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Muscle and Plasma Carnitine Levels and Urinary Carnitine excretion in multiply injured patients on total Parenteral Nutrition

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ABSTRACT Carnitine is necessary for the transport of long-chain fatty acids across the mitochondrial membrane. Thirteen severely injured patients on total parenteral nutrition were studied during days 2-8 post injury. Initially plasma and skeletal muscle carnitine values were within the range earlier found for normal subjects, whereas the urinary carnitine excretion was markedly increased. On day 4 there was a simultaneous decrease in the carnitine concentration in plasma ($\alpha < 0.01$) and urine ($\alpha < 0.05$) as well as in skeletal muscle tissue ($\alpha < 0.05$ using only the values that could be paired i.e. from eight subjects), whereas no difference was found between day 2 and 8. One explanation of this pattern might be that a redistribution of carnitine occurs to other organs not measured, for example the liver.

In skeletal muscle tissue, statistically significant positive correlations were found between the carnitine level and ATP ($\alpha < 0.01$) and phosphocreatine ($\alpha < 0.02$) as well as between carnitine and glycogen ($\alpha < 0.05$).

INTRODUCTION

Carnitine is essential for the oxidation of long-chain fatty acids by mammalian tissues because it is necessary for the entry of these substrates into the mitochondrial matrix, where the β-oxidation takes place [1]. Other functions for carnitine have been proposed although not yet established. Several investigators have identified acylcarnitine derivatives of oxoanalogs from branched-chain amino acids in animal tissues [2,3], and carnitine has been shown to increase their α-decarboxylation [4,5].

Carnitine is normally derived from the diet and from the endogenous synthesis from lysine and methionine in liver and kidney in human subjects [6]. Skeletal and cardiac muscle tissue, having a high concentration, are therefore dependent on transport via plasma. Solutions given during total parenteral nutrition (TPN) do not contain carnitine [7] and it has been anticipated that carnitine deficiency may develop in patients on TPN [8,9].

Severely injured patients have an increased energy requirement and it has been shown that they preferentially oxidize fat [10] and fat may also be given as part of their nutritional support. We have earlier shown that severely injured patients, both patients with burn and with multiple injuries, have a markedly increased urinary excretion of carnitine during the first week after trauma [7,11]. We have now extended our studies to include determination of plasma and skeletal muscle carnitine levels as well as urinary carnitine excretion in multiply injured patients.

MATERIALS AND METHODS

Subjects and protocol

A total of 13 patients, 9 men and 4 women, weighing 50-85 kg, aged 19-71 years, were studied. All patients had a major multiple injury with fracture of minimum two long bones or corresponding amount of trauma (Table 1).

Initial treatment included therapy for shock using balanced electrolyte solutions, packed red cells and albumin. They were also given analgetics and oxygen and most patients were given mechanical ventilation. Necessary surgical procedures such as homeostasis and stabilization of fractures were carried out early and the patients were in a stable condition in the morning the day after trauma (day 2).

The observation period started on day 2 and lasted for the 7 days through to day 8. Sampling for analysis of carnitine was performed on days 2, 4, and 8, but a few samples were taken for analysis of carnitine in blood, urine and muscle on days, 3, 5, and 7. Muscle biopsies were also taken on days 2, 4 and 8.

The patients received total parenteral nutrition, 45 kcal/kg/d (190 kJ) or about 3200 kcal (13.4 MJ) daily

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Table 1 Clinical data of the patients

Age (years)	Sex	Type of injury	Weight at admission (kg)
50	M	Fracture of tibia, hip, humerus, patella, radius and ulna	70
70	M	Fracture of pelvis, tibia and fibula	72
62	M	Fracture of multiple ribs, scapula, humerus and clavicle	73
33	M	Fracture of multiple ribs, pelvis, ulna and fibula	70
19	M	Fracture of both femurs, tibia, radius and ulna	64
69	M	Fracture of femur, multiple ribs and sternum	85
65	M	Fracture of femur and tibia	72
32	F	Fracture of femur, tibia and clavicle	68
24	M	Fracture of femur and cervical vertebra	70
25	F	Fracture of pelvis, femur and radius	50
38	F	Fracture of femur, tibia and humerus	57
71	F	Fracture of femur, ribs and hand	70
30	M	Fracture of femur and pelvis	64

provided as glucose, fat and amino acids solution as earlier described in detail for the nutritional groups 1–4, i.e. four nutritional regimes with which no statistically significant difference in urinary excretion of total carnitine was found [11]. Electrolytes were given according to need. Trace elements and vitamins were supplied in standard amounts.

The study was approved by the local Ethics Committee.

