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ORIGINAL CONTRIBUTION

Effect of carnitine, acetyl-, and propionylcarnitine supplementation on the body carnitine pool, skeletal muscle composition, and physical performance in mice

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Abstract
Purpose Pharmacokinetics and effects on skeletal muscle and physical performance of oral acetylcarnitine and propionylcarnitine are not well characterized. We therefore investigated the influence of oral acetylcarnitine, propionylcarnitine, and carnitine on body carnitine homeostasis, energy metabolism, and physical performance in mice and compared the findings to non-supplemented control animals.
Methods Mice were supplemented orally with 2 mmol/kg/day carnitine, acetylcarnitine, or propionylcarnitine for 4 weeks and studied either at rest or after exhaustive exercise.
Results In the supplemented groups, total plasma and urine carnitine concentrations were significantly higher than in the control group receiving no carnitine, whereas the skeletal muscle carnitine content remained unchanged. The supplemented acylcarnitines were hydrolyzed in intestine and liver and reached the systemic circulation as carnitine. Bioavailability of carnitine and acylcarnitines, determined as the urinary excretion of total carnitine, was in the range of 19 %. Skeletal muscle morphology, including fiber-type composition, was not affected, and oxygen consumption by soleus or gastrocnemius fibers was not different between the groups. Supplementation with carnitine or acylcarnitines had no significant impact on the running capacity, but was associated with lower plasma lactate levels and a higher glycogen content in white skeletal muscle after exhaustive exercise.
Conclusions Oral supplementation of carnitine, acetylcarnitine, or propionylcarnitine in mice is associated with increased plasma and urine total carnitine concentrations, but does not affect the skeletal muscle carnitine content. Despite better preservation of skeletal muscle glycogen and lower plasma lactate levels, physical performance was not improved by carnitine or acylcarnitine supplementation.

Keywords Carnitine and short-chain acylcarnitines · Bioavailability · Muscle composition and metabolism · Physical performance

R. Morand and J. Bouitbir have contributed equally to the study.

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Introduction

Carnitine is an amino acid derivative playing an essential role in cellular energy metabolism due to the acylation of its β-hydroxyl group. Carnitine is essential for the transport of long-chain fatty acids into the mitochondrial matrix [17], where they undergo β-oxidation. In addition, carnitine also acts as a buffer of the cellular pool of free coenzyme A (CoASH) [8, 16]. The major carnitine pool, accounting

for > 95 % of the total body stores, is located in skeletal muscle [22, 38].

Taking into account these important functions of carnitine, it is not surprising that carnitine supplementation has been studied intensively as a potential performance enhancer [6]. In ex vivo models, it has been reported that an elevation of the carnitine muscle content increases skeletal muscle force and delays fatigue [9, 14]. In humans, however, most studies failed to show a positive effect of carnitine supplementation on physical performance. This finding correlates with the fact that, despite elevated plasma concentrations, the skeletal muscle carnitine content could not be increased by oral or parenteral carnitine supplementation

High oral doses of carnitine during several days or intravenous infusion before exercise failed to show an impact on the physical performance of healthy volunteers or athletes [4, 31, 43]. Recently, however, Wall and colleagues reported an approximately 20 % increase in the carnitine muscle content in human subjects ingesting 80 g carbohydrate and 2 g carnitine tartrate per day [45]. This increase in the skeletal muscle carnitine content was associated with enhanced work output and maintained skeletal muscle glycogen stores as well as reduced lactate production during exercise.

Rodent studies investigating carnitine accumulation in skeletal muscle during or after carnitine supplementation led to controversial results. In several studies, an increase in the skeletal muscle carnitine content in supplemented rats was reported [2, 12, 21, 29, 40], whereas in other investigations in rats [24] or mice [34], no such increase could be detected. While the effect of exogenous carnitine on the carnitine skeletal muscle pool of rodents has been investigated intensively, studies about the effect of short-chain acylcarnitines, e.g., acetylcarnitine or propionylcarnitine, on the skeletal muscle carnitine content and morphology as well as on physical performance are rare. To the best of our knowledge, the pharmacokinetics of orally administered acetyl- and propionylcarnitine have so far not been described in detail. In humans, both acetylcarnitine [32] and propionylcarnitine [44] can intestinally be absorbed and can reach the systemic circulation, but the bioavailability is not known. In pigs, the relative bioavailability of oral acetylcarnitine (determined as free carnitine) was 61 % compared to L-carnitine [15]. In rodents, pharmacokinetic data are so far lacking for oral acetyl- and propionylcarnitine, but systemic effects have been described for both acylcarnitines [26], suggesting that intestinal absorption is possible. For carnitine, it has been shown that increased plasma concentrations are not necessarily associated with an increase in the skeletal muscle carnitine pool [7]. This can be explained by saturation of the transport into skeletal muscle already at physiological carnitine plasma concentrations [5] and by a much higher carnitine concentration in skeletal muscle compared to plasma [7, 16], rendering passive transport impossible. In comparison with carnitine, skeletal muscle concentrations of acetylcarnitine and propionylcarnitine are much lower under resting conditions. Similar to plasma, the acetylcarnitine concentration in skeletal muscle is in the range of 20 % of the free carnitine concentrations [16, 27], whereas, to the best of our knowledge, skeletal muscle propionylcarnitine concentrations have so far not been reported. In human plasma, the propionylcarnitine concentration is in the range of 0.5 mM [28]. Taking into account that short-chain acylcarnitines can cross biological membranes by passive diffusion [25], it might be possible to transport acetyl- and propionylcarnitine into skeletal muscle, if their plasma concentrations could be increased sufficiently.

In order to answer some of the open questions discussed above, we supplemented mice orally with high doses of carnitine, acetylcarnitine, or propionylcarnitine during 4 weeks, subjected some of them to exhaustive exercise and analyzed the carnitine pools and metabolic markers in skeletal muscle, plasma, and urine both at rest and after exhaustive exercise. The study was also designed to assess oral bioavailability of carnitine and acylcarnitines as well as the impact of carnitine supplementation skeletal muscle morphology and ex vivo oxidative capacity.

