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Evaluation of the Effect of Intravenous *L*-Carnitine Therapy on Function, Structure and Fatty Acid Metabolism of Skeletal Muscle in Patients Receiving Chronic Hemodialysis[1]

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Abstract. Chronic hemodialysis (HD) leads to significant losses of carnitine from plasma and muscle. Because *L*-carnitine is important in the production of energy from fatty acid oxidation (FAO) in muscle, we examined the role of carnitine replacement by administering therapeutic doses of intravenous carnitine to 14 male patients receiving HD. Placebo or carnitine was given 2 g i.v. 3 times weekly for 6 months in a double-blind manner. To evaluate long-term toxicity of carnitine, all patients subsequently received 1 g i.v. carnitine for 10 months. Patients were rated for muscle strength each week. After 6 months, definite improvement in strength occurred in 4 of 7 carnitine-treated patients and in none of 7 controls. During the subsequent 10 months of carnitine administration, no adverse effects were noted and muscle strength improved in 9 of 14 patients. Muscle biopsy was performed in 13 patients before and after the first 6 months of treatment and in 6 healthy controls. FAO and carnitine were measured in each muscle biopsy. FAO was significantly lower in both carnitine- and placebo-treated HD patients compared to healthy controls. Although carnitine therapy increased the muscle concentration of carnitine 3-fold in muscle of HD patients, muscle FAO did not increase significantly and never reached the level of healthy controls. Muscle histopathology and ultrastructure were not specific for HD myopathy. Carnitine may be useful in treating some patients with muscle weakness related to HD.

Introduction

L-Carnitine is a quaternary amine which plays an important role in β-oxidation of fatty acids (FAO) [1, 2]. Carnitine is an absolute requirement for the transport of long-chain fatty acids from cytoplasm into the matrix of mitochondria, the site of FAO [3]. Carnitine is essential in those tissues which preferentially use fatty acids for their energy needs, especially skeletal muscle and myocardium [4, 5]. Because the kidney is a major site of carnitine biosynthesis [6, 7], carnitine production may be reduced in end-stage renal disease. Carnitine is lost from plasma during hemodialysis (HD) and is lower in skeletal muscle of patients with end-stage renal disease than in healthy controls [8–10].

Carnitine in doses of 3 g/day may be associated with increased platelet aggregation and plasma triglyceride levels [11]. Therefore, in this study, only 2 g 3 times weekly was given followed by 1 g 3 times weekly after 6 months. Since *D,L*-carnitine may provoke a myasthenic-like syndrome [12–14], only *L*-carnitine was used in this study. Because depletion of plasma and muscle carnitine is much greater with HD than with peritoneal dialysis, and because skeletal muscle carnitine deficiency is more severe with long-term HD than with peritoneal dialysis [9], only patients receiving HD were selected. Although blood and muscle levels of carnitine have been shown to

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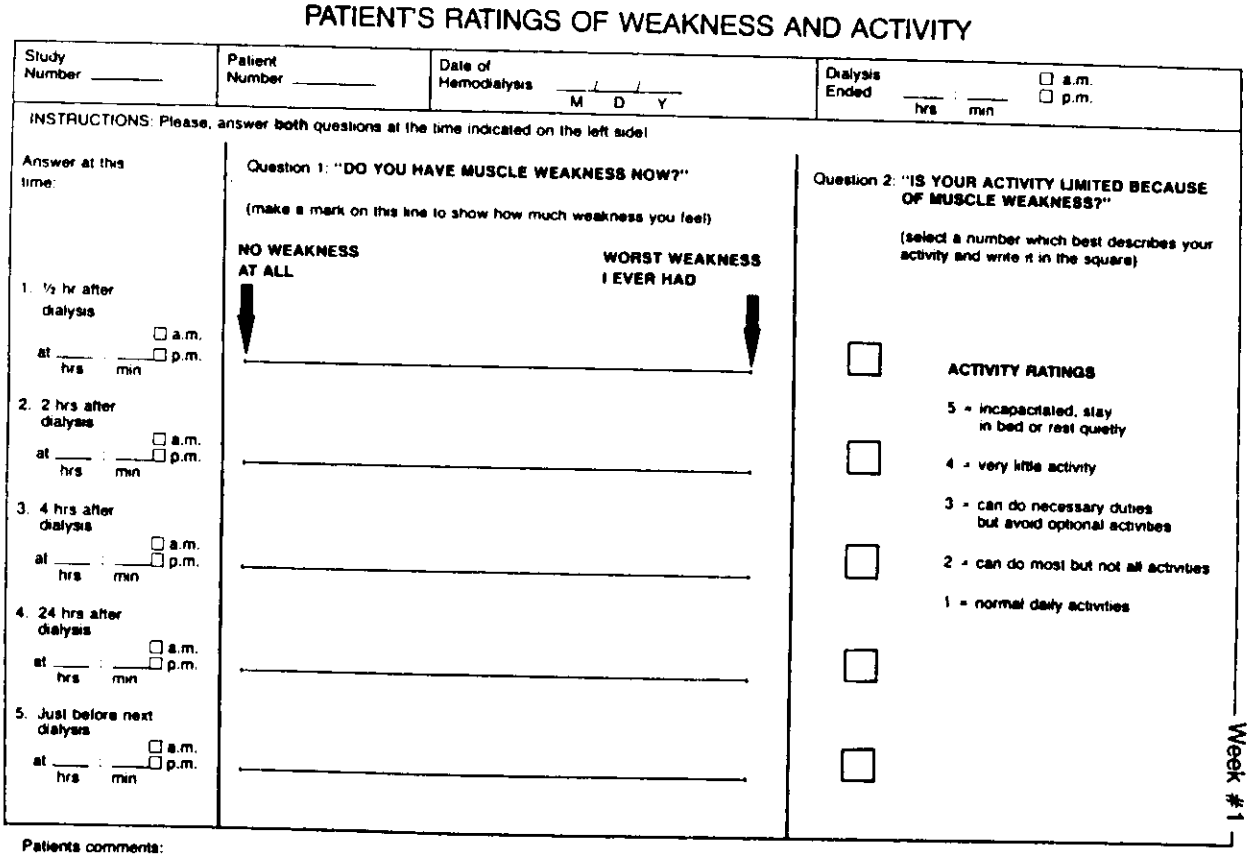

