Effect of intravenous L-carnitine on carnitine homeostasis and fuel metabolism during exercise in humans

This study was undertaken to challenge the hypothesis that short-term administration of carnitine during exercise can modify skeletal muscle carnitine homeostasis and fuel metabolism in normal humans. With a randomized, blinded, crossover design, subjects received carnitine or placebo at the start of a bicycle ergometer exercise session. During the 2 hours after intravenous administration of 185 μmol/kg carnitine, carnitine kinetics could be described with a central compartment volume of distribution of 200 ml/kg, a total clearance from this compartment of 1.9 ml/min/kg, and a renal clearance of 1.3 ml/min/kg. Carnitine administration had no effect on muscle total carnitine content or the workload-dependent accumulation of acylcarnitines in skeletal muscle. Carnitine had no effect on the respiratory exchange ratio, muscle lactate accumulation, plasma lactate concentration, muscle glycogen utilization, or plasma β-hydroxybutyrate concentration during exercise. Thus the skeletal muscle carnitine pool is segregated from dramatic changes in the plasma carnitine pool, and short-term administration of carnitine has no significant effect on fuel metabolism during exercise in humans. (CLIN PHARMACOL THER 1994;55:681-92.)

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Carnitine* is an endogenous compound found ubiquitously in mammalian cells. Carnitine is an important cofactor in cellular metabolism as an obligate for mitochondrial fatty acid oxidation[1] and buffering activated carboxylic acids as acylcarnitines.[1,2] Acylcarnitines (esters through the 3-hydroxy group on carnitine) are generated by reversible transfer of acyl moieties from acyl coenzyme A in reactions catalyzed by carnitine acyltransferases.[2] Although biosynthesis of carnitine in humans is adequate to meet the body's carnitine needs under physiologic conditions, therapeutic administration of supplemental carnitine is of proved efficacy in patients with renal carnitine wasting[3] and those with increased carnitine requirements for detoxification of accumulating acyl coenzyme A caused by metabolic defects.[4-6]

Carnitine has also been suggested as a therapeutic agent in a diverse group of disorders including chronic renal failure,[7,8] hyperlipidemias,[9] and peripheral arterial disease.[10] The potential for carnitine supplementation to improve exercise performance in humans has received considerable attention.[11-13] Skeletal muscle contains large amounts of carnitine (i.e., 3000 nmol/gm in skeletal muscle versus 40 nmol/ml in plasma), and the muscle carnitine pool is redistributed from carnitine to acylcarnitines in normal subjects at high-intensity exercise[14] and in patients with peripheral arterial disease at rest.[15] Studies have reported that long-term administration of carnitine improves exercise performance in patients receiving hemodialysis[7] or with peripheral arterial disease[10] and modifies the exercise capacity or ventilatory respiratory exchange ratio during exercise in normal subjects.[11,13,16,17] A carnitine-induced decrease in respiratory exchange ratio was interpreted as reflecting a

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Supported by Sigma Tau Pharmaceuticals (Rome, Italy).
Received for publication Sept. 28, 1993; accepted Dec. 23, 1993.
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change in fuel utilization toward lipids during the exercise test.[13,16] In addition, single doses of carnitine have been reported to produce similar results.[10,17] Carnitine also improved skeletal muscle function with in vitro[18] and in situ models.[19]

Despite these intriguing reports, many questions remain concerning the use of carnitine to modify exercise performance in humans. No clear mechanism has been established by which carnitine supplementation might improve exercise tolerance, although several have been proposed.[11,13,17,20] Attempts to identify effects of carnitine supplementation on metabolism have been limited and most often have failed to establish a carnitine-induced change in muscle metabolism.[12,21,22] The ability of supplemental carnitine to increase muscle carnitine content or modify carnitine homeostasis has been questioned, particularly in trials involving single doses of carnitine.[23] The muscle and plasma carnitine pools are segregated, muscle carnitine turnover is slow,[24-26] and acute carnitine administration in rats does not increase muscle carnitine content.[27]

This study was designed to challenge the hypothesis that single-dose administration of L-carnitine during exercise can modify skeletal muscle carnitine homeostasis and fuel metabolism in humans. The results show that after single-dose administration, carnitine is largely confined to a central compartment or excreted in the urine. No carnitine-associated changes in the physiologic responses to exercise were identified.

EXPERIMENTAL METHODS

Subjects and screening protocols. A total of 14 normal, healthy male subjects were recruited to participate in three protocols. All subjects were initially screened by history and physical examinations, and no subject was taking any medications, had any medical conditions, or was participating in competitive athletics. Subjects gave informed consent before the initiation of the studies, and all protocols were approved by the University of Colorado Health Sciences Center Institutional Review Board.

Subjects underwent screening exercise testing to define workloads corresponding to the individual's maximal oxygen consumption rate (VO_{2max}) and lactate threshold. After placement of a venous catheter in an upper extremity peripheral vein, the subject exercised on a bicycle ergometer (model Excalibur 600, Lode BV, Groningen, The Netherlands) with continuous electrocardiographic monitoring under an initial workload of 200 kg · m/min, with the workload increasing by 200 kg · m/min every 2 minutes until exhaustion. Gas exchange (rates of oxygen consumption and carbon dioxide production) was calculated on a breath-by-breath basis through a tight-fitting mouthpiece from measurements of ventilating volumes and respiratory gas tensions determined with an Applied Electrochemistry (Pittsburgh, Pa.) gas analyzer. Venous blood was sampled at 1-minute intervals for quantitation of lactate concentration. The lactate threshold was defined as the workload at which the plasma concentration of lactate initially increased based on extrapolation of the plasma lactate concentration-time plot.[28]