Biochemical analyses

Blood was collected for carnitine in the morning and daily 24-h urine collections were made. Muscle samples were obtained by a percutaneous needle biopsy technique from the lateral portion of the quadriceps femoris muscle as described by Bergström [12]. The samples were immediately frozen and stored in liquid N_2 until further treatment. The samples were then freeze-dried, extracted with perchloric acid and neutralized with $KHCO_3$. Analyses of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), phosphocreatine, and glycogen were performed as described by Hultman [13].

Carnitine was assayed as by the method of Cederblad and Lindstedt [14] modified as described Cederblad et al [7]. In muscle tissue, carnitine was anlysed in a perchloric extract after alkaline hydrolysis. Blood and urine samples were extracted with a chloroform-methanol mixture and assayed before and after alkaline hydrolysis giving free and total carnitine values. Acylcarnitine refers to the difference between these two values.

Statistics

The non-parametric tests, Wilcoxon's paired and unpaired two sample and Spearman's rank, were used for comparison and correlation calculations, respectively [15].

RESULTS

Total carnitine levels in plasma were normal on day 2, when compared to the reference range 30–72 μmol/l earlier found [14]. In most patients, the concentrations fell on day 4 and returned to the initial levels on day 8. The decrease on day 4 was statistically significant (Table 2). Free and acylcarnitine also decreased significantly from day 2 to day 4 but no differences were found between day 2 and 8. When expressed as an acyl/free carnitine ratio or as percentage free carnitine of total carnitine, no statistically significant change was found between the three observation days. A corresponding pattern was found for the urinary excretion of all carnitine derivatives with the lowest excretion on day 4 (Figure 1). There was no significant difference on day 2 when compared with 8 in any of the carnitine derivatives whereas the low values on day 4 were

Table 2 Plasma carnitine and its derivatives after multiple trauma

	Total μmol/l			Free μmol/l			Acyl μmol/l			Acyl/free ratio			Free (as % of total)		
Day	2	4	8	2	4	8	2	4	8	2	4	8	2	4	8
mean	51.4	37.8	48.8	47.5	35.1	44.1	4.0	2.3	4.8	0.09	0.07	0.11	92	94	90
SEM	4.5	4.8	1.7	4.3	4.6	1.9	0.5	0.4	1.1	0.01	0.01	0.03	1.0	0.8	2.2
n	9	8	9	9	8	9	9	8	9	9	8	9	9	8	9
α*															
Day 2 *vs* 4 (8)		0.01			0.025			< 0.025			ns			ns	
Day 2 *vs* 8 (9)		ns			ns			ns			ns			ns	
Day 4 *vs* (8)		< 0.02			< 0.05			0.1 > α > 0.05			ns			ns	

*Significance level (one-tailed) of Wilcoxon paired two sample test. Number of pairs within brackets.

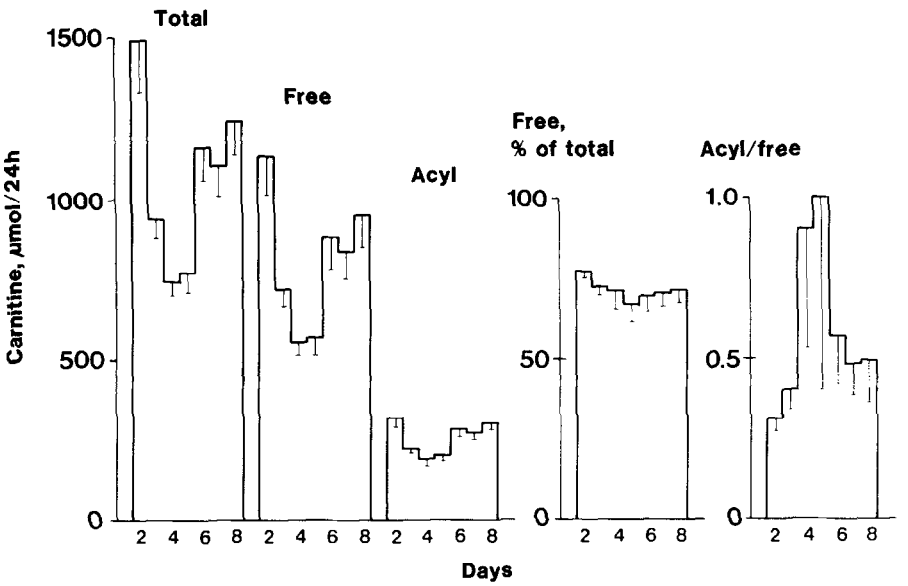

Fig. 1 Urinary excretion of carnitine and its derivatives during days 2–8 post injury.

significantly different from those on day 2 ($\alpha < 0.05$) and on day 8 ($\alpha < 0.05$). A statistically significant negative correlation was found between the ratio of acylcarnitine/free carnitine and the total amount of carnitine excreted in urine (Fig. 2).

