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ORIGINAL CONTRIBUTION

Effect of L-carnitine supplementation on the body carnitine pool, skeletal muscle energy metabolism and physical performance in male vegetarians

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Abstract
Purpose More than 95 % of the body carnitine is located in skeletal muscle, where it is essential for energy metabolism. Vegetarians ingest less carnitine and carnitine precursors and have lower plasma carnitine concentrations than omnivores. Principle aims of the current study were to assess the plasma and skeletal muscle carnitine content and physical performance of male vegetarians and matched omnivores under basal conditions and after L-carnitine supplementation.
Results Sixteen vegetarians and eight omnivores participated in this interventional study with oral supplementation of 2 g L-carnitine for 12 weeks. Before carnitine supplementation, vegetarians had a 10 % lower plasma carnitine concentration, but maintained skeletal muscle carnitine stores compared to omnivores. Skeletal muscle phosphocreatine, ATP, glycogen and lactate contents were also not different from omnivores. Maximal oxygen uptake (VO_2max) and workload (P_{max}) per bodyweight (bicycle spiroergometry) were not significantly different between vegetarians and omnivores. Sub-maximal exercise (75 % VO_2max for 1 h) revealed no significant differences between vegetarians and omnivores (respiratory exchange ratio, blood lactate and muscle metabolites). Supplementation with L-carnitine significantly increased the total plasma carnitine concentration (24 % in omnivores, 31 % in vegetarians) and the muscle carnitine content in vegetarians (13 %). Despite this increase, P_{max} and VO_2max as well as muscle phosphocreatine, lactate and glycogen were not significantly affected by carnitine administration.
Conclusions Vegetarians have lower plasma carnitine concentrations, but maintained muscle carnitine stores compared to omnivores. Oral L-carnitine supplementation normalizes the plasma carnitine stores and slightly increases the skeletal muscle carnitine content in vegetarians, but without affecting muscle function and energy metabolism.

Keywords Vegetarians · L-carnitine supplementation · Spiroergometry · Skeletal muscle · Energy metabolism

Katerina Novakova, Oliver Kummer and Jamal Bouitbir have contributed equally to this work.

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Introduction

Vegetarian diets, including diets devoid of meat and fish (vegetarians) or, in addition, devoid of eggs (lacto-vegetarians) or of eggs and all dairy products (vegans), are popular in industrialized countries mainly due to health considerations, concern for the environment and animal welfare factors [1]. Regarding health considerations, clinical studies have demonstrated that obesity and type 2 diabetes are less prevalent [2]

and cardiovascular mortality is reduced [3] in vegetarians as compared to non-vegetarians. Reasons for these findings may be lower ingestion of calories, saturated fatty acids, cholesterol and animal proteins as well as higher ingestion of plant sterols, polyunsaturated fatty acids and antioxidants [1].

However, it has also been recognized that vegetarian diets can be deficient in certain nutrients such as vitamin B_{12}, vitamin D, iron and the *n*-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), possibly requiring supplementation from non-animal-based sources to meet nutritional guidelines [1]. One nutrient that is almost totally devoid in a vegetarian diet is carnitine [4]. The consequences of limited carnitine ingestion upon the health of vegetarians have received relatively little attention to date [5–7]. This is surprising, as it well established that the carnitine requirements of the body are met predominantly from the consumption of meat [8, 9]. Indeed, long-term vegetarians have lower plasma carnitine concentrations than omnivores and a markedly reduced urinary carnitine excretion [6]. In a recent study, these findings have been confirmed and also a slight reduction in the skeletal muscle carnitine content of vegetarians has been reported [10].

L-carnitine, the biologically effective isomer of carnitine, is found ubiquitously in mammalian tissues and plays a key role within several cellular energy producing pathways [9, 11]. More than 95 % of the body's total carnitine is localized in skeletal muscle [9], where it is essential for the transport of long-chain fatty acids across the inner mitochondrial membrane into the mitochondrial matrix, the place of β-oxidation [12, 13]. Furthermore, carnitine is important for the removal of potentially toxic acyl-CoAs by facilitating the formation of acylcarnitines which can be exported from mitochondria [14, 15]. In addition, carnitine serves as a temporal acetyl group buffer during the oxidation of carbohydrates for periods of a high pathway flux [16, 17].

Considering the existing literature about carnitine homeostasis in vegetarians and the important role of carnitine in energy metabolism, we decided to study the effect of long-term oral treatment with carnitine on the body carnitine pool, fuel metabolism and physical performance of vegetarians and omnivores. We hypothesized that treatment with carnitine would increase plasma and possibly also skeletal muscle carnitine concentrations in vegetarians and would thereby improve skeletal muscle energy metabolism and physical performance.

Materials and methods

Ethics committee and subject recruitment

The study was approved by the Ethics Committee of the State of Basel Stadt. Twenty-four healthy male subjects aged between 18 and 40 years of age were recruited. Of these 24 subjects, eight were omnivores and served as a control group. The remaining 16 subjects were vegetarians needing to have consumed a vegetarian diet for a period of no less than 1.5 years. All subjects needed to be non-obese (BMI in the range of 19–25), non-highly athletically trained (no more than two trainings a week) and to have no history over the preceding 4 months of supplementary L-carnitine or creatine ingestion. Exclusion criteria were regular ingestion of medication, and any personal history of hypertension, mental illness, kidney and cardiopulmonary disease.