Materials and methods

Animals

The experiments were performed in agreement with the guidelines for the care and use of laboratory animals and were approved by the cantonal veterinary authority (License 2509). The animals were adult age-matched male C57BL/6 mice (Janvier, Le Genest-Saint-Isle, France), housed in a standard facility with 12-h light–dark cycles and controlled temperature (21–22 °C). The mice were fed with a standard pellet chow and water ad libitum. After 7 days of acclimatization, the mice were divided into four groups of 12 animals each: (1) Control group, (2) Carnitine group, (3) Acetylcarnitine group (Acetyl-Cn), and (4) Propionylcarnitine group (Propionyl-Cn). Half of the animals of each group ($n = 6$) were housed for 24 h in a metabolic cage for urine collection and performed an exhaustive exercise before killing (see below).

Carnitine or acylcarnitine supplementation

L-Carnitine and acetyl-L-Carnitine HCl were purchased from Sigma (St. Louis, MO, USA). Propionyl-L-Carnitine HCl was a kind gift of Sigma Tau (Pomezia, Rome, Italy).

The solutions of carnitine, acetylcarnitine, and propionylcarnitine were made in tap water once a week and were kept at 4 °C until use. After 1 week of acclimatization, the mice were supplemented with carnitine, acetylcarnitine, or propionylcarnitine via drinking water for 4 weeks. Carnitine and carnitine derivatives concentration in tap water were 10 mmol/L so that mice of about 25 g drinking 5 mL/day would be exposed to approximately 2 mmol/kg/day of each compound. Water consumption was monitored daily (see "Results"). Carnitine content in the standard chow and in the drinking water was determined using liquid chromatography–tandem mass spectrometry (LC–MS/MS) as previously described [28]. The mice received a standard pellet chow ad libitum (Kliba Futter 3436, Basel, Switzerland) with a carnitine content of 60 nmol/g.

Protocol

Between week 1 and 2 of supplementation, the mice spent 24 h individually in metabolic cages with supplemented water and food ad libitum. During this period, 24-h urine was collected to assess the excretion of carnitine and acylcarnitines.

After 4 weeks of supplementation, the mice were submitted to an exhaustive exercise on a treadmill with previous acclimatization to the apparatus (Exer-3/6, Columbus Instruments, Columbus, OH, USA). Two days and 1 day before initiation of the study, each mouse underwent a five-minute training session at a speed of 5 m/min. On the day of killing, the starting speed was 5 m/min; after 5 min, the speed was increased by 1 m/min every minute until the maximal speed of 20 m/min was reached. The rear of the treadmill was equipped with low-voltage, electric-stimulating grids, to encourage the mice to run. The grid delivered 0.4 mA at a frequency of 2 Hz, which caused an uncomfortable shock without injuring the animal. Exhaustion was defined as immobility for more than 5 s on the end lane grid despite electric stimulation.

Physical performance of the mice was calculated from the distance and the duration of the run according to the following equation:

$$\text{Performance(w)} = \frac{m\,(\text{kg}) \times a\,(\text{m/s}^2) \times d\,(\text{m})}{t\,(\text{s})} \qquad (1)$$

with m being the mass of the animal, a the gravitational acceleration, d the running distance, and t the running time.

Biological sample collection

After exhaustion, the mice were immediately anaesthetized with an intraperitoneal application of ketamine (100 mg/kg) and xylazine (10 mg/kg). The soleus and the superficial part of the gastrocnemius muscles were excised and conserved in ice-cold BIOPS buffer (10 mmol/L Ca-EGTA buffer, 0.1 μmol/L free calcium, 5.77 mmol/L ATP, 6.56 mmol/L $MgCl_2$, 20 mmol/L taurine, 15 mmol/L phosphocreatine, 0.5 mmol/L dithiothreitol, and 50 mmol/L K-MES, pH 7.1) until analysis.

For chemical analysis, muscle samples—red and white quadriceps biopsies—and liver samples were frozen in liquid nitrogen immediately after excision. Since the mice were anesthetized, tissues were obtained from living animals and the time between sampling and freezing was only a few seconds.

Blood was collected into heparin-coated tubes by an intracardiac puncture or a tail incision. Plasma was separated by centrifugation at 3,000*g* for 15 min. Plasma, urine, liver, and muscle samples were kept at −80 °C until analysis.

Carnitine and acylcarnitine bioavailability and biosynthesis

Carnitine and acylcarnitine bioavailability (F) and biosynthesis were calculated by solving equation for F or biosynthesis, respectively (2). Equation (2) represents the mass balance of carnitine and acylcarnitines, assuming that carnitine or acylcarnitine inputs into mice have to equal tissue accumulation plus carnitine excretion:

$$F \times (\text{intake}_{\text{water}} + \text{intake}_{\text{food}}) + \text{biosynthesis} = \text{tissue accumulation} + \text{excretion} \qquad (2)$$

Carnitine tissue accumulation was estimated using the average of the carnitine content of red and white muscle as the skeletal muscle carnitine content and assuming that skeletal muscle equals 35 % of the body weight in rodents [22]. Since skeletal muscle contains >90 % of tissue carnitine [22, 38], only the skeletal muscle carnitine content was used for estimating tissue accumulation. Carnitine excretion was determined using the 24-h urine samples obtained as described above. Carnitine intake by water or food was calculated by multiplying the amount of water or food ingested with the respective carnitine concentration.

F was calculated first for the mice treated with exogenous carnitine or acylcarnitines by solving Eq. (2) for F and assuming that intake of carnitine by food or water is much larger than carnitine biosynthesis [22]. The value for F obtained for mice treated with carnitine was then used to calculate carnitine biosynthesis in control mice using Eq. (2).

Skeletal muscle oxidative capacity

Mitochondrial respiration measured in situ allows the characterization of functional mitochondria in their normal

intracellular position and assembly, preserving essential interactions with other organelles. Mitochondrial oxygen consumption was studied in saponin-skinned fibers [23]. Fibers were separated under a binocular microscope in BIOPS buffer at 4 °C. After dissection, fibers were transferred into BIOPS buffer containing 50 µg/mL saponin and incubated at 4 °C for 30 min while shaking to complete permeabilization of the sarcolemma. Permeabilized fibers were then washed in BIOPS buffer for 10 min under intense shaking to completely remove saponin. Before oxygraphic measurements, the fibers were washed twice for 5 min in MiR05 buffer (EGTA 0.5 mmol/L, $MgCl_2$ 3 mmol/L, taurine 20 mmol/L, KH_2PO_4 10 mmol/L, HEPES 20 mmol/L, D-sucrose 110 mmol/L, BSA essentially fatty acid free 1 g/L, and lactobionic acid 60 mmol/L) to remove any trace amount of high-energy phosphates. All oxygen measurements were taken at 37 °C with an Oxygraph 2 k apparatus equipped with the Datlab software (OROBOROS, Innsbruck, Austria) [33]. Per respiration chamber, 1–2 mg of permeabilized fibers was added to 2.0 mL of MiR05 buffer.

