BIOCHEMICAL MEDICINE 32, 199–206 (1984)

Plasma Carnitine Concentrations in Cardiomyopathy Patients[1]

MARJORIE E. TRIPP*[2] AND AUSTIN L. SHUG†

*Department of Pediatrics, The University of Chicago, 950 East 59th Street, Chicago, Illinois 60637, and †Department of Neurology, University of Wisconsin, Middleton Veterans Hospital, Madison, Wisconsin 53706

Received August 1, 1983

Carnitine is an essential cofactor for the β-oxidation of free fatty acids, the predominant myocardial substrate (1). In the absence of carnitine, β-oxidation ceases, glycogen is depleted, lipid accumulates, and organ dysfunction results (2). Both hypertrophic and congestive cardiomyopathies have been associated with carnitine deficiency. In most cases, L-carnitine replacement normalizes cardiac function and morphology (3,4). Conversely, elevated plasma and tissue carnitine concentrations occur in avian cardiomyopathies such as turkey round cell disease and furazolidone toxicity (5,6). To determine the incidence of abnormal plasma carnitine concentrations in human cardiomyopathy, we assayed plasma in children and adults with cardiomyopathy.

METHODS

Plasma samples were obtained from 25 cardiomyopathy patients, ranging in age from 4 months to 54 years. Sixteen were female, 17 were white, and eight were black. Twenty patients had dilated, five had hypertrophic cardiomyopathy. In five the disease was familial. Patients with muscular dystrophy, alcoholism, culture or titer documented myocarditis were excluded. Six subsequently died from their heart disease.

Plasma samples were frozen until assay, then incubated with carnitine acetyltransferase and [^{14}C]acetyl-CoA. Unreacted labeled acetyl-CoA was

[1] Supported in part by the University of Wisconsin Graduate School Research Fund and by USPHS Grant RR00305, Clinical Research Center, The University of Chicago. L-carnitine was provided by Sigma-Tau, Inc., Holmdel, N.J.

[2] To whom correspondence and requests for reprints should be addressed at The University of Chicago, 950 East 59th Street, Box 132, Chicago, Ill. 60637.

0006-2944/84 $3.00

removed in an anion exchange chromatography column. Free carnitine concentration was determined by liquid scintillation counting of resultant [^{14}C]acetyl-carnitine. Total carnitine was determined in like manner after prior acid hydrolysis. Esterified carnitine was derived as the difference. Normal total carnitine values for the laboratory are 45 ± 10 μM for females; 50 ± 10 μM for males. Free carnitine normally accounts for 80 ± 10% of the total.

RESULTS

Of 25 patients screened, plasma carnitine values were below normal range in two, within normal in nine, and above normal in 14 patients (Table 1). For the eight males, the mean plasma carnitine was 77.0 ± 20.6 μM (differing from the normal 50 ± 10 at $P < 0.01$). For the 15 females, the mean of 61.5 ± 27.5 differed from the normal at $P < 0.05$. There was no significant difference between plasma carnitine concentrations in hypertrophic and dilated cardiomyopathies (69.6 and 65.7 μM, respectively). There was no correlation of plasma carnitine values with age or left ventricular ejection fraction. Both patients with low plasma carnitine, and five of nine with normal plasma carnitine had secondary cardiomyopathies. Both patients with presumptive but culture and titer negative myocarditis had normal plasma carnitines. Only one of 14 high plasma carnitine patients had a secondary cardiomyopathy (endocardial fibroelastosis associated with coarctation). All six patients who died had idiopathic disease and were in the high carnitine group.

