

Carnitine Ester Excretion in Pediatric Patients Receiving Parenteral Nutrition

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ABSTRACT. Carnitine plasma concentrations and the excretion of carnitine and individual carnitine esters were determined in 25 children and adolescents with gastrointestinal diseases receiving carnitine-free parenteral nutrition for at least 1 mo using radiochemical and radioisotopic exchange HPLC methods. Children <12-y-old usually had carnitine plasma concentrations <-2 SD from the normal mean for age, whereas patients >12-y-old had carnitine plasma concentrations within the normal range. Age was the only variable to correlate significantly with plasma carnitine concentrations during parenteral nutrition. Free carnitine (FC) excretion was closely correlated with plasma FC concentrations and minimal at values <25 $\mu\text{mol/L}$. The excretion of FC and short-chain acylcarnitines was reduced by an order of magnitude in younger compared with older patients and controls, but the excretion of "other" acylcarnitines was less affected. Some of the latter were tentatively identified using gas-liquid chromatographic and mass spectroscopic techniques as unsaturated and/or branched medium-chain carnitine esters with a carbon chain of C8-C10. The results suggest that FC and short-chain acylcarnitine are conserved by the kidney in nutritional carnitine deficiency but that there may be an obligatory renal excretion of other carnitine esters that contributes to the development of hypocarnitinemia in the younger age group. (*Pediatr Res* 28: 158-165, 1990)

Abbreviations

PN, parenteral nutrition
TPN, total parenteral nutrition
FC, free carnitine
TC, total carnitine
AC, acylcarnitine
SCAC, short-chain acylcarnitine
MCAC, medium-chain acylcarnitine
PEG, polyethylene glycol
IBD, inflammatory bowel disease

Carnitine (β -hydroxy- γ -trimethylamino-butyric acid) plays an important role in energy metabolism by facilitating the transport of FFA across the inner mitochondrial membrane for β -oxidation

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(1) and regenerating intramitochondrial coenzyme A through transport of acyl-groups out of the mitochondria, thereby maintaining conditions required for oxidative processes (2).

Although carnitine is synthesized *de novo* in mammalian tissues (3), nutritional carnitine deficiency may occur under certain circumstances if carnitine is not provided with the diet. Infants receiving carnitine-free TPN are particularly prone to develop carnitine deficiency, probably due to impaired carnitine biosynthesis (4) and low carnitine tissue stores (5). Moreover, infants on TPN appear to have a decreased capacity to oxidize fatty acids that can be improved by L-carnitine supplementation (6, 7). The question has therefore been raised whether carnitine should be considered an essential nutrient for the infant (8).

It is not known at what time during human development endogenous carnitine biosynthesis becomes adequate. Adults receiving TPN maintain a normal carnitine status over a longer period of time than infants (9). Nonetheless, abnormally low carnitine plasma concentrations were found in 35% of an adult population on long-term home PN (10). Some of them also had decreased liver carnitine content. The pathogenesis of this is not understood because, in the mature individual, endogenous carnitine biosynthesis should be adequate if precursors are provided with TPN. However, loss of carnitine through the kidney may be an important factor.

FC and/or AC excretion has been shown to be increased during TPN in adult patients with multiple injuries (11), with sepsis, and after surgery (12), but decreased in metabolically stable patients receiving long-term PN (13). Individual carnitine esters were not analyzed in the urine of these patients.

It has been suggested that carnitine plays a role in mitochondrial detoxification of excess or slowly metabolized coenzyme A-linked compounds of endogenous or exogenous origin (14). These acyl moieties are eventually excreted in the urine as AC. This has been well documented in inborn errors of organic acid metabolism. We hypothesized that such a mechanism may be operational even in metabolically normal humans. The associated carnitine loss may lead to decreased carnitine concentrations in susceptible individuals if carnitine is not provided with nutrition.

In the following, we report the carnitine status and excretion of carnitine and carnitine esters in children and adolescents with gastrointestinal diseases requiring PN for at least 1 mo using a radioisotopic exchange HPLC method recently adapted for human urine (15). In addition, L-carnitine absorption was studied in a child with a functional short bowel.

PATIENTS AND METHODS

Patients. Twenty-seven children and adolescents with gastrointestinal disease requiring PN for at least 1 mo were enrolled in the study. Informed consent was obtained from the parents and/

or patients and the study was approved by the Investigational Review Board of the Children's Hospital of Buffalo. Two children who were later diagnosed as having renal Fanconi syndrome with an abnormally high renal clearance of phosphate and bicarbonate were excluded from the study. The clinical data of the remaining 25 patients are given in Table 1. They were divided into two groups on the basis of their disease and age. Patients in group 1 ($n = 15$) were <12-y-old and had diseases involving large areas of the small intestine. Patients in group 2 ($n = 10$) were >12-y-old and had IBD affecting the colon and/or the terminal ileum. No patient in group 2 had resection of his/her intestine.

PN was administered through a central venous catheter and consisted of dextrose (15–25%), amino acids (1–3 g/kg), fat emulsion (1.5–3.0 g/kg), electrolytes, minerals, trace elements, vitamins, and heparin (1 IU/mL). Generally, 150–200 nonprotein kcal were given per g of nitrogen. The infusion solutions contained no carnitine. Seventeen patients received their total caloric intake parenterally and eight had variable oral intake during the time of the study (Table 1). About one half of the patients received PN for more than 3 mo at home and were brought to the hospital for catheter- or disease-related complications. The other half received PN for <3 mo while in the hospital. Three patients (all of them in group 1) had cirrhosis as diagnosed by liver biopsy, which was thought to be associated with their primary disease and/or long-term PN. Five had elevated aminotransferases (>3 times upper normal limit) without any other clinical or biochemical evidence of liver disease. One patient with cirrhosis had cholestasis with conjugated hyperbilirubinemia. Most of the patients with IBD received prednisone during the time of the study. All patients were metabolically stable and in good nutritional status at the time of the study.