The following protocol was used to evaluate the activity of the different mitochondrial complexes. The rate of basal respiration was measured with the complex I substrates glutamate (10 mM) and malate (2 mM), whereas active respiration was induced with 2 mM ADP. After inhibition of complex I with 0.5 mM rotenone, respiration was restored with the addition of complex II substrate succinate (10 mM). Inhibition of complex II with 20 mmol/L malonate was followed by the addition of the artificial substrates for complex IV, TMPD (0.5 mM), and ascorbate (2 mM). To verify the integrity of the outer mitochondrial membrane, cytochrome c (10 µmol/L) was added at the end of the respiration protocol. Respiration rates are expressed in picomoles O_2 per second per gram wet weight.

Skeletal muscle histology

Muscle biopsies for histological imaging were frozen in isopentane cooled in liquid nitrogen. Ten-micrometer serial sections were cut from the soleus and gastrocnemius muscle on a cryostat microtome (HYRAX C60, Carl Zeiss Microscopy, Jena, Germany) and mounted on glass slides. The histological structure of muscular fibers was determined by a routine hematoxylin/eosin staining. Soleus and gastrocnemius fiber-type composition was determined by histochemical staining of the myosin ATPase activity [3]. Stained fibers at pH 9.4 were counted from two non-overlapping visual fields using a microscope (Olympus BX43, Olympus corporation, Tokyo, Japan) at a 100-fold magnification. Quantification was performed with an empirically calibrated thresholding macro in imageJ [35], classifying and counting each pixel as either background, light or dark fibers, based on two cutoff values.

Urinary carnitine concentrations

A LC–MS/MS method previously described was adapted for the determination of carnitine and acylcarnitines in urine [28]. The method was extended to the analysis of propionylcarnitine (transitions 218/85 and 218/159) and creatinine (114/44 and 114/86). Hydrolysis of total carnitine was achieved with 0.5 mol/L potassium hydroxide, neutralization, and dilution with 0.1 % formic acid.

Plasma parameters

Plasma concentrations of carnitine were determined as previously described with adaptation to propionylcarnitine [28]. Venous lactate concentrations were analyzed with an enzymatic assay [30]. Creatine kinase activity in venous plasma was determined before and after exhaustive exercise with a kit according to the supplier's instructions (BioAssay Systems, Hayward, CA, USA).

Skeletal muscle and liver parameters

Muscle tissue was homogenized with a Micro-dismembrator during 1 min at 2,000 rpm (Sartorius Stedim Biotech, Göttingen, Germany). About 50 mg muscle was extracted with 1 mL of extracting solvent depending on the metabolites determined as described below. Liver tissue was homogenized during 30 s at 2,000 rpm and then extracted with 1 mL methanol per 100 mg tissue.

Phosphocreatine, creatine, and ATP were determined photometrically in an acidic extract (perchloric acid 0.5 mol/L, EDTA 1 mmol/L) of muscle powder as described [18]. Muscle glycogen content was analyzed in alkaline (NaOH 0.1 mmol/L) muscle extracts according to Harris [18]. Carnitine, acylcarnitines, and total carnitine were determined in aqueous muscle extracts with an established LC–MS/MS method [28]. This assay had an interassay precision of <6.0 % for all measured analytes, and the interday accuracies were between 92.9 and 105.5 %.

Statistical analysis

Data are expressed as mean ± SEM. Comparisons of two groups were performed using the unpaired two-tailed Student's *t* test. Multiple groups were compared using either one-way ANOVA or two-way ANOVA both followed by a Dunnett posttest. The software used was Prism version 5 (Graph Pad Software, San Diego, CA). Groups supplemented with carnitine or acylcarnitines were compared

individually with the untreated control group. For certain analyses, the groups treated with carnitine or acylcarnitines were also pooled for the comparison with the control group. Statistical significance was set at $*p < 0.05$.

Results

Characterization of the animals

During the study, the daily food intake was similar in all groups (mean ± SEM; 4.04 ± 0.18 g in the control group, 4.14 ± 0.27 g in the carnitine, 3.81 ± 0.41 g in the acetylcarnitine and 4.17 ± 0.12 g in the propionylcarnitine group) and we observed no weight differences between the groups (Fig. 1a). The body weight gain (mean ± SEM) was 2.78 ± 0.23 g in the control group, 3.26 ± 0.28 g in the carnitine, 3.10 ± 0.42 g in the acetylcarnitine, and 3.37 ± 0.24 g in the propionylcarnitine group. In contrast, the daily water intake was significantly increased in all groups treated with carnitine or acylcarnitines compared to the control group (Fig. 1b). The control animals ($n = 12$) drank 4.1 ± 0.2 mL/day, whereas the animals of the carnitine, acetylcarnitine, and propionylcarnitine group ingested 5.2 ± 0.1 mL/day, 5.7 ± 0.3 mL/day, and 5.6 ± 0.1 mL/day, respectively (mean ± SEM; no difference between treated animals). The mean daily exposure to exogenous carnitine, acetylcarnitine, or propionylcarnitine was 2.2 mmol/kg/day (range 1.9–2.7 mmol/kg/day) in the treated groups and below 10 µmol/kg/day in the control group, taking into account the carnitine ingested in drinking water and in food (Fig. 1c). Concentrations of carnitine, acetylcarnitine, and propionylcarnitine in drinking solutions remained stable during their storage at 4 °C and in the water bottles at room temperature (data not shown).

Carnitine and acylcarnitine pools in plasma, liver, and skeletal muscle and renal carnitine excretion

In plasma, total carnitine concentrations were 17–31 % higher in the treated groups compared to control animals, reaching statistical significance for all treatments (Table 1). This increase was mainly due to elevated free carnitine concentrations, whereas acetylcarnitine and palmitoylcarnitine plasma concentrations were similar between the groups. The propionylcarnitine concentration was approximately three times higher in mice treated with propionylcarnitine compared to the other groups but without reaching statistical significance.

In red or white skeletal muscle, we observed no difference in carnitine pools between the groups treated with carnitine or acylcarnitines and the control group.