Experimental protocol

The study consisted of five components, which were spread over a total time of 16 weeks (see Fig. 1). After completing the screening visit, plasma samples and a 24-hour urine collection were obtained to investigate renal handling and renal excretion of carnitine. Within the following 2 weeks, a maximal oxygen uptake (VO_2max) test was performed on an ergometer (see below) to assess the level of aerobic fitness of the participants and to calculate the workload for the sub-maximal exercise testing.

Subjects then reported back to the laboratory 1 week later after an overnight fast and after emptying their bladder. A blood sample was drawn, and a muscle biopsy was taken from the subjects non-dominant leg. After 30 min of recovery, subjects performed 60 min of sub-maximal cycling exercise at a workload of 75 % of VO_2max.

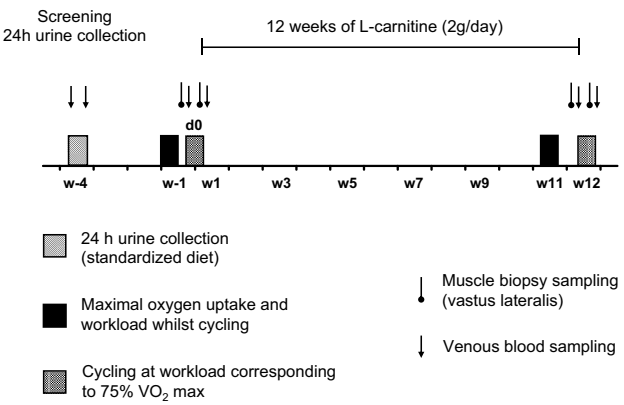

Fig. 1 Outline of the study. The study consisted of five parts spread over a period of 16 weeks. Part 1 was a screening visit, followed by a 24-hour urine collection. Part 2 was a maximal oxygen uptake (VO_2max) test on an ergometer (initial grading). Part 3 was an exercise test at 75 % of VO_2max. After part 3, the participants had to ingest 2 g of L-carnitine per day for 12 weeks. Part 4 was a VO_2max test on an ergometer performed 1 week before termination of supplementation with L-carnitine. Part 5 was an exercise test at 75 % of VO_2max immediately after termination of L-carnitine supplementation

Immediately following exercise, another blood sample and a second muscle biopsy were obtained from the dominant leg of the participants.

From this point on, the participants had to ingest 2 g of L-carnitine per day for the next 12 weeks (two capsules containing 500 mg L-carnitine as carnitine tartrate following breakfast and two capsules following the evening meal). The capsules (Carnipure™) were provided by Lonza Ltd, Basel, Switzerland. Compliance during this time was checked by regular phone calls and visits, during which the remaining capsules were counted. The dose of L-carnitine to be administered in the present study has previously been shown to be well tolerated by human volunteers [18, 19].

The participants returned to the laboratory 11 weeks later after an overnight fast. A second VO_2max test was performed to assess possible changes in their level of aerobic fitness and for the calculation of the workload for sub-maximal exercise testing. One week later, the participants underwent a second session of sub-maximal exercise testing at 75 % of VO_2max which was followed by blood collection and muscle biopsy as described above. After a final visit 1 week later, subjects had completed all aspects of the study.

Subjects were free to partake in exercise during the study period, provided that they did not exercise 48 h prior to their involvement in the study's exercise interventions. They had to record all exercise undertaken within their study diaries and to state the form of exercise, intensity (based on the maximal heart rate) and duration.

Primary outcome measure of the study was the effect of carnitine supplementation on the plasma and muscle total carnitine concentration, VO_2max, and on the muscle content of markers of energy metabolism (phosphocreatine, lactate and glycogen) before and 1 h after exercise at 75 % VO_2max in vegetarians. Secondary outcome measure was the comparison of these parameters with omnivores.

Spiroergometry was carried out at the Institute of Sport and Sport Science of the University of Basel and all clinical visits at the Phase 1 Research Unit of the University Hospital of Basel.

Maximal oxygen (VO_2max) uptake test (initial grading)

VO_2max was determined 1 week prior to the start and 1 week prior to the end of the study to determine the level of aerobic fitness of the participants (Fig. 1). During these visits, subjects were familiarized with the procedures to be used throughout the study, and their VO_2max was assessed. VO_2max is the highest rate at which oxygen can be used by the body during work; it is related directly to the peak capacity of the heart to deliver blood to the muscles. The VO_2max test involved a continuous incremental exercise protocol on a bicycle ergometer at a pedaling cadence of ~70 rpm [17]. All determinations of expired gas composition and volume during these tests were performed using breath-by-breath online computer-based analysis systems (Ergoline Ergometrics 800S; Leuenberger Medizin Technik AG, Glattbrugg, Switzerland).

Sub-maximal exercise performance (75 % VO_2max)

Subjects were asked to report to the laboratory, following an overnight fast, and performed 60 min of sub-maximal (two-legged) cycling exercise at a cadence of 70–85 rpm against a pre-determined resistance, which equated to 75 % of their VO_2max determined before. This workload and duration of exertion had been selected due to the high demand for carbohydrate oxidation toward energy production [20, 21] and thereby demand for carnitine-mediated buffering of the acetyl group pool in order to maintain the function of the pyruvate decarboxylase [16, 22–24]. Lactate accumulates under these conditions in skeletal muscle and plasma due to the limited capacity of the pyruvate decarboxylase [24–26]. By buffering the acetyl-CoA pool, which inhibits pyruvate decarboxylase, carnitine can decrease the accumulation of lactate [24]. Furthermore, by favoring fat metabolism, carnitine may be able to prevent the depletion of glycogen under these conditions [24].

