Urinary Excretion of Carnitine in Multiply Injured Patients on Different Regimens of Total Parenteral Nutrition

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Carnitine derives from intake of preformed exogenous carnitine and synthesis from lysine and methionine, but is absent in parenteral fluids. Urinary excretions of carnitine and its derivatives was measured in 30 patients 2–8 days after severe multiple injuries and compared with controls. The patients received five different isocaloric parenteral nutritional regimens; group 1 glucose and fat, group 2 glucose, fat and amino acids, group 3 glucose and insulin, group 4 glucose and amino acids, and group 5 branched-chain amino acids. The mean total carnitine excretion in healthy men was 420 μmol/24 h ± 57 (SEM), and in women 266 μmol/24 h ± 29, 41% of which was free carnitine. Mean excretion of total carnitine during days 2–8 after trauma for the five groups was: 900 ± 100, 1169 ± 112, 1251 ± 102, 1023 ± 117, and 668 ± 128 μmol/24 h, being significantly higher in groups 1–4 than in healthy men. The free carnitine fraction in the patients was significantly higher than in controlled healthy subjects. Total carnitine excretion was unaffected by different nutritional regimens in the very first days. During days 6–8, group 5, receiving branched-chain amino acids had lower excretion of total carnitine (compared to groups 2–4) and free carnitine (compared to groups 3–4). Groups 3 and 4 excreted a higher percentage as free carnitine compared to the other groups. A significant correlation was found between total carnitine and net nitrogen losses during the whole observation period and between total carnitine and 3-methylhistidine during days 6–8 after trauma.

CARNITINE, 3-hydroxy-4-trimethylaminobutyrate, facilitates the transport of long chain fatty acids into mitochondria. Other functions for carnitine have been proposed although not yet established. Several investigators have identified acylcarnitine derivatives or ketoanalogs from branched-chain amino acids in animal tissues,[1,2] and carnitine has been shown to increase their α-decarboxylation.[3,4]

Carnitine is normally derived from the diet or from endogenous synthesis from lysine and methionine. Skeletal and cardiac muscle, having a high concentration, cannot synthesize carnitine and are therefore dependent on transport via plasma. Carnitine deficiency is a rather rare syndrome.[5] The clinical manifestation is a progressive lipid storage myopathy with muscle weakness and atrophy. Multi-systemic manifestations are also seen with hypoglycaemia, hepatic and cerebral dysfunction. Low tissue carnitine levels have also been found in advanced liver cirrhosis[6] and haemodialysis.[7]

Moreover, low plasma carnitine levels have been reported in protein malnutrition.[8,9] Solutions given during total parenteral nutrition (TPN) do not contain carnitine.[10] Severely injured patients have an increased energy requirement. Fat is preferentially oxidized[11] and may also be given as part of their nutritional support, indicating the need of carnitine. However, we have shown that such patients have a markedly increased excretion of carnitine.[10] Moreover, Tao and Yoshimura,[12] reviewing carnitine metabolism in parenteral nutrition pointed out the apparent need to study the therapeutic role of carnitine in patients receiving intravenous nutrition.

In the present study thirty patients with severe multiple trauma were investigated with the aim of studying the influence of different nutritional regimens on carnitine metabolism and its relationship to net nitrogen losses and 3-methylhistidine excretion.

MATERIALS AND METHODS

Subjects

Twenty-four hour samples of urine were collected from thirty patients admitted with multiple injuries to the Intensive Care Unit, University Hospital of Linköping. They entered the study on the following criteria: multiple trauma with fractures of minimum two long bones or corresponding amount of trauma, age 15–70 yr, body weight 60–80 kg, previously healthy and without head injuries or systemic disease. On admission the patients were subjected to shock treatment based on Ringer's acetate solution, plasma and concentrated red cells according to their need. Surgical procedures were performed after resuscitation but before the study was started. The aim was to have the patients in a stable condition in the morning of the day after accident, when they entered the study (=day 2). Urine samples were also collected from healthy men (aged 17–51 yr, n = 11) and women (aged 23–40 yr, n = 9) on a free diet.