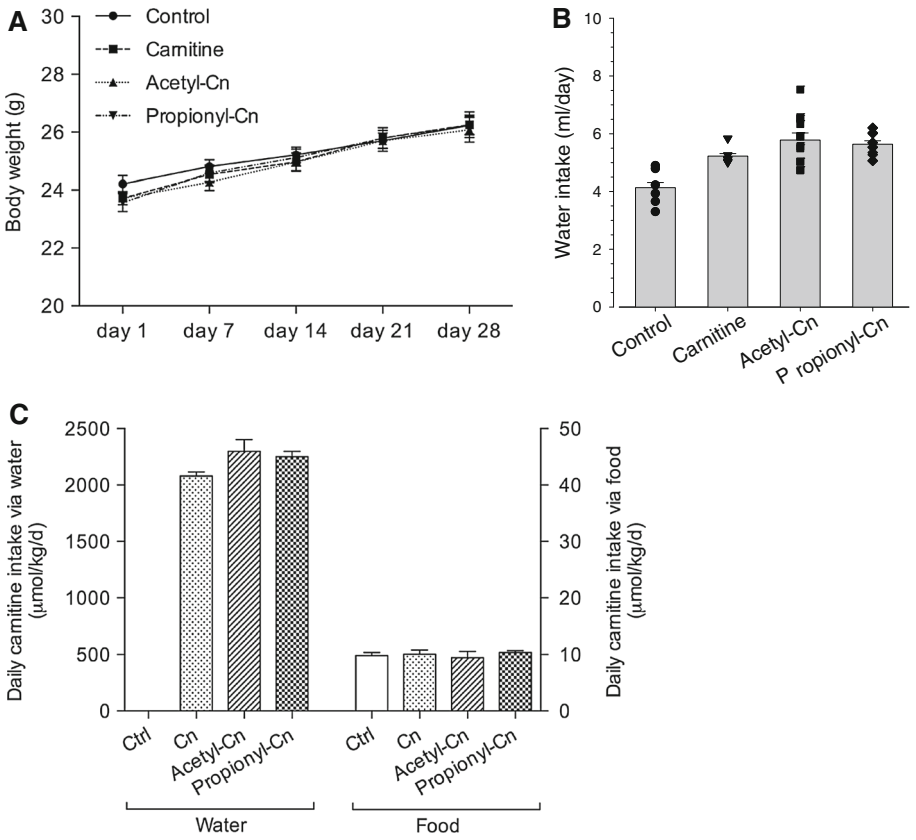

Fig. 1 Characterization of the animals. The weight gain (*in grams*) was similar in the treatment groups compared to control (**a**). Water consumption (in mL/mouse/day) was significantly higher in the treated groups (**b**). Daily consumption of carnitine or acylcarnitines was below 10 µmol/kg/day in the control group and in the range of 2,000 µmol/kg/day in the supplemented groups (**c**). Data are represented as mean ± SEM of 12 animals per group

Table 1 Concentrations of carnitine and acylcarnitines in plasma and skeletal muscle under resting conditions

	Control	Carnitine	Acetyl-Cn	Propionyl-Cn
Plasma concentrations (μmol/L)				
Carnitine	31.0 ± 0.5	37.2 ± 2.2	36.1 ± 1.4	35.6 ± 3.4
Acetylcarnitine	7.8 ± 0.8	8.5 ± 1.3	9.3 ± 0.6	7.6 ± 0.7
Propionylcarnitine	0.30 ± 0.04	0.24 ± 0.07	0.27 ± 0.04	0.76 ± 0.38
Palmitoylcarnitine	0.25 ± 0.01	0.29 ± 0.04	0.25 ± 0.02	0.30 ± 0.03
Total carnitine	36.4 ± 1.4	47.7 ± 1.8*	47.6 ± 1.6*	42.7 ± 1.6*
Skeletal muscle concentrations (μmol/kg wet weight)				
Red quadriceps				
Free carnitine	205 ± 29	272 ± 32	216 ± 25	189 ± 14
Acetylcarnitine	66 ± 7	69 ± 11	70 ± 3	62 ± 9
Propionylcarnitine	<0.5	<0.5	<0.5	<0.5
Palmitoylcarnitine	44 ± 8	55 ± 6	32 ± 12	28 ± 5
Total carnitine	291 ± 31	309 ± 59	359 ± 56	249 ± 43
White quadriceps				
Free carnitine	110 ± 7	115 ± 10	127 ± 11	130 ± 11
Acetylcarnitine	39 ± 5	37 ± 10	26 ± 3	28 ± 5
Propionylcarnitine	<0.5	<0.5	<0.5	<0.5
Palmitoylcarnitine	15 ± 5	20 ± 3	14 ± 3	19 ± 5
Total carnitine	195 ± 17	207 ± 14	169 ± 20	152 ± 14

Mean values ± SEM of six animals are represented

* $p < 0.05$ compared to control

Acetylcarnitine concentrations accounted for 20 % of total pool in red fibers and for 15 % in white fibers. Total carnitine, free carnitine, acetylcarnitine, and palmitoylcarnitine concentrations were by trend lower in the white compared to the red quadriceps. Propionylcarnitine concentrations in skeletal muscle were below detection limits (<0.5 μmol/kg), also in mice treated with propionylcarnitine (Table 1).

In liver samples of animals supplemented with propionylcarnitine, the free carnitine content (vs. non-supplemented control animals) was 449 ± 46 versus 314 ± 16 μmol/kg ($p < 0.05$), the acetylcarnitine content 231 ± 22 versus 195 ± 8 μmol/kg (not significant), and the propionylcarnitine content 4.7 ± 0.5 versus 3.1 ± 0.2 μmol/kg ($p < 0.05$).

Mice treated with carnitine or acylcarnitines excreted 16–18 times more total carnitine than control animals (Table 2). The major part of excreted carnitine was found in the form of free carnitine and acetylcarnitine. In all treated groups, independently of the supplemented form of carnitine, carnitine and acetylcarnitine excretion was significantly higher than in the untreated control group. Urinary excretion of propionylcarnitine could only be detected in the three supplemented groups, whereas propionylcarnitine concentrations were below detection limits in urine of control mice. The highest urinary propionylcarnitine excretion was found in the group treated with propionylcarnitine. Due to the high variability, this value was not significantly different from the propionylcarnitine concentration in the urines of mice treated with carnitine or acetylcarnitine.