The heart rate was monitored throughout each exercise bout, and the subject's perceived exertion was assessed using the Borg Scale [27].

Sample collection

Twenty-four-hour urine samples were obtained 2 weeks before sub-maximal exercise testing. After mixing the urine and measuring the volume, a sample was stored at −20 °C until analysis.

Blood samples were obtained from the subject's non-dominant arm before and after the 24-hour urine collection and before and after the sub-maximal exercise by venipuncture into heparinized. All blood samples were drawn between 8:00 and 10:00 a.m. while the subjects were in the fasted state. After centrifugation, plasma was obtained and stored at −20 °C until analysis.

Muscle biopsy samples were obtained from the vastus lateralis by using the Bergström needle technique [28] under local anesthesia with lidocaine pre-exercise and immediately post-sub-maximal exercise. In one participant, there was bleeding from the biopsy puncture after exercise, which could be stopped by compression. There was no permanent damage associated with the muscle biopsies, and the biopsies were generally well tolerated by the subjects.

Food diary and estimation of nutrient ingestion

Subjects were required to keep a structured diary throughout their involvement in the study and to note down all

food and drinks consumed with details on their weight. These data were used to calculate the amount of calories and nutrients consumed per day using EBISpro Version 7.0 (EBISpro, Willstätt-Legelshurst, Germany). The completeness and plausibility of the information in the diet diary was checked regularly by phone calls and visits.

For carnitine, the daily ingestion was estimated as described by Rebouche et al. [7], since the computer program used did not contain this information. The carnitine content in food was obtained from the publication of Steiber et al. [9]. For foods not contained in the tables, the carnitine content was estimated based either on their composition or on the carnitine content of similar foods as described by Rebouche et al. [7].

Sample analysis

Urine samples were analyzed to determine the creatinine, carnitine and acylcarnitine concentrations. Blood samples were analyzed for the carnitine and acylcarnitine concentrations as well as lactate and creatinine. Samples were processed and analyzed as described previously [17].

Muscle samples were rapidly frozen by immersion into liquid nitrogen and stored at −80 °C. They were freeze-dried and processed as described by Harris et al. [29] for the determination of the ATP, phosphocreatine, glycogen, lactate, carnitine and acylcarnitines content. In the perchloric acid extracts, ATP and phosphocreatine were determined using spectrophotometric methods as described by Harris et al. [29]. Lactate was determined enzymatically as described by Olsen [30]. Carnitine and acylcarnitines (including acetylcarnitine) were determined in the same perchloric acid extracts using the methods described previously [17]. For the determination of glycogen, 2- to 4-mg freeze-dried skeletal muscle powder was processed and analyzed as described by Harris et al. [29].

The technicians performing the analyses were blinded regarding the study participants.

Calculations and statistical analysis of the results

Renal clearances were calculated by dividing the amount of a metabolite excreted per 24 h by its plasma concentration (average of two measurements at the beginning and at the end of the urine collection period). Renal excretion rates were calculated by dividing the renal clearance of a metabolite by the respective creatinine clearance obtained from the same plasma and urine samples.

Two groups were compared using Student's *t* test. More than two groups were compared using two-way ANOVA with repeated measures. In the case of significant F-values in the ANOVA, two-sided Student's *t* tests with Bonferroni correction were used (unpaired *t* tests for the comparison between vegetarians and omnivores and paired *t* tests for the comparisons between baseline and end of L-carnitine supplementation as well as between pre- and post-exercise). Analyses were performed using Sigma-Stat version 3.5 (Scientific Solutions, Pully Lausanne, Switzerland).

Based on the study by Wall et al. [24], we wanted to be able to detect a difference of 15 % in the total carnitine skeletal muscle content in vegetarians (the primary endpoint of the study) with a power of 0.8. Considering the data of a previous study using the same methods [16, 17], we assumed a relative standard deviation of 20 % for the determination of the skeletal muscle carnitine content. With 16 vegetarians included, we calculated to reach a power of 0.801. In omnivores, where we included only 8 subjects, we calculated to be able to detect a 23 % difference with a power of 0.8. Between omnivores and vegetarians, we calculated to be able to detect a difference of 25 % with a power of 0.8.

Statistical significance was accepted at $p < 0.05$. All the values presented in text, tables and figures represent mean ± SD.

Results

Subject characteristics

The subjects recruited are characterized in Table 1. Among the 16 vegetarians, there were two vegans. Since these two participants were not significantly different from the other vegetarians regarding anthropometric and biochemical parameters as well as physical performance, they were analyzed together with the 14 lacto-ovo-vegetarians. In comparison with omnivores, vegetarians had a significantly lower body weight and less lean body mass at entry, whereas age, height, BMI and body fat mass were not different. There was no significant change in these variables during the study (data not shown). Vegetarians consumed significantly fewer calories than omnivores, but only when expressed as an absolute value and not when expressed per kg bodyweight. They also consumed less protein, carnitine and carnitine precursor lysine than omnivores, whereas their consumption of carbohydrates and fat was not different from omnivores. None of the participants reported any adverse event regarding the ingestion of the carnitine capsules.

