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Myocardial carnitine and carnitine palmitoyltransferase deficiencies in patients with severe heart failure

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Abstract

We studied myocardial tissue from 25 cardiac transplant recipients, who had end-stage congestive heart failure (CHF), and from 21 control donor hearts. Concentrations of total carnitine (TC), free carnitine (FC), short-chain acylcarnitines, long-chain acylcarnitines (LCAC) as well as carnitine palmitoyltransferase (CPT) activities were measured in myocardial tissue homogenates and referred to the concentration of non-collagen protein. Compared to controls, the concentrations of TC and FC as well as total CPT activities were significantly lower in patients. LCAC levels and the LCAC to FC ratio values were significantly greater in patients than in controls. While the malonyl-CoA sensitive fraction of CPT, which represents CPT I activity, was similar in patients and controls, the residual CPT activity after inhibition by malonyl-CoA, representing CPT II activity, was significantly reduced in patients compared to controls. Moreover, the activity of CPT in the presence of Triton X-100, which also represents the activity of CPT II, was significantly lower in patients than in controls. Malonyl-CoA concentrations required for half-maximal inhibition of CPT activity were significantly greater in patients than in controls. There was a linear relationship between ejection fraction (EF) values and concentrations of TC, FC, or total CPT activities. Values for LCAC and the LCAC to FC ratio were inversely related to EF values. We conclude that failing heart shows decreased total CPT and CPT II activities and carnitine deficiency that may be related to ventricle function.

Keywords: Carnitine; Carnitine palmitoyltransferase; Myocardium; Heart failure

1. Introduction

Carnitine is synthesized chiefly by the liver, and to a lesser degree, by the kidney from the amino acid precursors lysine and methionine [1]. In plasma as well as in the other tissues, total carnitine (TC) consists of three main fractions: free carnitine (FC), short-chain acylcarnitines (SCAC), and long-chain acylcarnitines (LCAC). Myocardial carnitine levels

Abbreviations: CPT, carnitine palmitoyltransferase; TC, total carnitine; FC, free carnitine; SCAC, short-chain acylcarnitines; LCAC, long-chain acylcarnitines; LCFA, long-chain fatty acids; CHF, congestive heart failure; NCP, noncollagen protein

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greatly exceed plasma levels as the heart can extract carnitine against a sixty-fold concentration gradient. Myocardial uptake of carnitine occurs slowly by means of a specific sodium-dependent carnitine transporter [2,3]. The mammalian heart primarily meets its requirements for energy through the oxidation of long-chain fatty acids (LCFA), where carnitine plays a key role as a carrier [4]. An important step in the oxidation of fatty acids is the translocation of long-chain acyl-CoA into the inner mitochondrial space. This is achieved by a carnitine-mediated translocation involving carnitine palmitoyltransferase (CPT) I, carnitine acylcarnitine translocase and CPT II [5]. While CPT I exists as two distinct isoforms each with unique physiologic and kinetic properties, CPT II is a widespread protein [6,7]. The liver contains predominantly the L-isoform which has a lower K_m of about 30 mM. This K_m corresponds to the relatively low carnitine content found in the liver [6,7]. In skeletal muscle, the main form is M-CPT showing a K_m as high as 500 mM, which corresponds to its greater carnitine content [6,7]. The heart contains both L- and M-CPT isoenzymes, and has an intermediate K_m, but the tissue levels of carnitine are even higher than those found in skeletal muscle [6,7]. These greater levels of carnitine in heart may be required for other functions such as the regulation of carbohydrate metabolism [7]. This effect, which is carried out by stimulation of the pyruvate dehydrogenase complex [8], appears to account for partly the beneficial effects of L-carnitine in treating certain cardiac diseases [8,9]. Other important functions of L-carnitine in heart have been extensively reviewed [7,10–12].