Ingestion and bioavailability of carnitine and acylcarnitines and carnitine biosynthesis

Total carnitine ingestion, bioavailability, and biosynthesis are presented in Table 3. Total carnitine intake was approximately 200 times higher in animals supplemented with carnitine, acetylcarnitine, or propionylcarnitine as compared to untreated control animals. Carnitine tissue accumulation, calculated from the mean total carnitine concentration in red and white muscles and from the muscle weight gain during the observation period of 1 month, was not different between the groups and was quantitatively negligible compared to oral ingestion of carnitine. Excretion of total carnitine was, as mentioned in the previous section, 16–18 times higher in the treated groups as compared to the control group. Carnitine bioavailability, calculated according to Eq. 2 and assuming that carnitine biosynthesis is much lower than the ingestion of exogenous carnitine or acylcarnitines by the drinking water, ranged between 18.6 and 19.8 % for carnitine and acylcarnitines without a significant difference between the supplemented groups. It is important to realize that bioavailability was calculated from the urinary excretion of carnitine (also for the acylcarnitines), since the acylcarnitines were hydrolyzed in the gut and/or the liver and reached the systemic circulation only in the form of carnitine. Carnitine biosynthesis, calculated for control mice using Eq. (2) and the value for carnitine bioavailability obtained for mice treated with carnitine, was in a similar range as reported for rats [22].

Table 2 Urinary excretion of carnitine, acetylcarnitine (Acetyl-Cn), propionylcarnitine (Propionyl-Cn), and total carnitine

	Control	Carnitine	Acetyl-Cn	Propionyl-Cn
Free carnitine	19 ± 2	356 ± 71*	335 ± 68*	360 ± 34*
Acetylcarnitine	2 ± 0	41 ± 10*	54 ± 13*	46 ± 6*
Propionylcarnitine	<0.5	1.0 ± 0.3*	1.1 ± 0.3*	3.3 ± 1.1*
Total carnitine	24 ± 4	417 ± 143*	390 ± 76*	448 ± 36*

Excreted amounts are mean values ± SEM of six animals, given in μmol/kg/day

* $p < 0.05$ compared to control group

Table 3 Carnitine bioavailability and biosynthesis

Treatment groups ($n = 6$)	Intake (μmol/kg bw/day)		Tissue accumulation (μmol/kg bw/day)	Excretion (μmol/kg bw/day)	Bioavailability (%)	Biosynthesis (μmol/kg bw/day)
	Water	Food				
Control	0 ± 0	9.7 ± 0.5	0.28 ± 0.04	24 ± 4	n.a.	22.4 ± 0.1
Carnitine	2,100 ± 26*	10.1 ± 0.7	0.31 ± 0.05	417 ± 143*	19.8 ± 0.3	n.a.
Acetyl-Cn	2,102 ± 78*	9.4 ± 0.8	0.35 ± 0.08	390 ± 76*	18.6 ± 0.7	n.a.
Propionyl-Cn	2,338 ± 46*	10.1 ± 0.1	0.41 ± 0.05	448 ± 36*	19.1 ± 0.4	n.a.

Carnitine intake by water and food was determined as described in Methods. Carnitine tissue accumulation was calculated based on the carnitine content in skeletal muscle and the increase in skeletal muscle weight over 28 days. Carnitine excretion was determined in the 24-h urine of the animals. The bioavailability (F) of carnitine and acylcarnitines was calculated as carnitine by solving Eq. (2) for F and assuming that carnitine biosynthesis is ≪ carnitine ingestion by food and water and can therefore be neglected [22]. Carnitine biosynthesis was calculated by solving Eq. (2) for carnitine biosynthesis and using the previously determined value for carnitine bioavailability in mice treated with carnitine. Values are given as mean ± SEM for 6 animals

bw body weight, *n.a.* not available

* $p < 0.05$ versus control mice. There were no significant differences among the groups treated with carnitine or acylcarnitines

Skeletal muscle oxidative capacity

As expected, maximal respiration rate in the soleus muscle, a red muscle mainly composed of oxidative fibers, was higher than in the gastrocnemius, which consists mainly of glycolytic fibers (Fig. 2). For soleus and gastrocnemius muscle biopsies, we observed no significant difference in oxygen uptake between animals treated with carnitine or acylcarnitines and control animals. These findings indicate that mitochondrial content and capacity (the activity of complexes I, II, and IV of the respiratory chain) remained unchanged in the supplemented compared to the control group.

Skeletal muscle histology

As illustrated in Fig. 3a, we observed no difference in muscle fiber-type composition in soleus and gastrocnemius muscles of treated as compared to control animals. In soleus muscle, type I fibers represented about 70 % of the fibers in all treatment groups studied (Fig. 3b).

Exhaustive exercise

As shown in Fig. 4, the mean running distance until exhaustion of control mice was 561 ± 61 m ($n = 6$). The distance covered by treated mice was by trend increased in all the groups but no statistical significance was observed because of high interindividual variability (carnitine 699 ± 108 m, acetylcarnitine 772 ± 117 m, propionylcarnitine 820 ± 214 m). After pooling all treated animals, the running distance was 764 ± 84 m ($n = 18$) and also not significantly different from the control group. Performance of the animals was in the range of 67–70 mW and was not different between the groups.

Energy metabolism parameters in plasma and skeletal muscle at rest and after exhaustive exercise

As shown in Table 4, plasma lactate levels increased significantly with exercise in all groups, indicating that the mice exercised above the lactate threshold. The lactate concentrations after exercise were significantly lower in the

