

**L-carnitine and creatine in Friedreich's ataxia.
A randomized, placebo-controlled crossover trial**

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Summary. Impaired oxidative phosphorylation is a crucial factor in the pathogenesis of Friedreich's ataxia (FA). L-carnitine and creatine are natural compounds that can enhance cellular energy transduction. We performed a placebo-controlled triple-phase crossover trial of L-carnitine (3 g/d) and creatine (6.75 g/d) in 16 patients with genetically confirmed FA. Primary outcome measures were mitochondrial ATP production measured as phosphocreatine recovery by ³¹P magnetic resonance spectroscopy, neurological deficits assessed by the international co-operative ataxia rating scale and cardiac hypertrophy in echocardiography. After 4 months on L-carnitine phosphocreatine recovery was improved compared to baseline ($p < 0.03$, t-test) but comparison to placebo and creatine effects did not reach significance ($p = 0.06$, F-test). Ataxia rating scale and echocardiographic parameters remained unchanged. Creatine had no effect in FA patients. L-carnitine is a promising substance for the treatment of FA patients, and larger trials are warranted.

Keywords: Friedreich's ataxia, therapy, L-carnitine, oxidative phosphorylation, magnetic resonance spectroscopy.

* Both authors contributed equally to this work

Introduction

Friedreich's ataxia (FA) is the most frequent form of autosomal recessive ataxia. The clinical hallmarks of the disease are progressive ataxia starting in the first or second decade of life, dysarthria, pyramidal weakness, extensor plantar responses and axonal sensory neuropathy with areflexia (Harding, 1981; Schöls et al., 1997). Mutations consist of expanded GAA trinucleotide repeats in the first intron of the FRDA gene in 96% and various micromutations in 4% of alleles (Campuzano et al., 1996). Repeat expansion as well as point mutations lead to drastically reduced levels of frataxin. Frataxin is supposed to be essential for the assembly of Fe-S-clusters (e.g. in respiratory chain complexes I–III), for maintenance of iron homeostasis or as a mitochondrial anti-oxidant (Puccio et al., 2002). Whatever the primary function of frataxin is, impaired oxidative phosphorylation (OXPHOS) is a crucial factor in the pathogenesis of FA. This has been confirmed in *in vivo* studies using ^{31}P -magnetic resonance spectroscopy (^{31}P -MRS) to monitor energy metabolism. In skeletal muscle phosphocreatine (PCr) recovery after exercise is a direct measure of ATP formation and is significantly prolonged demonstrating impaired OXPHOS capacity in FA patients (Lodi et al., 1999; Vorgerd et al., 2000). Furthermore, PCr/ATP ratio is reduced in cardiac muscle of patients with FA compared to healthy controls (Lodi et al., 2001).

PCr is generated by phosphorylation of creatine via creatine kinase and functions as an intrinsic energy reservoir to regenerate ATP in times of high energy demand (Hemmer and Wallimann, 1993). Therefore, supplementation with creatine is supposed to enhance energy metabolism by increasing PCr levels. This may be of particular benefit in disorders with mitochondrial dysfunction like Friedreich's ataxia in which ATP levels are depleted and PCr reserves are exhausted.

L-carnitine may improve mitochondrial function in FA by compensation of secondary carnitine deficiency. Defective OXPHOS, as in FA, is often associated with impaired β -oxidation, preferentially affecting brain, heart and skeletal muscle. Reduced function of the respiratory chain generates an increased NADH/NAD(+) ratio that inhibits β -oxidation and produces secondary carnitine deficiency while increasing reactive oxygen species and depleting α -tocopherol (Infante and Huszagh, 2000).

Therefore, we investigated the therapeutical potential of L-carnitine and creatine as natural compounds that can enhance cellular energy transduction and used ^{31}P -MRS to assess OXPHOS in a placebo-controlled trial in patients with FA.

Patients and methods

Adult patients with genetically confirmed Friedreich's ataxia were recruited from the ataxia clinics at the Departments of Neurology in Bochum and Bonn. To prevent immobilization effects on the muscle metabolic measurements with ^{31}P -MRS, only ambulant patients were included in the study. Sixteen FA patients (5 male, 11 female; age 30.7 ± 10.0 years, range: 18–55; age at onset 19.1 ± 7.5 years, range: 12–36) homozygous for a GAA repeat expansion (GAA1: 403 ± 241 , range: 139–808; GAA2: 810 ± 223 , range: 323–1011) were included in this study. Thirty-eight healthy controls were matched for age (30.0 ± 9.9 years; 15–63 years) and sex.