Carnitine levels in skeletal muscle were within the range previously found, 6.5–24.1 μmol/g dry weight, median value 17.9 μmol/g dry weight [16]. The lowest value observed (9.7 μmol/g dry weight) was found in a woman in the 30th week of pregnancy. Using all values in a non-paired non-parametric test, no differences were found between the different days of observation. However, when calculations were performed using only the values that could be paired, i.e. from the same individual, the values on

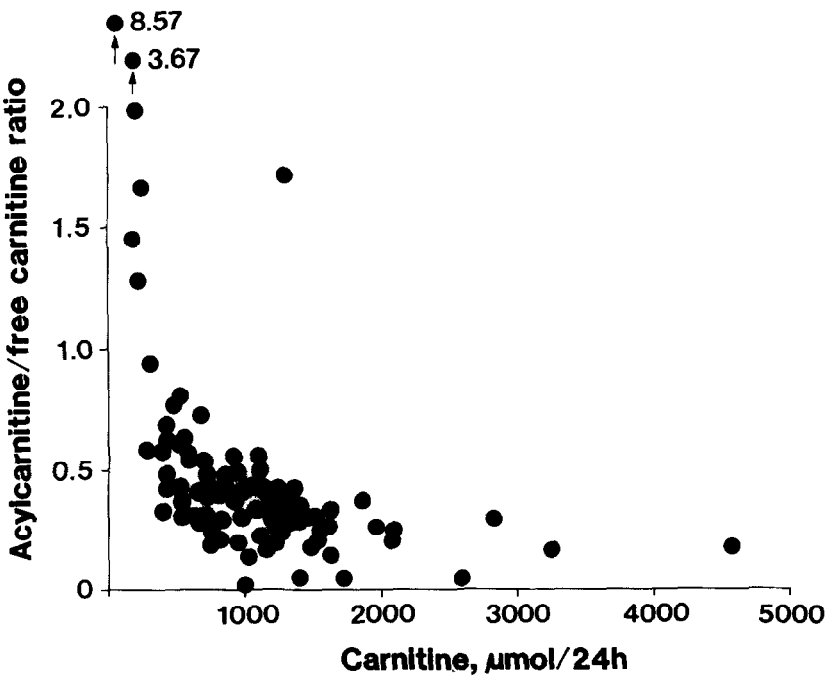

Fig. 2 Relationship between total urinary carnitine and the acylcarnitine/free carnitine ratio. The Spearman's rank coefficient was -0.6762, $\alpha < 0.001$, $n = 90$.

the days 4 and 30 but not 8 were significantly lower than the initial values on day 2 (Table 3). Thus, during the period of total parenteral nutrition the same pattern was again found with a dip on day 4. In spite of the fact that the patients have been fed orally, the carnitine concentration in skeletal muscle tissue on day 30–36 was still significantly lower than that measured initially but the magnitude of decrease was small. No relationship was found between the plasma carnitine and muscle tissue carnitine levels.

Table 3 Total acid soluble carnitine concentration in skeletal muscle tissue after multiple trauma

	Carnitine, μmol/g dry weight			
	Day			
	2	4	8	30–36
mean*	16.4	14.9	15.6	15.4
SEM	1.18	1.25	1.10	2.08
n	12	9	10	10
>**				
Day 2 *vs*		<0.05 (8)	ns (10)	0.05 (7)
Day 4 *vs*			0.0781(6) borderline significance	

* Mean of all values observed.
** Significance level (one-tailed) of Wilcoxon paired two sample test. Number of pairs within brackets.

In skeletal muscle tissue, statistically significant positive correlations were found between the carnitine level and the energy-rich phosphates, ATP and phosphocreatine as well as between carnitine and glycogen. All values obtained were used in the calculations and Spearman's correlation coefficient was between carnitine and ATP 0.4310, $\alpha < 0.01$, n=39, phosphocreatine 0.3996, $\alpha < 0.002$, n=40, and glycogen 0.3144, $\alpha < 0.005$, n=36, respectively. The mean values are given in Table 4.

Table 4 ATP, phosphocreatine and glycogen in skeletal muscle tissue

Component, per g dry weight	mean ± SD
ATP (μmol)	21.0±3.8
Phosphocreatine (μmol)	64.3±8.7
Glycogen (μmol glucosyl units)	339±137

n is between 36 – 40

DISCUSSION

We have earlier reported markedly increased excretion of carnitine during the first week after severe trauma [7,11]. After major thermal injury, regarded as the most severe injury, the mean carnitine excretion initially exceeed the mean value sixfold of normal men on a free diet (420 μmol/24 h), and was still twice that value on day 9 post burn. In severely multiply injured patients on total parenteral nutrition, we earlier observed a mean daily excretion of carnitine of 1,087 μmoles (the corresponding figure in the present study was 1,495 μmoles) during the first 7 days [11]. Normal subjects excreted 100 μmol/24 h when on a low carnitine diet [17], so these patients showed a daily loss of carnitine about 10 times higher than normal. This carnitine excretion also markedly exceeded the magnitude of the sex difference seen in healthy men and women on a free diet (420 μmol/24 h ± 57 (SEM) *vs* 266 ± 29 (SEM). In agreement with our findings in the present study, Harris *et al.*, have also observed a slight decrease in the plasma carnitine level during the first 10 days after burn injury [18].