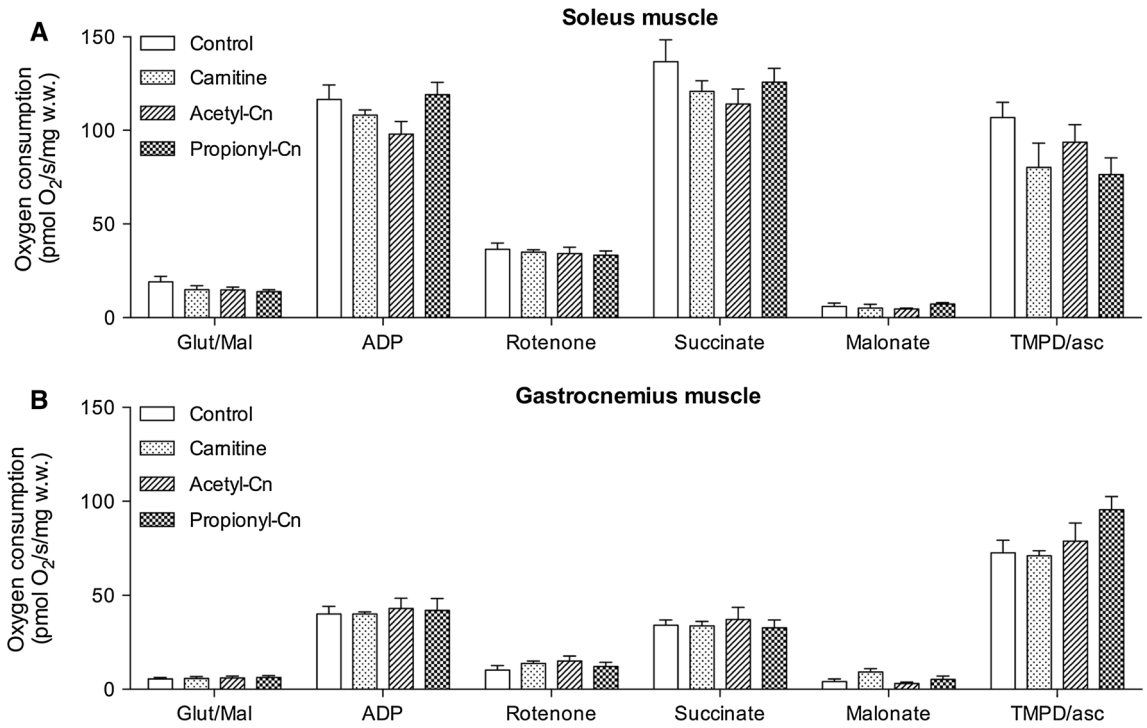

Fig. 2 Oxygen consumption by isolated muscle fibers. No differences were observed between carnitine or acylcarnitine supplemented mice and control mice. Data are represented as mean in pmol O_2/s/mg protein ± SEM of six animals

combined supplemented groups compared to the control group. Plasma creatine kinase activity was not different between supplemented and control animals and showed a slight elevation with exercise compared to resting conditions (results not shown).

Energy parameters were determined in separate samples of white and red quadriceps femoris (Table 4). Creatine concentrations were not different between supplemented and control groups, and were also not significantly influenced by exercise in both red and white quadriceps. Phosphocreatine concentrations increased by trend with exercise in red quadriceps and decreased in white quadriceps. The ATP concentrations were not influenced by exercise, neither in red, nor in white quadriceps. In red quadriceps, they were by trend higher in supplemented mice, reaching statistical significance for the combined supplemented groups both under resting conditions and after exhaustive exercise. Exercise was associated with a significant decrease in the glycogen content in white fibers of control animals and the combined supplemented animals, while it did not affect the glycogen content in red muscle fibers. After exercise, the glycogen content was approximately doubled in supplemented compared to control animals, reaching statistical significance for the combined supplemented mice.

Discussion

In contrast to reports in humans [32, 44], acetylcarnitine and propionylcarnitine showed no significant bioavailability as parent substance in mice. Both acylcarnitines were hydrolyzed before reaching the systemic circulation; as shown for propionylcarnitine, hydrolysis took place at least partially in the liver. These findings are in agreement with a study in pigs, also showing that most orally administered acetylcarnitine is hydrolyzed before reaching the systemic circulation [15]. Bioavailability, estimated as the excess urinary excretion of total carnitine, was in the range of 19 % for carnitine and also for the two acylcarnitines investigated. This value is in close agreement with the 14–18 % reported for carnitine in humans [36, 37]. It is important to realize that the carnitine excretion pattern was almost identical (15- to 20-fold increase in the excretion of total carnitine, approximately 15 % as acetylcarnitine and <1 % as propionylcarnitine) irrespective of the carnitine species administered. At least for propionylcarnitine, this means that the compound is taken up by tissues, hydrolyzed to carnitine, and then partially acetylated. This agrees well with studies in which labeled propionylcarnitine was administered intravenously to rats [13]. Thirty minutes after the injection, the labeled plasma carnitine pool contained <10 % propionylcarnitine, but