Experimental protocols. The three experimental protocols varied with respect to dose of carnitine and exercise workload. In each protocol, each subject was studied twice: once after placebo and once after carnitine administration. Studies were performed double blinded, in a randomized order at least 4 weeks apart. In all cases, studies were performed beginning at 6 to 9 AM after an overnight fast. Peripheral venous catheters were placed in each upper extremity for administration of test drug and sampling of blood. A muscle biopsy specimen was obtained at rest from the vastus lateralis with a Bergstrom biopsy cannula (DePuy; Boehringer Manneheim Corp., Warsaw, Ind.). The muscle sample was immediately frozen in liquid nitrogen and kept at −80° C for analysis. The subject was then assisted to the ergometer and, after an equilibration period, exercise was initiated at the defined workload based on the specific protocol and the individual subject's screening test. Gas exchange and ECG were monitored continuously throughout exercise as described for the screening exercise test. Drug (placebo or carnitine) was administered during the first 2 minutes of exercise. Plasma was sampled at rest, every 5 to 10 minutes through the first hour after drug administration, and at the end of the second hour. A second muscle biopsy specimen was obtained at the conclusion of exercise and, in some subjects, 30 minutes after completion of exercise. Urine was collected before initiation of exercise and at the 2-hour postdosing time point. The specific dosing and exercise regimens included the following protocols.

Protocol 1. Protocol 1 included 185 μmol/kg carnitine and high-intensity exercise; exercise was conducted for 30 minutes at a workload midway between the lactate threshold and VO_{2max} as determined by each subject's screening test. This design ensures that a metabolically equivalent high-intensity workload standardized to a similar relative intensity was obtained in each subject.[28,29] If the subject could not complete the 30-minute session at the desired workload, the workload was decreased by 100 kg · m/min

Table I. Characteristics of study populations

	Study group		
	High-intensity exercise (185 μmol/kg)	*High-intensity exercise (92.5 μmol/kg)*	*Low-intensity exercise (185 μmol/kg)*
No. subjects	8	3	3
Age (yr)			
Mean	26	26	23
Range	23-40	22-40	22-25
Weight (kg)			
Mean	75.4	71.4	79.9
Range	65.7-89.4	62.0-77.1	68.3-97.5
VO_{2max} (ml/min/kg)*	45.4 ± 1.7	42.5 ± 4.0	41.3 ± 1.3
Workload at lactate threshold (kg · m/min)*	700 ± 40	870 ± 70	800 ± 0
Workload used for protocol (kg · m/min)*	1090 ± 40	1120 ± 90	400 ± 0

*Mean ± SEM.

without interrupting the session. If adjustments in workload were made, they were reproduced in the individual's second test to ensure placebo-carnitine comparability.

Protocol 2. Protocol 2 included 92.5 μmol/kg carnitine and high-intensity exercise; protocol 2 was identical to protocol 1, except the carnitine dose was decreased by 50%.

Protocol 3. Protocol 3 included 185 μmol/kg carnitine and low-intensity exercise; protocol 3 was identical to protocol 1, except the workload was 50% of the workload corresponding to the lactate threshold and the exercise was maintained for 60 minutes. Blood samples were obtained 30 and 60 minutes after completion of exercise.

The physical and exercise characteristics of the 14 subjects used in the protocols are summarized in Table I.

Biochemical assays. Carnitine, short-chain acylcarnitines, and long-chain acylcarnitines were quantified in plasma, skeletal muscle, and urine by a previously validated and detailed radioenzymatic assay.[30] Carnitine refers to "free" or "unesterified" carnitine to which no acyl group is attached. Short-chain acylcarnitines are acylcarnitines in which the acyl moiety is shorter than approximately 10 carbons in length. Long-chain acylcarnitines are acylcarnitines in which the acyl moiety is approximately 10 or more carbons in length.

Lactate concentrations were quantified in plasma[31] and muscle[32] with enzymatic assays. Glycogen content in skeletal muscle was quantified by the method of Lust et al.,[33] in which free glucose in the muscle homogenate is initially destroyed, glycogen is enzymatically converted to glucose, and the glucose concentration is quantified by the glucose oxidase method adapted to kit form (Sigma Chemical Co., St. Louis, Mo.). The plasma β-hydroxybutyrate concentration was quantified with the fluorometric assay of Olsen.[32]

Pharmacokinetic analysis. The bulk of body-endogenous carnitine is in intracellular compartments. Transport of carnitine between intracellular and extracellular compartments is characterized by relatively long time constants.[24,26] Thus after short-term administration of carnitine, the short time–frame kinetics describe changes in carnitine concentration and content in a "central" compartment that does not reflect the true physiologic distribution of the compound.[26,34] Therefore the classic pharmacokinetic parameters used to describe the plasma carnitine time course and urinary elimination of the compound reflect kinetics of the central compartment. The fact that only the central compartment is being characterized means that removal of carnitine from this compartment by either urinary excretion or uptake into tissues, both of which are irreversible on the time scale of this study, will be reflected in the estimates of total clearance. Based on these concepts, the area under the plasma concentration–time curve (AUC) from 0 to 120 minutes [AUC(0-120)] was calculated according to the trapezoidal rule after subtraction of the time 0 (i.e., endogenous) carnitine concentration from all time points. The value of k was estimated as $-1 \times$ the slope of the linear least-square fit of the ln (plasma carnitine concentration) versus time plot from 45 to 120 minutes. Plasma carnitine concentrations were corrected for the endogenous carnitine concentrations before these calculations. The AUC for carnitine from zero to infinity was estimated as the AUC(0-120) plus the plasma concentration at 120 minutes (corrected for endoge-

nous carnitine) divided by k. Total clearance was then calculated as Dose/AUC(0-∞) and the volume of distribution (V_C) as Total clearance/k. Renal clearance was calculated with the AUC(0-120) and the quantitated urinary carnitine excretion during the 120 minutes after dosing.

