

## Carnitine and Acylcarnitine Metabolism during Exercise in Humans Dependence on Skeletal Muscle Metabolic State

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### Abstract

Carnitine metabolism has been previously shown to change with exercise in normal subjects, and in patients with ischemic muscle diseases. To characterize carnitine metabolism further during exercise, six normal male subjects performed constant-load exercise on a bicycle ergometer on two separate occasions. Low-intensity exercise was performed for 60 min at a work load equal to 50% of the lactate threshold, and high-intensity exercise was performed for 30 min at a work load between the lactate threshold and maximal work capacity for the individual. Low-intensity exercise was not associated with a change in muscle (vastus lateralis) carnitine metabolism. In contrast, from rest to 10 min of high-intensity exercise, muscle short-chain acylcarnitine content increased 5.5-fold while free carnitine content decreased 66%, and muscle total carnitine content decreased by 19% (all  $P < 0.01$ ). These changes in skeletal muscle carnitine metabolism were present at the completion of 30 min of high-intensity exercise, and persisted through a 60-min recovery period. With 30 min of high-intensity exercise, plasma short-chain and long-chain acylcarnitine concentrations increased by 46% and 23%, respectively. Neither exercise state was associated with a change in the urine excretion rates of free carnitine or acylcarnitines. Thus, alterations in skeletal muscle carnitine metabolism, characterized by an increase in acylcarnitines and a decrease in free and total carnitine, are dependent on the work load and, therefore, the metabolic state associated with the exercise, and are poorly reflected in the plasma and urine carnitine pools.

### Introduction

With exercise, there are fundamental changes in skeletal muscle energy metabolism that are dependent on exercise intensity and duration. When constant-load exercise is performed at a low intensity (< 35–45% of maximal oxygen consumption), there is an increase in both the plasma concentration and uptake of free fatty acids by exercising muscle (1, 2). As free fatty acids become the major substrate for energy metabolism, there is a fall in the plasma concentrations of insulin and glucose (3–5). With low-intensity exercise, blood lactate concentration

does not increase, and oxygen uptake kinetics remain at a steady state (1, 4–6).

In contrast, with high-intensity exercise at a constant work load (70–80% of maximal oxygen consumption), there is a decrease in the plasma concentration and turnover of free fatty acids, and an increase in the plasma concentration of glucose (1). At high work loads, carbohydrates serve as the primary substrate for exercising muscle, with an increase in the oxidation of amino acids also contributing to muscle energy metabolism (7, 8). At these high exercise loads, there is a rise in blood lactate concentration, oxygen uptake kinetics may not reach steady state, and exercise can be sustained for only short periods of time (1, 6, 9). Thus, high-intensity and low-intensity exercise reflect two qualitatively distinct states of skeletal muscle metabolism. The transition between these two exercise metabolic states occurs at a work load approximated by the lactate threshold, defined as the exercise intensity at which elevated plasma lactate concentrations are first seen (6, 9–11).

Exercise has been shown to alter carnitine metabolism. Carnitine is an essential cofactor for the transport of long-chain fatty acyl groups (fatty acids with 10 or more carbon atoms) into the mitochondria for  $\beta$ -oxidation to acetyl coenzyme A (CoA) (12). Under some metabolic conditions, carnitine also serves as a “buffer” of the metabolically critical mitochondrial acyl-CoA pool (13). Short-chain acylcarnitines (acyl groups less than 10 carbon atoms) are formed from, and are in equilibrium with, the corresponding intracellular short-chain acyl-CoA esters (14). Thus, changes in the distribution of total carnitine between acylcarnitines and free carnitine reflect similar changes in the acyl-CoA pool (14, 15).

In humans, the content of acylcarnitines in muscle has been shown to increase after exercise in some studies, but not in others (16, 17). Exercise has also been associated with increases in both the plasma concentration of acylcarnitines and the urine excretion of free carnitine (16, 18). However, in animals changes in the plasma and urine carnitine pools may reflect production from tissues other than muscle (15). As discussed above, the metabolic state of exercising muscle is fundamentally different at different work loads. In that changes in carnitine metabolism reflect changes in tissue metabolism, these differences in metabolic state must be incorporated into the design of studies examining carnitine metabolism in exercise and may explain the apparently discrepant results to date. An understanding of the relationship between carnitine metabolism and exercise is critical as studies are extended to the evaluation of carnitine metabolism in disease states (19, 20) and as carnitine is used as a therapeutic agent to improve exercise performance (21–23).

