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Effect of carnitine feeding on the levels of heart and skeletal muscle carnitine of elderly mice

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Aging has been associated with an increase in muscle dysfunction and weakness. We found a decrease in muscle carnitine with age [Biochem. Biophys. Res. Commun., 161 (1989) 1135–1143]. Prolonged oral administration to both young (2-month-old) and adult (7-month-old) mice with L-carnitine increased its content in blood by 50%. The levels of carnitine in skeletal and heart muscle of old treated animals became higher than in untreated mice of the same age. However, this extensive restoration did not reach the maximum values present in skeletal muscle of young mice. Our findings indicate that an alteration of the carnitine carrier in the sarcolemma could be responsible for the decrease with age of carnitine in skeletal but not in heart muscle.

Carnitine; Aging; Skeletal muscle; Heart; Acylcarnitine

1. INTRODUCTION

We reported decreased concentrations of carnitine in muscle of both old mice and humans [1]. However, the levels of carnitine in blood, liver and brain were not decreased with age [1]. Although carnitine can be totally synthesized in mammals, there are some metabolic and pathological circumstances where the endogenous synthesis is insufficient. In some of those situations, the oral administration of L-carnitine has been reported as beneficial to re-establish the depleted levels of the compound [2–7]. In relation to aging, administration of acetylcarnitine has been claimed to improve age-related deficits and cognitive function in aged rats [8,9] and humans [10], but it is not known if carnitine supplementation could avoid skeletal muscle carnitine reduction. The objective of this work was to determine if a prolonged oral administration of carnitine could prevent the muscle carnitine decrease with age.

2. MATERIALS AND METHODS

Male Swiss albino mice (Interfauna Iberica, Barcelona, Spain), fed a standard diet with a protein content of 18% (Sanders, Spain), were used. The animals were treated and killed in a manner consistent with the guide of the care and use of laboratory animals of the European Community. Groups of 75 animals each, of 2-month-old and 7-month-old, mice were maintained for as long as 5–10 months drinking a solution of L-carnitine (13 mmol/l in tap water) as the sole drinking fluid. The same number of animals of each age were maintained on drinking tap water alone, as controls. Six animals of each group were placed in metabolic cages, two per cage, and the urine was collected every 24 hours in 250 μl of 1.2 M perchloric acid. Mortality was not statistically different in any carnitine-treated group as compared to controls of the same age. At indicated intervals blood serum was collected. The animals were killed and heart and hind-limb skeletal muscle were removed, frozen in liquid nitrogen and stored below −70°C for less than 20 days. Esterified carnitine was measured after alkaline hydrolysis of the samples and free carnitine was determined [11]. Creatinine was determined by the colorimetric test of Boehringer. L-Carnitine was a commercial preparation from Sigma-Tau (Spain) and deoxycarnitine was a gift from Dr. N. Siliprandi (Dpto. di Chimica Biologica, Università degli Studi di Padova, Italy).

Statistical analysis. Results are reported as mean ± S.D. and the significance of differences between groups were determined using the Student's *t*-test.

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3. RESULTS AND DISCUSSION

Fig. 1 depicts the concentration of carnitine in muscle of young and adult mice fed on carnitine, compared with untreated animals of the same age. The concentration of free carnitine in skeletal muscle of controls decreased steadily with age. The decrease was very sharp during the first six months, reaching a plateau at 8–9 months. Thus, the concentration of carnitine at 9 months of age was approximately half that at 2 months of age. The daily supplementation of L-carnitine in the drinking water of young mice did not stop the decrease in free muscle carnitine for the first month of administration. However, by this time the supplementation of L-carnitine resulted in a stabilization of carnitine (at 70% of the initial levels of the young group). Notably, the same increase in muscle carnitine was also reached, when the administration of L-carnitine was started later (even at 7 months of age, see Fig. 1).