Material. L-Carnitine (1.1 mmol/ml) and placebo were provided in paired, randomized vials to the investigators (who remained blinded through the completion of each pair of studies) by Sigma Tau Pharmaceuticals (Rome, Italy). All enzymes, cofactors, and reagents used in the biochemical assays were obtained from Sigma Chemical Co. (St. Louis, Mo.). [1-^{14}C]-Acetyl coenzyme A used in the carnitine assay was purchased from New England Nuclear (Boston, Mass.).

Data analysis. Comparison of parameters during the placebo versus carnitine exercise tests was made on a paired basis with the Student *t* test, with $p < 0.05$ considered to be significant. Comparison of carnitine pharmacokinetic parameters between the three protocols was performed with the Student unpaired *t* test, with $p < 0.05$ considered to be significant. Analysis of time-dependent variables was conducted by ANOVA.

RESULTS

Kinetics of intravenous carnitine. Subjects performed 30 minutes of high-intensity exercise (workload midway between individual's lactate threshold and maximal capacity) on a bicycle ergometer and received either placebo or carnitine (185 or 92.5 μmol/kg) intravenously (delivered during 2 minutes) in a randomized fashion at the beginning of exercise. Consistent with previous studies,[14,35] high-intensity exercise after administration of placebo was associated with no change in the plasma carnitine concentration and a modest increase in the plasma short-chain acylcarnitine concentration (Fig. 1, *A*). The plasma short-chain acylcarnitine concentration partially normalized, whereas the plasma carnitine concentration decreased during the 90-minute recovery period after exercise in the placebo studies. After administration of 185 μmol/kg carnitine, the plasma carnitine concentration rose rapidly (Fig. 1, *B*), reaching a peak concentration of 1300 μmol/L (compared with predrug concentration of 42 μmol/L), and decreased slowly during the 120 minutes of observation (concentration 300 μmol/L at 120 minutes).

Carnitine uptake by tissues, with the exception of liver, is normally slow.[26,27] Thus the 120-minute period after carnitine administration reflects kinetic events describing drug behavior in a central compartment, rather than the drug's actual whole-body volume of distribution.[26,34] The time course of the plasma carnitine concentrations was therefore characterized by model-independent kinetics, recognizing that the central compartment was being described. Calculations of the AUC(0-120) and AUC(0-∞) after the 185 μmol/kg dose permitted estimation of a total clearance of 1.99 ± 0.11 ml/min/kg (Table II). Based on the rate constant (k) for the terminal portion of the 12-minute time course, the V_C was estimated as 198 ± 11 ml/kg, in good agreement with previous estimates.[34] The 2-hour urinary carnitine excretion was 77.4 μmol/kg, or 42% of the administered dose. Based on the urinary carnitine excretion, the renal carnitine clearance was 1.27 ± 0.09 ml/min/kg, again similar to previous estimates.[34,36] The plasma short-chain acylcarnitine concentration also increased after carnitine (185 μmol/kg) administration, reaching 33 μmol/L after 120 minutes versus 8 μmol/L for placebo (Fig. 2). Assuming a V_C for short-chain acylcarnitines similar to that for carnitine,[37] 4% of the dose could be accounted for by acylcarnitines. Increases in plasma short-chain acylcarnitine concentration were also observed when 92.5 μmol/kg carnitine was given (10 μmol/L versus 25 μmol/L at 120 minutes for placebo and carnitine administration, respectively) but not when 185 μmol/kg carnitine was given before 60 minutes of low-intensity exercise (10 μmol/L versus 10 μmol/L for placebo and carnitine treatments, respectively, at 120 minutes; $n = 3$) (Fig. 2). Thus at the end of 2 hours it could be estimated that 42% of the dose had been excreted in the urine, 32% of the dose was still in the V_C, and 4% was present as acylcarnitines in the V_C, with the remainder of the dose removed from the central compartment, presumably by tissue uptake (carnitine is not degraded in humans except in the intestine). The apparent pharmacokinetic parameters describing the time course after intravenous carnitine administration during exercise were not dependent on carnitine dose (185 μmol/L versus 92.5 μmol/L) or intensity of exercise (30 minutes of high-intensity exercise compared with 60 minutes of low-intensity exercise defined as a workload 50% of the lactate threshold, as detailed in Table II.

Skeletal muscle total carnitine content did not change during the 60-minute observation period in the placebo–high-intensity exercise group. However, after placebo administration, high-intensity exercise was associated with a redistribution of the muscle carnitine pool (Table III) from carnitine to short-chain acylcarnitines (8% of total carnitine as short-chain