Eight of 25 patients had skeletal muscle studied at either biopsy or autopsy. Skeletal muscle carnitine values were determined in six of these, and were normal in five. The remaining patient 1 described below had the lowest plasma carnities in this study. Muscle biopsy confirmed severe systemic carnitine deficiency (82.1 nmole/g wet wt—NL 2500–5000) with extensive lipid vacuolization. Five other patients had skeletal myopathies. Patients 4 and 8 had mitochondrial myopathies with Kearn–Sayres syndrome and congenital lactic acidosis, respectively. Patient 10 is followed elsewhere with a familial vacuolar myopathy and hypertrophic cardiomyopathy. Muscle carnitine was not measured. Patients 22 and 23 (see below) with high plasma carnitine concentrations had pathologic fiber size variation among type 1 and type 2 fibers.

Myocardial carnitine was determined in three patients with high plasma carnitine values—two at autopsy, one at biopsy. Patients 14 and 23 had normal myocardial values. Patient 25, with the highest plasma carnitine in this series had a myocardial carnitine of only 864 nmole/g wet wt (NL 1200–1800).

In no patients tested were skeletal muscle or myocardial carnitine levels elevated.

TABLE 1

PLASMA CARNITINE CONCENTRATIONS IN 25 CARDIOMYOPATHY PATIENTS

				EF	Total	Free	Esters	Diagnosis
Below normal range								
1.	W F	1.5 years	D	42%	16.2	15.4	0.8	Systemic carnitine deficiency
2.	B F	4 years	D	68%	31.1	22.8	8.3	Biliary atresia; malabsorption vitamins ADE&K deficiency
Normal range								
3.	B M	1 year	D	45%	40.7	30.5	10.2	EFE
4.	W F	0.4 year	D	31%	41.9	38.2	3.7	Chronic atrial tachycardia
5.	W M	1.5 years	D	59%	—	34.5	—	Chronic atrial tachycardia
6.	W F	12 years	H	—	42.1	33.5	8.6	Kearn-Sayres syndrome
7.	W F	1 year	D	—	—	40.3	—	Cx negative myocarditis
8.	W F	17 years	D	25%	47.7	47.1	0.6	Cong. lactic acidosis
9.	B F	17 years	H	62%	49.2	44.7	4.4	NOHCM
10.	W M	3 years	H	71%	51.1	52.4	—	Familial vacuolar myopathy and NOHCM
11.	W F	5 years	D	61%	53.3	35.9	17.4	Resolving cx negative myocarditis
Above normal range								
*12.	W F	1 year	D	—	56.4	33.7	22.7	EFE
13.	B F	6 years	D	—	59.3	55.9	4.4	IDCM
*14.	W F	0.7 year	D	42%	66.8	72.4	—	EFE
15.	W F	1 year	D	—	67.0	—	—	EFE
16.	W F	0.8 year	D	60%	73.2	44.1	29.1	Secondary EFE with coarctation
17.	B M	17 years	D	—	79.3	70.0	9.3	IDCM
18.	W M	45 years	D	—	81.4	75.6	5.8	Familial IDCM
*19.	W F	17 years	D	29%	81.5	69.7	11.8	IDCM; retardation
20.	B M	1.5 years	D	41%	87.3	62.1	25.2	IDCM
21.	B F	1 year	D	48%	89.6	74.5	15.1	Familial IDCM
*22.	W M	54 years	H	60%	95.3	81.7	13.6	Familial NOHCM
*23.	W M	2 years	D	41%	99.3	69.3	30.0	EFE
24.	W F	14 years	H	71%	110.4	107.4	2.9	Familial NOHCM
*25.	B F	32 years	D	32%	118.0	72.5	44.5	Familial IDCM

Note. Abbreviations: EF = ejection fraction; D = dilated; H = hypertrophic; EFE = endocardial fibroelastosis; NOHCM = nonobstructive hypertrophic cardiomyopathy; IDCM = idiopathic dilated cardiomyopathy; * = died.