Design of the Study

The patients were allocated at random (groups 1–3) and consecutively (groups 4 and 5) into five different isocaloric (13.4 MJ, 3200 kcal/day) nutrition programs (Table 1). TPN was given from day 2 to 8. Group 1 received half the energy as fat (20% Intralipid, Vitrum, Sweden) and half as glucose (20%). Group 2 was given fat, glucose

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Table 1. Daily Nutritional Supply Days 2–8

Group	Male	Female	Glucose MJ	Fat MJ	Amino acids MJ	Amino acids gN	MJ/kg Body Weight	gN/kg Body Weight	Volume l
1	6	1	6.7	6.7	0	0	0.19	0	3.85
2	6	1	6.7	6.7	2.6	24	0.19	0.33	3.98
3	6	1	13.4	0	0	0	0.20	0	4.00
4	6	3	11.7	0	2.6	24	0.18	0.31	4.98
5	6	1	6.7	6.7	0.6	6	0.15	0.08	4.80

and amino acids (Intraamin forte, Vitrum AB, Sweden). Group 3 received only glucose (20%) with 32 IU insulin per litre. Group 4 was given all non-protein energy as glucose (20%) and 24 g nitrogen (Intramin forte). Group 5 received half the energy from fat (Intralipid 20%), and the other half from glucose (10%) together with 6 g nitrogen as leucine (1.5%) (Table 1). Glucose was infused continuously during 24 hours. Fat and amino acids were given between 10 a.m. and midnight. Consecutive 24 hr urine samples, all aspirates and faeces were collected days 2–8.

Methods

Carnitine determinations were performed with the enzymatic radioisotopic method of Cederblad and Lindstedt[13] with N-ethylmaleimide included in the assay as described elsewhere.[10] All urine and faecal samples as well as drain fluids and gastric juice were analysed for total nitrogen using a semi-micro Kjeldahl method.[14] In the balance calculations losses to perspiration and skin were not taken into account. A correction of the nitrogen balance was made for changes in the total urea pool assuming the total body water to be 60% of the body weight in males and 53% in females. 3-Methylhistidine was determined (groups 1, 3–5) by amino acid analysis.[15]

Statistics

Nonparametric statistical tests were used in all comparisons and correlations: Kruskal-Wallis one-way analysis of variance, Mann-Whitney U-test for two-sample analysis and Spearman's rank correlation test.[16]

RESULTS

The healthy men had a higher urinary excretion of total and acylcarnitine than the healthy women, whereas the distribution between the free and acylated fractions was similar (Table 2). The distribution between the different carnitine forms was expressed both as free carnitine in % of total and as the acyl/free carnitine ratio to facilitate the comparison with previous reports.[10,17] The mean values of all observations of the daily excretion of carnitine and its derivatives during days 2–8 after trauma for the five groups are shown in Table 2. The men were used for comparisons of mean carnitine excretions of the patients, since most of the latter were men. The patients in groups 1–4 excreted significantly more total and free carnitine than controls. All five groups had a significantly different distribution of the carnitine forms than controls. Figure 1 shows the mean excretion of carnitine and its derivatives for each day and group. Total and free carnitine showed a similar pattern with initially high values, which declined on days 3 and 4 and rose again on days 6–8. This pattern was less obvious for acylcarnitine.

Comparisons between the five nutritional regimens were calculated separately for the initial days and the last days of the observation period. The nonparametric Kruskal-Wallis one-way analysis of variance was first used to test whether there was an over-all difference between the five groups. If so, it was sometimes possible to pick out which group differed significantly. In that case, the Kruskal-Wallis test was repeated for the remaining four groups in order to reduce the number of times two-sample statistical test has to be applied. No differences were found on days 2–3 between the five nutritional groups in total, free and acylcarnitine. During days 6–8, significant differences were found for total and free carnitine, which were no longer found if group 5 was excluded. When compared

Table 2. Urinary Excretion of Carnitine 2 to 8 Days After Severe Trauma and in Healthy Subjects

Group	Carnitine, μmole/24 hr Total	Free	Acyl	Free, % of Total	Acyl/Free Ratio
1	900 ± 100*	649 ± 82*	255 ± 22	57 ± 4*	2.03 ± 0.49*
2	1,169 ± 112†	839 ± 98†	379 ± 52*	64 ± 3†	0.82 ± 0.17†
3	1,251 ± 102†	985 ± 93†	266 ± 23	76 ± 2†	0.36 ± 0.04†
4	1,023 ± 117†	786 ± 93†	225 ± 20	74 ± 2†	1.31 ± 0.20†
5	668 ± 128	458 ± 96	210 ± 33*	59 ± 3†	0.96 ± 0.18†
Healthy subjects					
Men $n = 11$	420 ± 57	194 ± 39	226 ± 20	41 ± 4	1.67 ± 0.30
Women $n = 9$	266 ± 29*	122 ± 21	144 ± 11†	42 ± 4	1.81 ± 0.56

Values are mean ± SEM of all observations.