The new observation is the simultaneous decrease of carnitine levels on day 4 in both plasma and urine as well as in skeletal muscle tissue. The question arises as to where the carnitine has gone. An approximate calculation of the amount of carnitine lost can be made. The decrease observed in skeletal muscle tissue between day 2 and 4 was 1.5 μmol/g dry weight using all values or 2.1 using only the eight patients from which we had biopsies from the two days. Taking the first value and assuming a dry weight content of 22% and a skeletal muscles mass of 30 kg, gives a total amount of carnitine about 10 mmoles. The decrease in plasma carnitine was 13.6 μmol/l, assuming the same concentration in the extracellular water (40% of an assumed body weight of 70 kg) contributes only 0.4 mmoles. Of this amount 2.4 mmoles was recovered in urine, i.e. the sum of the carnitine excretion on day 2 and 3 and approximately 8 mmoles are left to be explained.

There are several possible explanations to account for the missing carnitine. Firstly, carnitine might have been metabolized to β-methylcholine. However, in experiments where labelled L-carnitine was given to rats no metabolite could be detected on chromatography of urine and other tissues [19,20] and there are doubts as to whether carnitine decarboxylation occurs in mammalian systems [21]. Secondly, carnitine might be redistributed to other organs of which the liver must be the most quantitatively important. An increase of the liver carnitine was first observed by McGarry *et al.* [22] during experimental induction of ketogenesis by fasting and alloxan diabetes in rats and a striking correlation was noted between the ketone body production and the carnitine level. The total carnitine content in the livers of fasting and alloxan diabetic rats were increased approximately three- and fivefold, respectively, compared to nonketotic (fed) rats. In sheep liver a sevenfold increase following alloxan treatment has been observed [23]. Brass et Hoppel [24] observed a twofold increase in carnitine liver concentration in the fasting rat. However, when liver total carnitine was expressed per mg DNA no change occurred except at 96 h indicating that the change in carnitine concentration resulted from loss of liver

constituents such as glycogen. In the fasting rat total carnitine in skeletal muscle increased steadily thoughout the fast. In human, Rudman *et al.* [17] reported carnitine concentrations of 2.9 μmol/g wet tissue in postmortem livers from normal subjects, which gives a total liver content of 4.4 mmol assuming a liver weight of 1.5 kg. This would imply that the liver content of carnitine would have to be increased about threefold to account for the 8 mmoles earlier calculated. However, there exists pronounced species differences in carnitine concentrations and biosynthesis in different tissues and observations in experimental animals are not necessarily applicable to human carnitine metabolism. Furthermore, it must be stressed that the calculations made of the amount of carnitine involved are only approximate. However, the simultaneous decrease in plasma, urine and skeletal muscle tissue offers some evidence for a redistribution of carnitine and the amount involved is of the same order of magnitude as the liver carnitine increase seen in experimental animals in a ketotic state. It is well known that the carnitine concentration varies greatly in the same tissue from different species and also in different tissues of a given species. Long *et al.* [25] have recently shown that for any given tissue the carnitine content seems to be set at a level necessary for optimal rates of fatty acid oxidation in the rat and that the rat liver responded to a wide range of carnitine concentrations by varying rates of fatty acid oxidation.

The negative relationship found between the acylcarnitine/free carnitine ratio and total carnitine excretion in urine has also been observed in burned patients and normal subjects [7]. One interpretation might be that during enhanced fatty acid oxidation more acylcarnitine is formed and due to its higher renal clearance it is excreted into urine and at the same time free carnitine is taken up into the tissues, conceivably the liver and is not excreted to the same extend.

It is notable that statistically significant positive correlations were found between the carnitine level and the energy-rich phosphates. A positive correlation between carnitine and glycogen levels have been found earlier [26,27]. In these latter studies, carnitine was also found to correlate to enzymes of importance for long chain fatty acid oxidation and to variables of physical performance, i.e. to heart rate and to running time. These findings might be taken as indirect evidence of the necessity to maintain adequate tissue concentrations of carnitine, which may be important in several cellular processes and in which the role of carnitine is less documented. Further studies are needed to evaluate the risk for a relative carnitine deficiency in severely injured patients and its functional consequences and also to evaluate the suggested beneficial effects of carnitine supplementation.

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