Seven patients (mean age 203 mo, range 146–252 mo) with IBD not requiring PN (group 3) were also studied for comparison with group 2. In addition, eight healthy children (mean age 46 mo, range 2.5–96 mo) were studied as controls for group 1.

Sample collection. Spontaneously voided urine was collected over a period of 24 h (12 h for children <3-y-old). During the collection period, the urine was stored in a refrigerator without preservative. Immediately upon completion of the urine collection, several portions were frozen at -70°C until analysis. During the urine collection, a blood sample was collected and centrifuged, and the plasma frozen at -70°C . In 11 patients, a blood sample was collected before the onset of PN and at one or more time points during PN.

Carnitine absorption study. In one patient (no. 7) who had a jejunostomy because of aganglionosis extending from the mid-jejunum, absorption of L-carnitine in the jejunum was studied after parental consent. After collecting the ostomy output for 1 h and obtaining a blood sample, 100 mg L-carnitine (Vitacarn, Kendall McGaw, Irvine, CA), 8 g D-xylose, and 1 g PEG 4000 dissolved in 100 mL water were infused through a nasogastric tube over a period of 5 min. Blood samples were obtained 1 and 4 h after the infusion, and the ostomy output was collected in hourly increments over a period of 7 h. Carnitine and D-xylose recovered from the ostomy output were related to the recovered nonabsorbable marker PEG 4000 to correct for possible inaccuracies in output collection or spillage. Carnitine and D-xylose absorption were determined by calculating the difference between the amount given and that recovered from the ostomy. In addition, both carnitine and D-xylose were measured in blood.

Carnitine determination. Plasma carnitine was measured by a modification (16) of the radiochemical method of McGarry and Foster (17). The same method was used for urinary carnitine except that urine samples were diluted 1:2 or 1:6 with water. To correct for possible spillage during the collection, the results for urine were expressed as $\mu\text{mol/g}$ creatinine. Carnitine in ostomy output was determined after precipitation of proteins with an equal volume of 6% perchloric acid.

Carnitine excretion. Excretion (E) and fractional tubular reabsorption (FTR) of FC were calculated from the concentrations

of FC and creatinine (Cr) in the collected urine (U) and in the plasma sample (P) obtained at midcollection using the following formulas:

$$E (\text{nmol}/100 \text{ mL GF}) = 100 \times \frac{U_{\text{FC}} \times P_{\text{Cr}}}{U_{\text{Cr}}}$$

where GF = glomerular filtrate, and

$$\text{FTR} (\%) = \left(1 - \frac{U_{\text{FC}} \times P_{\text{Cr}}}{P_{\text{FC}} \times U_{\text{Cr}}} \right) \times 100$$

Individual carnitine esters were determined by a modification (15) of the radioisotopic exchange HPLC method originally described by Kerner and Bieber (18). Urine (0.2 mL) was partially purified and subjected to the radioisotopic exchange reaction in which ^3H -L-carnitine is enzymatically exchanged into the AC pool using carnitine acetyltransferase. The labeled carnitine esters were subsequently separated by HPLC, detected by a radioactive flow detector, and the peak areas were integrated by computer.

Peaks of acetyl-, propionyl-, isobutyryl-, and 5-carbon branched-chain AC were identified by comparing their relative retention times with those of authentic standards (Sigma Chemical Co., St. Louis, MO, or synthesized according to ref. 19). Because urinary MCAC that are branched and/or unsaturated are poor substrates for carnitine acetyltransferase (20), they were indirectly estimated together with other carnitine esters presumably present in the urine but not detectable with the described method by subtracting the concentrations of the known carnitine esters from the total AC (as determined radiochemically). In the following, they are referred to as "other" carnitine esters.

In addition, acetylcarnitine was measured in 15 urine samples by an enzymatic spectrophotometric method (15, 21). With this method, acetylcarnitine was unmeasurable in five urine samples with a concentration of <7 nmol/mL (as determined by the radioisotopic exchange/HPLC method). There was a strong correlation between the two methods ($r = 0.98$).

Identification of isobutyrylcarnitine and medium-chain carnitine esters. From a patient in group 1 and a control child, 20 mL urine were lyophilized, dissolved in 2 mL water, extracted with 20 vol chloroform/methanol (3:2, vol/vol) and purified on a 50-mL column of Bio-Gel P-2. For marker purposes, the isotopic exchange reaction was performed with a small portion of these urine samples containing 1–2 nmol of carnitine. The remaining samples were freeze-dried and added to the labeled portion. The samples were then applied in 12 portions of 0.2 mL each to the HPLC column and eluted as previously described (18). The compounds in question were repeatedly collected from the column using a fraction collector. The combined fractions were further purified with a Dowex 1 (chloride form) column (30 \times 5 mm), lyophilized, applied again to the HPLC column and eluted under isocratic conditions with 90% 10 mM ammonium formate (pH 7.0) and 10% methanol. The labeled compounds in question were collected and lyophilized. Small portions were hydrolyzed with 1 N KOH and the fatty acid residue analyzed by gas-liquid chromatography (22). The remaining samples were subjected to fast atom bombardment mass spectrometry (22). In one sample, gas-liquid chromatography electron impact mass spectral analysis of the acyl moiety was performed after hydrolysis (22).