Fig. 1. Rating scales for muscle strength (left) and for activity (right) in hemodialysis patients during the carnitine study. All assessments of patient activity were done by one of the authors (J.G.) in conducted interviews.

increase after carnitine treatment in HD patients, histologic improvement may not be seen on muscle biopsy [15]. Therefore, an assay for muscle FAO was also performed. To evaluate the clinical benefits of carnitine therapy on dialysis patients with myopathy [16, 17], we also assessed muscle weakness and level of daily motor activity.

Methods

Fourteen patients were evaluated in this double-blind, parallel, placebo-controlled trial. Criteria for patient selection included presence of muscle weakness, long-term HD, and stable medical status. Approval from the Committee for the Protection of Human Subjects, Vanderbilt University, and written informed consent from each patient were obtained. Placebo or carnitine, supplied by A.H. Robins Co., Richmond, VA, was given intravenously at 2 g 3 times a week following each HD for 6 months. To evaluate the long-term safety of carnitine, all patients were subsequently given no carnitine for 1 month, and then all received carnitine 1 g i.v. 3 times a week for an additional 10 months. This lower dose was chosen because 2 g i.v. 3 times a week was found to produce twice normal levels of muscle carnitine in this study. The code for the placebo-controlled phase of the study was not broken until the biochemical and histologic data were compiled for both phases of the study, but to insure safety, reports were given to the investigators that some unidentified patient's plasma carnitines were several times normal. Complete blood counts and serum studies for electrolytes, calcium, phosphate, albumin, GOT, LDH, BUN, and creatinine were done by automated methods at monthly intervals. Electrocardiograms (ECG) were performed monthly by Phone-a-gram System, provided by America's ECG Network, San Francisco, Calif., USA.

Muscle biopsies were taken from the vastus lateralis by a staff surgeon in 13 patients both before and after the initial 6 months of therapy. One of the 14 participants refused muscle biopsy. Biopsies were taken in a similar manner from 6 healthy, untreated volunteer controls who had no known illnesses. Only 4 patients, all of whom were in the initial placebo group, agreed to a third muscle biopsy at the end of 10 months of carnitine. Muscle was analyzed for carnitine and FAO levels. Histochemical stains were graded and electron microscopy was performed.

Because removal of ascorbate and vitamin B_6 by HD may also play a role in decreased carnitine biosynthesis since these vitamins are required along with iron in carnitine production [18], all of our patients received supplemental ascorbate, vitamin B_6 and iron. All patients also received testosterone enanthate, 200 mg i.m. every 2–4

Table 1. Comparative listing of raw data for muscle biopsy grade, muscle carnitine level (n*M*/mg non-collagen protein, NCP, and patient activity scores

Patient	Muscle biopsy grade			Muscle carnitine nM/mg NCP		Patient activity score		
	baseline	6 months	10 months	baseline	6 months	baseline	6 months	change
Placebo group								
AW	IV	IV	IV	16.6	23.7	4.1	2.9	1.2
BH	Va	IV	IV	24.5	31.9	2.9	3.0	-.1
CC	II	IV	III	17.9	14.7	2.8	1.4	1.4
OJ	IV	III	IV	15.1	12.6	3.8	3.1	.7
WE	IV	IV		12.6	30.3	2.0	1.9	.1
ME	V	IV		12.6	25.1	5.0	4.9	.1
CH	no biopsy					4.0	4.4	-.4
Mean				18.3	22.0	3.5	3.1	.48
SD				5.6	7.8	1.0	1.24	.6
Carnitine group								
BW	IV	IV		13.6	57.1	4.0	3.3	.7
BF	II	II		12.6	53.7	3.8	2.0	1.8
CW	Vb	Vb		16.9	42.0	4.0	1.5	2.5
HJ	IV	III		15.5	41.0	3.6	1.7	1.9
JW	I	IV		20.3	82.9	3.4	1.6	1.8
WH	IV	IV		22.0	53.5	2.2	2.3	.1
SJ	Va	IV		19.7	37.7	2.5	1.7	.8
Mean				17.2	52.6	3.4	2.0	1.3
SD				3.56	15.3	0.73	0.65	0.85