The current studies evaluate the hypothesis that alterations in skeletal muscle metabolism during exercise lead to changes in carnitine metabolism which reflect the metabolic state of muscle. The results demonstrate a rapid, large redistribution of total skeletal muscle carnitine towards acylcarnitines with short-duration, high-intensity, but not with long-duration, low-intensity exercise. Changes in skeletal muscle carnitine

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Table I. Description of Subjects on Entry

Subject	Maximal heart rate	Maximal work load	Maximal VO <sub>2</sub>	Lact load	Lact VO <sub>2</sub>	Lact percent	Selected	
							HI load	LO load
	beats/min	kg · m/min	ml/(kg · min)	kg · m/min	ml (kg · min)	%		
1	175	1,400	41.4	800	23.2	56	1,000	400
2	175	1,800	42.1	1,000	27.8	66	1,400	600
3	190	2,000	52.9	1,200	30.8	58	1,500	500
4	190	1,400	37.9	800	22.0	58	1,100	—
5	193	2,000	53.6	1,000	31.9	60	1,500	500
6	182	1,800	45.9	800	25.0	55	1,300	300
Mean	184±3	1,733±96	45.6±2.6	933±67	26.8±1.7	59±2	1,300±86	460±51

Six normal subjects underwent a graded bicycle ergometer test to maximal exertion in 200-kg · m/min, 2-min stages. Maximal heart rate, work load, and oxygen consumption [Max VO<sub>2</sub> in ml/(kg · min)] were determined. The work load (*lact load*), oxygen consumption (*Lact VO<sub>2</sub>*), and percentage of maximal oxygen consumption (*Lact percent*) at which the concentration of blood lactate increased was estimated. From this initial evaluation, a work load was selected for high-intensity (*HI load*) and low-intensity (*LO load*) exercise as described in the text. One subject was not studied with the low-intensity protocol. Data represent mean±SE.

metabolism were poorly reflected in the plasma and urine carnitine pools.

## Methods

**Subjects.** Six male subjects, who were on no medications and in good health, were enrolled in the study. The subjects were physically active and performed exercise on a regular basis, but none was a competitive athlete. Their mean age was 25±2 yr, and they weighed 76±2 kg. The study protocols were approved by the University of Colorado School of Medicine Human Subjects Committee and informed consent was obtained from all subjects.

**Initial evaluation.** All subjects were familiarized with the testing procedures including an initial bicycle exercise test. A second exercise evaluation was performed for each subject to characterize each individual's metabolic response as a function of work load. Before exercise, an intravenous catheter was placed in a forearm vein. Maximal exercise testing was performed to exhaustion on a bicycle ergometer (Uniworke ergometer model 845, Quinton Instruments, Seattle, WA) with 200-kg · m/min increments in work load every 2 min. At rest and during each minute of exercise, heart rate was monitored by 12-lead electrocardiogram and blood pressure taken by auscultation. Ventilation (VE),<sup>1</sup> oxygen consumption (VO<sub>2</sub>), carbon dioxide production (VCO<sub>2</sub>), and the respiratory exchange ratio (RER) determined as VCO<sub>2</sub>/VO<sub>2</sub> were measured continuously with a metabolic cart (Thermo, Ametek Inc., Pittsburgh, PA). This system was calibrated with known concentrations of O<sub>2</sub> and CO<sub>2</sub> before each exercise test. Blood was drawn at the end of each minute of exercise for measurements of blood lactate.

From the initial studies, the work load and VO<sub>2</sub> at which blood lactate concentration increased was estimated (Table I). At low exercise intensities, blood lactate concentration remains unchanged from resting values, but as the work intensity is increased, there is a point where blood lactate concentration increases progressively (9–11). The lactate threshold can be defined as the inflection point for the increase in blood lactate concentration (9, 10). For the study group, the lactate threshold occurred at a work load that was 59% of the maximal work load, and at a VO<sub>2</sub> that was 60% of the maximal VO<sub>2</sub> (Table I). For subsequent testing, the low-intensity work load in an individual was

determined as a work load that was 50% of the lactate threshold. The high-intensity work load was defined as the lactate threshold plus 50% of the difference between the lactate threshold and the maximal work load. This method allows for a uniform selection of exercise intensity for each subject in regards to the metabolic differences between low- and high-intensity exercise (6, 9).

**Exercise testing.** The order of the subsequent low- and high-intensity exercise protocols was randomized, and the tests were separated by an average of 7 d. Subjects were studied after an overnight fast. Urine was obtained for analysis of creatinine, free carnitine, and acylcarnitines. An 18-g intravenous catheter, with a three-way stopcock, was placed in a large vein in the forearm and patency was maintained by flushing with heparinized saline. Blood was drawn at 10 min, 5 min, and immediately before exercise for determination of free carnitine, acylcarnitines, β-hydroxybutyrate, and lactate concentrations, with the results averaged to determine baseline values for these parameters. Baseline measurements of VO<sub>2</sub>, VCO<sub>2</sub>, and RER were obtained for 6 min at rest. At rest, a needle biopsy was performed in the vastus lateralis muscle in the lateral aspect of the thigh using a 5-mm needle (Bergstrom muscle biopsy cannula, DePuy, Boehringer Mannheim Corp., Warsaw, IN). Muscle tissue was assayed for free carnitine and acylcarnitines. The thigh was wrapped with a nonconstricting elastic bandage and exercise was begun within 15 min of the biopsy.