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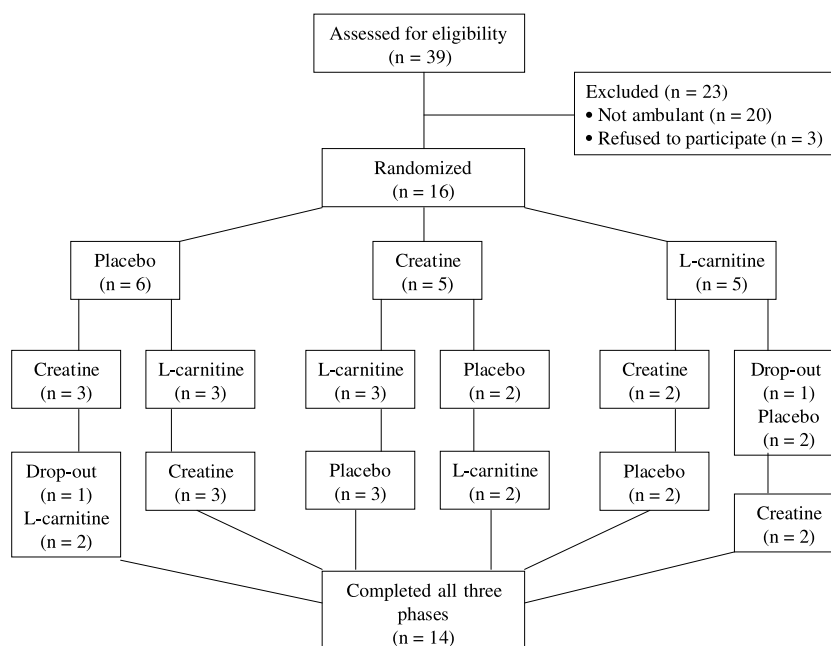


Fig. 1. Flow diagram of subject progress in the randomized triple-phase crossover trial

L-carnitine (3 g/d tid), creatine (6.75 g/d tid) and placebo were prepared in identical capsules at the central pharmacy of the Elisabeth Stiftung in Bochum. Medication was given in a randomized order in a triple-phase crossover design (Fig. 1). Participants and assessors remained blinded for allocation sequence until the end of the study. Each study period lasted 4 months with wash-out periods of 4 weeks in between.

Baseline and endpoint measurements were performed for each study period and enabled us to compare drug effects (baseline minus endpoint data of each respective period) as well as endpoint comparisons (creatine vs. L-carnitine vs. placebo). Drug effects in comparison to placebo were calculated by F-test including analyses of time effects and period effects.

Primary outcome measures were mitochondrial ATP production measured as phosphocreatine recovery by ^{31}P -MRS, neurological deficits assessed by the international co-operative ataxia rating scale (ICARS; Trouillas et al., 1997) and cardiac hypertrophy in echocardiography. Left ventricular mass has been calculated according to the formula of Devereux and Reichek (1977). Shortening fraction (%) has been calculated as $(\text{LVEDD} - \text{LVESD})/\text{LVEDD} \times 100$ where LVEDD is the enddiastolic diameter and LVESD the endsystolic diameter of the left ventricle.

OXPPOS was assessed *in vivo* using ^{31}P -MRS to measure PCr recovery in the standardized calf muscle exercise test as described in detail before (Vorgerd et al., 2000). Shortly, MR spectra were obtained in a 4.7 Tesla spectrometer (Bruker-Biospec 47/40, Bruker-Medizintechnik, Karlsruhe, Germany). Patients were asked to perform isometric exercise with 30% of their maximum test force by pressing a pedal under the right foot. Isometric exercise was performed for 3 minutes first under normal perfusion of the leg (aerobic test) and again after 7 minutes rest for further 3 minutes under arterial occlusion applied by a cuff at the thigh (ischemic test). Isometric exercise resulted in PCr depletion of calf muscle measured by ^{31}P -MRS. During recovery from exercise the time constant tau determined from the pseudo mono exponential time course of PCr resynthesis was used as a direct measure of OXPPOS capacity. The maximum rate of oxidative ATP formation (V_{\max}) was calculated by model based equations previously used for FA patients (Lodi et al., 1999, 2001). Absolute concentrations of metabolites were calculated assuming that at initial rest [ATP] is 8.2 mM.