Fig. 2 shows the concentration of free carnitine in

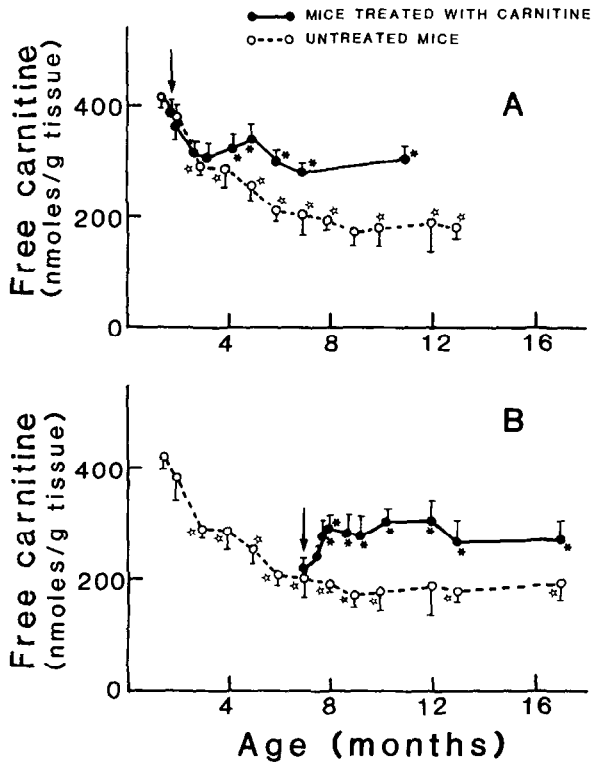

Fig. 1. Concentration of free carnitine in muscle of young and adult mice treated orally with carnitine. Free carnitine was measured in hind-limb muscle of mice at different ages. Animals were treated orally with 13 mmol/l L-carnitine starting from either: two months of age (A, ● filled symbols) or from 7 months of age (B, ● filled symbols). The control group (open symbols) was not treated with carnitine. Arrows indicate when carnitine treatment was started. Results are the mean value ± S.D. of 5–10 animals. (*) Treatment effect: treated values significantly different from untreated group of the same age ($P < 0.001$). (☆) Age effect: untreated group values significantly different from young untreated group (2-month-old) ($P < 0.001$).

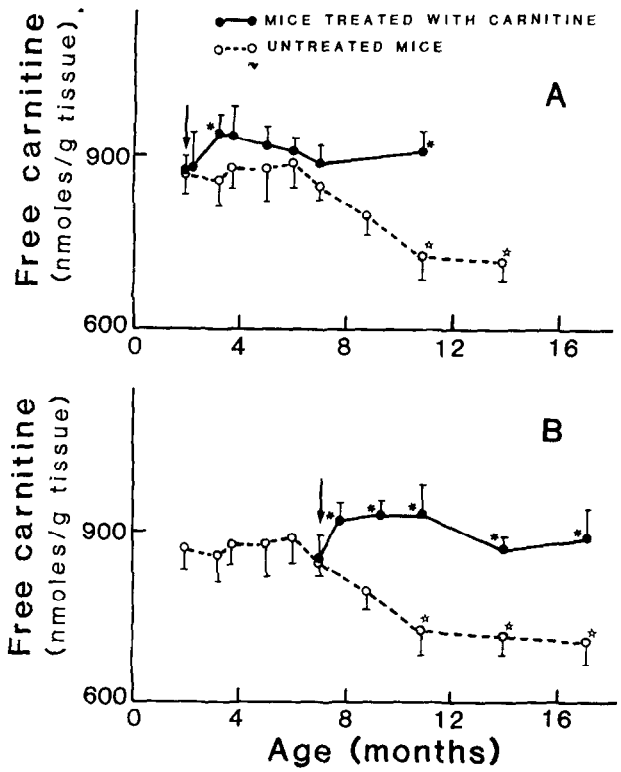

Fig. 2. Free carnitine content in heart of young and adult mice treated orally with L-carnitine. The animals studied and statistical analysis were the same as in Fig. 1. Results are the mean ± S.D. of 5–10 animals.

heart of young and adult mice treated with L-carnitine. Although the levels of carnitine in heart decreased slightly with age (levels in 1-year-old mice were ~80% of those of 2-month-old animals; with a net decrease of 180 nmol/g tissue), treatment of both young and old animals with carnitine produced an increase in heart (Fig. 2), above the free carnitine levels in young controls and prevented the decrease in older mice.

Table I shows the levels of carnitine in serum and urine of young, adult and old animals after being fed L-carnitine for up to 5 months. The amount of L-carnitine ingested daily was 1.37 ± 0.27 mmol/kg body weight. There was no significant difference between young and adult animals, in the amount ingested. Only in the 12-month-old mice was there a slight decrease, which could explain the decrease in carnitine excretion in urine. In all treated animals there was an increase in plasma carnitine levels, up to 1.5-fold of the starting levels, and in urine excretion. Urinary excretion of both free and esterified carnitine of mice fed L-carnitine was higher in young animals than in the older. From the data of esterified carnitine excreted in the urine, of particular interest is the ~5-fold higher elimination, under basal conditions (no carnitine treatment), by the old group over the younger, although the total amount of carnitine excreted was quite similar in the two groups. It seems possible that older animals need to excrete more acyl compounds which have not been oxidized in the mitochondria; carnitine would facilitate their transport across cellular membranes and subsequent elimination in urine, implying a detoxifying role for carnitine in old animals.

As shown, the response to carnitine treatment was different in skeletal from cardiac muscle. While heart reached young levels of carnitine, or higher, after less than one month of carnitine administration, in skeletal muscle those were always below the 70% of young concentrations. In some muscle carnitine deficiencies, it has been reported that carnitine administration failed to raise skeletal muscle levels, even though the plasma concentrations were elevated, claiming an impairment of carnitine transport into muscle cells [12–14].