*$p < 0.05$ compared with healthy men.

†$p < 0.01$ compared with healthy men.

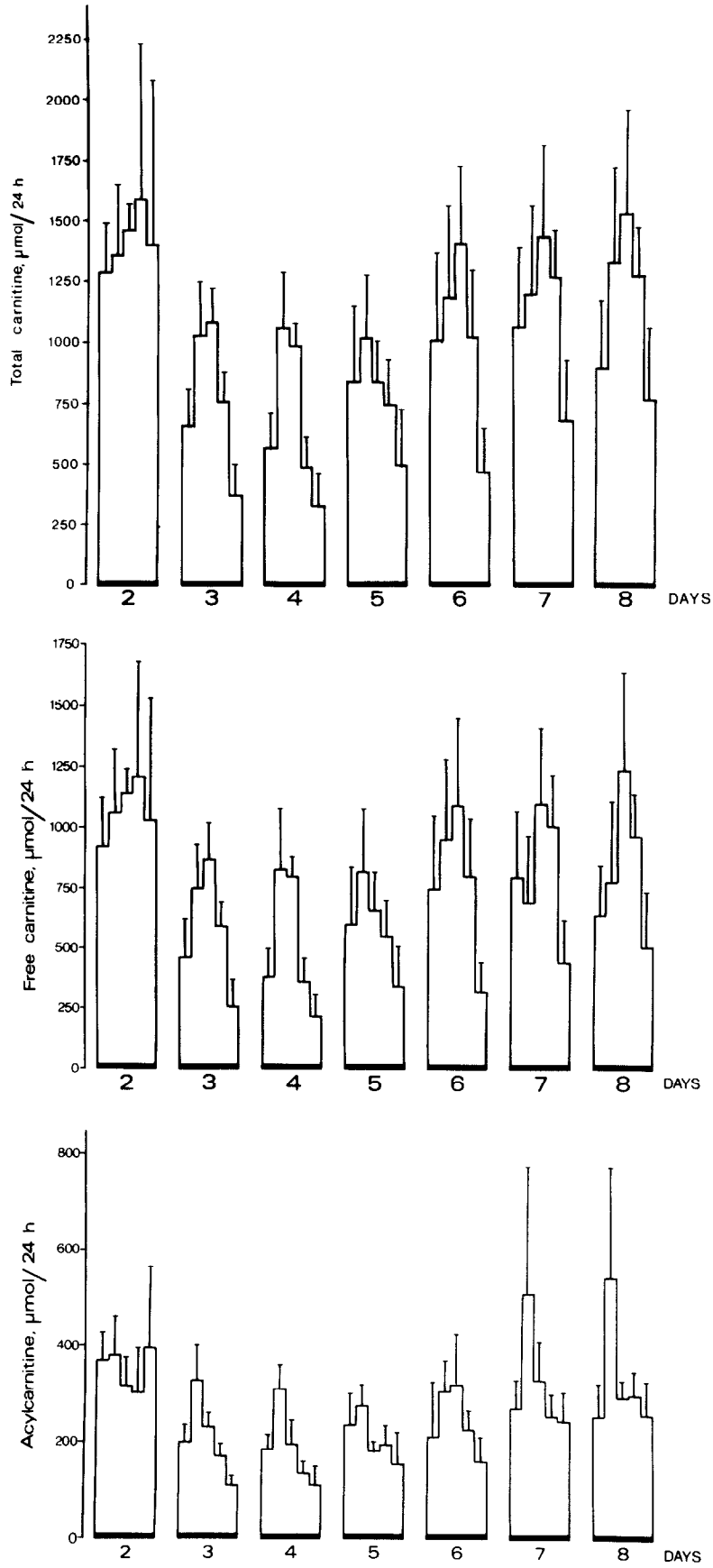

Fig. 1. Urinary excretion of carnitine of patients on five nutritional regimes followed for 7 days after trauma. Values are mean and SEM for groups 1–5 in that order.

with the other groups by Mann-Whitney two-sample test, group 5 showed a significantly lower excretion of total carnitine than group 2 ($p < 0.05$), group 3 ($p < 0.01$) and group 4 ($p < 0.01$) and of free carnitine than group 3 ($p < 0.01$) and group 4 ($p < 0.01$).