Previous studies, both in humans and in animals, have shown that decreased levels of carnitine in myocardium are associated with heart failure [7,10–13]. In this work, we measured the concentrations of carnitine fractions and the activities of CPT I and CPT II in myocardial cells from patients with end-stage congestive cardiac failure (CHF) in order to evaluate whether the carnitine-related mechanisms of transport of LCFA across the mitochondrion are altered in failing heart. Two earlier reports have dealt with carnitine metabolism in myocardial tissue from CHF patients. While the study by Regitz et al. focused solely on carnitine levels [13], the report by Maurer and Zierz [14] only analyzed CPT activities. Our study first documents both carnitine levels and CPT activities in heart tissue from CHF patients

2. Material and methods

2.1. Patients

Myocardial biopsy specimens were obtained from 25 cardiac transplant recipients (20 men and five women, aged 52.5 ± 6.1 years, mean ± S.D.): five had idiopathic dilated cardiomyopathy (DC), 18 had coronary artery disease (CAD), and the remaining two rheumatic heart disease. Hemodynamic data are shown in Table 1.

Twelve patients had New York Heart Association (NYHA) class IV congestive heart failure, and 13 had class III. The average left ventricular ejection fraction (EF), as measured by radionuclide ventriculography, was 20.5 ± 9.5. All patients with DC were shown to have normal coronary arteries or minimal atherosclerosis by coronary angiography or pathologic examination. Global ventricular dilatation was seen in all cases with DC. There was no evidence of systemic hypertension or concomitant cardiac valvular lesions. Patients with heart muscle diseases caused by known infective agents, metabolic alterations, sensitivity or toxic reactions, or specific heart muscle diseases associated with systemic or storage diseases were not included in the study; neither were patients with hypertrophic or restrictive cardiomyopathy. All

Table 1
Clinical and hemodynamic data

Age (years)	52.5 ± 6.1
Sex	20 male, 5 female
Functional class (NYHA)	13 (III), 12 (IV)
Etiology	18 CAD, 5 DC, 2 RHD
Mean evolution time heart failure (months)	55.1 ± 44.2
EF (%)	20.5 ± 9.5
PCP (mmHg)	18.9 ± 9.8
MPAP (mmHg)	28.2 ± 12.1
PVR (Wood Units)	2.4 ± 1.0

Values are mean ± S.D. EF, left ventricular ejection fraction; PCP, mean pulmonary capillary pressure; MPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance; CAD, coronary artery disease; DC, dilated cardiomyopathy; RHD, rheumatic heart disease.

subjects with CAD had severe multivessel disease documented by cardiac catheterization.

All patients, irrespective of the cause of the disease, had CHF and were considered candidates to heart transplantation.

Left ventricular myocardial samples of both transplant recipients (after cross-clamping of the aorta before beginning cardiectomy) and control donor hearts ($n=21$; 16 men and five women, aged 32 ± 16 years) were taken, immediately frozen in liquid nitrogen, and stored for subsequent biochemical studies.

Since the amount of sample available from both controls and patients was variable, especially in controls in whom the quantity of tissue obtained was low, it was not possible to measure CPT activity and carnitine content in each myocardial sample. Carnitine content alone was determined in 10 controls and six patients, both carnitine concentrations and CPT activities in 13 patients, and CPT activities alone in 11 controls and six patients.