Representative Case Summaries

Patient 1 (low plasma and tissue carnitine). KC, a 1½-year-old white female presented with a history of grunting respirations at 6 months of age, massive cardiomegaly, and congestive failure at 1 year of age. Cardiac catheterization had confirmed dilated cardiomyopathy without anatomic defect. Response to medical management was poor. The patient developed bizarre dietary habits and was subsisting almost entirely on carbohydrates including Coca-Cola every 1 to 2 hr day and night. There was no muscle weakness; development and tone were normal. Plasma carnitine values on three occasions were below normal (16.2, 20.5, and 8.4 μM). Muscle biopsy confirmed a vacuolar lipid myopathy with a tissue carnitine of 82.1 nmole/g wet wt (NL 2500–5000). L-carnitine replacement was instituted at 100 mg/kg/day. Within 2 weeks there was a marked decrease in cardiac size by chest X-ray and echocardiogram. Left ventricular dimensions and ventricular ejection fraction normalized within 3 months (see Table 2). Repeat muscle biopsy after 1 year of L-carnitine replacement was histologically normal. Skeletal muscle total carnitine was 3103 nmole/g wet wt, free 2892 and esterified 211, all within normal range. Chest X-ray, electrocardiogram, and echocardiogram at 1 year were also normal.

Patient 23 (high plasma, low normal tissue carnitine). JM, a 2½-year-old white male had been noted to have borderline cardiomegaly on chest X-ray at 9 months of age during an admission for viral meningitis. He developed congestive heart failure at 2 years of age. Cardiac catheterization excluded anatomic abnormalities. Gallium scanning was negative for cardiac inflamation, and viral titers were unremarkable. Myocardial function declined despite intensive medical management. Massive hepatosplenomegaly with ascites developed at 30 months of age. Marked wasting of shoulder girdle musculature and a positive Gowers sign were noted at that time. Creatine phosphokinase activity was normal. Plasma carnitine was markedly elevated at 99.3 μM. Muscle biopsy showed a slight decrease in tissue carnitine (2380.9 nmole/g wet wt) with marked fiber size variation in both type 1 and 2 fibers. No lipid vacuoles were seen and mitochondria

TABLE 2
RESPONSE OF PATIENT 1 TO ORAL L-CARNITINE REPLACEMENT BY ECHOCARDIOGRAPHY

	Pre-Rx	1 Week Rx	3 Weeks Rx	2 Months Rx
LV_D	4.2 cm (>90%)	4.0 cm (>90%)	3.9 cm (>90%)	3.6 cm (80%)
LV_S	3.5 cm (>90%)	3.3 cm (>90%)	2.9 cm (>90%)	2.2 cm (80%)
LVEF	31%	44%	59%	77%

Note. LV_D = Left ventricular diastolic dimension. LV_S = Left ventricular systolic dimension. LVEF = Left ventricular ejection fraction.

were normal. At 34 months, anasarca and respiratory distress occurred. A repeat plasma carnitine was 182 μM. When oliguria ensued, the patient was begun on L-carnitine at 100 mg/kg/day and on a tapering 5-day course of steroids. Appetite improved markedly after a single carnitine dose and before the first steroid dose. Despite this a 2-kg weight loss occurred with vigorous diuresis. The patient began ambulating for the first time in several weeks, and was discharged after steroids were stopped.

Several days later he developed otitis media. Pneumonia ensued despite oral antibiotics. He died of asystolic cardiac arrest during that illness. Autopsy demonstrated a massively dilated heart with endocardial fibroelastosis. There were multiple large antemortem thrombi within the left ventricle. The tricuspid valve had disrupted and was freely insufficient. Although there were no inflammatory cells on light microscopy, viral particles were seen in electron microscopy of the myocardium.

DISCUSSION

Carnitine, an essential cofactor for β-oxidation of fats is present in the diet and synthesized endogenously by the liver and kidney. Skeletal and cardiac muscle are highly dependent on free fatty acids as substrate, and require adequate carnitine concentrations for normal function. Neither muscle nor heart are capable of carnitine synthesis. Both depend on a combination of active transport and facilitated diffusion for maintenance of carnitine concentrations (7).