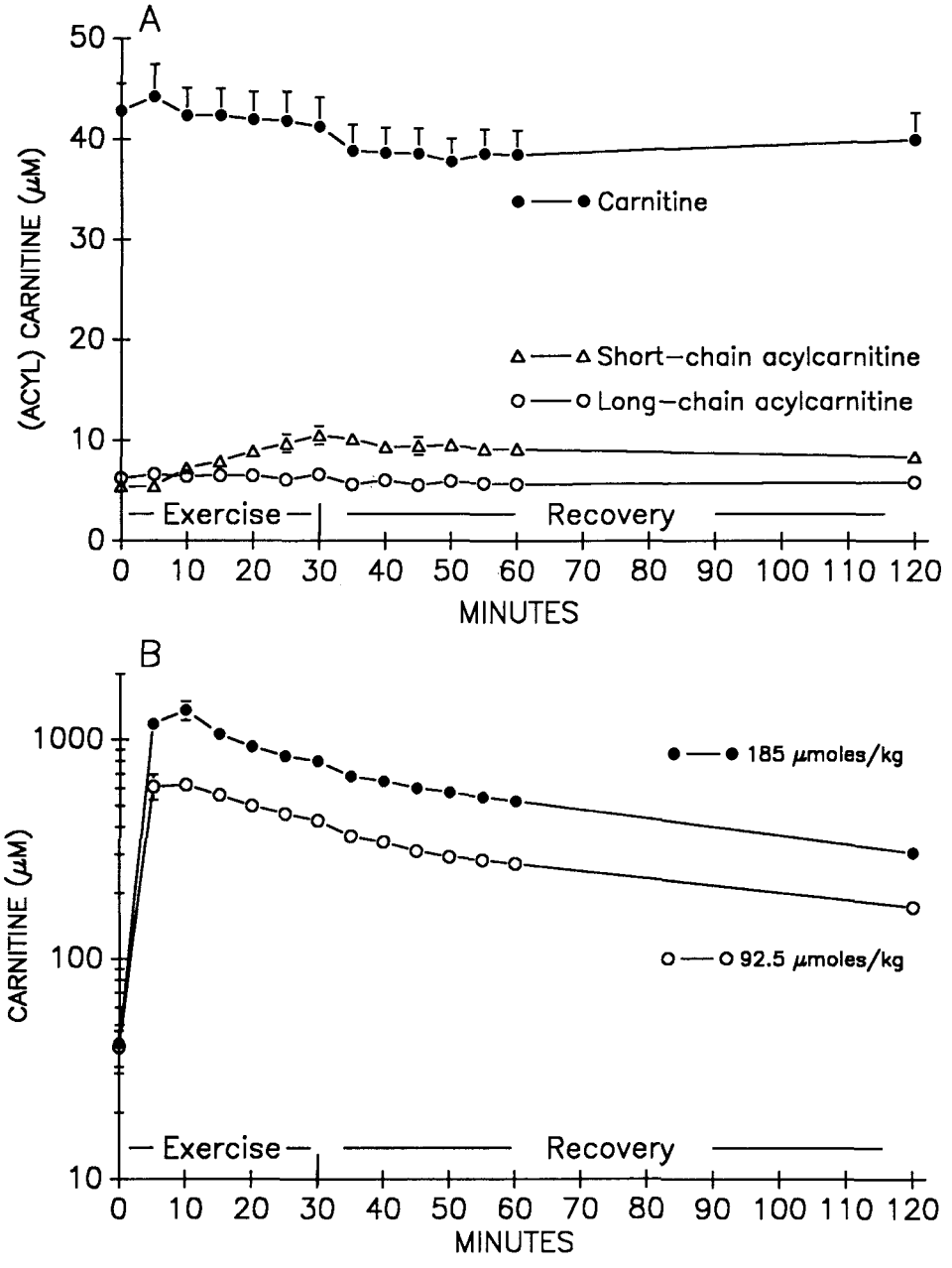

Fig. 1. Plasma carnitine concentrations after intravenous carnitine administration. **A,** Placebo was administered during first 2 minutes of 30-minute bicycle ergometer exercise session at high workload (midpoint between lactate threshold and maximal workload), and plasma carnitine *(solid circles),* short-chain acylcarnitine *(triangles),* and long-chain acylcarnitine *(open circles)* concentrations were quantitated. Values are mean ± SEM with $n = 11$ (placebo studies from both high-intensity exercise protocols). Compared with time zero, carnitine concentrations were decreased from minutes 35 to 120. Short-chain acylcarnitine concentrations were increased compared with time zero from minutes 10 through 120. **B,** Carnitine, 185 μmol/kg *(solid circles; n = 8)* or 92.5 μmol/kg *(open circles; n = 3),* was administered intravenously during first 2 minutes of 30-minute bicycle ergometer exercise session at high workload as in *panel A.* Plasma carnitine concentrations (mean ± SEM) were measured at times indicated.