2.2. CPT activities and carnitine concentrations

Concentrations of TC, FC, SCAC and LCAC were measured in myocardial tissue homogenates as described [13], and referred to the concentration of noncollagen protein (NCP) [15]. For determining CPT activities [14,16], frozen myocardial biopsies were homogenized with 19 vol of a solution containing 50 mM Tris buffer (pH 7.6), 100 mM KCl, 5 mM $MgSO_4$, and 1 mM EDTA using a hand-driven all-glass homogenizer. Homogenates were used in a final dilution of 1:60 (w/v). The efficiency of homogenization to disrupt the mitochondria was assessed by determining the latency of citrate synthase activity, a marker enzyme of the mitochondrial matrix. The addition of the detergent Triton X-100 (0.5%, v/v) to the homogenate led to an increase in citrate synthase activity of $<10\%$ compared to the enzyme activity without the addition of Triton X-100. This indicated that the mitochondria were nearly completely disrupted during the homogenization. In the disrupted organelles, both CPT I and CPT II, associated with the outer and inner mitochondrial membranes respectively, would have access to substrate in the enzyme assays [14]. CPT activity was measured using the isotope forward assay in the direction of palmitoylcarnitine formation [16], and referred to the concentration of NCP. The reaction mixture contained 100 mM Tris buffer (pH 7.6), 2 mM KCN, 0.1% fatty-acid-free bovine serum albumin, 1 mM dithiothreitol, 0.08 mM palmitoyl-CoA, and 5 mM DL-carnitine (0.04 μCi of DL-[methyl-^{14}C]carnitine/μmol). The linearity regarding time and amount of sample was checked. In some experiments, the inhibitor of CPT I malonyl-CoA was added directly to the assay mixture in varying concentrations ranging from 10 to 500 μM. Activity of CPT I was progressively inhibited by increasing concentrations of malonyl-CoA reaching a plateau between 200 and 400 μM malonyl-CoA. According to these findings, we used 400 μM malonyl-CoA to inhibit the malonyl-CoA sensitive fraction of CPT. The concentration required for half-maximal inhibition (IC_{50}) of CPT activity was determined by double-reciprocal plots of the malonyl-CoA concentration versus the percent inhibition of CPT activity as described [16]. In some experiments, Triton X-100 was added to homogenates to give a final concentration of 0.5% (v/v), the homogenate was kept on ice for 15 min and CPT was then measured [16]. The malonyl-CoA-sensitive fraction would represent the activity of CPT I, while the residual fraction or the Triton X-100-treated activity would indicate the activity of CPT II. To evaluate the statistical significance unpaired *t*-test and regression analysis were used.

3. Results

Levels of TC, FC, SCAC, LCAC, and activities of CPT I and II in patients with CHF and in controls are shown in Table 2.

Compared to controls, the concentrations of TC and FC were significantly lower in patients ($P<0.001$), but SCAC levels remained similar. Conversely, LCAC levels and the LCAC to FC ratio values were significantly greater in patients than in controls ($P<0.001$). The activities of total CPT were significantly lower in patients than in controls ($P<0.05$). While the malonyl-CoA sensitive fraction of CPT, which represents CPT I activity, was similar in patients and controls, the residual CPT activity after inhibition by malonyl-CoA, representing CPT II activity, was significantly reduced in patients com-

pared to controls. Moreover, we measured the activity of CPT in the presence of Triton X-100, which represents the activity of CPT II, in four controls and in six patients. These residual activities were also significantly lower in patients than in controls. Malonyl-CoA concentrations required for half-maximal inhibition of CPT activity (IC_{50}) were significantly greater ($P<0.05$) in patients (6.7 ± 2.1; $n=6$) than in controls (4.1 ± 1.0; $n=4$).

There was a lineal relationship between EF values and concentrations of TC ($r^2=0.614$, $P<0.001$; $y=3.4x-2.7$), FC, or CPT activities (Fig. 1). Values for LCAC ($r^2=0.234$, $P<0.05$; $y=-16.2x+30.5$) and the LCAC to FC ratio were inversely related to EF values (Fig. 1). Moreover, FC concentrations significantly correlated to total CPT activities ($r^2=0.632$, $P<0.01$; $y=0.77x-0.33$), whereas this correlation was inverse when we plotted such activities against the values for the ratio of LCAC to FC ($r^2=0.525$, $P<0.01$; $y=-3.0x+39$). No correlation was found among the rest of clinical and hemodynamic data and carnitine concentrations or CPT activities.

