

Effects of Oral L-Carnitine Supplementation on In Vivo Long-Chain Fatty Acid Oxidation in Healthy Adults

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Despite an abundance of literature describing the basic mechanisms of action of L-carnitine metabolism, there remains some uncertainty regarding the effects of oral L-carnitine supplementation on in vivo fatty acid oxidation in normal subjects under normal conditions. It is well known that L-carnitine normalizes the metabolism of long-chain fatty acids in cases of carnitine deficiency. However, it has not yet been shown that L-carnitine influences the metabolism of long-chain fatty acids in subjects without disturbances in fatty acid metabolism. Therefore, we investigated the effects of oral L-carnitine supplementation on in vivo long-chain fatty acid oxidation by measuring 1- ^{13}C palmitic acid oxidation in healthy subjects before and after L-carnitine supplementation ($3 \times 1 \text{ g/d}$ for 10 days). We observed a significant increase in $^{13}\text{CO}_2$ exhalation. This is the first investigation to conclusively demonstrate that oral L-carnitine supplementation results in an increase in long-chain fatty acid oxidation in vivo in subjects without L-carnitine deficiency or without prolonged fatty acid metabolism.

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DURING β -OXIDATION, long-chain fatty acids are broken down into 2-carbon acetyl fragments. The acetyl groups subsequently combine with coenzyme A (CoA) to form acetyl CoA, which is metabolized in the Krebs' cycle and respiratory chain with the production of H_2O and CO_2 . The resulting product of these processes is adenosine triphosphate (ATP) to realize the energy metabolism of the cell.

Activation of long-chain fatty acids occurs at the outer mitochondrial membrane (acyl-CoA-synthetase), while β -oxidation takes place inside the mitochondria. Long-chain fatty acids, however, are unable to penetrate the inner mitochondrial membrane. It was one of the most important discoveries regarding the energy metabolism of the cell that the long-chain fatty acids, the main source of cellular energy, required L-carnitine as a cofactor for penetrating the inner mitochondrial membrane.¹ The fatty acid intake is achieved by the cooperation of the enzymes carnitine-palmitoyltransferases I and II² and the carnitine-acylcarnitine translocase.³ The activity of these enzymes are responsible for the function of the fatty acid-carnitine exchange through the inner mitochondrial membrane. Because L-carnitine in this way plays an important role in these transport processes, we investigated whether a pharmacological dose of L-carnitine would influence the fatty acid oxidation of normal subjects without carnitine deficiency⁴ or disturbances in fatty acid oxidation.⁵

MATERIALS AND METHODS

Subjects

Ten healthy, untrained subjects (5 men and 5 women) participated in the study. In agreement with the ethical commission of the medical faculty of the University of Leipzig, pregnant and lactating women were excluded from the study. Age, body weight, and body mass index ($\text{BMI} = \text{body weight}/[\text{height}^2]$) of the subjects are shown in Table 1.

Test Material

Commercially available L-carnitine ampuls (Haleko, Hamburg, Germany) were used for L-carnitine supplementation. Each ampul contained 1 g L-carnitine/20 mL solution, citric acid, peach aroma, ace-sulfam-K, aspartame, and riboflavin. Before and after L-carnitine supplementation, each subject was given 1 g of ^{13}C -labeled palmitic acid (^{13}C -content: 3.76 mmol; 99% purity; Cambridge Isotope Laboratories Inc, Andover, MA) to determine fatty acid oxidation rate.

Experimental Protocol

At 7:30 AM on the test day, each subject was given a standardized breakfast consisting of 2 cups of coffee and 2 pieces of toast. A baseline value was recorded 10 and 20 minutes thereafter. At 8 AM, each subject was given 1 yogurt together with 1 g of ^{13}C -labeled palmitic acid. Breath samples were collected every 15 minutes for the following 15 hours. Lunch was given at 12:30 PM, coffee at 4:30 PM, and dinner at 7 PM. Twenty-four-hour urine samples were collected starting at 7 AM of the test day. Serum fasting samples were taken at 7 AM and stored at -30°C until assayed. Three weeks later, L-carnitine supplementation ($3 \times 1 \text{ g}$ L-carnitine) was started. The supplement was given with breakfast, lunch, and dinner for 10 days. On the 10th day of supplementation, 1 g of ^{13}C -labeled palmitic acid was given 30 minutes after the first L-carnitine dosage and CO_2 exhalation was determined as previously described. Twenty-four-hour urine collection started prior to the first L-carnitine dosage of the test day. Serum fasting samples were taken at 7 AM (14 hours after the last L-carnitine dosage) and stored at -30°C until assayed.