Other methods. Urinary creatinine was measured by the picric acid method using a commercially available kit (Sigma Chemical Co.). D-xylose in plasma and ostomy outputs was measured according to Roe and Rice (23), and PEG in ostomy outputs according to Malawer (24).

Statistical evaluation. The Mann-Whitney nonparametric test was used to evaluate differences between patient groups. To test the relationship between FC plasma concentration and urinary excretion, regression analysis was performed after logarithmic transformation of the FC excretion data that provided the best fitting curve. Multiple regression analysis was performed with

Table 1. Clinical data of 25 children and adolescents receiving >1 mo of PN.*

Patient No.	Age (mo)	Primary disease	Indication for PN	Duration of PN (mo)	Parenteral calories (% total)	Liver disease	Steroid therapy
Group 1:							
1	48	Intestinal neuro-gangliomatosis	Chronic diarrhea	45	100		
2	16	Intestinal pseudoobstruction	Hypomotility	7	100		
3	96	Volvulus	Obstruction	2	75		
4	78	Gastroschisis	Short bowel	78	100	Cirrhosis, cholestasis	
5	19	Chronic diarrhea		4	100		
6	42	Immune deficiency	Chronic diarrhea	1	75	AST, ALT elevated	
7	18	Aganglionosis	Hypomotility	18	100		
8	102	Jejunal atresias	Short bowel	102	100		
9	2.5	Chronic diarrhea		1	100		
10	7.5	Congenital chylus ascites		3	100	Cirrhosis	
11	9	Necrotizing enterocolitis	Obstruction	1	100		
12	75	Gastroschisis	Short bowel	75	50	Cirrhosis	
13	1.5	Gastroschisis	Short bowel	1	100		
14	3	Chronic diarrhea		1	100	AST, ALT elevated	
15	51	Intestinal pseudoobstruction	Hypomotility	36	100		
Mean	38			25	93		
Group 2:							
16	228	Ulcerative colitis	Bowel rest	1	100		+
17	228	Crohn's disease	Obstruction	7	60		+
18	264	Crohn's disease	Obstruction	1	100		+
19	180	Crohn's disease	Growth failure	1	100		-
20	156	Crohn's disease	Growth failure	5	50		+
21	264	Crohn's disease	Obstruction	60	100	AST, ALT elevated	-
22	144	Ulcerative colitis	Bowel rest	1	90		+
23	264	Crohn's disease	Fistulas	24	90	AST, ALT elevated	-
24	169	Crohn's disease	Obstruction	1	100		+
25	168	Crohn's disease	Malnutrition	1	90	AST, ALT elevated	+
Mean	207			10	88		

* Patients were divided into two groups on basis of age and type of disease (see text). Aspartate (AST) and alanine (ALT) aminotransferases were elevated >3 times upper normal limit where indicated.

plasma TC as the dependent variable and age, duration of PN, and oral intake as the independent variables.

RESULTS

Plasma carnitine. TC, FC, and AC plasma concentrations were lower in patients in group 1 than in those in group 2 (Table 2). Differences in TC and FC plasma concentrations between groups 2 and 3 were much less pronounced. All children except one (no. 11) of group 1 had TC plasma concentrations <-2 SD from the normal mean for age (16). With one exception (no. 16), all patients in group 2 had TC and FC plasma concentrations within 2 SD of the normal mean for age.

Among the variables tested by multiple regression analysis, only age was significantly correlated with TC plasma concentrations (expressed as % of the normal mean, $r = 0.68$, $p < 0.001$, Fig. 1). This correlation was still significant even if only patients in group 1 were considered ($r = 0.52$, $p < 0.05$).

In 10 children (four in group 1 and six in group 2), plasma

carnitine concentrations could be measured before the onset of PN (Fig. 2). Of the group 1 patients, two (nos. 9 and 14) had decreased and two (nos. 5 and 6) had normal carnitine plasma concentrations at that time. All were malnourished with wt/ht < 3rd percentile and hypoalbuminemia. All six patients of group 2 had normal carnitine plasma concentrations before the onset of PN. Four of them were also malnourished. In eight of 10 patients, carnitine plasma concentrations dropped after 1 mo of PN. They remained relatively constant thereafter in those patients who were followed for up to 4 mo (Fig. 2).

Carnitine excretion. Because of exceptionally low carnitine concentrations in plasma and urine, patient no. 16, who had massive blood loss from his colon requiring several transfusions of red blood cells, was excluded from the statistical evaluation of the carnitine excretion data.

Urinary excretion of TC, FC, and AC was lower and the percentage of TC excreted as AC was higher in group 1 compared with group 2 patients or control children (Table 3). Carnitine excretion was not different between patients in groups 2 and 3.