Exercise performance

The subjects were first tested on the ergometer until exhaustion (initial grading) [17] to determine their maximal physical capacity (P_{max}), maximal oxygen uptake (VO_2max) and maximal respiratory exchange ratio (RER_{max}) (Table 2). In

Table 1 Characterization of the patients at study entry

	Omnivores ($n = 8$)	Vegetarians ($n = 16$)
Age (years)	26.1 ± 3.0	27.2 ± 3.4
Body weight (kg)	79.1 ± 5.1	72.0 ± 6.5[a]
Height (cm)	184 ± 6	180 ± 7
BMI (kg/m^2)	23.4 ± 2.5	22.0 ± 3.1
Lean body mass (kg)	68.8 ± 4.1	62.6 ± 4.5[a]
Fat body mass (kg)	10.5 ± 3.4	9.4 ± 5.4
Energy intake (kcal/day)	2,200 ± 230	1,920 ± 200[a]
Energy intake (kcal/kg/day)	27.8 ± 2.4	26.7 ± 2.6
Protein intake (g/day)	83 ± 31	60 ± 13[a]
Protein intake (% of energy)	16 ± 2	13 ± 2[a]
Fat intake (g/day)	78 ± 22	76 ± 6
Fat intake (% of energy)	32 ± 7	34 ± 5
Carbohydrate intake (g/day)	267 ± 48	250 ± 59
Carbohydrate intake (% of energy)	49 ± 7	52 ± 8
Lysine (mmol/day)	12.8 ± 2.6	8.9 ± 3.1[a]
Carnitine		
μmol/day	317 ± 84	26 ± 4[a]
mg/day	51 ± 14	4.2 ± 0.6[a]

Intake of nutrients was estimated using the food diaries from the participants and the software (EBISpro Version 7.0) or, for carnitine, using published values in different nutrients. Data are given as mean ± SD

[a] $p < 0.05$ versus omnivores by unpaired t tests

comparison with omnivores, vegetarians had a significantly lower absolute P_{max} at study entry. There was no significant difference in P_{max} between vegetarians and omnivores, however, when expressed per kg bodyweight and after treatment with carnitine. VO_2max, RER_{max} and the blood lactate concentration at exhaustion were not significantly different between vegetarians and omnivores before and after treatment with carnitine. Carnitine supplementation had no significant effect on VO_2max in vegetarians [difference post- to pre-carnitine supplementation −0.2 (95 % CI −1.8–1.4) mL × min^{-1} × kg^{-1}] or omnivores [difference post- to pre-carnitine supplementation −1.2 (95 % CI −4.6–2.2) mL × min^{-1} × kg^{-1}]. Carnitine supplementation had also no significant effect on P_{max}, RER at exhaustion or the blood lactate concentration.

After the initial grading, subjects performed a sub-maximal cycling exercise test for 1 h at the power corresponding to 75 % of their VO_2max during grading. As shown in Table 2, VO_2, RER and the blood lactate concentrations were not significantly different between vegetarians and omnivores at 30 min (only VO_2 and RER) and at the end of spiroergometry, both before and after supplementation with L-carnitine. Supplementation with L-carnitine affected these variables neither in omnivores nor in vegetarians.

Plasma carnitine concentration

At baseline, vegetarians had significantly lower plasma free, total acid soluble and total carnitine concentrations (43.1 ± 4.3 μmol/L in vegetarians and 47.9 ± 4.6 μmol/L in omnivores, $p < 0.05$) compared to omnivores (see supplementary Table 1 for detailed results). Supplementation with L-carnitine significantly increased the plasma carnitine concentrations both in omnivores and vegetarians and eliminated the difference between omnivores and vegetarians observed before supplementation. Regarding the total carnitine plasma concentration before exercise, carnitine supplementation was associated with an increase of 13.5 μmol/L (95 % CI 9.3–17.7 μmol/L) in vegetarians and of 11.6 μmol/L (95 % CI 6.3–16.9 μmol/L) in omnivores. Exercise at 75 % VO_2max was associated with a significant increase in the plasma short-chain acylcarnitine and a corresponding drop in the free carnitine concentration both in omnivores and vegetarians at baseline and also after supplementation with L-carnitine (supplementary Table 1).

Renal excretion and clearance of carnitine

The creatinine clearance of the omnivores was 167 ± 27 mL/min and of the vegetarians 122 ± 19 mL/min ($p < 0.05$). Compared to omnivores, vegetarians excreted significantly less free carnitine (see supplementary Table 2 for detailed results), short-chain acylcarnitine and total carnitine (96 ± 30 μmol/day in vegetarians and 192 ± 52 μmol/day in omnivores, $p < 0.05$), primarily reflecting reduced ingestion of carnitine (see Table 1). Both the renal excretion fraction (data not shown) and the renal clearance of free carnitine were significantly lower in vegetarians than in omnivores (0.46 ± 0.31 mL/min in vegetarians and 1.95 ± 1.49 mL/min in omnivores, $p < 0.05$). In contrast, renal clearance and excretion fraction of short-chain acylcarnitines were not significantly different between vegetarians and omnivores (supplementary Table 1).