Analysis

Determination of $^{13}\text{CO}_2$ breath exhalation was performed using an isotope-selective nondispersive infrared spectrometry (Fischer Analyt-ics, Leipzig, Germany) providing test results which are comparable to measurements obtained with isotope mass spectrometry.⁶ Breath samples were taken every 15 minutes for 15 hours (starting at 8 AM) and $^{13}\text{CO}_2$ -exhalation rates were calculated using the modified method of Ghooes et al.⁷ being based on a supposed total $^{13}\text{CO}_2$ -exhalation ($300 \text{ mmol/h} \times \text{m}^2 \text{ body surface area}$) as described by Haycock et al.⁸ In parallel, 24-hour urine samples were collected for determination of total excreted L-carnitine. Also, serum total and free L-carnitine were determined before and after chemical hydrolysis using a manifold modified radioenzymatic test method: a liquid scintillation counter (Tri-Carb, 2500 TR; Canberra Packard, Meriden, CT) was used to measure ^{14}C -labeled acetylcarnitine as formed by carnitine-acetyltrans-

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Table 1. Age, Body Weight, and BMI of Healthy Subjects

	Mean $\pm$ SD	Range
Age (yr)	36.4 $\pm$ 12.8	22-56
Body weight (kg)	69.4 $\pm$ 13.0	50-96
BMI (body weight/[height ²])	23.6 $\pm$ 2.9	20.6-30.3

NOTE. Each value represents the mean $\pm$ SD of 10 observations.

ferase (E.C. 2.3.17) starting from [¹⁴C-acetyl]CoA. Serum acylcarnitine was calculated based on the difference between the serum total and free carnitine.⁹

Statistics

Statistical analysis was performed using Student's *t* test and the Mann-Whitney test. Normality distribution ($P < .05$) was tested for by the Kolmogorov-Smirnov test. SPSS statistical software (version 3.0; SPSS, Inc, Chicaco, IL) was used.

RESULTS

L-carnitine substitution resulted in an increased ¹³CO₂ production in 9 of 10 subjects. Figure 1 depicts the cumulative ¹³CO₂ exhalation mean and standard deviation values prior and after L-carnitine supplementation calculated as percentage of applied ¹³CO₂. Table 2 summarizes the most important results of the investigation: without L-carnitine supplementation, cumulative ¹³CO₂ exhalation averages at 5.12%. L-carnitine supplementation (3 $\times$ 1g/d for 10 days) significantly increased cumulative ¹³CO₂ exhalation up to 7% ($P < .01$). L-carnitine supplementation raised serum total L-carnitine levels (placebo: 47.1 $\pm$ 6.8 μ mol/l *v* L-carnitine: 59.9 $\pm$ 9.5 μ mol/L) and increased urinary excretion of total L-carnitine (placebo: 36.8 $\pm$ 33.3 mg/24 hours *v* L-carnitine: 367.4 $\pm$ 202.1 mg/24 hours).

We found no changes in the serum levels of glucose, hemoglobin A_{1c} (HbA_{1c}), triglycerides, cholesterol, high-density lipoprotein (HDL)-cholesterol, and low-density lipoprotein