Figure 2 shows the distribution of the carnitine derivatives. On day 2 variance analysis showed no overall difference between the groups in contrast to days 6–8. Pair-wise comparisons showed that group 4 differed from groups 1, 2, 5 and group 3 differed from groups 2, 5 in both variables (Table 3 and Fig. 2).

Mean values of net nitrogen losses and 3-methylhistidine are shown in Figure 3. Total urinary carnitine correlated significantly with net nitrogen losses in four of ten comparisons calculated (Table 4). This relationship was not significant in groups 2 and 5 on days 2–4 and in groups 3 and 4 on days 5–8. An overall correlation was made using the mean of each patient's carnitine excretion and the cumulative nitrogen excretion. The Spearman's R was 0.4108, and $p < 0.05$.

The relationship between 3-methylhistidine and the total carnitine excretion is shown in Table 4. No significant differences were found on days 2–4, in contrast to the significantly positively relationships that were noted for groups 3–5 on days 5–8.

DISCUSSION

The present study shows that severely injured patients had increased carnitine excretion after trauma compared to healthy men. On the second day after the trauma the mean excretion of total carnitine was increased 3 to 4 times. This increase is caused by the free fraction, being 4 to 6 times higher, whereas acylcarnitine is only increased about 50% above control levels. Furthermore, when the means of total carnitine excretion of the whole observation period were compared to the daily mean of healthy men, groups 1 to 4 but not 5 had significantly higher values. We have here not taken into account that the healthy subjects were not on a carnitine-free diet, which would magnify the observed changes. We have demonstrated an even more marked increase in urinary carnitine in burned patients.[10] Two days after burn the patients had a sixfold increase and the mean value was still

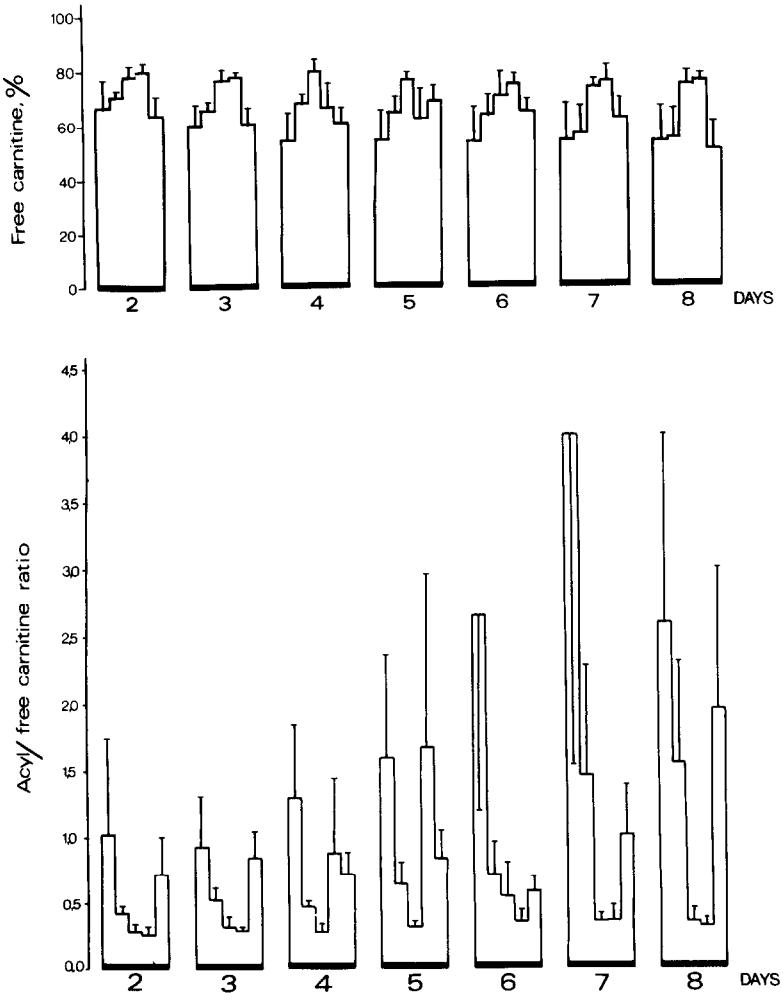

Fig. 2. Distribution of carnitine derivatives in urine of patients under conditions as in Fig. 1.