The hydroxylation of deoxycarnitine to carnitine is restricted to liver and kidney [15]. Other tissues, including skeletal and cardiac muscles, are entirely dependent on carnitine uptake from the blood. These tissues do export deoxycarnitine to the hydroxylating organs. The

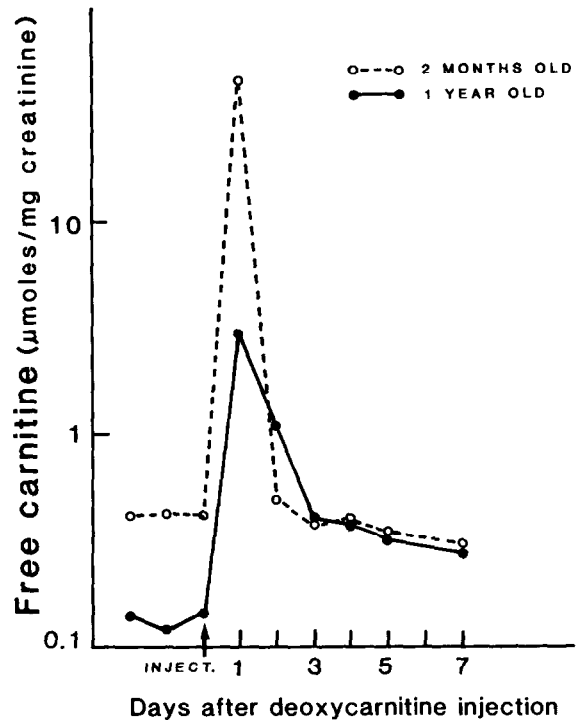

Fig. 3. Carnitine excretion in urine of young and adult mice injected intraperitoneally with deoxycarnitine. Mice 2-month-old (○) and 12-month (●) were injected i.p. with 1 mmol of deoxycarnitine/kg body weight, on the day indicated by the arrow. Free carnitine was determined in the urine. Values are shown for several days following the injection. Data are the mean of 4 experiments.

existence of a sarcolemmal translocator for carnitine has been demonstrated in in vitro and in vivo experiments [16–18]. It was shown that an exchange occurs bidirectionally, in a ratio close to 1:1, between deoxycarnitine and carnitine. Although under physiological conditions the exchange in muscle will be between endogenous deoxycarnitine and external carnitine, it was demonstrated by Sartorelli et al. [18] that the intraperitoneal administration of high doses of deoxycarnitine will revert the direction of interchange, inducing a massive exit of free carnitine from the tissues, proportional to the rate of this carrier, and an entry of deoxycarnitine. Of the total carnitine pool in the body, 98% is in skeletal muscle. Thus, the rate of increase of carnitine levels in urine immediately after deoxycarnitine injection will reflect the activity of the sarcolemma carnitine/deoxycarnitine carrier.

As shown in Fig. 3, the administration of deoxycarnitine (intraperitoneal injection of 1 mmol deoxycarnitine per kg body weight), to either young (2-month-old) or old (1-year-old) mice, produced an immediate excretion of free carnitine in the urine. On the first day after the injection of deoxycarnitine the excretion of free carnitine was significantly slower in old than in young mice (46 μmol/mg creatinine in the 2-month-old mice vs. 3 μmol/mg creatinine in the 12-month-old mice; Fig. 3, note log scale). The extent of such elimination was much greater in young than in old animals, although on the 2nd day carnitine excretion in the young was nearly

Table I

Serum and urine carnitine concentrations in mice treated with L-carnitine

Age of mice	Carnitine levels		
	Controls (untreated mice) (a)	Period of treatment with L-carnitine 1-week (b)	5-months (c)
2-month-old			
Serum (μmol/l)	38.1 ± 3.4	55.2 ± 5.9*	–
Urine (μmol/mg creat.)			
Free	0.415 ± 0.053	18.0 ± 1.02*	–
Esterified	0.052 ± 0.013	6.2 ± 0.58*	–
7-month-old			
Serum (μmol/l)	35.3 ± 3.7	52.3 ± 1.3*	49.9 ± 7.5*
Urine (μmol/mg creat.)			
Free	0.280 ± 0.065^{+}	12.2 ± 0.87^{*+}	13.2 ± 0.58^{*+}
Esterified	0.200 ± 0.034^{+}	4.1 ± 0.77^{*+}	3.8 ± 0.72^{*+}
12-month-old			
Serum (μmol/l)	36.4 ± 3.7	–	58.8 ± 7.0*
Urine (μmol/mg creat.)			
Free	0.140 ± 0.032^{+}	–	11.2 ± 0.43^{*+}
Esterified	0.250 ± 0.027^{+}	–	3.2 ± 0.39^{*+}

The data given for free and esterified carnitine excreted in urine are referred to the creatinine content in the urine. Values are the mean ± S.E. of 6 animals for serum and the mean ± S.E. of 6 animals for 5 consecutive days for urine. The age effect has been indicated by $^{+}$($P < 0.001$): statistically significant difference versus 2-month-old group (group 'a' for controls and group 'b' for all treated groups). The treatment effect has been indicated by *($P < 0.001$): each treated group was referred to controls of the same age.

basal, while in the older it was about 1 μmol/mg creatinine, and was maintained above basal levels for a week.

The data shown indicate that the decrease in skeletal muscle carnitine with age may be due to a functional alteration of the carrier responsible for the entry of carnitine into the myocyte. However, a partial restoration of carnitine levels can be achieved after 1 month of oral administration, at any age. Another implication of our observations is the potential detoxifying role of carnitine in old individuals, facilitating the excretion of acyl compounds.

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