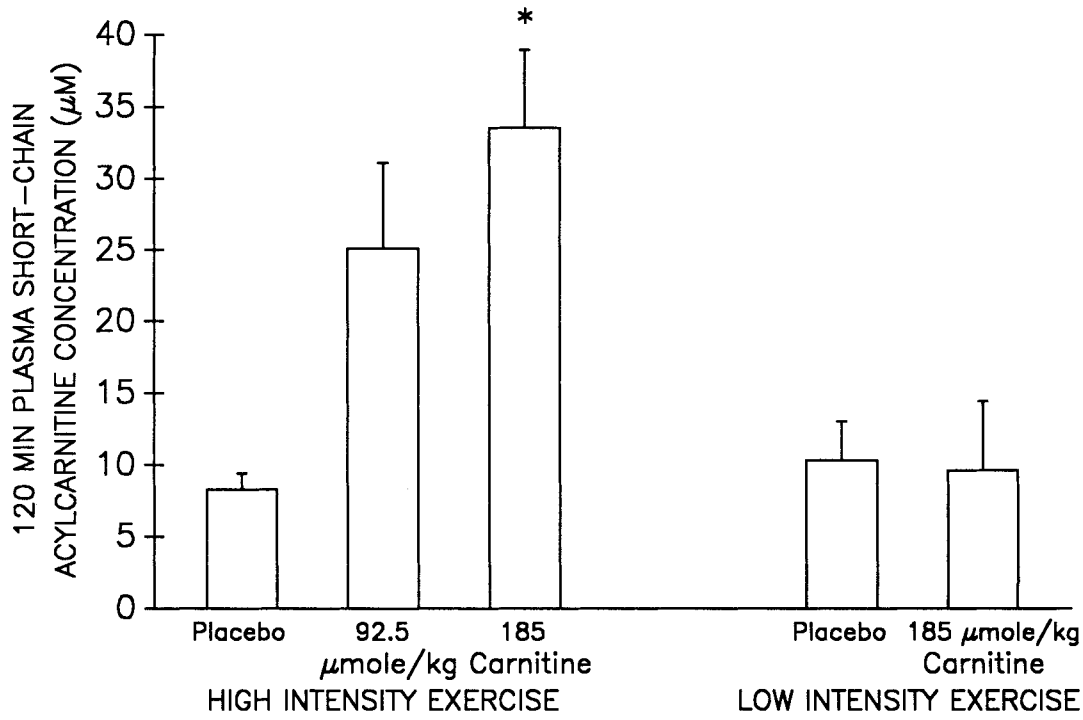

Fig. 2. Plasma short-chain acylcarnitine concentration during exercise. Subjects performed high- or low-intensity exercise after administration of placebo or carnitine at doses indicated (see text for details). Plasma short-chain acylcarnitine concentrations were assessed at conclusion of 120-minute protocol. Values are mean ± SEM; $*p < 0.05$ versus placebo.

Table II. Apparent pharmacokinetic parameters describing acute time course after intravenous carnitine administration during exercise

	Study group		
	High-intensity exercise (185 μmol/kg)	*High-intensity exercise (92.5 μmol/kg)*	*Low-intensity exercise (185 μmol/kg)*
AUC(0-120) (μmol · min/L)	67,500 ± 2,600	33,300 ± 1,400	70,300 ± 2,600
AUC(0-∞) (μmol · min/L)	94,800 ± 4,800	47,600 ± 2,800	105,000 ± 6,600
k (min^{-1})	0.0102 ± 0.0006	0.0094 ± 0.0006	0.0092 ± 0.0006
Urinary carnitine from 0 to 120 min (μmol/kg)	77.4 ± 11.1	35.4 ± 0.8	98.8 ± 2.8
Total clearance (ml/min/kg)	1.99 ± 0.11	1.96 ± 0.12	1.78 ± 0.11
Renal clearance (ml/min/kg)	1.27 ± 0.09	1.06 ± 0.09	1.41 ± 0.09
V_C (ml/kg)	198 ± 11	208 ± 9	194 ± 6

Parameters were calculated as described in the text after administration of carnitine at the doses and under the exercise conditions indicated.
AUC(0-120), Area under the plasma concentration–time curve from 0 to 120 minutes; AUC(0-∞), area under the plasma concentration–time curve from 0 to infinity; k, rate constant; V_C, volume of distribution.

acylcarnitines at rest increased to 39% after 30 minutes of exercise). This redistribution was only partially reversed during 90 minutes of postexercise recovery. In contrast to high-intensity exercise, 60 minutes of low-intensity exercise was associated with a smaller redistribution of the muscle carnitine pool despite the longer exercise duration (Table III). Administration of carnitine at the beginning of exercise had no effect on the muscle carnitine or total carnitine contents 30 or 60 minutes after administration, nor did carnitine modify the short-chain acylcarnitine response to exercise or the postexercise recovery of the muscle carnitine pool (Table III).

Table III. Skeletal muscle carnitine pool during exercise after placebo or carnitine

Treatment	*Workload*	*Time*	*Carnitine (μmol/gm wet weight)*	*Short-chain acylcarnitines (μmol/gm wet weight)*	*Long-chain acylcarnitines (μmol/gm wet weight)*	*Total carnitine (μmol/gm wet weight)*
Placebo	High intensity	Rest (10)	4.06 ± 0.35	0.37 ± 0.06	0.12 ± 0.01	4.54 ± 0.36
		30 Min (10)	2.73 ± 0.12	1.86 ± 0.28	0.12 ± 0.01	4.71 ± 0.30
		60 Min (8)	2.76 ± 0.38	1.41 ± 0.26	0.12 ± 0.02	4.29 ± 0.26
Carnitine, 185 μmol/kg	High intensity	Rest (7)	4.05 ± 0.28	0.26 ± 0.09	0.18 ± 0.05	4.49 ± 0.29
		30 Min (7)	2.70 ± 0.20	1.98 ± 0.43	0.13 ± 0.01	4.81 ± 0.51
		60 Min (5)	2.75 ± 0.24	0.79 ± 0.25	0.16 ± 0.03	3.70 ± 0.37
Carnitine, 92.5 μmol/kg	High intensity	Rest (3)	4.15 ± 0.61	0.21 ± 0.05	0.13 ± 0.02	4.48 ± 0.67
		30 Min (3)	2.82 ± 0.21	1.48 ± 0.34	0.12 ± 0.02	4.42 ± 0.21
		60 Min (3)	3.60 ± 0.93	1.19 ± 0.43	0.15 ± 0.03	4.94 ± 0.93
Placebo	Low intensity	Rest (3)	3.88 ± 0.37	0.38 ± 0.04	0.08 ± 0.01	4.35 ± 0.37
		60 Min (3)	3.70 ± 0.30	1.25 ± 0.39	0.12 ± 0.00	5.07 ± 0.23
Carnitine, 185 μmol/kg	Low intensity	Rest (3)	4.36 ± 0.58	0.44 ± 0.09	0.10 ± 0.01	4.90 ± 0.57
		60 Min (3)	3.68 ± 0.42	1.28 ± 0.11	0.13 ± 0.01	4.99 ± 0.52

Subjects performed high- or low-intensity exercise bicycle ergometer protocols as described in Experimental Procedures after administration of placebo or carnitine at the dose indicated. Skeletal muscle biopsy specimens were obtained at rest, the end of exercise (30 minutes for high-intensity exercise and 60 minutes for low-intensity exercise), and 120 minutes after initiation of exercise. Total carnitine refers to the sum of carnitine and all acylcarnitines. Data are mean values ± SEM. Note that the placebo studies from the two high-intensity exercise protocols have been combined for clarity.
*$p < 0.05$ versus placebo.