V_{\max} was calculated as:

$$V_{\max} = V_i \{1 + (K_m/[ADP]_{\text{end}})\}$$

This model assumes a hyperbolic control of mitochondrial ATP synthesis by [ADP] and a Km value of 30 μM. The initial rate of ATP synthesis (V_i) was given as

$$V_i = \Delta[\text{PCr}]_{\text{contraction}} \tau$$

[ADP] was calculated from the creatine kinase equilibrium reaction assuming an equilibrium constant (K_{eq}) of $1.66 \times 10^9 \text{ M}^{-1}$. Total creatine [TCr] was individually calculated from the initial spectra recorded at rest assuming $[\text{PCr}] = 0.8 [\text{TCr}]$. Current creatine concentration [Cr] was calculated as $[\text{TCr}] - [\text{PCr}]$.

$$[\text{ADP}] = ([\text{ATP}][\text{Cr}]) / ([\text{PCr}][\text{H}^+]K_{\text{eq}})$$

Additionally, fine motor skills were assessed by the subtest “plugging” (25-hole pegboard) of the motor performance test according to Schoppe (1974).

All patients gave their written fully informed consent. The study has been performed according to the ethical standards of the Declaration of Helsinki and was approved by the Ethic Committee of the Ruhr-University, Bochum.

Results

L-carnitine as well as creatine were well tolerated without any side effects. Two patients discontinued the study. One patient refused follow-up after the first study phase because further visits appeared to be too time-consuming. A second patient dropped out after the second study phase. She experienced a substantial benefit from the medication in phase I in comparison to phase II and wanted to continue the phase I medication which turned out to be placebo.

PCr recovery was impaired in FA patients after aerobic (τ at baseline: $87.7 \pm 55.5 \text{ s}$, range: 37–251 s; controls: $37.8 \pm 15.9 \text{ s}$; 16.4–70.0 s, $p < 0.05$; t-test) as well as after ischemic exercise (τ at baseline: $95.7 \pm 55.7 \text{ s}$, range: 46–228 s; controls: $43.3 \pm 16.2 \text{ s}$; 18.9–90 s; $p < 0.05$; t-test) confirming earlier studies (Lodi et al., 1999; Vorgerd et al., 2000).

After 4 months on L-carnitine, PCr recovery improved after exercise under aerobic conditions from $84 \pm 32 \text{ s}$ to $68 \pm 29 \text{ s}$ (not significant; t-test) and after ischemic exercise from $103 \pm 48 \text{ s}$ to $69 \pm 15 \text{ s}$ ($p < 0.03$; t-test; Fig. 2).

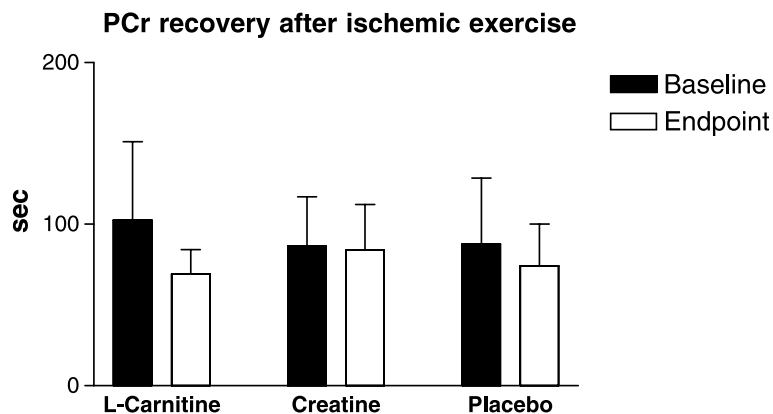


Fig. 2. Effect of L-carnitine, creatine and placebo on energy metabolism in Friedreich’s ataxia. Phosphocreatine (PCr) recovery in ^{31}P -magnetic resonance spectroscopy is improved after 4 months on L-carnitine compared to baseline ($p < 0.03$, t-test), but comparison to placebo and creatine effects did not reach significance ($p = 0.06$, F-test)