Skeletal muscle carnitine content

Muscle biopsies were obtained before and after sub-maximal exercise testing both at baseline and at the end of supplementation with L-carnitine. The skeletal muscle carnitine content of the participants is given in Table 3. Before carnitine treatment, there were no significant differences between the skeletal muscle carnitine content of omnivores and vegetarians before or after exercise. Sub-maximal exercise at 75 % VO_2max was associated with a significant increase in the skeletal muscle acetylcarnitine content and a corresponding decrease in the free carnitine content in both vegetarians and omnivores. As shown in Fig. 2, long-term

Table 2 Exercise testing

	Baseline		End of supplementation with L-carnitine	
	Omnivores ($n = 8$)	Vegetarians ($n = 16$)	Omnivores ($n = 8$)	Vegetarians ($n = 16$)
Exhaustive exercise				
P_{max} (W)	276 ± 25	245 ± 30[a]	263 ± 16	243 ± 30
P_{max} (W/kg)	3.49 ± 0.32	3.40 ± 0.45	3.29 ± 0.25	3.36 ± 0.47
VO_2max (mL x min^{-1} × kg^{-1})	46.3 ± 3.7	45.3 ± 6.0	45.1 ± 6.2	45.1 ± 5.7
RER	1.17 ± 0.05	1.20 ± 0.08	1.15 ± 0.09	1.19 ± 0.06
Blood lactate (mmol/L)	13.8 ± 2.7	14.1 ± 1.6	12.2 ± 3.3	13.4 ± 2.8
Sub-maximal exercise				
VO_2 (mL × min^{-1} × kg^{-1})				
0 min	3.6 ± 0.4	3.7 ± 0.8	3.8 ± 0.8	3.7 ± 1.3
30 min	36.5 ± 2.0	36.6 ± 2.3	34.1 ± 4.4	35.3 ± 3.8
60 min	37.7 ± 2.3	37.3 ± 2.4	35.7 ± 4.1	35.4 ± 4.0
RER (VCO_2/VO_2)				
0 min	0.82 ± 0.03	0.85 ± 0.04	0.83 ± 0.06	0.81 ± 0.08
30 min	0.96 ± 0.03	0.97 ± 0.05	0.97 ± 0.03	0.95 ± 0.02
60 min	0.92 ± 0.02	0.95 ± 0.04	0.95 ± 0.02	0.94 ± 0.03
Blood lactate (mmol/L)				
0 min	1.1 ± 0.2	1.2 ± 0.3	1.3 ± 0.4	1.5 ± 0.5
60 min	5.6 ± 2.3	6.1 ± 1.9	5.6 ± 2.6	6.3 ± 2.4

Exhaustive exercise testing (initial grading) and sub-maximal exercise testing (at 75 % of VO_{2max} for 1 h) were conducted on a bicycle ergometer as described in Methods. Data are given as mean ± SD

Supplementation with L-carnitine had no significant effects in either omnivores or vegetarians

Means were compared by ANOVA followed by *t* tests with Bonferroni correction to localize differences detected by ANOVA

[a] $p < 0.05$ versus omnivores

supplementation with L-carnitine did not significantly affect the skeletal muscle carnitine content in omnivores [difference post- to pre-carnitine supplementation before exercise −0.8 (95 % CI −6.5–4.9) mmol × kg^{-1} dry weight]. In vegetarians, however, supplementation with L-carnitine was associated with a significant 11 % (pre-exercise) or 13 % increase (post-exercise) in the skeletal muscle total carnitine content [difference post- to pre-carnitine supplementation 2.5 (95 % CI −0.9–4.1) and 2.8 (95 % CI 1.0–4.6) mmol × kg^{-1} dry weight pre- and post-exercise, respectively].

Skeletal muscle metabolites

The skeletal muscle content of different markers of energy metabolism is given in Table 4. The phosphocreatine content was not significantly different between vegetarians and omnivores, both before and after carnitine supplementation. Carnitine supplementation had no significant effect on the phosphocreatine content in vegetarians [difference post- to pre-carnitine supplementation −7 (95 % CI −24–10) and −2 (95 % CI −21–17) mmol × kg^{-1} dry weight pre- and post-exercise, respectively] or omnivores [difference post- to pre-carnitine supplementation −5 (95 % CI −18–8) and −3 (95 % CI −20–17) mmol × kg^{-1} dry weight pre- and post-exercise, respectively]. The phosphocreatine content showed a drop with sub-maximal exercise, reaching significance in omnivores, but not in vegetarians. Similarly, also the ATP content was not significantly different between vegetarians and omnivores and was not affected by carnitine ingestion or exercise.

The skeletal muscle glycogen and lactate contents were also not different between vegetarians and omnivores, both before and after supplementation with carnitine. Carnitine supplementation had no significant effect on the muscle glycogen content in vegetarians [difference post- to pre-carnitine supplementation −6 (95 % CI −42–30) and 0 (95 % CI −30–30) mmol × kg^{-1} dry weight pre- and post-exercise, respectively] or omnivores [difference post- to pre-carnitine supplementation −27 (95 % CI −121–67) and 27 (95 % CI −2–56) mmol × kg^{-1} dry weight pre- and post-exercise, respectively]. Similarly, carnitine supplementation had no significant effect on the muscle lactate content in vegetarians [difference post- to pre-carnitine supplementation −0.4 [95 % CI −1.2–0.4) and −0.2 (95 % CI −5.6–5.2) mmol × kg^{-1} dry weight pre- and