Physiologic response to exercise after carnitine administration. After placebo administration, high-intensity exercise was associated with a rapid increase in respiratory exchange ratio (0.82 ± 0.02 at rest versus 1.03 ± 0.02 after 5 minutes of exercise), which then stabilized at approximately 1.00 (Table IV). The response in respiratory exchange ratio to high-intensity exercise was unaffected by 185 μmol/kg carnitine (Fig. 3) or 92.5 μmol/kg carnitine (Table IV). Similarly, the respiratory exchange ratio during low-intensity exercise was unaffected by 185 μmol/kg carnitine. The rate of oxygen consumption (VO_2) during exercise was also unaffected by carnitine in all three protocols used (Table IV).

Lactate accumulation during exercise reflects anaerobic glycolysis and may contribute to fatigue during exercise.[38,39] Neither muscle content nor plasma concentration of lactate was different at peak exercise when comparing placebo with carnitine treatments with either high- or low-intensity exercise (Table IV). The partial normalization of the muscle lactate content after high-intensity exercise was not affected by 185 μmol/kg carnitine (Table IV).

Muscle glycogen is an important fuel source during exercise, and the rate of glycogen depletion may be a determinant of exercise capacity.[40] Muscle glycogen content was decreased by 55%, 71%, and 4% during exercise in the placebo-high-intensity-185 μmol/kg carnitine, placebo-high-intensity-92.5 μmol/kg carnitine, and placebo-low-intensity-185 μmol/kg carnitine studies, respectively (Table IV). Glycogen utilization during exercise was not modified by carnitine administration in any of the protocols used (Table IV).

The plasma β-hydroxybutyrate concentration provides a useful index of hepatic fatty acid oxidation.[41] Plasma β-hydroxybutyrate concentrations were not modified by administration of carnitine (Table IV).

DISCUSSION

Although numerous studies suggest therapeutic actions of carnitine in humans, detailed integrative information on carnitine's pharmacokinetics and pharmacodynamics is limited in clinical studies of the compound. In part, this limitation is the result of the complexity of carnitine homeostasis in humans, including tissue-specific transport kinetics,[42] saturable renal reabsorption,[43,44] intraconversion between carnitine and acylcarnitines,[45] and the discreet compartmentalization of potential sites of action such as skeletal muscle.[24] This study illustrates the importance of these concepts and demonstrates that short-term intravenous administration of carnitine has no effect on skeletal muscle carnitine homeostasis during exercise. In addition, carnitine does not modify any of several

Table IV. Physiologic response to exercise after placebo versus carnitine administration

Protocol	*Parameter*	*Placebo*		
		Rest	*End exercise*	*Recovery*
High-intensity work load, 185 μmol/kg carnitine (n = 8)	RER	0.81 ± 0.02	1.01 ± 0.04	0.84 ± 0.03
	VO_2 (ml/min/kg)	5.1 ± 0.7	35.6 ± 1.9	4.8 ± 1.1
	Muscle lactate (μmol/gm)	5.9 ± 1.7	9.2 ± 1.4	5.1 ± 1.1
	Plasma lactate (mmol/L)	0.8 ± 0.1	5.7 ± 0.6	0.9 ± 0.1
	Muscle glycogen (mg glucose/gm wet wt)	12.9 ± 1.9	5.8 ± 1.4	5.0 ± 0.8
	Plasma β-hydroxybutyrate (μmol/L)	70 ± 10	80 ± 10	90 ± 30
High-intensity work load, 92.5 μmol/kg carnitine (n = 3)	RER	0.84 ± 0.04	0.95 ± 0.02	0.78 ± 0.04
	VO_2 (ml/min/kg)	5.0 ± 0.5	39.3 ± 2.8	5.6 ± 1.3
	Muscle lactate (μmol/gm)	2.9 ± 0.8	10.6 ± 3.0	3.7 ± 0.5
	Plasma lactate (mmol/L)	0.6 ± 0.0	7.6 ± 0.5	1.3 ± 0.4
	Muscle glycogen (mg glucose/gm wet wt)	7.2 ± 2.2	2.1 ± 0.2	3.5 ± 1.1
	Plasma β-hydroxybutyrate (μmol/L)	160 ± 60	130 ± 10	160 ± 80
Low-intensity work load, 185 μmol/kg carnitine (n = 3)	RER	0.77 ± 0.01	0.84 ± 0.02	0.83 ± 0.02
	VO_2 (ml/min/kg)	4.4 ± 0.5	15.9 ± 1.4	3.0 ± 0.3
	Muscle lactate (μmol/gm)	4.3 ± 0.5	6.1 ± 1.2	ND
	Plasma lactate (mmol/L)	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.1
	Muscle glycogen (mg glucose/gm wet wt)	7.1 ± 0.5	6.8 ± 0.2	ND
	Plasma β-hydroxybutyrate (μmol/L)	50 ± 30	70 ± 30	110 ± 40

RER, Respiratory exchange ratio; *ND*, not determined.
Values are mean ± SEM.
Placebo and carnitine treatment exercise studies were performed as described and detailed in the text, and each of the metabolic variables was quantitated. Recovery refers to 60 minutes after drug administration for muscle lactate and glycogen in the high-intensity protocols and 120 minutes after drug administration for all other variables.
*$p < 0.05$ placebo versus carnitine.
VO_2, maximal oxygen consumption rate.

important parameters of fuel metabolism during exercise in healthy subjects.