Table 1. Effects of L-carnitine, creatine and placebo on ataxia, fine motor skills, cardiomyopathy and energy metabolism

	Placebo		Creatine		L-carnitine		P value (F-test)
	Baseline	Endpoint	Baseline	Endpoint	Baseline	Endpoint	
International co-operative ataxia rating scale	30.5 ± 12.2	30.7 ± 10.6	34.1 ± 8.8	33.1 ± 9.3	33.2 ± 9.0	34.2 ± 11.0	0.56
Pegboard test: Right hand [s]	86.9 ± 19.2	85.8 ± 19.3	87.0 ± 17.0	88.3 ± 18.3	85.6 ± 15.8	81.7 ± 17.0	0.08
Left hand [s]	95.8 ± 24.7	98.9 ± 24.9	94.6 ± 24.7	96.4 ± 22.2	92.5 ± 23.5	94.8 ± 24.1	0.79
Echocardiography							
LV EDD [mm]	44.0 ± 6.0	43.6 ± 6.6	42.5 ± 5.4	42.4 ± 5.7	42.5 ± 5.8	44.1 ± 6.1	0.14
LV ESD [mm]	27.7 ± 5.1	27.8 ± 5.7	28.5 ± 6.8	27.9 ± 6.5	28.4 ± 4.9	29.8 ± 5.3	0.33
Interventricular septum [mm]	10.8 ± 1.8	10.5 ± 1.6	11.3 ± 2.1	10.6 ± 1.9	10.6 ± 1.9	10.1 ± 1.8	0.78
Posterior wall [mm]	11.0 ± 2.0	10.9 ± 2.2	11.7 ± 2.3	11.1 ± 1.5	10.8 ± 1.8	10.7 ± 1.4	0.53
Shortening fraction [%]	37.1 ± 8.4	36.2 ± 9.0	33.4 ± 10.4	34.6 ± 10.6	33.3 ± 8.1	32.4 ± 9.1	0.68
LV mass [g]	194 ± 45	187 ± 52	200 ± 65	182 ± 40	180 ± 59	183 ± 44	0.20
³¹ P-magnetic resonance spectroscopy							
PCr, aerobic exercise [%PCr _i]*	21.1 ± 13.9	13.4 ± 8.8	18.7 ± 9.1	20.0 ± 18.5	23.0 ± 10.5	19.0 ± 9.3	0.27
PCr, ischemic exercise [%PCr _i]*	27.8 ± 19.4	25.5 ± 12.4	27.3 ± 7.5	28.8 ± 18.8	29.2 ± 14.5	29.0 ± 14.6	0.53
Phosphate, aerobic exercise [%Pi _i]*	19.4 ± 13.4	14.8 ± 6.7	18.3 ± 13.2	19.3 ± 20.6	22.9 ± 12.2	18.3 ± 11.7	0.38
Phosphate, ischemic exercise [%Pi _i]*	29.4 ± 17.0	29.3 ± 12.0	26.3 ± 9.2	27.7 ± 18.9	29.6 ± 15.3	24.6 ± 12.3	0.52
ATP, aerobic exercise [%ATP _i]*	0.0 ± 2.7	0.8 ± 1.9	-1.3 ± 4.0	0.2 ± 1.1	0.1 ± 2.6	0.1 ± 1.3	0.30
ATP, ischemic exercise [%ATP _i]*	-0.2 ± 1.0	-0.2 ± 2.2	-0.1 ± 3.8	-0.5 ± 1.9	0.4 ± 2.9	0.3 ± 1.7	0.91
PCr/ATP ratio at initial rest	3.7 ± 0.5	3.9 ± 0.9	4.1 ± 0.6	4.2 ± 0.5	3.6 ± 0.4	3.9 ± 0.5	0.85
Tau1, PCr recovery from aerobic exercise [s]	85.7 ± 59.3	77.2 ± 31.5	61.4 ± 17.6	75.8 ± 37.2	83.7 ± 32.2	67.5 ± 29.6	0.68
Tau2, PCr recovery from ischemic exercise [s]	87.8 ± 40.8	74.3 ± 25.7	86.2 ± 30.7	84.1 ± 28.1	102.6 ± 48.4	69.2 ± 15.1	0.06
Vmax, recovery from aerobic exercise [mM/min]	17.3 ± 14.8	10.4 ± 4.8	14.3 ± 6.6	15.3 ± 9.2	12.8 ± 6.5	14.1 ± 8.3	0.17
Vmax, recovery from ischemic test [mM/min]	13.6 ± 8.9	16.6 ± 8.9	12.7 ± 7.0	14.7 ± 6.8	12.9 ± 7.5	16.4 ± 5.6	0.87
Maximum test force [N]	774 ± 248	794 ± 277	807 ± 224	776 ± 162	788 ± 183	810 ± 193	0.54