weeks, to stimulate red blood cell production. Patients were dialyzed in the hospital in a uniform manner using an acetate dialysate with 135 mEq/l Na^+. Hypotension was treated with saline infusion until symptoms resolved and the patient's blood pressure normalized.

Biochemical Studies

FAO was measured using 1-^{14}C-palmitic acid, modified Krebs-Henseleit phosphate buffer, ATP, CoA and muscle homogenate [19]. Muscle homogenates were prepared using sucrose to protect mitochondria [20, 21]. $^{14}CO_2$ was collected and measured in a liquid scintillation counter and DPM calculated. Carnitine-stimulated FAO was measured by adding exogenous carnitine to a final concentration of 0.5 m*M*. Protein determination was carried out according to the microbiuret method of Goa [22]. Sample testing was carried out in duplicate.

Total muscle carnitine was measured before the treatment period, after the initial 6 months of therapy (n = 13) and after the subsequent 10 months of therapy (n = 4) using the method of Cederblad and Lindstedt [23]. Biopsied muscle was homogenized in water followed by alkaline hydrolysis of acetyl carnitine and acyl carnitine. Extraction of muscle was performed with perchloric acid. The supernatant, containing free, short-chain, and long-chain carnitine esters, was then used. Samples were incubated in ^{14}C-acetyl CoA and carnitine acyl transferase. The radioactive acetyl carnitine was separated using a Dowex 2 × 8 column and counted [23]. Total carnitine was expressed as nanomoles per milligram of noncollagen protein in muscle [24]. Plasma carnitine was measured by the same method. Plasma aluminum was measured by flameless atomic absorption spectrometry, and plasma immunoreactive parathyroid hormone was determined by radioimmunoassay for the N-terminal end using a kit provided by Nichols Institute, San Juan Capistrano, Calif., USA.

Muscle Architecture

Histochemical staining of muscle was performed according to the method of Dubowitz [25]. Hematoxylin and eosin, modified Gomori trichrome, NADPH-tetrazolium reductase, succinic dehydrogenase, actomyosin ATPase, periodic acid-Schiff, oil red 0, acid phosphatase and myophosphorylase stains were completed on each muscle. Biopsies were reviewed in a blind manner and were graded according to the following criteria: 1 = normal; 2 = increased variation in fiber sizes and type II fiber atrophy; 3 = features of grade 2 plus increased central nuclei (> 3%) and increased connective tissue; 4 = features of grades 2 and 3 plus histochemical alteration of the intramyofibrillary network including one or more of the following: moth-eaten fibers, whorled fibers, non-specific interruption of the intramyofibrillary network, or target fibers; 5 = necrosis and phagocytosis either mild, 5a, or severe, 5b.

For electron microscopy, small (2–3 mm diameter) cylinders of muscle were fixed in phosphate-buffered 1% osmium tetroxide, dehydrated in a series of graduated ethanol solutions, transferred to propylene oxide and embedded in Epon 812. Sections were post-stained with uranyl acetate and lead citrate and examined in a Jeol 100 CX electron microscope. X-ray microanalysis was performed using a TN 2000 energy dispersive detector and analyzer [26] (Tracor

Northern, Madison, Wisc.). The specimen was placed in a graphite holder, tilted 30°, and analyzed at an accelerating voltage of 60 kV. A Jeol JEM-100 CX Temscan scanning electron microscopy unit was used on spot mode to selectively analyze myofibrillar inclusions.

Patient Assessment

Carnitine mediates lipid metabolism so that improvement in performance is more likely to be reflected by resistance to fatigue over hours. Therefore muscle performance could not be assessed on the basis of the usual standardized measures typified by the Medical Research Council [27] defining strength graded 1–5. Hence, a scale was designed to rate overall activity over the course of a day.

Patient activity was assessed once weekly according to criteria shown in figure 1. Limitation of daily motor activity was rated by a member of the research team who interviewed the patients weekly (JG). Scores were given on the following scale: 1 = normal daily activity; 2 = can do most but not all activities; 3 = can do necessary duties but avoids optional activities; 4 = limited to very basic activity (i.e. personal toilet and transfer to chair); 5 = incapacitated and bed-bound. Data were gathered from the first 2 weeks of the study, prior to treatment, and the last 2 weeks of the 6-month placebo-controlled phase of the study. Daily scores for 2 weeks before and then after treatment were recorded as integers 1–5 and averaged.