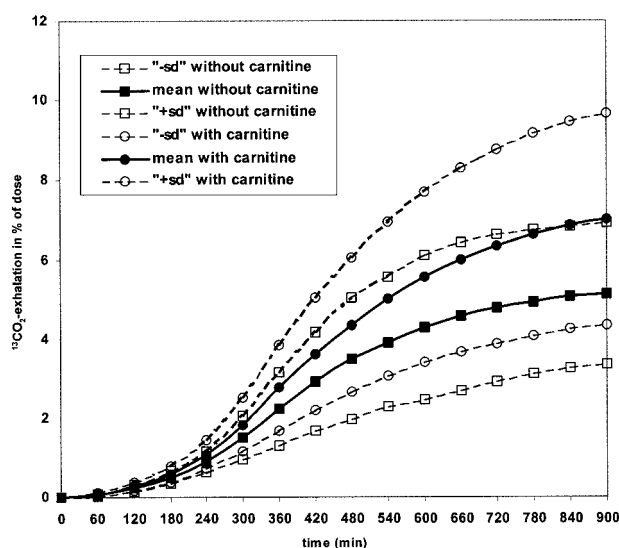


Fig 1. ¹³CO breath test before and after L-carnitine supplementation (3 $\times$ 1 g/d for 10 days). Each value represents the mean SD of 10 observations.

Table 2. Calculated Cumulative 15-Hour ¹³CO₂ Production, Percentage of ¹³CO₂ Exhalation, Serum L-Carnitine Concentration, and 24-Hour Urinary L-Carnitine Excretion in 10 Healthy Subjects Before and After L-Carnitine Supplementation (3 $\times$ 1 g/d for 10 days)

Variable	L-Carnitine Supplementation	Mean $\pm$ SD	<i>P</i>	Comparison of Pairs
¹³ CO ₂ -DOB	B	1,927 $\pm$ 559	.007	9 higher
	A	2,878 $\pm$ 929		1 lower
¹³ CO ₂ %*	B	5.12 $\pm$ 1.88	.009	9 higher
	A	7.00 $\pm$ 2.81		1 lower
Serum total L-carnitine (μ mol/L)	B	47.07 $\pm$ 6.82	.007	9 higher
	A	59.86 $\pm$ 9.52		1 lower
Serum free L-carnitine (μ mol/L)	B	41.10 $\pm$ 6.54	.005	10 higher
	A	52.74 $\pm$ 9.23		0 lower
Serum acyl-L-carnitine (μ mol/l)	B	5.98 $\pm$ 3.64	.285	8 higher
	A	7.11 $\pm$ 1.61		2 lower
Urinary total L-carnitine (mg/24 h)	B	36.8 $\pm$ 33.3	.0002	10 higher
	A	367.4 $\pm$ 202.1		0 lower
Urinary free L-carnitine (mg/24 h)	B	26.6 $\pm$ 23.0	.0002	10 higher
	A	270.0 $\pm$ 165.0		0 lower

NOTE. Each value represents the mean $\pm$ SD of 10 observations.

Abbreviations: ¹³CO₂-DOB, 15-hour CO₂ production; ¹³CO₂%, percentage CO₂ exhalation; B, before L-carnitine supplementation; A, after L-carnitine supplementation.

*Calculated based on the applied amount of 3.76 mmol ¹³C.

(LDL)-cholesterol in our test group without and with L-carnitine supplementation (10 days, 3 $\times$ 1 g/d) (Table 3).

DISCUSSION

Currently, there are only few investigations describing the determination of long-chain fatty acid metabolism using the in vivo ¹³CO₂ exhalation method. The results of these studies are inconsistent, most probably due to methodological differences. For example, Murphy et al¹⁰ reported that in 6 healthy females, cumulative ¹³CO₂ exhalation percentage calculated by indirect calorimetric measurement over 15 hours reached 18.7% $\pm$ 7.4% of the initially applied ¹³C palmitic acid (10 mg/kg body weight). Other researchers report an increased cumulative 24-hour ¹³CO₂ exhalation percentage (30.7% $\pm$ 6.7% in women,

Table 3. Blood Serum Levels of Glucose, HbA_{1c}, Triglycerides, Cholesterol, HDL-Cholesterol, and LDL-Cholesterol in 10 Healthy Adults Without and With L-Carnitine Supplementation (10 days, 3 $\times$ 1 g/d)