Table 3. Distribution of Carnitine Derivatives in Patients on Five Nutritional Regimes During Days 6–8 After the Trauma

Group Compared	Group 1	2	3	4	5
	Free carnitine, % of total				
3	ns	<0.05		ns	<0.01
4	<0.025	<0.01	ns		<0.001
	Acyl/free carnitine ratio				
3	ns	<0.05		ns	<0.01
4	<0.025	<0.01	ns		<0.001

p is the significance level of Mann-Whitney two-sample test.

twice the control excretion on day 8. The present results are also in agreement with the report of Tanphaichitr and Lerdvuthisopon, who studied seven patients with protein-energy malnutrition on TPN pre- and post-operatively and noted a 2 to 7 fold increase in carnitine excretion during periods of operation and/or infection.[18]

During the observation period, the carnitine excretion showed a pattern with initially high values then a decrease on the third and fourth days, followed by an increase on days 5 to 8. Tanphaichitr and Lerdvuthisopon observed a second rise in three of the seven patients, which occurred concurrently with infections.[18] Our results seem to indicate that this secondary rise could be a more general pattern although not seen in every patient. It was also found that the free fraction of carnitine contributed most to this pattern. The excretion pattern initiated calculations on an early and late phase as different factors may influence the carnitine excretion during these two phases.

Regarding the net nitrogen losses, most groups showed significant relationships with total carnitine excretion during both phases. This finding supports that of others.[18] However, for 3-methylhistidine none of the four groups analyzed showed a significant relationship with total carnitine during the early phase, but three of four did during the late phase. Taking 3-methylhistidine as an index for muscle tissue breakdown, this seems to contribute more to the carnitine losses during the late than the early phase.

Both the hormonal milieu and the substrate utilization following major injury are very complex. Askanazi et al.[11] have shown that in septic/injured patients even a large carbohydrate intake does not prevent continued utilization of fat for energy in contrast to nutritionally depleted patients. Furthermore a lipid load, Intralipid 10% 500 ml over 4–6 hr, has been shown to decrease the excretion of carnitine from 1431 to 820 μmole/24 hr.[19] Carnitine excretion is also augmented by administration of adrenocorticotrophic and thyroid hormones.[20] Further studies are needed to elucidate the interplay between substrate and hormonal influences on carnitine metabolism and their consequences. It should be pointed out that the even higher mean values of carnitine excretion in the burned patients showed a progressive decrease during the corresponding observation period. In patients followed during a longer time, increases of carnitine were sometimes noted. This may well correspond to periods of septicemia. A significant positive relationship was also found between the individual mean value during days 2–8 and the percentage burned area.[10]