Table 2
Carnitine levels and CPT activities

	Controls	End-stage congestive heart failure
TC	10.8 ± 1.8 (10)	6.5 ± 2.2[a] (19)
FC	10.3 ± 1.8 (10)	5.4 ± 2.2[a] (19)
SCAC	0.40 ± 0.12 (10)	0.42 ± 0.24 (19)
LCAC	0.15 ± 0.09 (10)	0.69 ± 0.27[a] (19)
LCAC/FC	1.6 ± 1.1 (10)	16.5 ± 10.1[a] (19)
Total CPT	9.9 ± 1.5 (11)	7.6 ± 2.3[b] (19)
CPT I (mCoA sens)	5.8 ± 0.8 (11)	5.1 ± 1.9 (19)
CPT II (mCoA insens)	4.2 ± 0.6 (11)	2.5 ± 1.3[b] (19)
CPT II (Triton X-100)	5.6 ± 1.0 (4)	4.1 ± 1.3[b] (6)

Values indicate mean ± S.D. and subjects analyzed (in parentheses). Levels of TC, FC, SCAC and LCAC are expressed as nmol (mg NCP)$^{-1}$. LCAC/FC ratio is expressed as percentage. CPT activities are expressed as nmol min^{-1} (mg NCP)$^{-1}$. TC, total carnitine; FC, free carnitine; SCAC, short-chain acylcarnitines; LCAC, long-chain acylcarnitines; CPT, carnitine palmitoyltransferase; NCP, non-collagen protein; mCoA sens, malonyl-CoA sensitive; mCoA insens, malonyl-CoA insensitive.
[a] $P<0.001$, significant difference vs. controls.
[b] $P<0.05$, significant difference vs controls.