Table 2. Plasma concentrations ($\mu\text{mol/L}$) of TC, FC, and AC in three groups of patients*

	<i>n</i>		TC	FC	AC	AC/FC
Group 1	15	\bar{x}	22.3 [†]	16.6 [†]	3.8 [‡]	0.21
		R	8.8–40.4	8.4–34.3	0.4–8.9	0.05–0.54
		\bar{x}	21.0	17.0	3.9	0.23
Group 2	9	\bar{x}	42.4 [§]	33.7 [§]	10.4	0.31
		R	25.8–61.7	20.9–41.9	2.8–22.4	0.08–0.64
		\bar{x}	43.4	32.3	11.4	0.35
Group 3	7	\bar{x}	52.3	38.0	15.8	0.43
		R	43.3–75.0	36.1–48.1	3.3–33.0	0.07–0.79
		\bar{x}	56.1	40.5	15.5	0.39

* Group 1: PN, <12-y-old; group 2: PN, >12-y-old, IBD; group 3: oral nutrition, >12-y-old, IBD. \bar{x} , median; R, range; \bar{x} , mean. Normal range (16): 1 mo–10 y: TC, 28–84; FC, 22–66; AC, 3–32. 10–17 y: TC, 34–77; FC, 22–65; AC, 4–29.

[†] $p < 0.0001$ compared with group 2.

[‡] $p < 0.002$ compared with group 2.

[§] $p < 0.03$ compared with group 3.

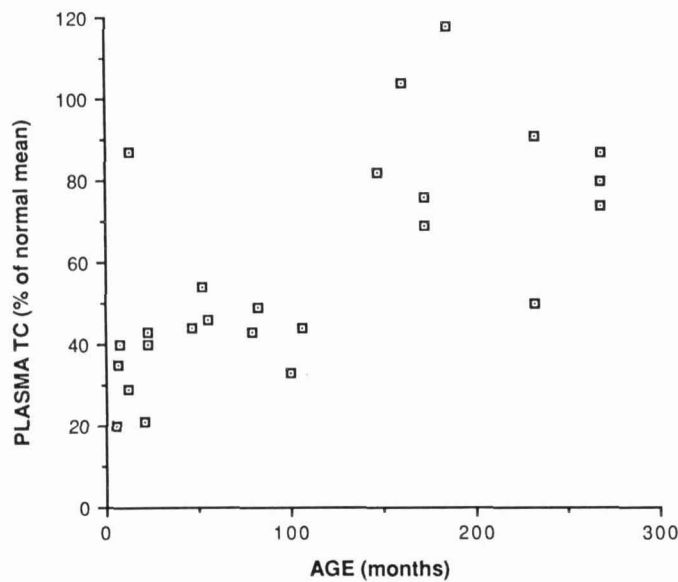


Fig. 1. Relationship between TC plasma concentrations (expressed as % of the normal mean for age) and age of 25 children and adolescents receiving PN for >1 mo. $y = 36.00 \pm 0.19x$, $r = 0.68$, $p < 0.001$.

As shown in Figure 3, there was a positive correlation ($r = 0.80$, $p < 0.001$) between plasma FC concentrations and FC excretion (expressed as nmol/100 mL glomerular filtrate). At FC plasma concentrations of $<25 \mu\text{mol/L}$, FC excretion was minimal. The two patients with renal Fanconi syndrome had a high excretion of FC despite low FC plasma concentrations. The mean fractional tubular reabsorption of FC was 99.7% in group 1, 96.8% in group 2, and 97.1% in group 3 patients. The difference between groups 1 and 2 was significant ($p < 0.015$), indicating the ability to conserve FC in the former.

The urinary excretion of all SCAC and other AC was lower in group 1 than in group 2 or controls (Tables 4 and 5). However, the differences were more pronounced for SCAC (~10-fold) than for other AC (2- to 3-fold). The percentage of TC excreted as SCAC was lower and that excreted as other AC higher in group 1 compared with group 2 or controls (Table 5). Carnitine ester excretion was not different between patients in groups 2 and 3.

Identity of carnitine esters. Figure 4 shows HPLC chromatograms of urinary carnitine esters of patient no. 11 of group 1 and no. 18 of group 2. In the former (Fig. 4A), peaks in the area of MCAC were more pronounced relative to SCAC peaks (C-2 to C-5) than in the latter (Fig. 4B).

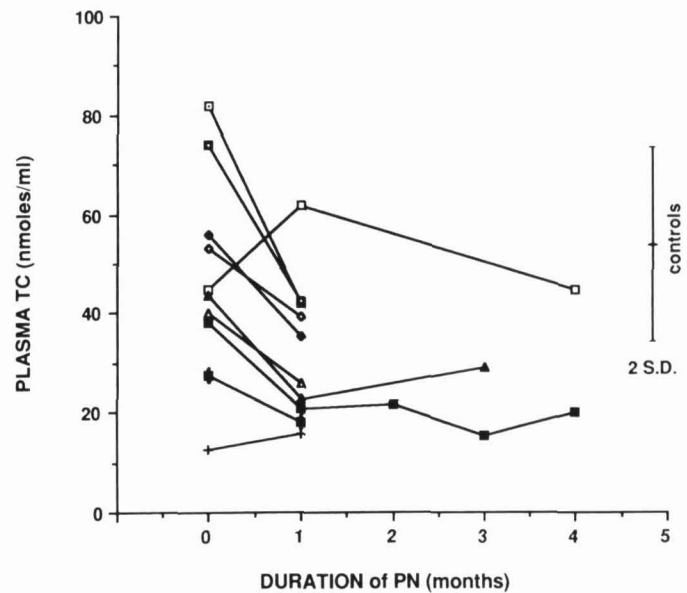


Fig. 2. Plasma TC concentrations of 10 patients before and during PN. Age-matched controls (mean + 2 SD, $n = 20$) are given for comparison. \square , no. 18; \square , no. 22; \diamond , no. 25; \diamond , no. 24; \square , no. 19; \triangle , no. 6; \triangle , no. 16; \blacksquare , no. 5; \blacksquare , no. 14; $+$, no. 9.

