Kidney International, Vol. 46 (1994), pp. 1616–1619

ND: 19605

Selective trophic effect of L-carnitine in type I and IIa skeletal muscle fibers

PAOLO GIOVENALI, DANIELA FENOCCHIO, GIUSEPPE MONTANARI, CORRADO CANCELLOTTI, STEFANIA D'IDDIO, UMBERTO BUONCRISTIANI, MAURIZIO PELAGAGGIA, and RODOLFO RIBACCHI

II Cattedra Anatomia Istologia Patologica, Università di Perugia, Perugia; Centro di Fisiologia dell'Esercizio, Gubbio, Università di Chieti, Chieti; Sigma Tau, Direzione Ricerche Cliniche, Pomezia; and Divisione Nefrologia, Ospedale "R. Silvestrini", Perugia, Italy

Selective trophic effect of L-carnitine in type I and IIa skeletal muscle fibers. Biopsies were taken from the vastus lateralis muscle of 26 chronic uremic patients before and after a 24-week treatment with L-carnitine given at the dose of 2 g i.v. at the end of hemodialysis, or in dialysis solution, or per os twice daily. The aim of the study was to evaluate both the muscle morphology in dialyzed subjects and the modification provoked by the therapy. All patients manifested a significant, even if variable, degree of muscular atrophy which involved all types of muscle fibers. After the treatment there was an increase of about 7% in the diameter of type I and type IIa fibers, which can utilize carnitine for fatty acid oxidation to produce energy, and a reduction in the atrophic fibers. No noteworthy changes were documented in type IIb fibers, which depend on glycolysis for energy production.

L-carnitine, a quaternary amine, is a natural constituent of cells that plays a fundamental role in skeletal muscle metabolism by promoting mitochondrial oxidation of fatty acids [1–3]. It is mainly utilized by type I and IIa muscle fibers, which have a wide range of oxidative enzymes, whereas type IIb fibers are characteristically anaerobic and, therefore, do not generally use fatty acids and, in consequence, carnitine for metabolism [4–6].

Patients on chronic hemodialysis are reported to be deficient in carnitine [7, 8], probably due to the hemodialysis membrane being permeable to low molecular weight compounds [9]. Lack of carnitine causes atrophy in type I and IIa muscle fibers, and is associated with an accumulation of intracytoplasmic lipids [4, 5, 10]. Diffuse fiber atrophy, not directly correlated with carnitine deficiency, which is often more marked in type II fibers, has also been observed in uremic patients [11, 12].

It has been demonstrated that hemodialysis patients treated with 2 g/i.v./day L-carnitine for one year present type I fiber predominance and hypertrophy, while the type II fibers remain unaltered, and that the hypertrophy regresses markedly four months after the therapy is suspended [13].

We carried out a prospective study to determine whether L-carnitine supplementation was able to increase muscle carnitine levels, reduce muscle hypotrophy and improve muscle strength in patients on maintenance hemodialysis.

Received for publication January 3, 1994
and in revised form July 11, 1994
Accepted for publication July 18, 1994

Methods

Patients

Twenty-six patients who had been on maintenance hemodialytic treatment for at least one year (range 1 to 14 years) were enrolled in the study.

On enrollment, all patients had a GFR < 1 ml/min and both their nutritional and hematological conditions were satisfactory. Table 1 details the patient data.

Exclusion criteria were: malignancies, liver failure, severe hypertension, concomitant diseases affecting the skeletal muscle function, and treatment with anabolistic compounds and/or L-carnitine during the six months preceding the study.

Patients were divided into three groups on the basis of three different regimens of carnitine supplementation procedures.

Group 1. Eleven patients (7 males, 4 females, mean age 55.5 years) had 0.0725 mM/liter (2 g in 170 liter) added directly to the dialytic solution.

Group 2. Six patients (4 males, 2 females, mean age 50.9 years) were administered 2 g L-carnitine per os daily.

Group 3. Nine patients (6 males, 3 females, mean age 54.8 years) were given 2 g L-carnitine i.v. at the end of each dialytic treatment.

L-carnitine doses, supplemented for 24 weeks in all cases, were comparable.

Morphometry

Muscle biopsies were obtained from the upper-third of the musculus vastus lateralis of the femoral quadriceps at basal time and after 24 weeks.

Biopsies were snap frozen in isopenthane brought to freezing point in liquid nitrogen and sectioned in a cryostat. Sections were stained by the ATPase technique at pH 9.4, 4.6 and 4.3 to distinguish the various types of fiber and by the Red-O oil method to visualize intracytoplasmic lipids.