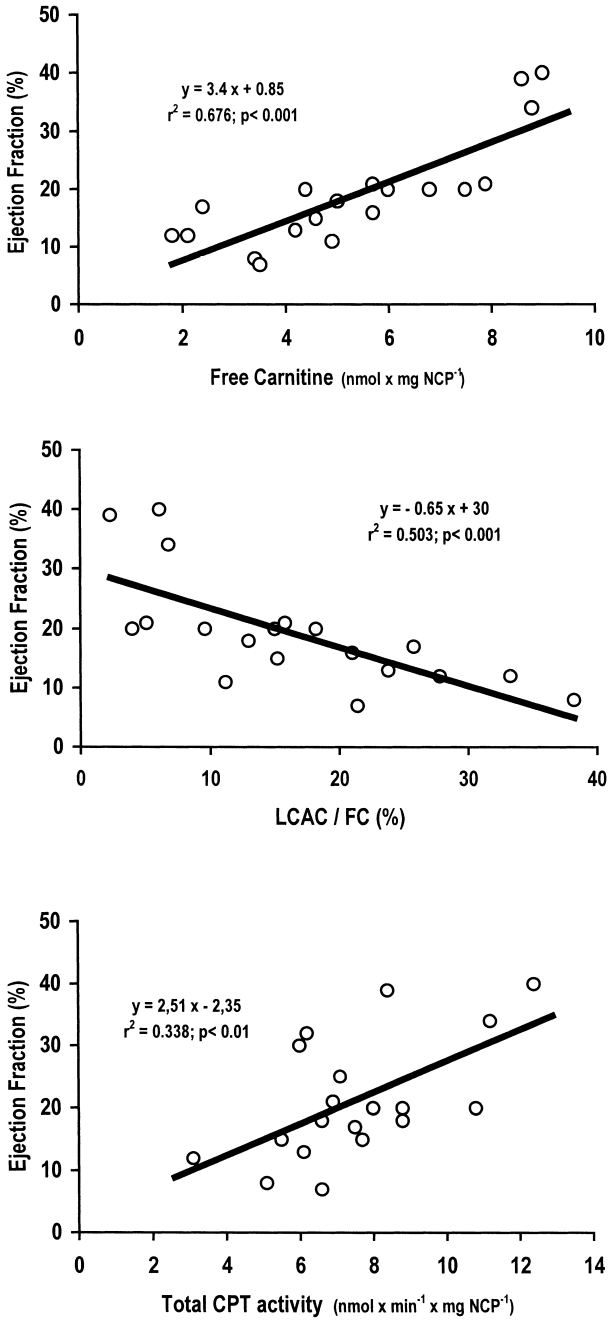

Fig. 1. Correlation between left ventricular ejection fraction (EF) and myocardial content of free carnitine (FC), long-chain acylcarnitines (LCAC) to FC ratio, and total carnitine palmitoyltransferase (CPT) activity. Linear regression equation, determination coefficient and P values are shown.

4. Discussion

In homogenates of frozen tissue containing dis-

rupted mitochondria, the differentiation between CPT I and CPT II is indirectly possible by means of the inhibition of CPT I activity by malonyl-CoA and by the influence of the detergent Triton X-100 on CPT activity. It is known that only CPT I but not CPT II is inhibited by malonyl-CoA and that CPT II is solubilized by Triton X-100 in the enzymatically active form whereas the activity of CPT I is abolished by Triton X-100 [17].

CPT I in heart has been noted to display kinetics intermediate between those of liver and skeletal muscle (in terms of IC_{50} for malonyl-CoA and K_m for carnitine) [18]. This behavior is consistent with the finding that although this tissue expresses M-CPT I predominantly, it also contains sufficient L-CPT I to account for the kinetic data[19,20]. That L-CPT I and M-CPT I are present in the human heart has been confirmed in tissue obtained during surgery or transplant [6]. We found a 50% decrease in carnitine content together with normal levels of CPT I in myocardium from patients with CHF. Given that the heart contains high levels of carnitine and that this tissue has a K_m for carnitine around 200 μM [7], it is not surprising that the marked reduction in carnitine we observed does not result in any substantial variation in CPT I activity. Moreover, recent reports have revealed that the CPT I isoform expression in heart failure shifts from the M- to the L-isoform of CPT I [7,21]. In support of this view, we found a rise in the IC_{50} values for malonyl-CoA of CPT I in CHF patients compared with controls, which is consistent with the possibility of a shifting ratio of M- to L-CPT I in failing heart. Since the L-CPT I has a lower K_m for carnitine, it makes it less likely that a 50% decrease in carnitine levels would affect CPT I activity. In this regard, the group of Paulson, by using the pivalate-induced model of secondary carnitine deficiency, documented that a heart with a 50% decrease in carnitine content maintain normal rates of fatty acid oxidation at a low workload, and exhibits a moderate decrease in fatty acid oxidation at a higher workload [22,23]. They suggested that the heart compensated for the loss of L-carnitine by increasing the expression of the L-isoform of CPT I. Since this isoform has a lower K_m, the heart may be able to maintain fatty acid oxidation. Of interest, a rise in L-CPT I has been documented in the heart of carnitine-deficient juvenile visceral steatosis mice [21]

Our results show that in failing heart there is a reduction in total CPT activity, as well as in the values of residual CPT activities both in the presence of malonyl-CoA and after the addition of Triton X-100. These data unequivocally indicate that the decrease in overall CPT activity in heart from patients with CHF is mainly contributed by a fall in the activity of CPT II. Interestingly, the 'infantile' form of CPT II deficiency, an inherited disorder of long-chain fatty acid oxidation, is a life-threatening disease characterized by cardiomyopathy, myopathy, and hepatopathy, whose severity is related to a difference in levels of residual CPT II activity [24]. The drop in CPT II activities is consistent with the marked increase in myocardial LCAC levels, because CPT II catalyzes the formation of long-chain acyl-CoA from LCAC. In addition, CPT activities were inversely correlated to values for the LCAC to FC ratio. Our enzyme activity results contradict in part those documented by Maurer and Zierz [14], who found similar CPT activities in patients with CHF and in controls with the same method we used in the present study. It is remarkable that in the study conducted by Maurer and Zierz the values for CPT in CHF patients were in the same range as those observed by us. By contrast, our CPT activities in heart controls were substantially greater than those documented by them. The reasons of these discrepancies are unknown, but might be related to preanalytical variations in control samples (e.g., how samples were drawn or collected, time elapsed until freezing, etc) or to differences in the enzyme content of the myocardial tissue analyzed.