Endogenous carnitine homeostasis is dependent on dietary carnitine intake and hepatic carnitine biosynthesis to meet tissue demands for this important cofactor. Carnitine uptake into tissue is mediated through an active transport system with a Michaelis-Menten constant of 5 to 50 μmol/L in skeletal and cardiac muscle,[42,46,47] and carnitine turnover in these tissues is slow (i.e., days).[24] Because the normal plasma carnitine concentration of 40 μmol/L is sufficient to yield near-maximal rates of muscle carnitine uptake, and is only slightly lower than the transport maximum for carnitine reabsorption by the kidney,[42-44] it would be anticipated that short-term administration of carnitine would have little impact on muscle carnitine metabolism. These concepts were confirmed in this study. Exogenous carnitine is distributed into a central compartment of approximately 200 ml/kg. Similar estimates for the V_C for carnitine have been published[34] and presumably represent extracellular water. Carnitine is removed from this compartment with a clearance of 1.9 ml/min/kg, of which 60% represents renal clearance (Table II). Two hours after carnitine administration, fully 42% of the dose could be recovered in the urine, whereas an additional 32% remained in the central compartment. These estimates of mass balance are similar to those obtained in studies in animals.[27] The nonrenal clearance is most likely attributable to net uptake by tissues (which will appear as nonreversible removal from the central compartment during the short 2-hour period of observation). The previously published muscle transport kinetics,[42,46] the muscle biopsy data from the current study (Table III), and studies in animals[27] suggest that muscle is not a site of significant acute net uptake of exogenous carnitine. In contrast, liver has a transport uptake Michaelis-Menten constant of 5 mmol/L[48] and demonstrates net uptake of exogenous carnitine in animal studies.[27,45] If extended to humans, the 10% to 20% uptake by liver of an intravenous carnitine dose in rats[27] would account for most of the administered carnitine not present in urine or the central compartment 2 hours after intravenous administration.

The plasma short-chain acylcarnitine concentration was increased in a dose-dependent manner by carnitine during the high-intensity, but not low-intensity, exercise protocol (Fig. 2). Because acylcarnitine accumulation in muscle is also workload dependent, muscle may be the source of these plasma acylcarnitines.

Carnitine		
Rest	*End exercise*	*Recovery*
0.79 ± 0.02	0.96 ± 0.02	0.83 ± 0.04
4.5 ± 0.2	35.1 ± 1.7	4.2 ± 0.4
3.2 ± 0.5	8.7 ± 1.2	5.9 ± 2.1
0.7 ± 0.1	5.2 ± 0.5	0.9 ± 0.10
10.0 ± 2.5	5.9 ± 1.3	4.8 ± 1.2
190 ± 130	130 ± 50	300 ± 210
0.84 ± 0.03	0.95 ± 0.01	0.76 ± 0.01
4.2 ± 0.2	38.1 ± 2.0	3.8 ± 0.3
3.0 ± 0.5	8.3 ± 1.0	4.7 ± 0.4
0.8 ± 0.1	7.9 ± 1.4	1.2 ± 0.3
8.5 ± 1.5	3.2 ± 0.9	4.8 ± 1.8
70 ± 0.00	90 ± 10	90 ± 10
0.80 ± 0.06	0.87 ± 0.01	0.79 ± 0.07
4.2 ± 0.6	16.7 ± 1.8	3.4 ± 0.8
5.7 ± 2.7	7.1 ± 3.3	ND
1.1 ± 0.1	0.8 ± 0.1	0.8 ± 0.2
8.0 ± 0.7	6.0 ± 0.2	ND
50 ± 10	50 ± 20	120 ± 50

However, carnitine did not accentuate the accumulation of acylcarnitines in muscle (Table III), and thus simple net efflux of acylcarnitines is an unlikely mechanism for this effect. High concentrations of extracellular carnitine can exchange with intracellular acylcarnitines across the plasma membrane.[49,50] This mechanism would explain the carnitine- and workload-dependent appearance of acylcarnitines with no net change in muscle total carnitine content. A similar exchange has been postulated in animals after carnitine administration.[27] Despite this increase in plasma acylcarnitine concentration, the amount of acylcarnitine in the plasma (or the V_C) is very small compared with the large amounts of acylcarnitines generated in skeletal muscle during high-intensity exercise (20 nmol/ml plasma versus 1500 nmol/gm muscle).

The apparent pharmacokinetic parameters for carnitine were not dose dependent, nor were they influenced by the exercise intensity (Table II). The absence of dose-dependent kinetics is consistent with the known kinetics of the dominant processes involved, including renal clearance by filtration and a saturated reuptake system, as well as saturation of muscle transport systems at the high plasma carnitine concentrations achieved. Exercise at high workloads is known to decrease the glomerular filtration rate,[51] but the magnitude of this effect is small and was insufficient to modify the renal clearance of carnitine significantly when comparing low versus high-intensity exercise.

Because short-term carnitine administration had no effect on skeletal muscle carnitine homeostasis, its lack of effect on the physiologic response to exercise was not surprising. Indexes of fuel metabolism during exercise were characterized under two different exercise regimens. The respiratory exchange ratio reflects the relative amount of lipid and carbohydrate being oxidized, as well as carbon dioxide generated to buffer lactic acid production,[52] and was unaffected by carnitine (Table III; Fig. 3). Veechiet et al.[17] reported that 2 gm (approximately 150 μmol/kg) carnitine administered orally 1 hour before exercise (oral carnitine has a bioavailability of 5% to 20% and a time to maximal concentrate of 1 to 4 hours[36]) increased maximal VO_2 consumption and decreased carbon dioxide production at a given workload. These changes in gas exchange were suggested to reflect a decrease in the respiratory exchange quotient and an increase in lipid oxidation. However, no measurements of muscle carnitine homeostasis were included in this study, and the biochemical basis for the hypothesis by Veechiet et al. was challenged by Hultman et al.[23] This study confirms the lack of effect on muscle carnitine metabolism predicted by Hultman et al. Veechiet et al. used a graded work load protocol, resulting in non-steady state conditions at intermediate workloads,[17] hence making assessment of ventilatory parameters difficult. This study clearly demonstrates the lack of change in respiratory exchange quotient during exercise at two standardized work intensities (Table III). Other studies reporting changes in respiratory exchange ratio in response to carnitine[13,16] have used 7 to 28 days of oral carnitine administration. However, the resting respiratory exchange ratios were not reported in detail in these studies and in one case[16] appeared to be lowered by carnitine. Because a lower resting respiratory exchange ratio results in a lower exercise respiratory exchange ratio, the lower respiratory exchange ratios reported in this study may reflect a modification of the resting state and a nonmuscle site of carnitine action.

