Carnitine intake and excretion in neuromuscular diseases[1-3]

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ABSTRACT Free and total carnitine serum concentrations and urinary excretion were examined in patients with various neuromuscular diseases. On a measured, low carnitine diet and during fasting, the patients did not differ from controls. Carnitine excretion in patients (3.08 ± 1.87 μmol/kg/day) and controls (2.99 ± 1.12) exceeded carnitine intake (patients, 2.35 ± 0.94 μmol/kg/day; controls, 1.33 ± 0.70). Because of heterogeneity in the patient population, carnitine excretion was assessed according to creatinine excretion, chosen as an indicator of muscle mass. Those patients with daily creatinine excretion <1 g/day had significantly lower carnitine excretion (106 ± 47 versus 205 ± 95 μmol/day, $p < 0.05$), and there was a positive correlation between creatinine excretion and carnitine excretion ($r = 0.82$) and between muscle carnitine and carnitine excretion ($r = 0.67$). Urinary clearances for acylcarnitine were 10 to 20 times higher than those for free carnitine. Two patients with carnitine palmityl transferase deficiency were similar to the other patients, but the carnitine-deficient patient lost excessive carnitine during fasting, probably secondary to an elevated acylcarnitine fraction in the blood. *Am. J. Clin. Nutr.* 34: 2693–2698, 1981.

KEY WORDS Carnitine, neuromuscular diseases, muscle, carnitine deficiency, carnitine palmityl transferase deficiency

Introduction

Carnitine participates in the transfer of long-chain fatty acids across the mitochondrial membrane for oxidation. Because of its relatively high concentration in skeletal muscle, carnitine serum concentrations and urinary excretion have been widely investigated in a number of neuromuscular diseases. As documented in a series of reviews by Mitchell (1–3), most of these studies have neglected the influence of dietary intake on the data (4–7). Many studies have measured only free carnitine and not total carnitine (4, 6).

The goal of this study was to examine the influence of muscle disease on carnitine serum concentrations and excretion during a period of known, low carnitine intake. In order to place stress on homeostatic mechanisms that maintain carnitine concentrations, a brief fast was used in the protocol. In addition to a variety of neuromuscular diseases and normal controls, two disorders thought to affect carnitine metabolism, muscle carnitine deficiency (8) and carnitine palmityl transferase deficiency (CPT) (9), were studied. We also examined the effect of muscle mass, as estimated by urinary creatinine excretion (10), on carnitine excretion.

Methods

Subjects

Nineteen patients with various neuromuscular diseases (**Table 1**) and six adult volunteers (four males and two females) participated in the fasting and low carnitine diet studies. Diagnoses were established by clinical examination, electromyography, and laboratory evaluation, including muscle biopsy, histochemistry, and biochemical analysis where necessary. Four more adult volunteers (two males and two females) took part in the fasting portion of the protocol only. Informed consent was obtained from all subjects, and the study was approved by the Washington University Human Studies Committee.

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TABLE 1
Patients

Diagnosis (males)	Age
	yr
1. Myopathy, creatine kinase > 10000 mμ/ml (normal < 70), mild weakness. Muscle biopsy showed fiber degeneration and internal nuclei.	15
2. Myopathy, same picture as patient 1.	19
3. Myopathy, creatine kinase > 1000 mμ/ml, mild weakness. Muscle biopsy showed tubular aggregates.	19
4. Becker's muscular dystrophy	16
5. Becker's muscular dystrophy	14
6. Limb girdle dystrophy	44
7. Limb girdle dystrophy	37
8. Facioscapulohumeral dystrophy	18
9. Myotonic dystrophy	49
10. Spinal muscular atrophy	24
11. Carnitine palmityl transferase deficiency	22
12. Carnitine palmityl transferase deficiency	25
Diagnosis (females)	
13. Limb girdle dystrophy	58
14. Limb girdle dystrophy	50
15. Limb girdle dystrophy	28
16. Limb girdle dystrophy	27
17. Hereditary sensory and motor neuropathy	30
18. Muscle carnitine deficiency	30
19. Ragged red fiber disease	30

Protocol

All subjects consumed a meat-free diet for 2 days. After the evening meal at 1800 h of the 2nd day, they began a 38-h fast. After the fast the study continued for 5 days on a diet of known carnitine content. For carnitine data analysis on a meat-free diet, only the last 4 days of the study were used. Each subject was allowed to choose his own diet according to the defined guidelines. Mean caloric intake for the group was 36 ± 10 cal/kg/day (mean ± SD). For all subjects on the average, the diet contained 44 ± 5% fat, 42 ± 6% carbohydrate, and 14 ± 2% protein. No meat or meat products were used, and the diet contained dairy products. Protein intake was approximately 1 g/kg ideal body weight per day. The subjects ate the same three meals each day of the protocol and did not participate in sustained exercise during the study. One of the volunteers also consumed a normal, meat-containing diet during another 4-day period.