Statistics

Statistical analysis of data included paired and unpaired Student's t tests for parametric data. For nonparametric data, Wilcoxon signed rank, Wilcoxon rank sum and Spearman's rank correlation tests were used where indicated below.

Results

In table 1, all raw data are tabulated. By the Wilcoxon signed rank test, there was no difference in patient activity at the beginning of the study with mean grades of 3.5 for those on carnitine and 3.4 for those on placebo. A lower score indicates better muscle activity. Following 6 months of treatment, mean daily activity score was 3.1 for the placebo group and 2.0 for the carnitine group. The mean gain of the placebo group was 0.48, whereas the carnitine group had a greater mean gain at 1.3. By the Wilcoxon signed rank test, significance was closely missed. There was, however, a cluster of clear responders, 4 of the 7, in the carnitine group who improved daily activity by 2 or nearly 2 grades (fig. 2). Neither the other 3 in the carnitine group nor any of the 7 in the placebo group improved to that degree. This suggests that there are two groups of HD patients, one that is improved by carnitine therapy and one that is not. It is also possible that significance would be reached if patient numbers were greater.

Each HD treatment produced approximately a 50% decline in plasma carnitine levels, dropping from a mean of 70 ± 20 to a mean of 33 ± 11 n*M*/ml in patients on placebo and from a mean of 894 ± 15 to a mean of 375 ± 134 n*M*/ml ($p < 0.001$) in those receiving carnitine. Four weeks following cessation of the initial 6 months of therapy, patients who had received carnitine had sustained plasma carnitine levels 3-fold higher (185 ± 96.8 n*M*/ml) than those who had received placebo (60 ± 17.5 n*M*/ml) ($p < 0.01$). Following the subsequent 10 months of carnitine therapy (1 g i.v. 3 times a week), plasma carnitine was 857 ± 294 n*M*/ml in patients who had initially received carnitine and 493 ± 115 n*M*/ml in patients who had originally received placebo ($p < 0.01$). Plasma carnitine levels differed between these two groups prior to the final 10 months of therapy: 185 ± 96 n*M*/ml for

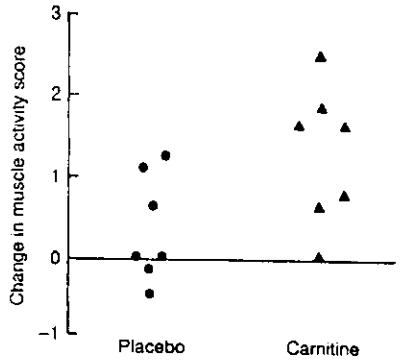

Fig. 2. Results of muscle strength rating after 6 months of treatment. Results are tabulated as the change in muscle strength. Note the clustering of responders in the carnitine group who improved by 2 grades or more.

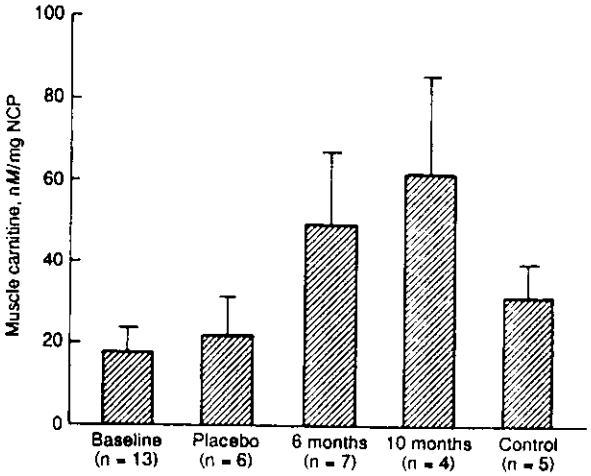

Fig. 3. Muscle carnitine concentrations are shown for patients at baseline, after 6 months of placebo, after 6 months of carnitine, and following 10 more months of carnitine, and for controls. Numbers of patients are shown in parentheses. The bars represent the means ± SD. At 10 months only 4 patients, all from the initial placebo group, agreed to a third muscle biopsy.

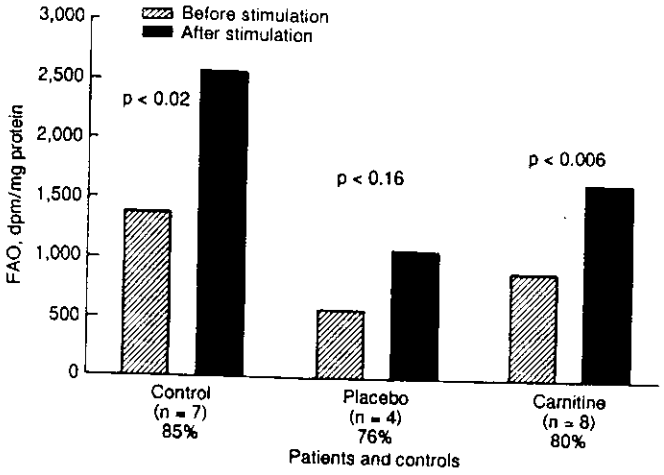

Fig. 4. Comparison of in vitro muscle FAO in three groups before and after carnitine stimulation. Percent stimulation is shown beneath each set of treatments and was similar in all three groups. p values are given for differences between unstimulated and stimulated values.