Table 3. Urinary excretion ($\mu\text{mol/g creatinine}$) of TC, FC, AC in three groups of patients and healthy controls (2.5- to 96-mo-old)*

	<i>n</i>		TC	FC	AC	AC $\times 100$ / TC
Group 1	13	\bar{x}	81.8 [†]	14.4 [†]	72.1 [‡]	83 [‡]
		R	43.2–254	4.9–59.6	30.5–195	68–944
		\bar{x}	105	18.3	86.9	84
Group 2	7	\bar{x}	351	170	205	52
		R	271–755	103–397	154–363	43–68
		\bar{x}	437	212	225	54
Group 3	5	\bar{x}	317	126	191	64
		R	106–3607	36–1288	70–2319	56–71
		\bar{x}	940	341	599	63
Controls	8	\bar{x}	512	272	235	52
		R	198–598	55–333	143–320	41–72
		\bar{x}	455	227	229	53

* The patient groups are the same as in Table 2. \bar{x} , median; R, range; \bar{x} , mean.

[†] $p < 0.001$ compared with group 2.

[‡] $p < 0.002$ compared with group 2.

\bar{x} $p < 0.001$ compared with controls.

In the urine of patient no. 11, the prominent peak C-4 (retention time, 20.1 min) was identified as isobutyrylcarnitine (Table 6). The MCAC in the urine of patient no. 11 and a control child were (partially) identified as shown in Table 6 and Figures 5 and 6. Due to limited amounts of material, only the structure of the compound with a retention time of 36.8 min on HPLC could be established as 2-methyloctanoylcarnitine. This peak was found in the urine of 60% of the control children, in all patients in group 3, and only occasionally in patients receiving PN (groups 1 and 2). The peaks with retention times of 33.7 and 35.3 min on HPLC were found in practically all urine samples studied. No octanoylcarnitine (retention time 36.1 min) was present in any specimen.

Carnitine absorption study. Absorption of L-carnitine was studied in patient no. 7, who had a jejunostomy. His TC and FC plasma concentrations were 20.4 and 18.0 $\mu\text{mol/L}$, respectively.

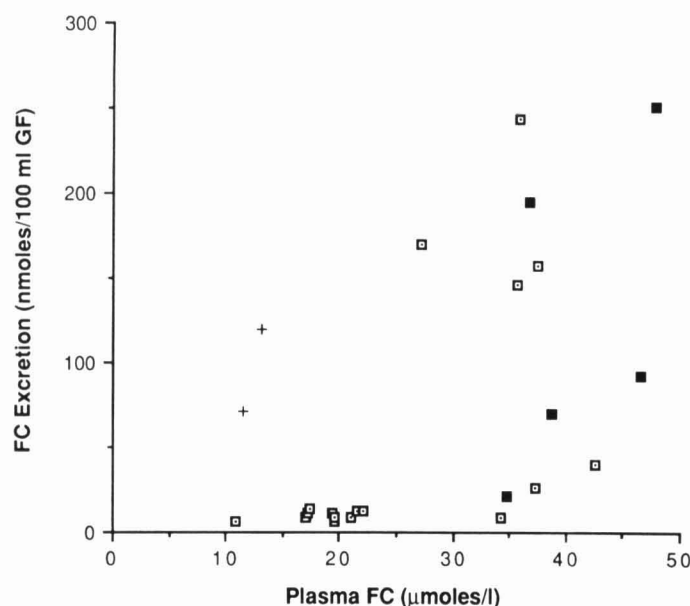


Fig. 3. Relationship between FC plasma concentrations and FC excretion (expressed as nmol/100 mL glomerular filtrate). □, patients receiving PN (groups 1 + 2); ■, patients with IBD on oral food (group 3); +, two patients with renal Fanconi syndrome receiving PN (not included in the statistical evaluation).

Carnitine concentration was minimal ($<2 \mu\text{mol/L}$) in the initial ostomy output. Four h after L-carnitine and D-xylose administration, all of the administered carnitine was recovered from the ostomy and plasma carnitine concentrations were unchanged. Twelve percent of the administered D-xylose was absorbed and the 1-h D-xylose blood level was $8 \mu\text{g/dL}$.

DISCUSSION

In the human, there is indirect evidence that low plasma carnitine concentrations reflect decreased carnitine tissue levels, at least in organs with a relatively high turnover rate for carnitine. Premature infants receiving carnitine-free TPN have decreased carnitine concentrations not only in plasma (25, 26), but also in liver, heart, and kidney (5). It has therefore been suggested that infants may have impaired endogenous carnitine biosynthesis. However, it is not known at what age the capacity for carnitine biosynthesis is fully developed in the human. Our finding that

age was the only variable to correlate significantly with plasma TC concentrations (expressed as % of normal mean for age) in our patients receiving >1 mo of carnitine-free PN suggests that the rate of endogenous carnitine biosynthesis and/or carnitine tissue reserves are insufficient in very young patients to meet carnitine requirements during rapid growth. This is supported by the study of Helms *et al.* (7), in which young infants (mean age 3 mo) receiving long-term PN had even lower TC plasma concentrations (mean $9 \mu\text{mol/L}$) than our patients in group 1.