Table 3 Skeletal muscle carnitine content

	Before exercise		After exercise	
	Omnivores ($n = 8$)	Vegetarians ($n = 16$)	Omnivores ($n = 8$)	Vegetarians ($n = 16$)
Baseline (before supplementation with L-carnitine)				
Free carnitine	15.3 ± 3.7	15.9 ± 1.4	9.5 ± 3.0[a]	9.1 ± 3.1[a]
Acetyl-carnitine	2.1 ± 1.2	2.5 ± 1.0	8.6 ± 1.9[a]	8.8 ± 1.9[a]
TAS carnitine	17.7 ± 4.5	18.6 ± 1.8	18.6 ± 3.0	18.9 ± 3.6
LCA carnitine	4.3 ± 1.2	3.6 ± 1.6	3.5 ± 0.7	3.4 ± 0.9
Total carnitine	22.0 ± 5.1	22.2 ± 2.1	22.1 ± 3.3	22.3 ± 3.8
End of supplementation with L-carnitine				
Free carnitine	14.1 ± 3.6	17.2 ± 2.4	10.3 ± 3.1	11.7 ± 2.1[a]
Acetyl- carnitine	2.4 ± 1.9	2.8 ± 0.9	7.1 ± 1.9[a]	8.4 ± 2.4[a]
TAS carnitine	17.4 ± 3.5	20.3 ± 2.4	17.0 ± 5.2	20.7 ± 2.5
LCA carnitine	3.8 ± 1.4	4.4 ± 1.0	3.5 ± 0.8	4.4 ± 1.5
Total carnitine	21.2 ± 4.0	24.7 ± 2.9[b]	20.5 ± 5.8	25.1 ± 3.3[b]

Muscle biopsies were performed at baseline (before supplementation with L-carnitine) and at the end of supplementation with L-carnitine. At both time points, the carnitine content was determined before and immediately after sub-maximal exercise (at 75% of VO_2max for 1 hour). The carnitine fractions were determined using a radioenzymatic method. *SCA* short-chain acyl, *TAS* total acid soluble, *LCA* long-chain acyl. Units are mmol/kg muscle dry weight. Data are given as mean ± SD

Means were compared by ANOVA with repeated measures followed by t tests with Bonferroni correction to localize differences detected by ANOVA

[a] $p < 0.05$ versus the respective group before exercise

[b] $p < 0.05$ versus the respective group at baseline

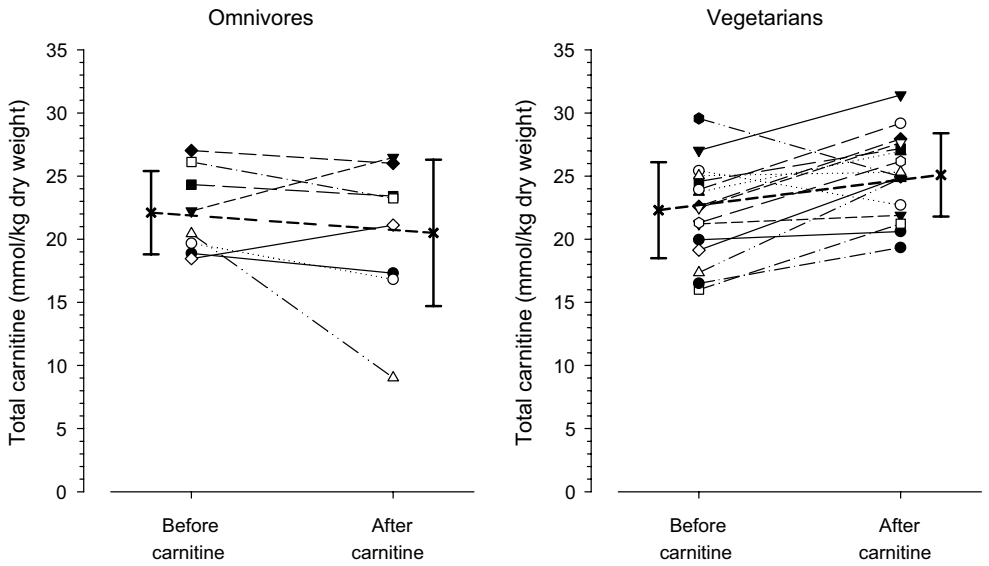

Fig. 2 Effect of L-carnitine supplementation on the total carnitine skeletal muscle content. A muscle biopsy was obtained after sub-maximal exercise at baseline (before supplementation with L-carnitine) and at the end of a 12 weeks period with L-carnitine supplementation (2 g per day). Treatment with carnitine significantly increased the muscle carnitine content in vegetarians, but not in omnivores. Participants were $n = 8$ omnivores and $n = 16$ vegetarians. Carnitine was determined using a radio enzymatic method. Units are mmol/kg skeletal muscle dry weight

post-exercise, respectively] or omnivores [difference post- to pre-carnitine supplementation −0.7 (95 % CI −2.7–1.3) and −0.6 (95 % CI −9.3–8.1) mmol × kg^{-1} dry weight pre- and post-exercise, respectively]. As shown in Fig. 3, sub-maximal exercise for 1 h at 75 % VO_2max was associated with a significant increase in muscle lactate and a drop