Substrate	Without Carnitine	With Carnitine	Normal Values
Glucose	4.40 $\pm$ 0.70	4.51 $\pm$ 0.61	4.13-6.38 mmol/L
HbA _{1c}	5.52 $\pm$ 0.27	5.64 $\pm$ 0.39	3.40-6.10 mmol/L
Cholesterol	5.13 $\pm$ 0.61	5.10 $\pm$ 0.50	3.60-6.20 mmol/L
Triglycerides	1.33 $\pm$ 0.38	1.44 $\pm$ 0.54	0.80-2.30 mmol/L
HDL-cholesterol	1.49 $\pm$ 0.37	1.50 $\pm$ 0.29	0.90-2.00 mmol/L
LDL-cholesterol	3.04 $\pm$ 0.50	2.94 $\pm$ 0.46	2.10-4.90 mmol/L

35.1% $\pm$ 9.3% in men, 61.0% $\pm$ 55.4% in boys, and 54.2% $\pm$ 17.9% in girls) using the same basal values.¹¹

Remarkably lower were the values obtained in similar studies that fixed the hourly CO₂ production to 300 mmol/m² body surface area: Akimoto et al¹² reported a cumulative 7-hour ¹³CO₂ exhalation percentage of 14.4% $\pm$ 2.3% in 4 healthy subjects. In 10 healthy subjects, Watkins et al¹³ showed a cumulative 6-hour ¹³CO₂ exhalation percentage of 6.6% $\pm$ 4.2% and Jakobs et al¹⁴ reported in 3 healthy subjects cumulative 8-hour ¹³CO₂ exhalation percentages of 6.6%, 6.7%, and 7.9%, respectively. Watkins et al¹³ observed that cumulative ¹³CO₂ production was strongly elevated by 11.7% $\pm$ 2.9% in 5 patients suffering from pancreatic insufficiency as compared to controls.

Obviously, there is no systematic approach in the available literature and the use of different experimental designs, different fatty acids, and different calculation methods lead to a certain data inconsistency that makes the interpretation difficult.

Our investigation revealed a cumulative 15-hour basal ¹³CO₂ production of 5.1%. Four weeks later, after L-carnitine supplementation (3 $\times$ 1 g/d for 10 days), the same subjects showed a significantly increased ¹³CO₂ production of 7.0%.

Under normal conditions, daily L-carnitine needs in humans are met by diet and by biosynthesis. Breast milk provides babies with the daily need of L-carnitine as their biosynthesis runs at a much lower capacity as compared to adults. It has been shown that lactating women have a higher L-carnitine concentration in breast milk than in serum (breast milk: 60 to 70 μ mol/L v serum: 30 to 40 μ mol/L).¹⁵ Therefore, infant formulas are normally fortified with up to 100 μ mol/L L-

carnitine. Infants and adults obtain their L-carnitine mainly from meat and milk products. Depending on the source of meat, 100 g of fresh meat can provide between 30 to 200 mg L-carnitine (sheep: 200 mg; beef: 60 mg; pork: 30 mg). In contrast, a strict vegetarian diet is almost void of L-carnitine.

In contrast to the number of in vitro investigations on the effects of L-carnitine on fatty acid oxidation, only very few in vivo data have been published so far. Jakobs et al¹⁴ described a positive effect of L-carnitine supplementation on the oxidation of ¹³C-palmitic acid in a patient with multiple acyl-CoA dehydrogenase deficiency.

To date, no in vivo investigation reports on the effects of L-carnitine supplementation on long chain fatty acid oxidation in healthy adults. Although higher serum L-carnitine levels have been reported after L-carnitine supplementation, there is still uncertainty whether this promotes an increase in fatty acid oxidation¹⁶ in healthy subjects.

Our results show that L-carnitine supplementation (3 $\times$ 1 g/d for 10 days) significantly increased serum L-carnitine concentrations, while the serum levels of glucose, HbA_{1c}, triglycerides, cholesterol, HDL-cholesterol, and LDL-cholesterol were not changed. The urinary excretion of L-carnitine was determined as 11% on the tenth day of supplementation. More importantly, our data show for the first time that supplementary L-carnitine significantly increases fatty acid oxidation as determined by the cumulative ¹³CO₂ exhalation method.

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