On the other hand, the type of disease was strikingly different between our two patient groups. Extensive small bowel disease resulting in diarrhea and malabsorption (group 1) may have led to decreased carnitine reserves even before the onset of PN due to impaired intestinal absorption of carnitine or its precursors. This is supported by the finding of abnormally low plasma carnitine concentrations before initiation of PN in two of the four longitudinally studied patients with malabsorption. Moreover, we recently found carnitine plasma concentrations to be <-1 SD from the normal mean for age in four of five children with total villous atrophy due to celiac disease (Hebel E and Schmidt-Sommerfeld E, unpublished data). Carnitine absorption has not been studied *in vivo* in the human, but it has been shown to be a sodium-dependent active process in biopsy specimens from duodenum and ileum (27). Our absorption study in a child with jejunostomy demonstrates that carnitine absorption can be severely impaired in a patient with a short small intestine.

In two children in group 1, we were able to observe carnitine plasma concentrations over a period of 3–4 mo of PN. In both children, levels dropped after 1 mo to subnormal values and then remained unchanged. This suggests that plasma carnitine concentrations attain a new steady state at a decreased level during carnitine-free nutrition.

Most of our children and adolescents >12 y of age with IBD maintained plasma carnitine concentrations in the normal range. Moreover, plasma carnitine concentrations differed only slightly from those in age-matched IBD patients not receiving PN. This indicates that, in this patient population, carnitine reserves and synthetic capacity are probably adequate to maintain a normal carnitine status over an extended period of time on PN (mean 10 mo). This finding is at variance with a study that reported that 35% of adult patients with gastrointestinal disease receiving home PN had decreased carnitine plasma concentrations (10). However, these patients were on PN for a longer period of time (mean 32 mo) and most of them, in contrast to our patients in group 2, had short bowel syndrome.

Carnitine excretion was markedly different between our two

Table 4. Urinary excretion ($\mu\text{mol/g creatinine}$) of short-chain carnitine esters*

	n		Acetyl-carnitine	Propionyl-carnitine	Isobutyryl-carnitine	C5-acyl-carnitine
Group 1	12	\bar{x}	1.7†‡	0.1†‡	0.8§	0.1†‡
		R	0.2–13	0–1.0	0–15	0–0.6
		\bar{x}	2.7	0.2	3.4	0.2
Group 2	7	\bar{x}	28.2	3.4	8.0	2.6
		R	13–87	1.3–9.7	3.2–25	0.9–6.2
		\bar{x}	39.4	4.5	10.1	2.8
Group 3	5	\bar{x}	28.6	1.9	4.9	1.2
		R	5.6–317	0.5–23.3	2.7–6.0	0.3–3.4
		\bar{x}	79	5.9	4.4	1.8
Controls	8	\bar{x}	38.7	2.8	13.5	3.0
		R	8.4–56	0.8–5.1	3.2–69	1.1–4.8
		\bar{x}	35	2.9	18	3.0

* The groups are the same as in Table 3. \bar{x} , median; R, range; \bar{x} , mean.

† $p < 0.001$ compared with group 2.

‡ $p < 0.001$ compared with controls.

§ $p < 0.02$ compared with group 2.

|| $p < 0.003$ compared with controls.

Table 5. Urinary excretion of SCAC and other carnitine esters expressed in $\mu\text{mol/g}$ creatinine and % of TC excreted*

	<i>n</i>		SCAC		Other AC	
			($\mu\text{mol/g}$)	(% TC)	($\mu\text{mol/g}$)	(% TC)
Group 1	12	\bar{x}	3.1 $\dagger\dagger$	4§	64§	75 $\dagger\dagger$
		R	0.2–23	0–20	30–172	54–94
		\bar{x}	6.5	6	82	76
Group 2	7	\bar{x}	47.2	12	158	43
		R	22.8–128	5–15	101–286	24–56
		\bar{x}	56.7	11	168	41
Group 3	5	\bar{x}	34.4	10	160	54
		R	13–348	6.7–12.5	57–1971	46–65
		\bar{x}	91	10	508	54
Controls	8	\bar{x}	71.6	12	178	40
		R	20.2–90.5	9–20	95–245	21–61
		\bar{x}	58.9	13	170	41

* The groups are the same as in Table 3. \bar{x} , median; R, range; \bar{x} , mean.

$\dagger p < 0.001$ compared with group 2.

$\dagger\dagger p < 0.001$ compared with controls.

§ $p < 0.02$ compared with group 2.

|| $p < 0.003$ compared with controls.

Table 6. Identification of SCAC and MCAC separated by HPLC

Urine from	HPLC retention time (min)	Compound identified	Figure
Patient no. 11	20.1	isobutyrylcarnitine* \dagger	
Patient no. 11 and control child	33.7	8-carbon AC with one double bond $\dagger\dagger$	5A
Patient no. 11	35.3	?10-carbon AC with a OH-group and one double bond \dagger ?10-carbon AC with three double bonds \dagger	5B
Control child	36.8	2-methyloctanoyl-carnitine $\dagger\dagger$ §	6A and B

* Gas-liquid chromatography of the acyl moiety.

\dagger Fast atom bombardment mass spectrometry.

$\dagger\dagger$ Previously identified in adult urine (22, 38).

§ Gas-liquid chromatography electron impact mass spectrometry of the acyl moiety.