Early after trauma no significant difference was

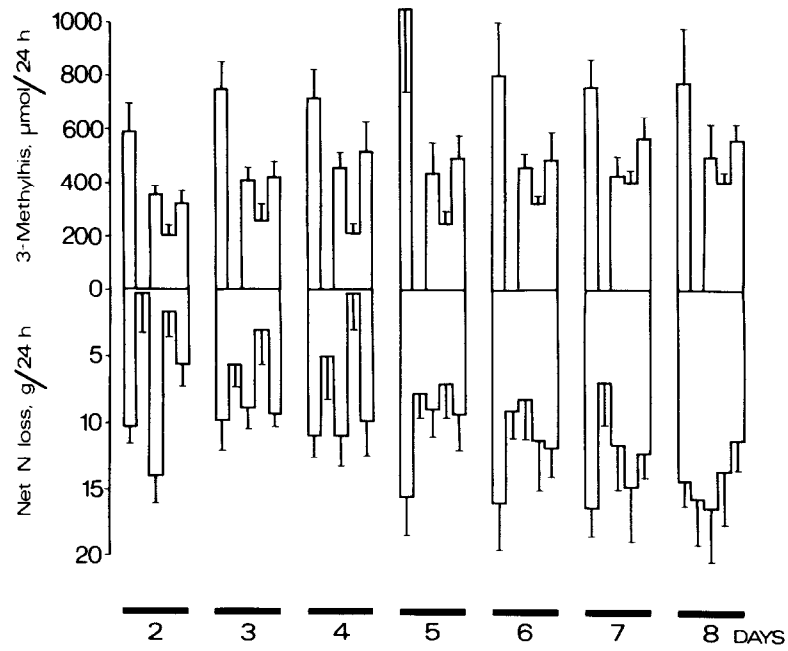

Fig. 3. Urinary excretion of 3-methylhistidine and net nitrogen losses in patients under conditions as in Fig. 1.

Table 4. Relationship between Total Carnitine Excretion and Net Nitrogen Losses and 3-Methylhistidine Excretion

Period	Group 1	2	3	4	5
	Net nitrogen losses				
Days 2–4					
R	0.6512	0.2912	0.4221	0.4304	0.3953
p	<0.0025	ns	<0.05	<0.0025	ns
Days 5–8					
R	0.8713	0.5146	0.1559	0.3107	0.8817
p	<0.00025	<0.01	ns	ns	<0.005
	3-Methylhistidine				
Days 2–4					
R	0.1104	—	0.0662	0.1868	0.1104
p	ns		ns	ns	ns
Days 5–8					
R	0.3079	—	0.5088	0.3780	0.7959
p	ns		<0.005	<0.05	<0.0005

p is the significance level of Spearman's coefficient (R) of rank.

found between the different nutritional groups in carnitine excretion. During days 5–8, group 5 excreted significantly less total and free carnitine than the other groups. It should be noted that neither the net nitrogen loss nor the 3-methylhistidine excretion of group 5 was less than in the other groups. Furthermore, there was a difference in the distribution of the carnitine derivatives. Groups 3 and 4, which did not receive fat and only a small number of branched-chain amino acids, had a lower degree of carnitine acylated. Group 3 differed significantly from groups 2 and 5 and group 4 from 1, 2 and 5. This finding might reflect the fact that carnitine esters are formed in the metabolism both of fatty acids[21,22] and branched-chain amino acids.[2,3] Furthermore, there is experimental evidence that the fat content of the diet[23] or parenteral regimens[19] lowers the plasma level and urinary excretion of carnitine. In a preliminary report including both multiple injuried and burned patients, we made the same observation.[24] Increased uptake of carnitine in tissues is one of the suggested possibilities to explain this decline. Increased uptake in the liver has been shown in rats during ketogenesis.[25]

Carnitine is included in the diet and also synthesized from lysine and methionine. In normal subjects, the carnitine excretion on a low carnitine diet (<10 μmol) was 100 μmole/day, which probably approximates to the rate of endogenous synthesis when the diet lacks carnitine.[6] In subjects given common foodstuffs, carnitine intake was 380–450 μmol and urinary excretion similar.[6] It is not known to what extent the endogenous synthetic capacity can be accelerated. In our patients, the intake of carnitine was zero during TPN and under normal conditions the excretion would be about 100 μmole/day. The mean excretion in all patients during this period was 1087 μmole/day, implying that the daily loss of carnitine was about 10 times higher than normal. On the other hand, the carnitine liberated from muscle tissue breakdown in these patients is very likely to be excreted immediately since an excess of carnitine intake is almost completely recovered in urine.[26] During the very first days, the carnitine excretion was high and showed no relationship to 3-methylhistidine excretion. Later there was a positive relationship to 3-methylhistidine, but it is not known if the carnitine loss from muscle is balanced with the muscle tissue loss or if the flux of carnitine between different tissues inevitably leads to excessive losses.

Speculatively, an immediate consequence, when the patient still is catabolic, could be a state of relative carnitine deficiency on prolonged TPN. It also seems as if different tissues react differently to a low intake of carnitine.[27,28] The more severe the injury the greater the risk of developing a carnitine deficiency due to the positive relationship between carnitine excretion and surface burned area that we have found earlier.[10] Subsequently, when the patient had reached an anabolic state, we do not know whether synthesis of carnitine and/or dietary intake can keep up with the need for carnitine. Further studies are needed to evaluate the risk for an acquired 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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