Fig. 5. Electron micrograph of degenerating muscle fiber. The fiber on the left shows dissolution of sarcomere structure. A large subsarcolemmal pool of glycogen is present.

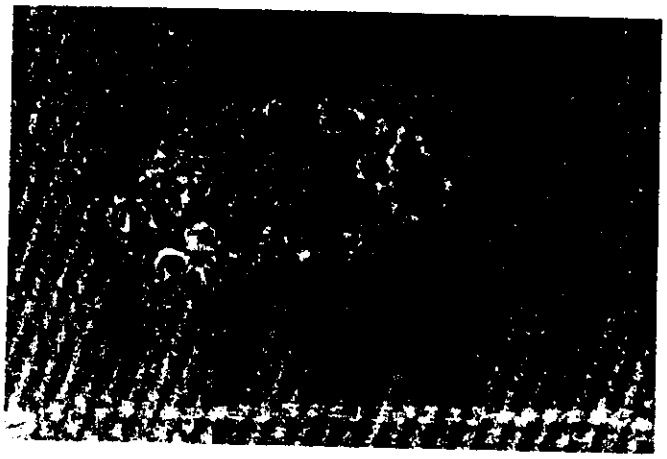

Fig. 6. Electron micrograph of muscle fiber with central necrosis. The vacuole borders abruptly on normal-appearing muscle sarcoplasm, without an intervening zone of filamentous and mitochondrial degeneration. × 4,000.

carnitine-treated versus 58 ± 24 n*M*/ml for placebo-treated ($p < 0.04$). Tissue saturation is the likely source for the continuing high plasma levels of carnitine, and it is probable that the initial carnitine group had maintained tissue saturation once carnitine was begun.

Skeletal muscle carnitine concentrations are shown in figure 3. Prior to therapy, HD patients had significantly lower values than controls: 17.8 ± 4.6 n*M*/mg non collagen protein (NCP) for HD patients compared with 31.2 ± 7.4 n*M*/mg NCP for healthy controls ($p < 0.001$). Patients who received carnitine for the 10-month second phase of the study had increased muscle carnitine, 62 ± 23.9 n*M*/mg NCP which was three time the baseline value and twice that of healthy controls ($p < 0.04$). It is notable that both muscle carnitine and level of patient activity increased in the carnitine group compared to placebo.

FAO in muscle homogenates was significantly higher for healthy controls compared to the patients on placebo ($1{,}487 \pm 267$ vs. 638 ± 285 dpm/mg protein; $p < 0.003$) (fig. 4). Although muscle FAO for the patients who received carnitine was greater than for patients on placebo (906 ± 294 vs. 639 ± 285 dpm/mg), the difference was not statistically significant. Carnitine-stimulated FAO was determined by adding exogenous carnitine to the incubation mixture and this activity was compared for healthy controls, patients on placebo, and patients who received carnitine (fig. 4). The percent stimulation of FAO by exogenous carnitine was similar for all three groups.

Microscopic examination of each muscle biopsy was graded as to the severity of pathologic and histochemical changes. Biopsies showed a spectrum of changes ranging from histologically and ultrastructurally normal to biopsies with numerous atrophic, degenerating or necrotic fibers. Degenerating fibers showed dissolution of myoplasmic filaments and loss of filaments with pallor of sarcoplasmic staining. Electron microscopy of these fibers showed that some contained prominent, irregular bodies with contents of heterogeneous electron density (fig. 5). X-ray microanalysis of these structures showed only lead and osmium attributable to specimen fixation and staining. This technique is capable of detecting all elements in the specimen heavier than fluorine (atomic number 9) [27]. In the more severe cases, an unusual form of central fiber degeneration was seen (fig. 6). These

fibers contained central vacuoles with glycogen, lipid, degenerating membranous whorls and other cytoplasmic debris. These vacuoles bordered abruptly on adjacent, normal myoplasm.

Although excess content of neutral lipid has been described in cases of carnitine deficiency, this abnormality is difficult to quantify by either light or electron microscopy. We did not find that muscle histology was useful in diagnosing a carnitine-deficient state. Muscle biopsies did not show any consistent changes following 6 months or 10 months of carnitine therapy in either group of patients. The sole value of muscle biopsy in the evaluation of weakness in uremic patients is to exclude other possible causes of myopathy. Carnitine deficiency cannot be diagnosed without biochemical analysis. In our study, the severity of changes on biopsy did not correlate with the degree of weakness nor activity limitation using Spearman's rank correlation test. In fact, one control patient, who was thought to be healthy on clinical examination, actually had myopathic changes on muscle histology. Despite this, muscle FAO and muscle carnitine levels for this individual were similar to those of other healthy controls.