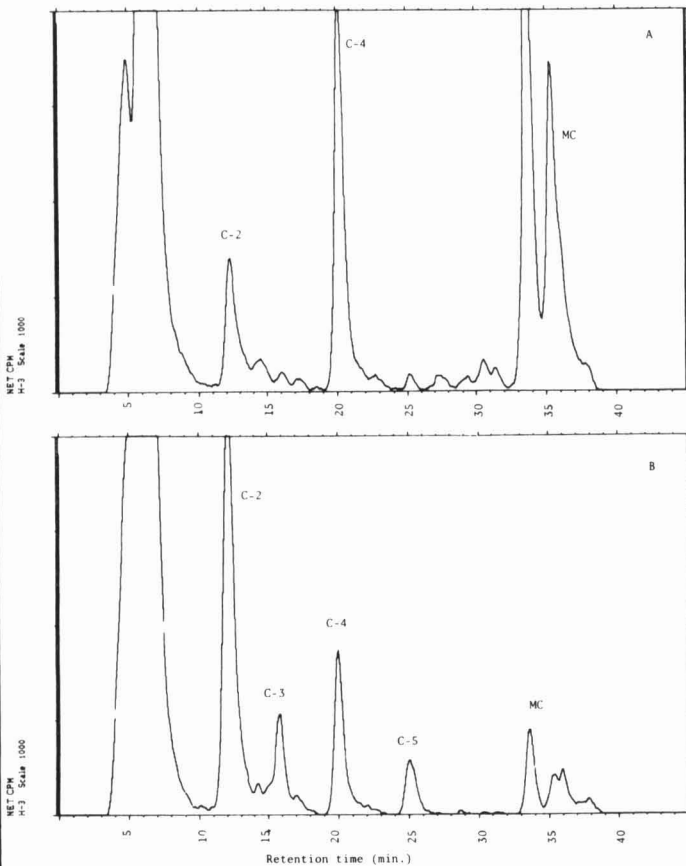


Fig. 4. Chromatograms of urinary carnitine esters of: A) a 9-mo-old infant (no. 11) with small bowel obstruction after extensive gut resection for necrotizing enterocolitis who received TPN for 1 mo, and B) a 22-y-old patient (no. 18) with small bowel obstruction due to Crohn's disease who received TPN for 1 mo. Carnitine excretion (in $\mu\text{mol/g}$ creatinine) in A: FC = 20.0; acetylcarnitine (C-2) = 3.0; isobutyrylcarnitine (C-4) = 4.8. In B: FC = 112; C-2 = 28.2; propionylcarnitine (C-3) = 6.6; C-4 = 9.0; 5-carbon AC (C-5) = 3.4. The amount of MCAC was not calculated because their complete exchange with the labeled FC pool could not be established.

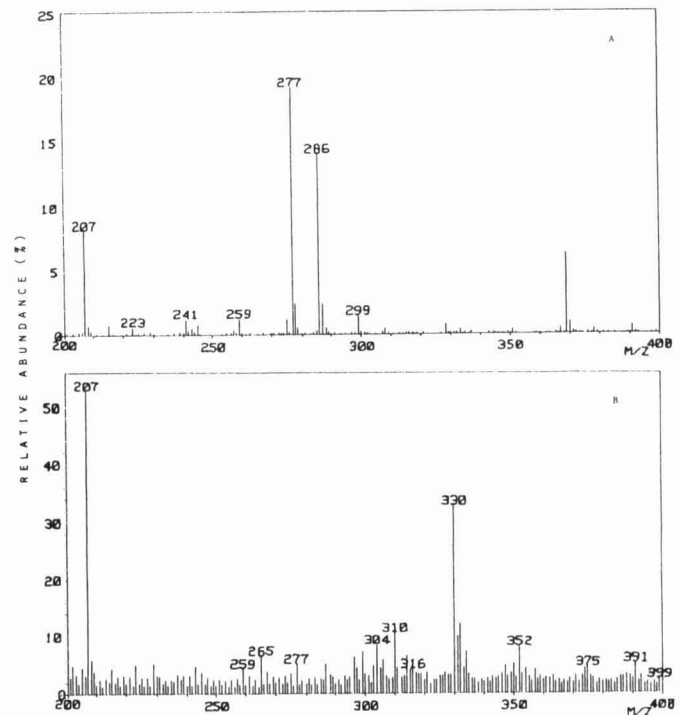


Fig. 5. Fast atom bombardment mass spectra of two peaks in Figure 4A with retention times of 33.7 (A) and 35.3 (B) min. A: Protonated mol wt = 286 (Na-adduct mol wt = 308) best fit an 8-carbon AC with one double bond. B: Protonated mol wt = 330 (Na-adduct mol wt = 352) best fit a 10-carbon AC with an OH-group and one double bond; protonated mol wt = 310 best fits a 10-carbon AC with three double bonds (see Table 6). Peaks at 207, 277, 299, 369, and 391 are due to the glycerol matrix used.

groups of patients receiving PN, but not different between groups 2 and 3 (IBD with and without PN). This suggests that carnitine excretion is not influenced by the type of nutrition or carnitine intake, but primarily by plasma carnitine concentrations. The