In carnitine deficiency, plasma carnitine may be normal or decreased but transmembrane gradient is maintained or increased. By contrast, cardiomyopathy patients in this study have elevated plasma carnitines without elevated tissue concentrations. Active transport into cells may be slowed in these patients; alternatively carnitine may be leaked into plasma through faulty membranes. Shug and co-workers (8) demonstrated that anoxic and ischemic hearts have a rapid drop in free carnitine with a coincident rise in esterified carnitine. As anoxia or ischemia are prolonged and damage becomes irreversible, total carnitine drops. Shug postulated that carnitine was leaked but these studies were short term and transport was not measured.

Borum *et al.* working with the cardiomyopathic UM-X 7.1 Syrian hamster, noted a decline in myocardial carnitine with progression of disease. This decline exceeded a lesser fall with age from a higher myocardial concentration baseline in control hamsters (9). In humans, hypocarnitinemia has been reported in a variety of organic acidemias and defects in neutral lipid metabolism. Both hypertrophic and congestive cardiomyopathies have been described in such patients (10–12).

Conversely, Pierpont reported marked elevation in both plasma and tissue carnitine concentrations in turkey round cell disease and in fur-

azolidone-induced cardiomyopathy—also in turkeys. The reason for the elevation is unclear. Just as the UM-X 7.1 hamsters began with low baseline tissue carnitine concentrations, round cell turkey hatchlings were found to have elevated plasma carnitines prior to the onset of demonstrable cardiac dysfunction (5).

Human cardiomyopathy is a heterogeneous group of disorders. The variation of plasma carnitines in our patients reflects this heterogeneity. In two patients, plasma carnitine concentrations were low. In patient 1, systemic carnitine deficiency was confirmed at muscle biopsy. As in our previously reported case (3), plasma and tissue carnitine concentrations and myocardial function normalized with L-carnitine replacement. In patient 2, carnitine deficiency accompanied multiple vitamin deficiencies (ADEK). An uncorrectable hemorrhagic disorder due to hepatic failure prevented diagnostic muscle biopsy, and results of L-carnitine replacement were equivocal.

Nine patients had normal plasma concentrations. In most, a definable secondary cardiomyopathy was present. In two, mitochondrial myopathy was diagnosed. In two, uncontrollable chronic atrial tachycardia contributed to cardiac dysfunction. In a fifth patient, familial vacuolar myopathy with hypertrophic cardiomyopathy was diagnosed elsewhere.

Fourteen patients had elevated plasma carnitines. All but one had idiopathic primary cardiomyopathy. Six of the fourteen died during the 2 years of study. Seven patients with high plasma carnitine values had tissue concentrations measured. In none of the seven were these elevated. In patient 25, plasma carnitine was three times normal while the myocardial level was decreased so that the transmembrane gradient was reduced from 30- to fivefold. Myocardial cell necrosis and membrane disruption were not seen at biopsy. In patient 23, an elevated plasma carnitine of 99.3 correlated with a borderline low skeletal muscle carnitine of 2380 (NL 2500–5000). As he deteriorated plasma carnitine rose to 182 μM. On the assumption that the elevated plasma carnitine was due to impaired transport into or increased leakage from cardiac and skeletal myocytes, he was begun on carnitine. He died with pneumonia after 4 weeks of L-carnitine at 100 mg/kg/day. His last plasma carnitine was 333 μM without evident improvement of function. There was no lipid deposition in the heart or skeletal muscle at autopsy.

Plasma carnitine determination in cardiomyopathy serves several purposes. It can establish the diagnosis of systemic carnitine deficiency, whether primary or secondary to related metabolic disorders such as acyl Co-A dehydrogenase deficiency or isovaleric acidemia (11,12). It suggests secondary cardiomyopathy due to arrhythmia or mitochondrial disorders when plasma carnitine concentration is normal. In patients

with elevated plasma carnitine, dysfunction of cytoplasmic membrane is suggested. Plasma carnitine elevation appears to correlate inversely with prognosis. We conclude that plasma carnitine determination may be useful adjunct in the diagnosis of cardiomyopathy.