Data are given as mean ± standard deviation. LV left ventricle, EDD end diastolic diameter, ESD end systolic diameter, PCr Phosphocreatine, ATP Adenosine triphosphate. * changes during exercise, relative concentrations in % of PCr at initial rest

However, compared to placebo and creatine this effect was not significant ($p = 0.06$; F-test). Echocardiographic data and ICARS did not show significant improvement with L-carnitine, neither in baseline versus endpoint analysis nor in comparison to placebo. No effect of creatine could be established in this study (Table 1). Baseline data did not show significant differences between study phases. Time effects or carry over effects were not observed.

Discussion

This study confirms earlier reports of delayed PCr recovery indicating impaired ATP production in FA (Lodi et al., 1999; Vorgerd et al., 2000). These findings further substantiate pathogenetic concepts of defective OXPHOS possibly due to impaired Fe-S-cluster assembly as major factor causing neurodegeneration in FA.

In this study, we tested the therapeutic effects of L-carnitine and creatine supplementation, endogenous substances potentially improving energy metabolism in different neuromuscular and neurodegenerative disorders (Tarnopolsky and Beal, 2001). Bioenergetic dysfunction is thought to be primarily or secondarily involved in the pathogenesis of cell death underlying neurodegeneration. Enhanced energy reserves may help to protect neurons against cytotoxic agents, in particular if the disease is due to impaired mitochondrial ATP production as in FA (Brewer and Wallimann, 2000). Neuroprotective effects of creatine have been observed in animal models of neurodegenerative disorders such as Huntington's disease and amyotrophic lateral sclerosis (Klivenyi et al., 1998; Ferrante et al., 2000), whereas L-carnitine attenuates neuronal damage induced by 3-nitropropionic acid, rotenone or MPTP *in vitro* (Virmani et al., 1995). Furthermore, L-acetyl-carnitine increased phosphocreatine and decreased lactate in a ^{31}P and ^1H -MRS study in the adult and aged rat brain (Aureli et al., 1990).

^{31}P -MRS is a powerful measure of OXPHOS *in vivo* and may detect relevant biological effects earlier than any clinical benefit can be expected in a chronic progressive disease like FA. During ischemic exercise, when phosphorylation deficits in FA patients were most pronounced, we found PCr recovery to be improved by 30% with L-carnitine. However, placebo turned out to have a mild (15%) though insignificant effect on ^{31}P -MRS performance as well (Table 1, Fig. 2). In the triple-phase crossover design, comparison with placebo and creatine did not confirm a significant beneficial effect of L-carnitine.

This study underscores the importance of placebo controls in chronic progressive diseases like FA and questions positive results of uncontrolled trials of antioxidants like idebenone and coenzyme Q10 in FA published recently (Lodi et al., 1999; Hausse et al., 2002). Placebo-controlled trials could not proof a significant effect of idebenone on energy metabolism in FA and only minor effects on cardiac hypertrophy (Schöls et al., 2001; Mariotti et al., 2003).

A recent placebo-controlled trial with L-acetylcarnitine in 15 FA patients demonstrated mild improvement in clinical scores of coordination after 3 and 6 months with L-acetylcarnitine. However, similar effects occurred under placebo, and L-acetylcarnitine versus placebo effects were not directly compared

(Sorbi et al., 2000). Nevertheless, this study in conjunction with the presented trial demonstrate that L-carnitine is a promising substance for the treatment of FA. Larger controlled trials with L-carnitine are desirable in FA.