Table 4 Skeletal muscle metabolites at rest and during exercise

	Before exercise		After exercise	
	Omnivores ($n = 8$)	Vegetarians ($n = 16$)	Omnivores ($n = 8$)	Vegetarians ($n = 16$)
Baseline (before supplementation with L-carnitine)				
Phosphocreatine (PCr)	69 ± 14	65 ± 13	44 ± 16[a]	50 ± 18
ATP	20.2 ± 4.1	21.3 ± 3.2	18.2 ± 5.9	21.0 ± 5.2
PCr/ATP	3.5 ± 0.8	3.1 ± 0.6	2.6 ± 0.8	2.6 ± 0.7
Glycogen	324 ± 109	308 ± 92	75 ± 34[a]	112 ± 61[a]
Lactate	4.9 ± 1.9	4.8 ± 1.0	32.9 ± 4.1[a]	34.9 ± 6.3[a]
End of supplementation with L-carnitine				
Phosphocreatine (PCr)	64 ± 18	58 ± 19	41 ± 11[a]	48 ± 25
ATP	20.4 ± 5.9	18.4 ± 5.4	18.8 ± 6.0	21.8 ± 5.7
PCr/ATP	3.0 ± 0.8	3.6 ± 2.0	2.2 ± 0.3	2.1 ± 1.2
Glycogen	297 ± 43	302 ± 79	109 ± 22[a]	112 ± 47[a]
Lactate	4.2 ± 1.9	4.4 ± 1.1	32.3 ± 8.8[a]	34.7 ± 5.8[a]

Muscle biopsies were performed at baseline (before supplementation with L-carnitine) and at the end of supplementation with L-carnitine. At both time points, the measurements were performed before and immediately after sub-maximal exercise (at 75 % VO_2max for 1 h). The metabolite contents were determined using spectrophotometric or fluorimetric methods. Units for ATP, phosphocreatine and lactate are mmol/kg muscle dry weight and for glycogen mmol/kg dry weight in the form of glucose. Data are given as mean ± SD

Means were compared by ANOVA with repeated measures followed by *t* tests with Bonferroni correction to localize differences detected by ANOVA

[a] $p < 0.05$ versus the respective group before exercise

in muscle glycogen, which were not different between vegetarians and omnivores.

Discussion

Our study shows that male vegetarians had maintained skeletal muscle carnitine stores despite lower plasma carnitine concentrations than omnivores. Exercise capacity and maximal oxygen uptake were not different between vegetarians and omnivores. Oral supplementation with L-carnitine for 12 weeks increased the skeletal muscle carnitine stores in vegetarians by approximately 13 %, but did not affect skeletal muscle energy metabolism or physical performance.

Considering the ingestion of calories and nutrients (Table 1), the amount of calories was below the range of the recommended 30–35 kcal/kg/day for physically active men of this age, body weight and height [31]. The fact that the body weight remained unchanged during the study therefore suggests that some underreporting of nutrient intake may have occurred. The significantly lower intake of carnitine and of the carnitine precursor lysine mainly reflects the abstinence from meat [7]. Regarding lysine, the daily ingestion was much larger in both omnivores and vegetarians than urinary excretion of total carnitine in the 24-hour urine, which, at least in vegetarians, mainly reflects carnitine biosynthesis.

In agreement with the current study, lower plasma carnitine concentrations in vegetarians compared to omnivores have been reported in previous publications [6, 7, 32]. In addition, we found a decrease in the renal clearance of free carnitine, which has also been reported previously [7, 32]. A possible interpretation of these findings is that the kidney conserves carnitine in vegetarians by up-regulation of the organic cation transporter OCTN2, the carnitine transporter responsible for renal carnitine reabsorption [33]. Since vegetarians ingest less carnitine than omnivores (see Table 1), renal conservation of carnitine is reasonable to conserve the carnitine body stores. In support of this interpretation, rats with secondary carnitine deficiency have an increased renal expression of OCTN2 mRNA and an increased carnitine transport activity into proximal tubular plasma membrane vesicles [34].

In contrast to the current study, Stephens et al. [32] found an approximately 20 % decrease in the carnitine skeletal muscle content in vegetarians compared to omnivores. This decrease was more pronounced in female than in male vegetarians and was independent of the duration of the vegetarian diet. This contrasts with an older study, where the skeletal muscle carnitine content was reported to be independent of carnitine ingestion in a population with different eating habits [35]. An at least partial explanation for the divergent findings in the current and Stephens' study is the fact that we included only male and not female vegetarians in our study.