SUMMARY

Carnitine is an essential cofactor for the β-oxidation of fats. Both hypertrophic and congestive cardiomyopathies have been reported in primary and secondary carnitine deficiency. Conversely in avian cardiomyopathy models abnormally elevated plasma and tissue carnitine concentrations have been described. We measured plasma carnitine concentrations in 25 cardiomyopathy patients. In 14 patients with either hypertrophic or congestive cardiomyopathy plasma carnitine concentrations were abnormally elevated. Patients with secondary cardiomyopathies tended to have normal carnitine values. One patient with systemic carnitine deficiency was diagnosed. Her cardiac function normalized with L-carnitine replacement. Six of 14 patients with high plasma carnitine concentrations died. None of the 10 with low or normal plasma carnitine have died. Plasma carnitine determination may be a useful adjunct in the diagnostic evaluation of idiopathic cardiomyopathy.

REFERENCES

1. Opie, L. H. Role of carnitine in fatty acid metabolism of normal and ischemic myocardium. *Amer. Heart J.* **97**, 375 (1979).
2. Engel, A. G. Possible causes and effects of carnitine deficiency in man, *in* "Carnitine Biosynthesis, Metabolism and Function" (R. A. Frankel and D. McGarry, Eds.), pp. 271–285. Academic Press, New York, 1980.
3. Tripp, M. E., Katcher, M. L., Peters, H. A., *et al.* Systemic carnitine deficiency presenting as familial endocardial fibroelastosis: A treatable cardiomyopathy. *N. Engl. J. Med.* **305**, 385 (1981).
4. Waber, L. J., Valle, D., Neill, C. A., DeMauro, S., and Shug, A. L. Carnitine deficiency presenting as familial cardiomyopathy: A treatable defect in carnitine transport. *J. Pediatr.* **101**, 700 (1982).
5. Pierpont, M. E., Judd, D., Borgwardt, B., Noren, G. R., Staley, M. A., and Einzig, S. Carnitine and acyl carnitines in turkey cardiomyopathy. *Circulation* **66** (Suppl. 2); 110 (1982).
6. Pierpont, M. E., Judd, D., Borgwardt, B., Staley, M. A., Noren, G., and Einzig, S. Comparison of carnitine and acyl carnitines in spontaneous and drug induced turkey cardiomyopathy. *Fed. Proc.* **42**(3), 598 (1983).
7. Neely, J. R., Robishaw, J. D., and Vary, J. C. Control of myocardial levels of Co-A and carnitine. *J. Mol. Cell Cardiol.* **14** (Suppl. 3), 37 (1982).
8. Shug, A. L., Hayes, B., Huth, P. J., *et al.* Changes in carnitine linked metabolism during ischemia, thermal injury and shock. *In* "Carnitine Biosynthesis, Metabolism and Function" (R. A. Frankel and D. McGarry, Eds.), pp. 321–340. Academic Press, New York, 1980.

9. Borum, P. R., Pak, J. H., Law, P. K., and Roclofs, R. I. Altered tissue carnitine levels in animals with hereditary muscle dystrophy. *J. Neurol. Sci.*38, 113 (1978).
10. Allen, R. J., Hausch, D. B. and Wu, H. L. C. Hypocarnitinemia in disorders of organic acid metabolism. *Lancet* 2, 500 (1982).
11. Stanley, C. R., Hale, D. E., *et al.* Systemic carnitine deficiency in isovaleric acidemia. *Pediatr. Res.* 17, 296A (1983).
12. Hale, D. E., Coates, P. M., Stanley, C. R., Cortina, J. A., and Hall, C. L. Long chain acyl co-A dehydrogenase deficiency. *Pediatr. Res.* 17, 290A (1982).