Morphometric analysis was performed with a Leitz MIAMED image analyzer. MODE (Leica) software was used for determining: (1) the number and percentage of type I and type II fibers; (2) the mean lesser diameters of fibers in transverse section; (3) atrophy and hypertrophy factors, respectively indicative of the proportion of fibers with a diameter of less than 40 μ in men and 30 μ in women and the proportion of fibers with a diameter greater than 80 μ in men and 70 μ in women. The atrophy factor

Table 1. Patient and hemodialytic data

Men	17
Women	9
Mean age *years*	55.3
Serum creatinine *mg/ml*	13.2
Mean dialytic time *months*	71
Dialysis treatment *min*	240
Blood flow rate *ml/min*	272
Dialyzer surface area m^2	1–1.7
Heparin *U/dialysis*	7500 ± 1500
Dialysate acetate *mml/liter*	17
Dialysate bicarbonate *g/liter*	9

is derived, in men, by multiplying the number of fibers with a diameter between 30 and 40 μ (20 to 30 μ in women) by one, the number of fibers with a diameter between 20 and 30 μ (10 to 20 μ in women) by two, the number of those from 10 and 20 μ (< 10 μ in women) by three, and the number of those with a diameter less than 10 μ by four. These products are added together, divided by the total number of computed fibers and then multiplied by 1000. Hypertrophy factor is computed dividing the number of fibers with a diameter larger than 80 μ (70 μ in women) by the total number of computed fibers and multiplying the result by 1000 [4].

Morphometric analysis was performed "blindly", without knowledge of group of treatment nor time of biopsy.

The overall morphometric features were evaluated in all subjects irrespective of the type of treatment.

Muscle strength

The force produced by isometric contractions of the quadriceps muscle was studied during maximal voluntary contraction, according to the method of Edwards et al [14], which allows the isometric strength value to be measured in kg. A force/time curve of the isometric strength course that reflects the performance effort was then constructed. The resulting graph allows certain fiber dependant (I, IIa, IIb) force features (rapid, maximal, resistant) and their predominant metabolism (aerobic, anaerobic and mixed) to be identified.

The first value obtained is the ascending velocity of the curve in the first second of the effort (AP), which expresses the rapid force of type II fibers.

The maximum isometric force peak (MIFP) value, which expresses the maximal voluntary force involving both type of fibers, follows.

The final value, the plateau phase (PP), is the curve length between the MIFP and the relaxing phase. This section of the curve represents the resistant force typical of type I aerobic fibers.

Plasma and muscle carnitine

Plasma levels of free carnitine were determined at basal time and every four weeks up to week 24 immediately before and after the dialytic treatment.

Muscle total carnitine, free carnitine and acetylcarnitine were assayed before and after treatment by the method described by McGarry and Foster [15], and Pande and Caramancion [16].

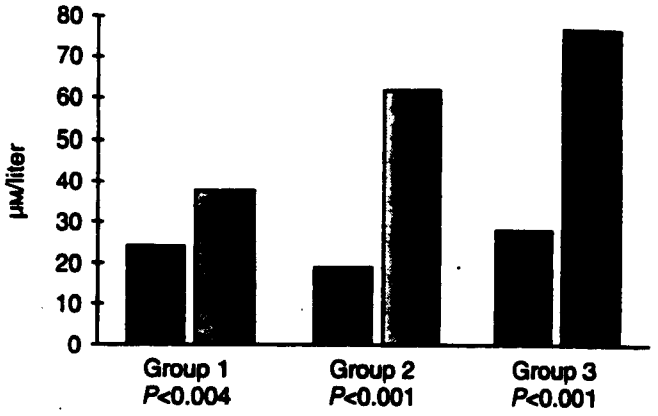

Fig. 1. *Free plasma carnitine before and after L-carnitine treatment.* Symbols are: (▨) basal; (□) 24th week.

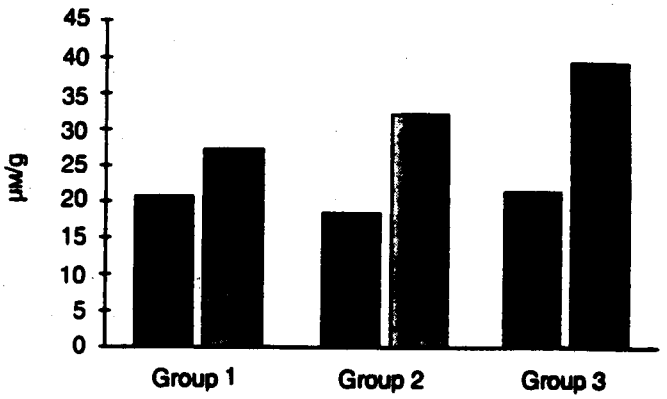

Fig. 2. *Free muscle carnitine before and after L-carnitine treatment.* Symbols are: (▨) basal; (□) 24th week.

Clinical symptoms and safety

Muscle related symptoms and general clinical symptoms were checked weekly. Standard safety laboratory tests and ECGs were performed at basal time and at the end of the study.