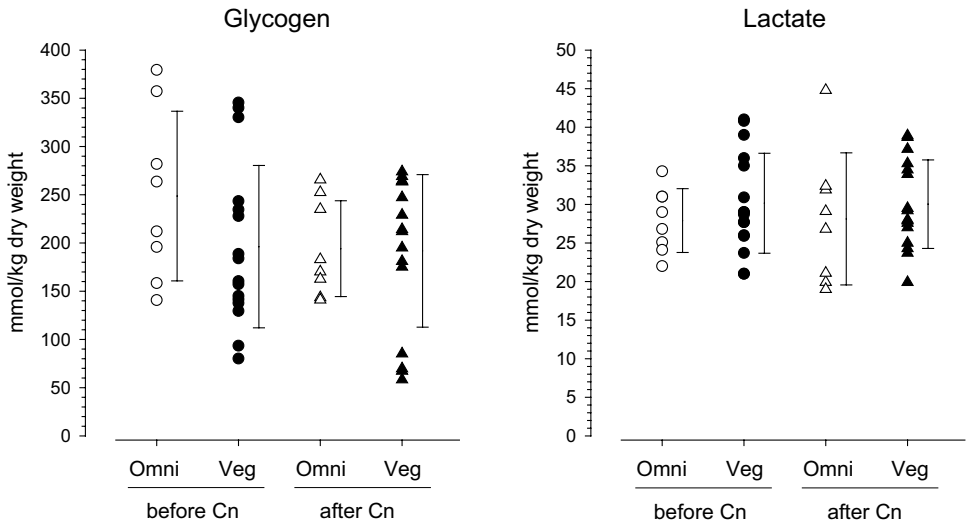

Fig. 3 Effect of sub-maximal exercise on the skeletal muscle glycogen and lactate content. Participants ($n = 8$ omnivores and $n = 16$ vegetarians) performed exercise on a bicycle ergometer at 75 % VO_{2max} for 1 h. Muscle biopsies were obtained before and immediately after exercise. The participants were studied under baseline conditions (before carnitine) and after supplementation with L-carnitine (2 g per day for 12 weeks). The data show the difference of the metabolite content before and after exercise under basal conditions and after L-carnitine supplementation. Units are mmol/kg skeletal muscle dry weight

As shown also in previous studies, ingestion of L-carnitine over 3 months did not affect the skeletal muscle carnitine content in omnivores, despite an increase in the carnitine plasma concentration [36, 37]. Taking into account the K_m of carnitine transport into skeletal muscle, which is in the range of 10 μM [38], this finding can be explained by saturation of carnitine transport at physiological carnitine plasma concentrations. Surprisingly, in the current study, vegetarians treated with carnitine showed not only the expected increase in the carnitine plasma concentration, but also an approximately 13 % increase in the skeletal muscle carnitine content. It has been shown that the skeletal muscle carnitine content can acutely be increased by approximately 20 % with the combination of high plasma carnitine concentrations and insulin [10, 39] or long-term with the ingestion of carnitine together with carbohydrates to increase serum insulin [24]. In the recent study of Stephens et al. [10], it was not possible, however, to acutely increase the skeletal muscle carnitine content in vegetarians with the combination of high carnitine plasma concentrations and insulin. In comparison, in the current study, we treated the participants with carnitine long-term for 12 weeks. Our study does not allow providing a mechanism for the observed increase in the skeletal muscle carnitine content of vegetarians by long-term carnitine supplementation. Nevertheless, as suggested also by the study of Stephens et al. [10], it appears that the regulation of carnitine transport into skeletal muscle is different in vegetarians and omnivores, which may be a consequence of the different composition of the diets.

Before the treatment with carnitine, there was no significant difference in VO_2max and the maximal working capacity adjusted per kg bodyweight as well as the blood lactate concentration at exhaustion between vegetarians and omnivores. Also during sub-maximal exercise at 75 % VO_2max, there was no significant difference in the RER or in the blood lactate concentrations between the two groups. The skeletal muscle parameters of energy metabolism showed the expected effects of work at 75 % VO_2max such as a drop in phosphocreatine and glycogen as well as an increase in acetylcarnitine and lactate, but again without a difference between the two groups. Although review articles about the effect of vegetarian diets in athletes state that there is no impairment in physical performance in vegetarians compared to omnivores [40, 41], physiological studies about this subject are rare. In support of our results, Raben et al. [42] showed that there was no difference in VO_2max or maximal working capacity in athletes ingesting a diet containing meat or an isocaloric vegetarian diet for 6 weeks each.

In omnivores, treatment with carnitine did not affect the skeletal muscle carnitine content and, therefore, had no effect on muscle energy metabolism or physical performance. In vegetarians, carnitine supplementation increased the skeletal muscle carnitine content up to 13 %, but also without having an effect of skeletal muscle energy metabolism or physical performance. At 75 % of the VO_2max, glucose is the preferred substrate for energy metabolism and fat plays only a minor role [20, 21]. As shown by Wall et al. [24], possible effects of carnitine at

this work intensity could have been a decrease in the skeletal muscle lactate content, an increase in the pyruvate decarboxylase (PDC) activity, and, possibly, also reduced glycogen consumption. Although we did not determine the PDC activity, the fact that carnitine administration had no significant effect on the skeletal muscle (and blood) lactate concentrations and the skeletal muscle glycogen content in vegetarians compared to omnivores indicates the observed increase in the skeletal muscle carnitine content in vegetarians was without effect on skeletal muscle carbohydrate metabolism. This is in contrast to studies in omnivores, where a 20 % increase in the skeletal muscle carnitine content was associated with effects on both skeletal muscle fatty acid and glucose metabolism [24]. The discrepancy to our findings can be explained primarily by the more accentuated increase in the skeletal muscle carnitine content in the study of Wall et al. compared to our study (21 % vs. 13 %).

In conclusion, in comparison with male omnivores, male vegetarians maintain their skeletal muscle carnitine pool despite lower plasma carnitine concentrations. Oral supplementation with L-carnitine is associated with a slight increase in the skeletal muscle carnitine content in vegetarians, but not in omnivores. Despite this increase, skeletal muscle function and energy metabolism are unaffected in vegetarians supplemented with 2 g L-carnitine per day for 12 weeks, indicating that a more pronounced increase in the skeletal muscle carnitine content is necessary to affect skeletal muscle energy metabolism and function.

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

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