Acute efficacy of a single dose of carnitine in improving exercise performance has been reported in peripheral arterial disease.[10] Again, the acute effects of carnitine on the muscle carnitine pool were not reported. This study showed no acute effects of carnitine on muscle glycogen utilization, lactate accumulation, or plasma β-hydroxybutyrate concentrations, indicating that no major changes in fuel utilization were induced by the compound. However, the muscle response to exogenous carnitine in peripheral arterial disease may be different than in normal subjects, because the endogenous muscle carnitine pool reflects an

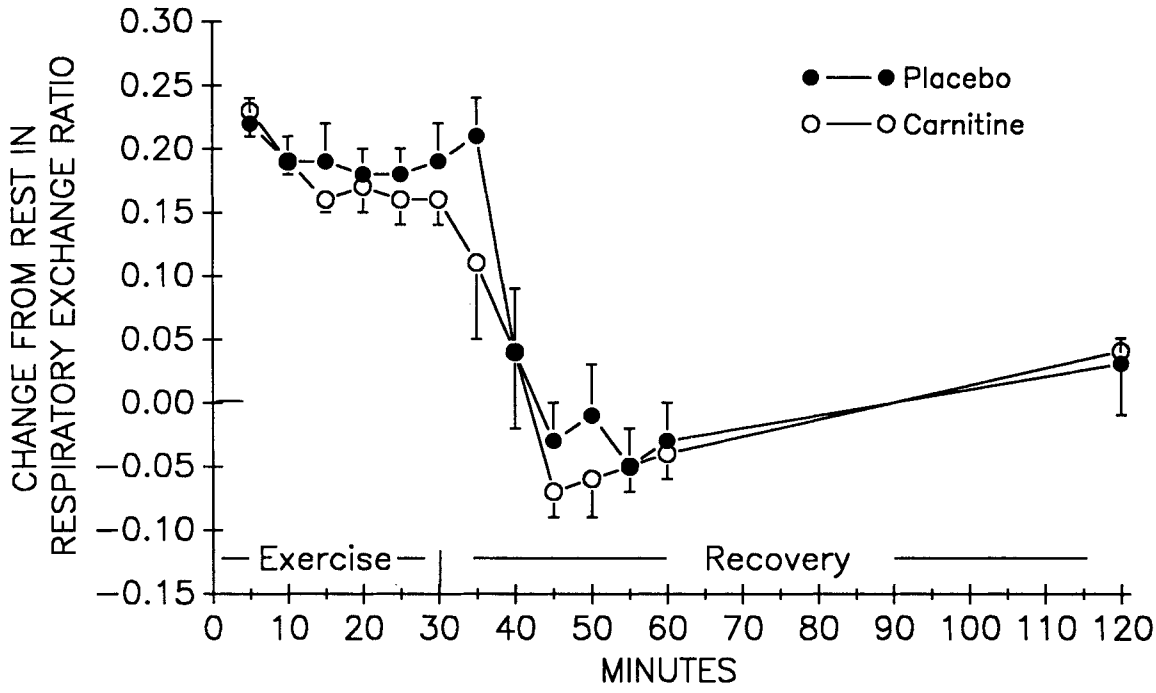

Fig. 3. Response of respiratory exchange ratio to high-intensity exercise. Eight subjects performed high-intensity exercise after administration of placebo or 185 μmol/kg carnitine. Change in respiratory exchange ratio from rest (see Table IV for baseline absolute values) was determined for each subject and averaged. *Solid circles,* Placebo; *open circles,* carnitine. Values are mean ± SEM.

abnormal accumulation of acylcarnitines at rest,[15] and carnitine has been shown to modify lactate efflux under these conditions.[10]

In vitro[18] and in situ[19] models support an acute effect of carnitine on skeletal muscle function. In the case of isolated rat muscle strips,[18] carnitine's beneficial effects on characteristics of fatigue were associated with an increase in muscle carnitine content. Thus carnitine may modify muscle metabolism if it reaches the intracellular compartment in a quantitatively meaningful amount. This is difficult to achieve in normal humans, particularly with short-term administration. Nonetheless, long-term oral dosing in patients with peripheral arterial disease[10] and patients receiving hemodialysis (who lack the rapid renal clearance mechanism)[7] can increase muscle total carnitine content and may explain the efficacy of carnitine in these conditions.

This study, consistent with previous work,[14] documents the relative segregation of the skeletal muscle carnitine pool from the plasma and extracellular fluid central compartment in humans. As a result, short-term administration of carnitine has no significant effect on exercise physiology in normal subjects. The efficacy of carnitine in modifying exercise performance must therefore reflect either a nonmuscle site of action, altered muscle carnitine content caused by long-term carnitine administration, or altered muscle carnitine handling in pathophysiologic states.

We thank Laura Ruff, Laurie Albers, and William Vetter for their excellent technical assistance. We appreciate the constructive comments of Dr. K. Sietsema.

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