted. But the short-chain acylcarnitine level increased significantly at 2 hours after deah. Its use as a control may not be appropriate.

Myocardial levels of carnitine and its derivatives in patients

Carnitine is an essential cofactor for activated long-chain acyl groups to be transported from the cytoplasm to the intramitochondrial sites of fatty acid oxidation[7,8,24]

It has been demonstrated in ischemic myocardium that the tissue levels of long-chain acyl CoA and long-chain acylcarnitine increased, but free carnitine decreased[4,9,10,13] The decline in free carnitine results in the accumulation of long-chain acyl CoA and long-chain acylcarnitine. High levels of long-chain acyl CoA inhibit several fatty acid metabolic enzymes such as adenine nucleotide translocase,[24,25] acyl CoA synthetase[26] and carnitine acyl transferase.[27] Besides, it inhibits myocardial mitochondrial oxygen uptake,[28] and interferes with membrane Na^+, K^+-ATPase. During whole heart ischemia, the accumulation of long-chain acylcarnitine has been found to be nearly three times as much as that of long-chain acyl CoA.[13,30] High levels of long-chain acylcarnitine inhibit carnitine acylcarnitine translocase.[8] It has also been reported that long-chain acylcarnitine is a more powerful inhibitor of cardiac plasma membrane Na^+, K^+-ATPase than long-chain acyl CoA, and is an inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase,[29,31,32] probably by its detergent-like action.[32] These reports indicate that the accumulation of long-chain acylcarnitine, like long-chain acyl CoA, may be implicated in the cellular damage in ischemic myocardium, result in impaired myocardial energy production and utilization. The infusion of exogenous L-carnitine protects the reduction of free carnitine as well as the accumulation of long-chain acyl CoA and long-chain acylcarnitine.[9,11,13] It also pre-

vents the decline of ischemic tissue levels of ATP and creatine phosphate, and of adenine nucleotide translocase activity[9,11–13] So the administration of L-carnitine can improve energy metabolism and mechanical performance.

Other reports have described how carnitine palmitoyltransferase I inhibitors, such as TDGA (2-tetraglycidic acid), POCA (sodium 2 [5- (4-chlorophenyl) -pentyl] oxirane-2-carboxylate), and oxfenicine, apparently protect the ischemic myocardium, perhaps due to a decrease in myocardial long-chain acylcarnitine[33–35]

Reduced carnitine has been reported in hypertrophic rat hearts[14,15] and in hypertrophied rabbit hearts associated with the increase in lipid stores[15] In the failing hearts of guinea pigs, long-chain fatty acid oxidation was characterized by a reduced level of free carnitine, a reduced rate of palmitate oxidation, and an increased rate of palmitate incorporation into triglyceride and lecithin[19] Exogenous carnitine restored the defective palmitate metabolism[19] Previous reports have shown depressed fatty acid oxidation in cardiomyopathic hamster hearts[16] and decreased carnitine[17,18] which was not a secondary effect of an advanced stage of the cardiomyopathy[17] Administration of L-carnitine improved fatty acid oxidation[16] and decreased the percentage of the area of necrosis, fibrosis and calcification in cardiomyopathic hamster hearts[18] Also in human cardiomyopathic patients with systemic carnitine deficiency, L-carnitine administration has been shown to be a successful treatment[20,21] In the present study, we found decreased myocardial free carnitine and increased long-chain acylcarnitine in patients with chronic heart failure. The mechanism of these changes in the myocardial carnitine and its derivatives in patients with chronic heart failure is not yet clear. Pierpont et al. also have measured left ventricular myocardial carnitine in patients undergoing orthotopic cardiac transplantation[36] Their study group included patients with idiopathic dilated cardiomyopathy,coronary artery disease, myocarditis and rheumatic heart disease. They have reported that left ventricular total carnitine for patients with severe chronic heart failure was not significantly different from control. This result is consistent with our result. However, Pierpont et al did not measure free carnitine and long-chain acylcarnitine. We found that decreased myocardial free carnitine and increased long-chain acylcarnitine in patients with chronic heart failure.

The status of chronic heart failure cannot be identified with acute ischemic heart, hypertrophic heart or cardiomyopathic heart. However, it has been considered that congestive heart failure is associated with hypoxia at the cellular levels due to impaired oxygen diffusion into the hypertrophied cells[37,38] The reduction in free carnitine and the accumulation of long-chain acylcarnitine in patients with chronic heart failure may impair fatty acid metabolism and may aggravate the chronically failing heart itself. As in the cases of ischemic heart, administration of L-carnitine would be worth trying to treat chronic heart failure.

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