Statistic

The Student's *t*-test for paired data was used for assessing the mean diameters of muscle fibers before and after treatment and to evaluate the difference of muscle and plasmatic carnitine levels before and after treatment. The Wilcoxon's signed rank test was used for comparing the proportion of type IIb atrophic muscle fibers separately with type I and IIa atrophic muscle fibers.

Results

Plasma and muscle carnitine

Free plasma carnitine (Fig. 1) was increased, after treatment, in all groups. The variations were from 24.2 ± 8.9 mM/liter to 38.0 ± 11.0 mM/liter in group 1 ($P < 0.004$); from 19.2 ± 5.6 mM/liter to 61.5 ± 22.9 mM/liter in group 2 ($P < 0.001$) and from 28.0 ± 11.8 mM/liter to 76.6 ± 31.7 mM/liter in group 3 ($P < 0.001$).

Free muscle carnitine (Fig. 2) followed a similar trend. It increased from 20.7 ± 7.4 mM/g to 27.2 ± 8.9 mM/g in group 1 ($P < 0.05$); from 18.4 ± 4.8 mM/g to 32.2 ± 4.3 mM/g in group 2 (P

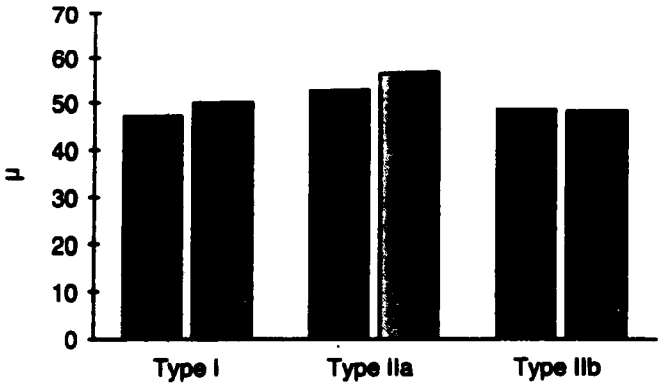

Fig. 3. *Mean fiber diameter before and after L-carnitine treatment.* Symbols are: (■) basal; (▣) 24th week.

< 0.001) and from 21.4 ± 3.6 mM/g to 39.6 ± 15.2 mM/g in group 3 ($P < 0.003$).

The trend of total and acetylate muscle carnitine variations was similar to free muscle carnitine.

Although muscle and free plasma carnitine was higher in group 3 subjects, there was no statistically significant difference between groups.

Muscle morphometry

As free plasma and muscle carnitine levels rose markedly, without significant intergroup differences, the morphometric results were evaluated taking the 26 patients as a single group.

Muscle fiber composition. There was no statistical difference in the proportion of single types of muscle fiber before and after treatment.

The percentage of muscle fibers before and after therapy were 48.3% ± 9.3 versus 50.5% ± 7.8 for type I fibers, 29.4% ± 8.9 versus 30.1% ± 9.1 for type IIa fibers and 22.1% ± 8.8 versus 19.3% ± 6.5 for type IIb fibers.

Diameter of muscle fibers. Mean diameter values after treatment were significantly greater in both sexes than pre-therapy values in type I and IIa ($P < 0.001$), but not in type IIb fibers.

The before and after L-carnitine mean lesser diameters were 47.2 μ ± 13.8 and 50.1 μ ± 12.1 for type I fibers, 52.6 μ ± 14.4 and 56.2 μ ± 13.9 for type IIa, 48.1 μ ± 14.4 and 47.8 μ ± 14.4 for type IIb fibers (Fig. 3).

Atrophic muscle fibers. Dubowitz [4] defines as atrophic those fibers that have a diameter of less than 40 μ in men and 30 μ in women. Numerous atrophic fibers were present in patients' biopsies. However, the percentage of atrophic type I fibers fell after therapy from 22.0% to 10.5% and type IIa from 15.3% to 9.1%, while no noteworthy change was recorded in type IIb fibers (from 22.8% to 20.7%; Table 2).

The atrophic factor calculated for the different types of fiber decreased after treatment from 251.4 to 133.2 for type I, from 153.2 to 96.3 for type IIa and varied from 241.2 to 257.1 for type IIb fibers.

Because the modification in type IIb fibers, which are not equipped with enzymes that utilize carnitine, was negligible, they acted as an internal control group for statistical evaluation.

Statistical analysis showed that the reduction in the proportion of atrophic fibers was significant for type I fibers ($P < 0.025$) and at the limits of significance for type IIa fibers ($P < 0.07$).