Patients who had high plasma aluminum levels had somewhat lower muscle FAO activity than did patients with normal serum aluminum levels (631 ± 290 vs. 1,079 ± 497 dpm/mg protein) but not significantly so. Serum PTH levels had no relation to muscle FAO in our patients. Long-term *L*-carnitine therapy produced no changes in other serum chemistries, hematologic values or ECG's.

Discussion

Whether carnitine is an essential nutrient in man has recently been questioned and discussed [28, 29]. Because hemodialysis membranes are very permeable to compounds with a molecular weight under 300, dialysis patients may be at special risk of carnitine deficiency. The small molecular weights of carnitine (161), of its essential amino acid precursor lysine (146), and of the co-factors ascorbate (176) and pyridoxine (205) may predispose dialysis patients to carnitine deficiency [8–10]. The renal diet consists of 45 g of protein of which 3 g is lysine. The combination of marginal dietary carnitine caused by low-protein diets and reduced carnitine biosynthesis may not be sufficient to meet the requirements of dialysis patients who have enhanced losses of these small-molecular-weight nutrients through the dialysis membrane [8, 34]. It appears that tight regulation of plasma carnitine is at the expense of tissue stores, especially muscle, in this group of patients.

Although one short-term (6 week) study did not find obvious deficiency of carnitine in the muscle of hemodialysis patients [30], our results demonstrated that muscle carnitine concentrations in HD patients were half those of healthy controls. Although a previous study [10] had shown that 6 g/day of oral *D,L*-carnitine did not increase muscle levels of this nutrient, our HD patients responded to 2 g i.v. thrice weekly of *L*-carnitine by tripling their muscle carnitine concentrations. Plasma carnitine levels in these patients were still twice normal after carnitine had been discontinued for 1 month, demonstrating a marked capacity for tissue storage of supplemented carnitine in HD patients.

This is the first report to our knowledge, of FAO determinations in skeletal muscle of uremic patients. Tissue FAO may be an indirect measure of the presence of carnitine in otherwise healthy individuals, but in our HD patients, FAO levels did not correlate with muscle carnitine levels. FAO could be stimulated by adding carnitine in vitro both in healthy controls and dialysis subjects. Patients who showed improvement in muscle strength during this study had greater baseline and carnitine-stimulated FAO activity in muscle samples taken following 6 months of carnitine than did patients not showing improvement in strength. Despite muscle carnitine levels that were twice those of healthy control subjects (fig. 3), muscle FAO activity in carnitine-treated HD patients was still significantly less than in controls at baseline and following in vitro stimulation by carnitine (fig. 4). Although carnitine must be important in muscle function, these results demonstrate that even supraphysiologic concentrations of carnitine do not restore uremic skeletal muscle to a normal state.

Improvement in motor performance is the clinical objective for administering carnitine therapy. During the first 6 months of treatment, there was a clear clustering in the carnitine group where 4 of 7 patients improved by 2 grades in their muscle activity scores. Furthermore, after 10 months of carnitine therapy, 9 of 14 patients improved by at least one grade compared with initial values. These results suggest that there are two subgroups of HD patients with muscle weakness: one group in which carnitine deficiency constitutes a major cause for the weakness, and a second group in which carnitine deficiency is not a major cause of muscle weakness. Muscle weakness in these latter patients may be attributable to other

metabolic abnormalities involving utilization of fatty acids, to mitochondrial membrane dysfunction or to other factors.

We also looked at histologic changes in muscle before and after prolonged carnitine administration. The most common microscopic abnormality described in uremic muscle is type II fiber atrophy [31, 32], and this was found in all of our patients. In our study, 5 of 13 patients (38%) had muscle necrosis on biopsy, a figure which agrees with a previous study of HD patients [33]. Numerous lipid droplets were found in some biopsies, as has been reported in carnitine deficiency [34], although carnitine deficiency may be present when excess neutral lipid staining is not recognized on histology. Additionally, muscle degeneration due to any cause may result in accumulation of neutral lipid in some muscle fibers while other fibers are depleted of lipid stores. This is presumably due to defects in metabolic systems, probably involving mitochondria, which utilize lipid for energy production. Lipid storage in muscle is also influenced by the state of nutrition and physical exertion at the time of the biopsy. In none of our patients, except those with overt necrosis, could excess neutral lipid, as seen on oil red 0 staining and by electron microscopy, be determined with certainty. Biochemical analysis is essential in determining abnormalities of lipid metabolism since stored neutral lipids are difficult to quantify morphologically.