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References

- Aureli T, Miccheli A, Ricciolini R, et al. (1990) Aging brain; effect of acetyl-L-carnitine treatment on rat brain energy and phospholipid metabolism: a study by ^{31}P and ^1H NMR spectroscopy. *Brain Res* 526: 108–112
- Brewer GJ, Wallimann TW (2000) Protective effect of the energy precursor creatine against toxicity of glutamate and beta-amyloid in rat hippocampal neurons. *J Neurochem* 74: 1968–1978
- Campuzano V, Montermini L, Moltò MD, et al. (1996) Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* 271: 1423–1427
- Devereux RB, Reichek N (1977) Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. *Circulation* 55: 613–618
- Ferrante RJ, Andreassen OA, Jenkins BG, et al. (2000) Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. *J Neurosci* 20: 4389–4397
- Harding AE (1981) Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. *Brain* 104: 589–620
- Hausse AO, Aggoun Y, Bonnet D, et al. (2002) Idebenone and reduced cardiac hypertrophy in Friedreich's ataxia. *Heart* 87: 346–349
- Hemmer W, Wallimann T (1993) Functional aspects of creatine kinase in brain. *Dev Neurosci* 15: 249–260
- Infanate JP, Huszagh VA (2000) Secondary carnitine deficiency and impaired docosahexaenoic (22:6n-3) acid synthesis: a common denominator in the pathophysiology of diseases of oxidative phosphorylation and beta-oxidation. *FEBS Lett* 468: 1–5
- Klivenyi P, Ferrante RJ, Matthews ST, et al. (1998) Neuroprotective effects of creatine in a transgenic animal model of ALS. *Nat Med* 5: 347–350
- Lodi R, Cooper JM, Bradley JL, et al. (1999) Deficit of in vivo mitochondrial ATP production in Patients with Friedreich's ataxia. *Proc Natl Acad Sci USA* 96: 11492–11495
- Lodi R, Hart PE, Rajagopalan B, et al. (2001) Antioxidant treatment improves in vivo cardiac and skeletal muscle bioenergetics in patients with Friedreich's ataxia. *Ann Neurol* 49: 590–596
- Mariotti C, Solari A, Torta D, Marano L, Fiorentini C, Di Donato S (2003) Idebenone treatment in Friedreich patients: one-year-long randomized placebo-controlled trial. *Neurology* 60: 1676–1679
- Puccio H, Koenig M (2002) Friedreich ataxia: a paradigm for mitochondrial diseases. *Curr Opin Genet Dev* 12: 272–277
- Schoppe KP (1974) Das MLS-Gerät. Ein neuer Testapparat zur Messung feinmotorischer Leistungen. *Diagnostica* 20: 43–46
- Schöls L, Amoiridis G, Przuntek H, Frank G, Epplen JT, Epplen C (1997) Friedreich's ataxia: revision of the phenotype according to molecular genetics. *Brain* 120: 2131–2140
- Schöls L, Vorgerd M, Schillings M, Skipka G, Zange J (2001) Idebenone in patients with Friedreich ataxia. *Neurosci Lett* 306: 169–172
- Sorbi S, Forleo P, Fani C, Piacentini S (2000) Double-blind, crossover, placebo-controlled clinical trial with L-acetylcarnitine in patients with degenerative cerebellar ataxias. *Clin Neuropharmacol* 23: 114–118

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- Tarnopolsky MA, Beal MF (2001) Potential for creatine and other therapies targeting cellular energy dysfunction in neurological disorders. *Ann Neurol* 49: 561–574
- Trouillas P, Takayanagi T, Hallett M, et al. (1997) International cooperative ataxia rating scale for pharmacological assessment of the cerebellar syndrome. The ataxia neuropharmacology committee of the world federation of neurology. *J Neurol Sci* 145: 205–211
- Virmani MA, Biselli R, Spadoni A, et al. (1995) Protective actions of L-carnitine and acetyl-L-carnitine on the neurotoxicity evoked by mitochondrial uncoupling or inhibitors. *Pharmacol Res* 32: 383–389
- Vorgerd M, Schöls L, Hardt C, Ristow M, Epplen JT, Zange J (2000) Mitochondrial impairment of human muscle in Friedreich ataxia *in vivo*. *Neuromuscular Disord* 10: 430–435

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