Biochemical analyses

Blood was collected for carnitine and free fatty acids at 0800 h of each day and at 4-h intervals during the fast. Total 24-h urine collections were made throughout the protocol for carnitine and creatinine. For each subject, an extra day's food allowance was prepared (including cooking), homogenized, weighed, and analyzed for total carnitine content. In 14 of the patients skeletal muscle was analyzed for total carnitine. Carnitine was assayed by the method of McGarry and Foster (11) except for the substitution of Hepes for Tris buffer (12). Separation of carnitine into long-chain, short-chain, and free fractions was done using perchloric acid extraction (13). Free carnitine was determined in samples before hydrolysis and total carnitine after alkaline hydrolysis at 30°C for 1 h. The difference between these two values is acylcarnitine, which is primarily acetylcarnitine in the acid soluble fraction. Free fatty acids were estimated by the colorimetric method of Novak (14). Creatinine was analyzed enzymatically as creatine after preincubating with creatininase (15).

Carnitine urinary clearances were calculated using the formula: clearance in ml of serum/min = Ux/Sx · (ml urine/min), where Ux = urine concentration and Sx = serum concentration. Ux was determined from specimens collected over 24-h periods, and Sx was the mean of the serum concentrations obtained over each collection period.

Statistics

Student's *t* tests were performed to determine levels of statistical significance. Linear regressions were determined with a Texas Instruments model 59 calculator, and the best fit was obtained from among linear, exponential, power, or logarithmic transformations of data.

Results

Table 2 shows the carnitine intake, excretion, and the mean of the 0800 h serum concentrations for patients and controls. Long-chain acylcarnitine carnitine was measured in many of the serum samples, but is not reported because of its consistently low concentration. The two groups differed significantly only in carnitine excretion per milligram creatinine. This difference was due to lower creatinine excretion in the patients and not to carnitine excretion. Overall, carnitine excretion exceeded carnitine intake. Among all the subjects there was a positive correlation (Fig. 1) between urinary creatinine and total urinary carnitine while on the low carnitine diet. Probably because of their higher creatinine excretion all of the controls fell above the regression line. In the 10 neuromuscular disease patients with daily urinary creatinine excretion less than 1 g, total carnitine excretion was significantly lower than in the controls (106 ± 47 versus 205 ± 95 μmol/day, mean ± SD, $p < 0.05$). In those patients with known muscle carnitine, total urinary carnitine also correlated with muscle carnitine concentrations **(Fig. 2)**.

Long-chain acylcarnitine was found to constitute less than 3% of the total carnitine intake and thus was not measured in most subjects. Fecal carnitine constituted less than 2% of the carnitine ingested (preliminary studies).

TABLE 2
Low carnitine diet

	Neuromuscular diseases (16)		Controls (10)	Carnitine deficiency (6)	CPT deficiency (2)
	mean ± SD				*mean*
Carnitine intake/kg body wt (μmol/kg/day)	2.35 ± 0.94	NS*	1.33 ± 0.70	3.60	1.67
Excretion/kg body wt (μmol/kg/day)	3.08 ± 1.87	NS	2.99 ± 1.12	2.43	5.15
Net excretion/kg body wt (excretion-intake) (μmol/kg/day)	1.02 ± 1.82	NS	1.39 ± 1.87	0	3.48
Excretion/cm height (μmol/cm/day)	1.18 ± 0.84	NS	1.17 ± 0.49	0.90	2.17
Excretion/mg urinary creatinine (μmol/mg/day)	0.269 ± 0.08	$p < 0.01$	0.141 ± 0.04	0.141†	0.226†
Percentage urine carnitine as acylcarnitine	67 ± 18	NS	70 ± 15	70	88
Free carnitine clearance (ml/min)	1.31 ± 1.19	NS	1.19 ± 0.69	0.78	2.00
Acylcarnitine clearance (ml/min)	20.4 ± 20.6	NS	24.6 ± 2.18	9.36	18.4
Total serum carnitine (μM)	48 ± 9	NS	45 ± 15	34	66
Percentage serum carnitine as acylcarnitine	13 ± 6	NS	12 ± 4	29†	12.5

* Not significantly different from controls.
† Outside 2 SD from subjects with neuromuscular diseases.