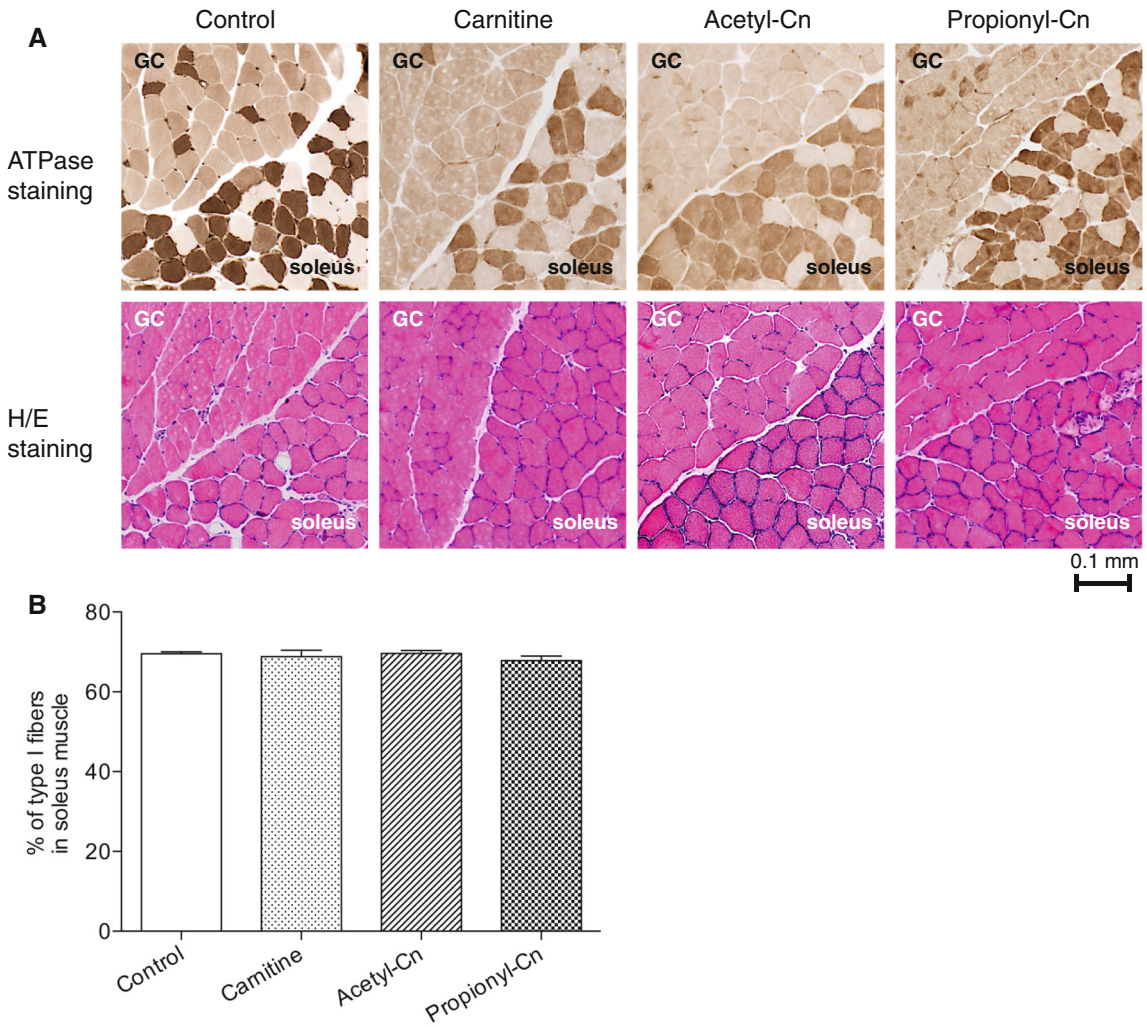

Fig. 3 Histochemical determination of muscle fiber-type composition. **a** Representative ATPase and hematoxylin/eosin (H/E) staining of soleus and gastrocnemius (GC) biopsies. **b** In soleus muscle, we observed no difference in muscle fiber-type composition between treated and control animals. Mean values ± SEM of six animals are represented

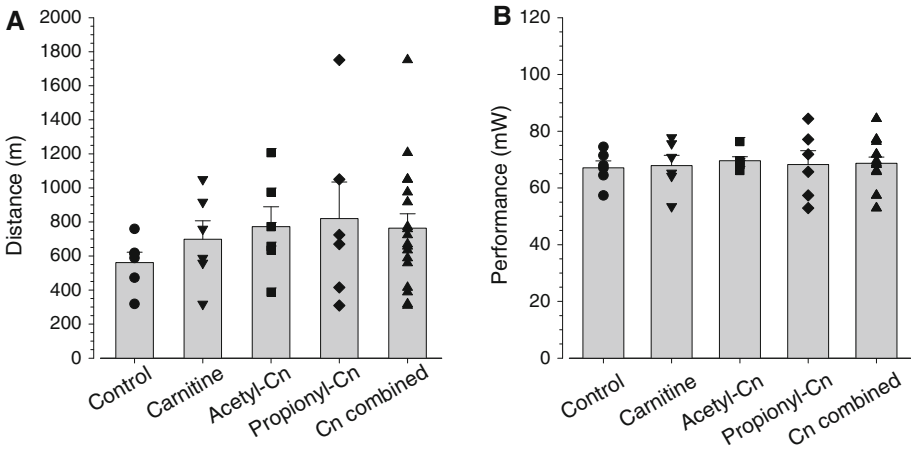

Fig. 4 Running capacity of mice completing a treadmill exercise until exhaustion. A slight upward trend for the running distance **a** was observed in treated groups. Performance expressed in mW **b** did not differ between the groups. Mean values ± SEM of six animals are represented

approximately 15 % acetylcarnitine and 80 % carnitine. Similarly, in humans treated with oral carnitine, elevated concentrations of acetylcarnitine and propionylcarnitine have been observed in plasma and urine [10]. Furthermore, in patients with end-stage renal failure undergoing hemodialysis, intravenous administration of carnitine was associated

Table 4 Effect of exercise on skeletal muscle and plasma parameters

	Control ($n = 6$)	Carnitine ($n = 6$)	Acetyl-Cn ($n = 6$)	Propionyl-Cn ($n = 6$)	Carnitine combined ($n = 18$)	Control ($n = 6$)	Carnitine ($n = 6$)	Acetyl-Cn ($n = 6$)	Propionyl-Cn ($n = 6$)	Carnitine combined ($n = 18$)
	Resting conditions					Exhaustive exercise				
Red quadriceps										
Creatine (nmol/g)	25.4 ± 1.4	23.6 ± 1.3	28.2 ± 0.8	25.2 ± 2.1	25.6 ± 0.9	25.8 ± 1.2	26.6 ± 1.3	24.8 ± 1.6	28.3 ± 1.4	26.6 ± 0.8
Phosphocreatine (nmol/g)	3.1 ± 1.1	7.5 ± 1.6	7.1 ± 0.9*	6.1 ± 1.8	6.9 ± 0.8*	9.0 ± 1.4^{+}	7.7 ± 2.2	12.8 ± 0.9^{+}	9.3 ± 1.3	9.9 ± 1.0^{+}
ATP (nmol/g)	4.3 ± 0.6	8.6 ± 1.5	9.9 ± 0.8	8.5 ± 1.4	9.0 ± 0.7*	6.6 ± 1.2	11.0 ± 1.6	8.8 ± 0.9	9.3 ± 1.2	9.7 ± 0.7*
Glycogen (μmol/g)	9.5 ± 1.1	11.8 ± 0.9	12.8 ± 0.9	11.5 ± 2.6	12.0 ± 0.9	7.3 ± 1.7	14.7 ± 2.3	14.8 ± 3.1	8.5 ± 1.9	12.7 ± 1.5
White quadriceps										
Creatine (nmol/g)	23.7 ± 2.5	21.8 ± 2.5	26.6 ± 2.3	21.6 ± 1.5	23.3 ± 1.3	26.1 ± 2.0	26.1 ± 1.4	23.0 ± 1.9	24.5 ± 1.3	24.5 ± 0.9
Phosphocreatine (nmol/g)	11.8 ± 2.0	12.1 ± 0.3	10.9 ± 2.5	10.3 ± 1.8	11.1 ± 1.0	4.4 ± 1.1^{+}	7.7 ± 1.8	7.8 ± 2.1	7.0 ± 1.2	7.5 ± 1.0^{+}
ATP (nmol/g)	8.2 ± 1.0	9.7 ± 0.9	9.6 ± 0.6	9.2 ± 0.9	9.5 ± 0.4	9.4 ± 1.0	9.1 ± 2.0	10.9 ± 1.0	9.3 ± 0.7	9.8 ± 0.7
Glycogen (μmol/g)	17.0 ± 2.9	13.6 ± 1.3	20.9 ± 1.7	15.1 ± 1.1	16.6 ± 1.1	5.6 ± 1.8^{+}	9.7 ± 1.4	14.4 ± 2.7	12.6 ± 2.5	12.3 ± 1.3*$^{+}$
Plasma										
Lactate (mmol/L)	2.2 ± 0.1	2.2 ± 0.2	2.1 ± 0.2	1.9 ± 0.1	2.1 ± 0.1	6.2 ± 0.3^{+}	4.8 ± 0.2^{+}	5.0 ± 0.2^{+}	5.2 ± 0.5^{+}	5.0 ± 0.2*$^{+}$