The data presented here show that patients with CHF have a great relative proportion of LCAC to FC in myocardium, suggesting that FC concentrations are probably insufficient to cope with the metabolic demands of failing myocardium. Our carnitine levels results are consistent with those by Regitz et al. [13,25], who found a loss of myocardial carnitine and an alteration in the TC to FC ratio in patients with heart failure. Moreover, various animal cardiac models showed depletion of myocardial carnitine resulting in depression of fatty acid oxidation and cardiac function [7,10–12,21]. We suggest that carnitine deficiency in myocardium may result from a leakage of carnitine from the heart and a defect in myocardial uptake via specific carrier protein. In favor of this

hypothesis, plasma carnitine concentrations have shown to be increased in patients with heart failure [13,25]. Also, experimental evidence for an alteration in the carnitine carrier-mediated transport in diseased myocardium is accumulating [26].

We found a significant relationship between levels of TC, FC, or total CPT activities and EF values. Consistently, Regitz et al. [13,25] documented a significant correlation, between myocardial FC and EF values. Moreover, when we plotted EF values against LCAC concentrations or the LCAC to FC ratio values, the relationship proved to be inverse. The correlations observed do not necessarily mean that a decreased carnitine content and CPT activity is responsible for the dysfunction observed in the failing heart. In fact, recent reports suggest that etomoxir, an irreversible CPT I inhibitor, actually improves the symptoms of heart failure [27,28]. Although evidence for LCAC as mediator of impaired contractile function is still lacking, there is experimental evidence for arrhythmogenicity of increased LCAC [29]. In this regard, Bonet et al. [30] suggested that the accumulation of LCAC may be responsible for arrhythmias in patients with inborn errors of fatty acid oxidation. Our results support the hypothesis that heart carnitine system is related to ventricular function. Yet, all data presented here must be interpreted with caution, because a correlation between EF and either CPT activities or carnitine levels is not direct proof that an alteration in the carnitine-related supply of LCFA to mitochondria is contributing to the decrease in EF in these patients. Moreover, other subcellular organelles, in particular microsomes or peroxisomes,also contain CPT-like enzyme activities that may contribute in some degree to the findings of the present study [6].

Recently, we documented a marked increase in the activities of respiratory chain enzymes in myocardium from patients with heart failure [31]. Although a decrease in both myocardial CPT activities and carnitine levels is apparently unrelated to an increase in mitochondrial oxidative phosphorylation, it is tempting to hypothesize some degree of relationship between both findings. We can speculate that a reduction in the LCFA availability to myocardial cell mitochondria might trigger an increase in the capacity of the mitochondrial respiratory chain in an attempt to compensate for the shortage in energy. In fact, Wang et al. [32] recently demonstrated that CPT I is activated upon ATP depletion, suggesting that phosphorylation may modulate the activity of the L-CPT I isoform.

The data in this study confirm previous reports by Regitz et al. [13,25] showing that myocardial carnitine levels are decreased in patients with heart failure and that there is a significant correlation between myocardial carnitine concentrations and EF in these patients. These authors did not measure CPT activities in heart. The novel aspects documented here are the observations that myocardial CPT activities are decreased in heart failure patients compared to control hearts and that EF in these patients are related to CPT activities.

Recently, two reviews drew attention to the potential therapeutical applications of carnitine and its derivatives in heart disease [11,12]. They highlighted that most published data are favorable, but clinical trials are lacking, and that these substances are virtually devoid of significant side effects. Further work will help to clarify the potential beneficial effects of these substances in heart failure.

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