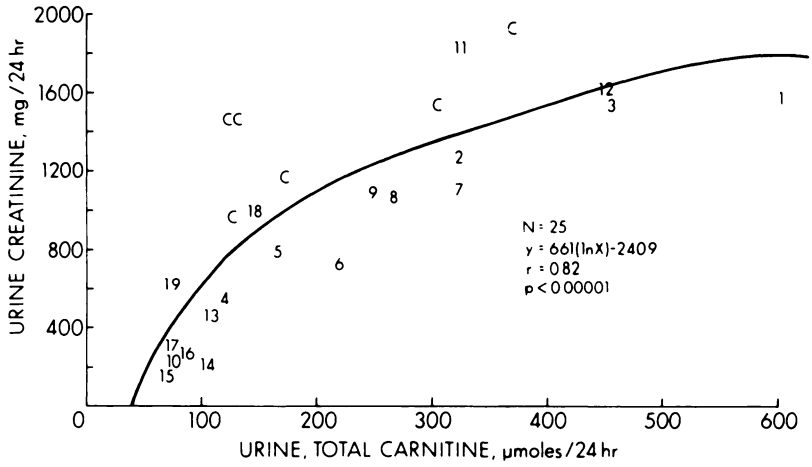

FIG. 1. Daily urinary creatinine excretion in patients and controls correlated positively with daily total carnitine excretion. Patients are designated by *number* (see Table 1) and controls by *C*.

In the subject studied on a meat containing diet, carnitine intake was 13.3 μmol/kg/day and excretion was 5.1 μmol/kg/day (range 4.3 to 6.7). In the same subject on the meat-free diet, carnitine intake was 0.7 μmol/kg/day, and excretion was 1.7 μmol/kg/day (range 1.6 to 1.9).

As compared to the low carnitine diet, overall carnitine excretion did not change during the brief fast (**Table 3**). Neuromuscular disease patients and controls differed significantly only in carnitine excretion per milligram creatinine. There was a positive correlation between serum free fatty acids and percentage serum carnitine as acylcarnitine at the end of the fast ($r = 0.49$, $p < 0.01$).

In the fed state both the carnitine deficient patient and the CPT deficient patients showed a lower carnitine excretion per milligram creatinine (outside 2 SD) than the other neuromuscular disease patients. The carnitine deficient patient had a higher percentage serum carnitine as acylcarnitine. During fasting the carnitine-deficient patient showed higher carnitine excretion per kilogram body weight and per centimeter height while also having a lower serum total carnitine.

Discussion

We found essentially no differences between the neuromuscular disease patients as

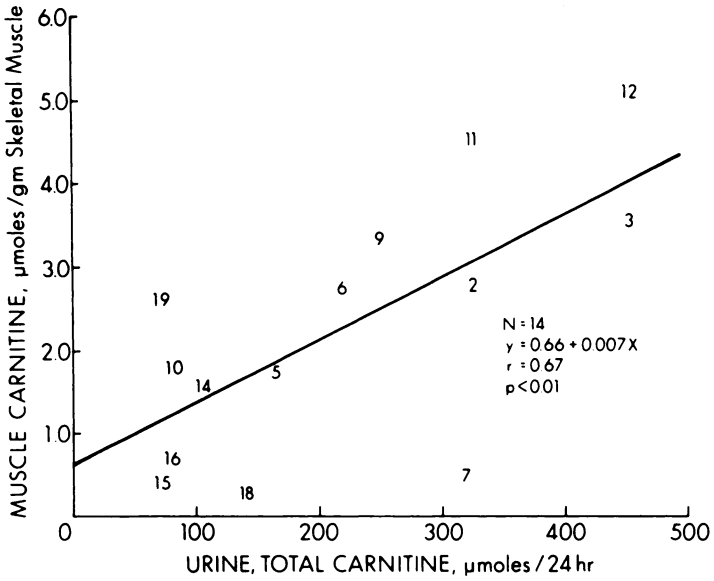

FIG. 2. Total muscle carnitine concentrations in 14 patients correlated positively with their daily total carnitine excretion.