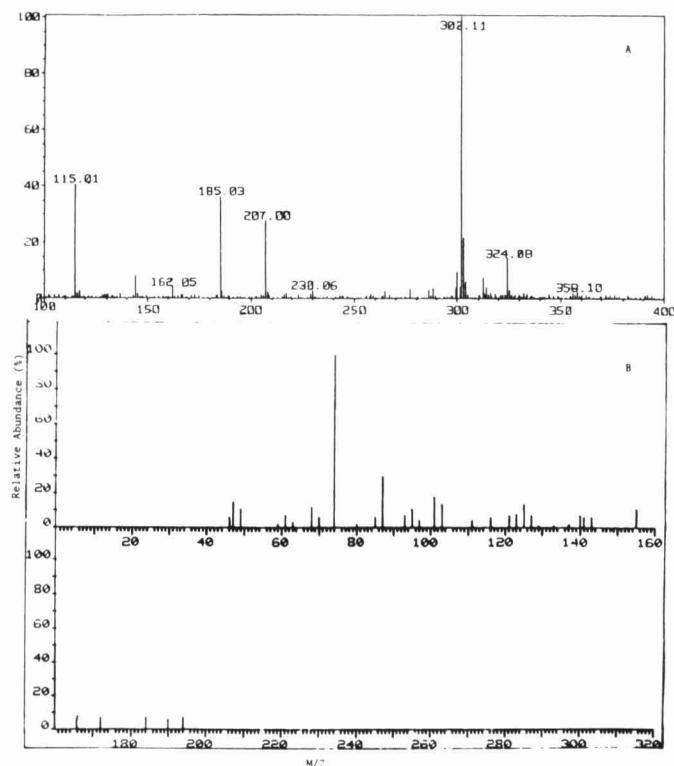


Fig. 6. Identification of MCAC from the urine of a 5-y-old control child. Retention time on HPLC: 36.8 min. A, Fast atom bombardment mass spectral analysis. Protonated mol wt = 302 (Na-adduct mol wt = 324) best fit a 9-carbon saturated AC (see Table 6). B, Gas-liquid chromatographic electron impact mass spectral analysis of the acyl moiety after hydrolysis: base peak 74 and fragment 87 indicate a methyl branch in position 2.

apparent renal threshold for FC was $\sim 25 \mu\text{mol/L}$ in our patient population. This value is lower than the extrapolated value ($46 \mu\text{mol/L}$) reported by Engel *et al.* (28) in normal adults receiving i.v. L-carnitine. Because all our children with low FC blood concentrations and low urinary FC excretion received PN and were in the younger age group, we cannot comment as to whether the apparent renal threshold for FC is influenced by PN or age. Although the kidney was capable of conserving FC when plasma levels were decreased, FC was always detected in the urine. This may reflect secretion of FC by the kidney (29).

Quantitation of individual urinary carnitine esters revealed that the excretion of short (branched)-chain AC was also sharply reduced in our patients in group 1, suggesting that they are reabsorbed by the kidney tubule to a similar extent as FC. This is in agreement with studies by Hokland and Bremer (30) using the perfused rat kidney. However, the excretion of other carnitine metabolites including MCAC was reduced to a much lesser extent in group 1 patients and was appreciable in all patient groups and controls. These AC made up a significantly higher percentage of TC excreted by patients in group 1 compared with group 2 or age-matched controls.

We were able to identify tentatively some of these compounds as unsaturated and/or branched medium-chain carnitine esters with a carbon chain of C-8 to C-10. Kerner *et al.* (22) have previously identified three medium-chain carnitine esters in urine from adults as 2-methyl- Δ^6 -octenoyl-, Δ^6 -octenoyl-, and 2-methyloctanoylcarnitine. The latter two compounds are probably identical to those found in our children.

The origin of these medium-chain carnitine esters, which are distinctly different from octanoyl- and hexanoylcarnitine excreted by patients with medium-chain acyl-CoA dehydrogenase deficiency (31), is unknown. They could be derived from exogenous or endogenous fats. The finding that some of them were excreted in all of our subjects independent of the type of nutrition

they received suggests that an endogenous origin is likely. This is supported by our recent finding of complex medium-chain carnitine esters in the urine of newborn infants collected before the onset of feedings (Schmidt-Sommerfeld E, unpublished data). It is also not known in which organs and cell organelles they are formed and whether they are transported through the blood stream. Nor is it known which carnitine acyltransferases are involved in their formation. Some of them may be secreted directly into urine by the kidney. Hokland and Bremer (30) found unidentified carnitine metabolites in rat urine after perfusion of the kidney with labeled carnitine. Judging from their R_f values on thin-layer chromatography, these metabolites could have been medium-chain carnitine esters.

It has been suggested that, in renal Fanconi syndrome (32, 33), organic acidurias (34), and primary disturbances of carnitine transport (35), carnitine deficiency results from carnitine loss in the urine. Apart from our two patients with renal Fanconi syndrome, this was not apparent in our hypocarnitinemic patients in group 1. Their kidneys were able to conserve FC and SCAC. However, they lost appreciable amounts of carnitine in the form of MCAC and other yet to be identified carnitine esters with the urine.

On the basis of these findings, one could speculate that carnitine has a role in the removal of presumably slowly metabolized acyl moieties from the body. Such a role has been suggested before for the elimination of fatty acid-like drugs such as valproic and pivalic acids, which are excreted as their respective carnitine esters (36, 37). This normal metabolic pathway proceeds despite hypocarnitinemia and contributes to a state of carnitine deficiency in young children who receive carnitine-free nutrition and in whom rates of endogenous carnitine biosynthesis may be inadequate. It remains to be established whether carnitine supplementation of PN is warranted in this situation.

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