Ultrastructural examination of degenerating and atrophic fibers showed filamentous dissolution and disappearance similar to changes reported by Bundschu and Scholte [35] for muscle biopsies from patients with chronic renal failure. Some of these atrophic fibers contained accumulations of heterogeneous, irregular bodies with the ultrastructural appearance of tertiary lysosomes (fig. 5). Bregman et al. [36] described similar inclusions in muscle fibers of chronic hemodialysis patients with iron overload myopathy and found histochemically demonstrable iron in these inclusions. However, in our patients, no iron was detectable in these inclusions by energy-dispersive X-ray microanalysis, a technique much more sensitive than histochemical stains [27]. In the more severe cases, there was an unusual form of central muscle degeneration with membranous whorls, glycogen, and cytoplasmic debris (fig. 6). These structures resembled the central autophagic vacuoles seen in target fibers, but lacked the margin of degenerating muscle filaments and mitochondria found in target fibers [37] usually associated with denervation. Membranous whorls and glycogen-containing vacuoles have been described in muscle biopsies from dialysis patients [38], but these were isolated subsarcolemmal structures in contrast to the large, central, heterogeneous vacuoles seen here. Because there are no specific histologic and electron-microscopic changes in muscle that are diagnostic of carnitine deficiency, biochemical confirmation is necessary.

In conclusion, long-term supplementation of chronic HD patients with intravenous *L*-carnitine, 1–2 g after each dialysis, increased plasma and muscle carnitine levels to supranormal values in all patients. Nine of 14 treated subjects noted increased muscle strength. Muscle FAO activity was improved in 6 of these 9 patients. *L*-carnitine administration at this dosage resulted in no noticeable adverse side effects. A therapeutic effect of carnitine on myocardial ischemia [39–41], ventricular arrhythmias [42], or anemia [43] was not demonstrated in our study by routine hematologic and ECG testing. Parenteral *L*-carnitine supplementation may improve muscle strength and function in a subgroup of HD patients with muscle weakness.

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References

1 Fritz IB: The effects of muscle extracts on the oxidation of palmitic acid by liver slices and homogenates. Acta Physiol Scand 1953;34:367–385.
2 Fritz IB: Action of carnitine on long chain fatty acid oxidation by liver. Am J Physiol 1959;197:297–304.
3 Fritz IB: Advances in Lipid Research. New York, Academic Press, 1963.
4 Engel AG, Angelini C: Carnitine deficiency of human skeletal muscle with associated lipid storage myopathy: A new syndrome. Science 1973;179:899–902.
5 Borum PR: Clinical Aspects of Human Carnitine Deficiency. New York, Pergamon Books, 1986.
6 Carter AL, Frenkel R: The role of the kidney in the biosynthesis of carnitine in the rat. J Biol Chem 1979;254:10670–10674.
7 Rebouche CJ: Sites and regulation of carnitine biosynthesis in mammals. Fred Proc 1982;41:2848–2852.
8 Leschke M, Rumpf KW, Eisenhauer T, Cristoph I, Fuchs C, Becker K, Kisthe U, Sheler F: Quantitative assessment of carnitine loss during hemodialysis and hemofiltration. Kidney Int 1983;24:S143–S146.
9 Moorthy AV, Rosenblum M, Rajaram R, Shug AL: A compar-