TABLE 3
38-Hour fast

	Neuromuscular diseases (16)		Controls (10)	Carnitine deficiency (1)	CPT deficiency (2)
	mean ± SD				*mean*
Carnitine excretion/kg body wt (μmol/kg/day)	3.01 ± 1.68	NS*	3.03 ± 1.38	8.02†	2.32
Excretion/cm height (μmol/cm/day)	1.03 ± 0.49	NS	1.11 ± 1.38	2.95†	0.97
Excretion/mg urinary creatinine (μmol/mg/day)	0.320 ± 0.260	$p < 0.05$	0.144 ± 0.05	0.465	0.100
Percentage urine carnitine as acylcarnitine	85 ± 15	NS	91 ± 10	92	95
Total serum carnitine (μM)	49 ± 13	NS	45 ± 16	16†	68
Percentage serum carnitine as acylcarnitine	36 ± 15	NS	28 ± 12	63	27

* Not significantly different from controls.
† Outside 2 SD from subjects with neuromuscular diseases.

a group and the controls in regard to carnitine excretion or serum concentrations during the low carnitine diet. In the subject studied on both a meat-containing and meat-free diet, urinary carnitine excretion was lower and more stable without meat. This result is in contrast to that of Cederblad (16), who found that carnitine excretion did not change in the same subject on different diets. We chose to use a low carnitine diet for this study so that differences among the subjects would be less obscured by exogenous carnitine. In comparison to a meat-containing diet, all of the subjects' carnitine intakes during the study were quite low. Although probably not the case on a meat-containing diet, urinary carnitine excretion usually exceeded intake on the meat-free diet, underlining the role of endogenous production. Rudman et al. (17) also found that normal controls excreted more carnitine than that ingested on a low carnitine diet. The effect of carnitine synthesis on the data is unknown, but presumably this factor did not differ in controls and the group of heterogenous neuromuscular disease patients. Fecal excretion was minimal, but carnitine may have been degraded in the gut. Possible urinary metabolites of carnitine, such as β-methyl choline (18) and trimethylamine oxide (19), were also not examined.

Although the relationship is less well-defined for patients with muscle diseases, daily

urinary creatinine excretion has been used to estimate muscle mass (10). When carnitine excretion was plotted against urinary creatinine, choosen as representative of muscle mass, there was a positive correlation. In Figure 1, the regression line intersects the X-axis at 38 μmol/24 h, perhaps indicating a minimum excretion for carnitine irrespective of muscle mass. Several previous investigators have noted that males tended to have higher serum carnitine concentrations and urinary excretions than females (4, 7). In this study, because of the apparent importance of muscle mass as estimated by urinary creatinine excretion and the fact that some female patients had larger muscle mass than some males, the data were not segregated according to sex. Among those patients with daily urinary creatinine excretion less than 1 g/day, carnitine excretion was significantly lower than in controls. In those subjects where muscle carnitine was known, urinary carnitine was also positively related to muscle carnitine. These data suggest that urinary carnitine is positively related to the amount of skeletal muscle and the carnitine content of that muscle. This finding probably explains the discrepancy between the work of DiMauro and Rowland (6) and Maebashi et al. (5), where the latter group failed to control for body size. The former workers found no difference between muscular dystrophy patients' and controls' carnitine excretion, while the latter noted a significant difference. Neither of these studies was diet controlled.

Neuromuscular disease patients and controls also did not differ during the fast other than carnitine excretion per milligram urinary creatinine. Among the group as a whole, the brief fast did not bring about any change in carnitine excretion. Others have previously found an increase in excretion with fasting (7, 20), but the duration of these fasts was longer than in the present study.

Since there was reason to believe that carnitine metabolism might be specifically abnormal in carnitine deficiency and in CPT deficiency, we analyzed their data separately. While the CPT-deficient patients were much like the other patients, the carnitine-deficient patient tended to have a higher percentage serum carnitine as acylcarnitine, and a lower serum carnitine during fasting with higher excretion. These results indicate that this patient was losing excessive carnitine through the kidney during fasting. There may, however, be numerous causes for carnitine deficiency (21).

Short-chain acylcarnitine was the main excreted form of carnitine detected in this study. Calculated clearance rates of acylcarnitine were approximately 10 to 20 times that for free carnitine. Acylcarnitine in the serum rose during fasting along with fatty acids, perhaps making some subjects more prone to carnitine loss during fasting. This chain of events seemed to be the case for the carnitine-deficient patient. More information is needed about the specifics of the renal excretion mechanism for carnitine, but the data suggest that high concentrations of acylcarnitine in the blood would make the individual more subject to renal carnitine loss. Although this hypothesis was not tested in the present study, elevation of free fatty acids, as in disturbances of lipid metabolism, could lead to increased carnitine loss by increasing the acylcarnitine fraction.

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