Table 2. Variability of atrophic, normal and hypertrophic fibers before and after L-carnitine treatment

Type of fiber	Atrophic fibers %		Normal fibers %		Hypertrophic fibers %	
	basal	24th week	basal	24th week	basal	24th week
I	22.0[a]	10.5[a]	77.6	88.6	0.4	0.9
IIa	15.3[b]	9.1[b]	83.2	85.9	1.4	4.9
IIb	22.8	20.7	74.0	77.4	3.3	2.0

[a] $P < 0.02$
[b] $P < 0.07$

Hypertrophic muscle fibers. Hypertrophic fibers are, according to Dubowitz [4], those with a diameter greater than 80 μ and 70 μ in women. Although some hypertrophic muscle fibers were found in patients' biopsies, the proportion was negligible and there were no significant differences between the first and second biopsies.

Muscle strength

The basal values of the isometric force (AP, MIFP, PP) were in the low normal range or significantly below the normal range in all the patients, according to the model of force measurement proposed by Edwards et al [14]. After treatment there was an increase for AP, MIFP and PP in all patients compared to the basal values.

Paired comparison within each treatment group showed a significant increase of isometric force values in group 1 ($P < 0.04$) and in group 3 ($P < 0.001$).

Detailed analysis of isometric force parameters will be referred in another paper [17].

Clinical symptoms and safety

Muscle strength improved in 7 patients out of 16; muscle pain regressed in 2 out of 5 and muscle cramps in 6 out of 10.

Neither the laboratory tests nor the ECGs revealed any significant difference between the basal and end of treatment values.

Discussion

Several interesting findings emerge from this study.

All groups of dialyzed patients had significant, even if variable, muscular atrophy.

Various muscular alteration patterns have been documented in patients on chronic hemodialysis. Muscle morphology characterized by carnitine deficiency, that is, type I fiber atrophy associated with an intracytoplasmic accumulation of lipids, has rarely been seen even when muscle carnitine dropped to 50% of the normal value [18, 19]. Diffuse muscular atrophy is more frequent and particularly evident in type II fibers, probably due to secondary hyperparathyroidism or intracellular acidosis [5, 11, 12].

However, our patients manifested no significant difference in the percent of the various types of atrophic fibers (type I, 22.0%; type IIa, 15.3%; type IIb, 22.8%) and there was no evidence of intracytoplasmic lipid accumulation.

Muscular atrophy seems, therefore, not to be associated with a carnitine deficiency.

Despite the fact that no patient presented a pathologically low carnitine level, administration of L-carnitine determined a

marked rise in free plasma and muscle carnitine and, in consequence, a greater availability of the molecule for muscle oxidative metabolism, irrespective of the administration route.

The 24-week treatment with L-carnitine seems to have been responsible for the 7% increase in the diameter of type I and IIa fibers, but to have provoked no appreciable modification in type IIb fibers, which is what would be expected from the metabolic characteristics of muscle fibers [4–6]. Type I fibers, mainly oxidative and type IIa, which are mixed oxidative-glycolytic, can utilize fatty acids and so take advantage of carnitine uptake to produce energy. In contrast, as type IIb fibers are strictly glycolytic, they were not modified by the treatment. The specific trophic effect of L-carnitine was also confirmed by the fact that all hematochemical and hemocytometric indices remained unaltered during treatment, thereby indicating that L-carnitine exerts a direct effect on muscle.

The increase in the diameter of type I and IIa fibers seems to depend on the reduction in atrophic fibers, since after therapy the atrophic factor was about half the basal value for both fiber types, and the percentage of type I atrophic fibers dropped from 22.0% to 10.5% and that of type IIa from 15.3% to 9.1%. When type IIb fibers were used as an internal control, that is, presuming that variations in this type of fiber were treatment independent, the reduction in the proportion of atrophic type I fibers was significant ($P < 0.025$), and the decrease in type IIa atrophic fibers was at the limits of significance ($P < 0.07$). A greater responsiveness of the more oxidative fiber to L-carnitine could account for this difference. The increase in fiber diameter together with a reduction of frequency of atrophic fibers also agrees well with the improved muscular force.

No significant modifications in the relative percentages of the different fiber types were documented at the second biopsy. As far as we know, there is only one other study [13] of muscle morphometry in dialyzed patients supplemented with L-carnitine. In that study, after long-term treatment with L-carnitine, Spagnoli et al found not only type I fiber hypertrophy and regression of hypertrophy after therapy suspension, but also a significant predominance of type I fibers, which was presumed to be due to phenotypic transformation of type IIa to type I fibers as a result of the great availability of lipidic substrates for oxidative metabolism. A possible explanation for this discrepancy would be that our treatment was sufficient to obtain a decrease in oxidative fiber atrophy, but not continued long enough to modify the percentage of the fibers.

Reprint requests to dr. Claudio Romano, Sigma-Tau, Industrie Farmaceutiche Riunite S.P.A., 47, viale Shakespeare, 00144 Rome, Italy.

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