Carnitine combined represents the mean of all animals treated with carnitine, acetylcarnitine, or propionylcarnitine. Concentrations are given per g wet weight. Mean values ± SEM are presented

* $p < 0.05$ compared to respective control animals

$^{+}$ $p < 0.05$ compared to respective resting values

with increased plasma concentrations of many acylcarnitines and increased elimination of acylcarnitines by dialysis [41]. These findings provide evidence for an intense exchange between the plasma and tissue carnitine pools. In tissues, carnitine can be acylated (depending on the cellular acyl-CoA pool), transported back into plasma as acylcarnitine and eventually be excreted via the urine.

The current investigation is in agreement with other animal studies reporting that oral supplementation of carnitine or acetylcarnitine has no significant impact on the skeletal muscle carnitine content. In rats, low oral doses of carnitine or acetylcarnitine (200 μmol/kg/day) for 14 days did not increase the skeletal muscle carnitine pool [24]. In mice, a carnitine dose of 200 mg/kg/day (approximately 1 mmol/kg/day) for 5 weeks also failed to show an effect on the skeletal muscle carnitine pool [34]. In contrast, several studies in rats have shown that the skeletal muscle carnitine content can be increased with oral carnitine supplementation [2, 12, 21, 29, 40]. The reasons for the divergent findings regarding the effect of exogenous carnitine on the skeletal muscle carnitine pool are so far not clear; important variables may be the skeletal muscle carnitine content before the administration of exogenous carnitine [12, 40] and nutritional factors.

To the best of our knowledge, data concerning oral bioavailability were so far lacking for propionylcarnitine. In humans, propionylcarnitine can be absorbed intestinally and can reach the systemic circulation [44]. However, after a single dose of 500 mg, the plasma concentration was only doubled (from 0.3 to 0.6 mM), suggesting a low bioavailability as propionylcarnitine. In the current study, we also observed a doubling of the plasma propionylcarnitine concentration, but this increase did not reach statistical significance. Although the propionylcarnitine concentration was higher in plasma than in skeletal muscle of the mice supplemented with propionylcarnitine, we did not observe an increase in the skeletal muscle propionylcarnitine and/or total carnitine concentration. Obviously, even higher plasma propionylcarnitine concentrations would be necessary to have an impact on the skeletal muscle carnitine pool. As suggested by the current study, such concentrations could only be reached by parenteral application.

Consistent with our findings in the current study, supplementation with high doses of carnitine or acylcarnitines had no influence on muscle fiber-type composition also in other studies [11]. This is in contrast to recent findings in obese Zucker rats, where oral supplementation of carnitine not only increased the skeletal muscle carnitine content, but also prevented the obesity-associated shift from type I to type II muscle fibers [12]. In agreement with the lacking effect on skeletal muscle morphology in the current study, treatment with carnitine or acylcarnitines had also no significant impact on oxygen consumption by and mitochondrial function in isolated skeletal muscle fibers (Fig. 2). These findings agree with most studies in humans in which the effect of oral carnitine supplementation on the total muscle carnitine content and on physical performance has been assessed [4, 43]. On the other hand, the results of the current study disagree with two studies in long-distance runners, in which oral supplementation of 2 g of carnitine per day for 1 month was associated with an approximately 10 % increase in the skeletal muscle carnitine content and with an increased activity of mitochondrial enzymes, including complexes of the respiratory chain [1, 19]. An explanation for these discrepancies may be interactions between nutrition and expression of the carnitine carrier OCTN2. It is well established that carnitine supplementation in humans treated with a high amount of carbohydrates can increase skeletal muscle carnitine content and physical performance due to increased expression of OCTN2 [39, 45]. In addition, PPARα has been reported to be a regulator of OCTN2 expression, suggesting that also lipids may affect carnitine transport into tissues [46].

Surprisingly, despite the lacking effect of carnitine or acylcarnitine supplementation on the skeletal muscle carnitine pool, carnitine supplementation was associated with a lower plasma lactate concentration and a better maintenance of the glycogen stores in white skeletal muscle after exhaustive exercise (Table 4). Lower post-exercise blood lactate concentrations have recently also been reported in humans treated with 4.5 g glycine propionylcarnitine before exercise [20]. These findings indicate that the animals supplemented with carnitine or acylcarnitines tolerated exhaustive exercise metabolically all in all better than the control animals. This may at least partially explain the beneficial effects of carnitine supplementation on physical recovery after intense exercise [42]. In a review about the pharmacokinetics and metabolism of carnitine and acetylcarnitine, Rebouche [36] asked the question whether an increased carnitine content in target tissues is necessary for a pharmacological effect of carnitine or whether an increased exchange between the plasma and tissue carnitine pools could be sufficient. As discussed above, the current study provides evidence that there is an intense exchange between the plasma and tissue carnitine pools. The current study also suggests that an increase in the plasma carnitine pool may be sufficient to influence certain metabolic pathways in skeletal muscle, e.g., lactate production and glycogen breakdown. The molecular mechanisms responsible for this effect remain speculative, however. One possibility is the export of potentially toxic acyl groups from skeletal muscle, as shown in patients on hemodialysis supplemented with carnitine [41]. Another possibility is an effect of carnitine and/or acylcarnitines on capillary endothelial cells, possibly resulting in

vasodilation and improved skeletal muscle perfusion and nutrient supply during high-intensity exercise [20].

Conclusions

Oral supplementation of carnitine, acetylcarnitine, or propionylcarnitine is associated with increased plasma concentrations of total carnitine and increased urinary excretion of carnitine, but does not affect the skeletal muscle carnitine content. Skeletal muscle morphology, oxidative capacity, and physical performance are also not affected by long-term carnitine supplementation. On the other hand, white skeletal muscle glycogen stores were maintained better and plasma lactate concentrations were lower in supplemented animals after exhaustive exercise, indicating that mice with carnitine supplementation could achieve the same physical performance as control mice with less metabolic perturbance.

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Conflict of interest None of the authors reports any conflict of interest regarding this study.

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