ison of plasma and muscle carnitine levels in patients on peritoneal or hemodialysis for chronic renal failure. Am J Nephrol 1983;3:205–208.
10 Mingardi G, Bizzi A, Cini M, Licini R, Mecca G, Garattini S: Carnitine balance in hemodialyzed patients. Clin Nephrol 1980;13:269–270.
11 Weschler A, Arivam M, Levin M, Better OS, Brook JG: High dose of *L*-carnitine increases platelet aggregation and plasma triglyceride levels in uremic patients on hemodialysis. Nephron 1984;38:120–124.
12 Bazzato G, Mezzina C, Ciman M, Guarnieri G: Myasthenia-like syndrome associated with carnitine in patients on long-term hemodialysis (letter). Lancet 1979;i:1041–1042.
13 Bazzato G, Coli U, Landini S, Mezzina C, Ciman M: Myasthenia-like syndrome after *D,L*- but not *L*-carnitine (letter). Lancet 1981;i:1209.
14 Rossini PM, Marchionno L, Gambi D, Pirchio M, Del Rosso G, Albertazzi A: EMG changes in chronically dialyzed uremic subjects undergoing *D,L*-carnitine treatment. Ital J Neurol Sci 1981;2:255–262.
15 Savica V, Bellinghieri G, Di Stefano C, Corvaja E, Consolo F, Corsi M, Maccari F, Spagnoli LG, Villaschi S, Palmieri G: Plasma and muscle carnitine levels in hemodialysis patients with morphological-ultrastructural examination of muscle samples. Nephron 1983;35:232–236.
16 Bellinghieri G, Savica V, Mallamace A, Di Stefano C, Consolo F, Spagnoli LG, Villaschi S, Palmieri G, Corsi M, Maccari F: Correlation between increased serum and tissue *L*-carnitine levels and improved muscle symptoms in hemodialyzed patients. Am J Clin Nutr 1983;38:523–531.
17 Casciani CU, Caruso U, Cravotto E, Corsi M, Maccari F: Beneficial effects of *L*-carnitine in post-dialysis syndrome. Curr Ther Res 1982;32:116–127.
18 Broquist HP, Borum PR: Carnitine biosynthesis, Nutritional implications, in Draper HH (ed): Advances in Nutritional Research. New York, Plenum Press, 1982, pp 181–204.
19 Mannaerb GP, Debeer LJ, Thomas J, De Schepper PJ: Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate-treated rats. J Biol Chem 1979;254:4585–4595.
20 Fritz IB, Schultz SK, Srere PA: Properties of partially purified carnitine acetyltransferase. J Biol Chem 1963;238:2509–2517.
21 McGarry JD, Mannaerts GP, Foster DW: A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. J Clin Invest 1977;60:265–270.
22 Goa J: A microbiuret method for protein determination. Scand J Clin Lab Invest 1953;5:218–222.
23 Cederblad G, Lindstedt S: A method for the determination of carnitine in the picomole range. Clin Chim Acta 1972;37:235–243.
24 Borum PR, Broquist HP, Roelofs RI: Muscle carnitine in neuromuscular disease. J Neurol Sci 1977;34:279–286.
25 Dubowitz V: Muscle Biopsy: A Practical Approach, ed 2. Philadelphia, Baillière-Tindall, 1985, pp 3–220.
26 Hutchinson TE: Determination of subcellular elemental concentration through ultrahigh resolution electron microprobe analysis. Int Rev Cytol 1979;58:115–158.
27 Medical Research Council of the United Kingdom: Aids to the Examination of the Peripheral Nervous System. London, Pendragon House, 1978.
28 Rebouche CJ: Is carnitine an essential nutrient for humans? J Nutr 1986;116:704–706.
29 Borum PR, Bennett SG: Carnitine as an essential nutrient. J Am Coll Nutr 1986;5:177–182.
30 Fagher B, Cederblad G, Eriksson M, Monti M, Moritz U, Nilsson-Ehle P, Thysell H: *L*-Carnitine and haemodialysis: Double-blind study on muscle function and metabolism and peripheral nerve function. Scand J Clin Lab Invest 1985;45:169–178.
31 Lazaro RP, Kirshner HS: Proximal muscle weakness in uremia: Case reports and review of the literature. Arch Neurol 1980;37:555–558.
32 Bautista J, Gil-Necija E, Castilla J, Chinchom I, Rafel E: Dialysis myopathy: Report of 13 cases. Acta Neuropathol (Berl) 1983;61:71–75.
33 Ahonen RE: Light microscopic study of striated muscle in uremia. Acta Neuropathol (Berl) 1980;49:51–55.
34 Bertoli M, Battistella PA, Vergani L, Naso A, Gasparotto MC, Romagnoli GF, Angelini C: Carnitine deficiency induced during hemodialysis and hyperlipidemia: Effect of replacement therapy. Am J Clin Nutr 1981;34:1496–1500.
35 Bundschu HD, Scholte W: Elektronenmikroskopische Untersuchung der Skelettmuskulatur bei terminaler Niereninsuffizienz. J Neurol Sci 1974;23:243–254.
36 Bregman H, Gelfand MC, Winchester JF, Manz HJ, Knepshield JH, Schreiner GE: Iron-overload-associated myopathy in patients on maintenance hemodialysis: A histocompatibility-linked disorder. Lancet 1980;ii:882–885.
37 Mrak RE, Saito A, Evans OB, Fleischer S: Autophagic degradation in human skeletal muscle fibers. Muscle Nerve 1982;5:745–753.
38 Floyd M, Ayyar DR, Barwick DD, Hudgson P, Weightman D: Myopathy in chronic renal failure. Q J Med 1974;43:509–524.
39 Folts JD, Shug AL, Koke JR, Bittar N: Protection of the ischemic dog myocardium with carnitine. Am J Cardiol 1978;41:1209–1214.
40 Suzuki Y, Kamikawa T, Yamazaki N: Protective effect of *L*-carnitine on ischemic heart; in Frankel RA, McGarry JD (eds): Carnitine Biosynthesis, Metabolism and Functions. Academic Press, New York, 1980, pp 341–352.
41 Spagnoli LH, Crosi M, Villaschi S, Palmieri G, Maccari F: Myocardial carnitine deficiency in acute myocardial infarction (letter). Lancet 1982;i:1419–1420.
42 Suzuki Y, Kamikawa T, Yamazaki N: Effects of *L*-carnitine on ventricular arrhythmias in dogs with acute myocardial ischemia and a supplement of excess free fatty acids. Jpn Circ J 1981;45:552–559.
43 Trovato GM, Ginardi V, Di Marco V, Dell'Aira AE, Corsi M: Long-term *L*-carnitine treatment of chronic anemia of patients with end-stage renal failure. Curr Ther Res 1982;31:1042–1049.

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