Exercise was performed on the bicycle ergometer for 60 min (low intensity) or for 30 min (high intensity) at a constant work load (Table I). However, in three subjects the load during high-intensity exercise was reduced 100–200 kg · m/min during the last 10 min of exercise because of fatigue. Respiratory measurements were obtained continuously for the first 10 min of exercise, and at the time points indicated in Table II. Blood samples were drawn at 10, 30, 40, 50, and 60 min of low-intensity exercise and at 5, 10, 15, 20, and 30 min of high-intensity exercise (Table II). After 10 min of exercise, subjects had a second biopsy performed at a site within 1 cm of the previous biopsy. This procedure interrupted the exercise session for not more than 2 min. A final biopsy was performed at the end of the exercise period, and then a urine sample was obtained.

At the completion of exercise, subjects returned to the supine position. Respiratory measurements and blood samples were obtained at 10, 30, and 60 min after exercise (i.e., recovery), and urine was obtained after 60 min of recovery. In four subjects after high-intensity exercise, an additional muscle biopsy was obtained after 60 min of recovery.

**Sample preparation.** To each urine specimen, 25 μl of 1.2 M-HCl was added, the volume was measured, and samples were then frozen in

1. Abbreviations used in this paper: RER, respiratory exchange ratio; VCO<sub>2</sub>, carbon dioxide production; VE, ventilation; VO<sub>2</sub>, oxygen production.

liquid nitrogen. Blood samples (5 ml) were withdrawn and immediately placed in a heparinized tube on ice. The sample was then centrifuged at 600 *g* for 3 min in chilled centrifuge tubes, and plasma aliquots frozen in liquid nitrogen. Muscle samples of between 30 and 100 mg were immediately frozen in liquid nitrogen. All samples were stored at  $-70^{\circ}\text{C}$  for subsequent analysis. Blood samples for lactate determination (25  $\mu\text{l}$ ) were immediately placed in chilled perchloric acid, vortexed, and stored on ice until assayed.

**Assay methods.** Carnitine was measured by a radioenzymatic assay (24), as previously described by Brass and Hoppel (15). Plasma and muscle samples were prepared in perchloric acid and centrifuged at 10,000 *g* for 2 min. The acid-soluble fraction (supernatant) was assayed for free carnitine and short-chain acylcarnitines, and the acid-insoluble fraction (pellet) was used to determine the long-chain acylcarnitine content. Total acid-soluble carnitine concentration is the sum of the free carnitine and short-chain acylcarnitine concentrations. The ratio of short-chain acylcarnitine content to total acid soluble carnitine content was determined from these measurements. Total carnitine is the sum of free carnitine, short-chain acylcarnitine, and long-chain acylcarnitine concentrations. Each sample was assayed in duplicate, with the average result reported.

Urine samples were assayed for free carnitine and short-chain acylcarnitines as described above except that the perchloric acid fractionation was omitted. The urinary excretion rate of carnitine was

estimated as the concentration of free carnitine or short-chain acylcarnitine in micromolar, normalized to the urine concentration of creatinine in millimolar. As creatinine clearance changes with exercise (25), this normalization corrects for changes in renal function not specific for carnitine.

The plasma concentration of  $\beta$ -hydroxybutyrate was measured by the method of Olsen (26). Blood lactate concentration was assayed by a lactate dehydrogenase method (27).

**Statistical analysis.** Values are reported as mean  $\pm$  standard error of the mean. Subjects served as their own controls in that measurements at rest were compared with changes during exercise and recovery. Changes during exercise or recovery were compared with rest values by an analysis of variance for repeated measures. Post-hoc testing was performed with Dunnett's test (28). Values between groups were compared with Student's *t* test for unpaired data. Significance was defined as  $P < 0.05$ .

## Results

**Low-intensity exercise state.** Low-intensity exercise was characterized by increases in heart rate, systolic blood pressure,  $\text{VO}_2$ , VE, and RER that were similar to those observed in previous studies (Table II) (1, 4). There was no change in

Table II. Hemodynamic, Respiratory, and Metabolic Changes with Exercise

Measurement	Low intensity								
	Rest	Exercise					Recovery		
		10	30	40	50	60	10	30	60
Heart rate, <i>beats/min</i>	70 $\pm$ 4	105 $\pm$ 3*	104 $\pm$ 3*	105 $\pm$ 5*	108 $\pm$ 4*	108 $\pm$ 5*	69 $\pm$ 3	71 $\pm$ 5	68 $\pm$ 3
Systolic blood pressure, <i>mmHg</i>	121 $\pm$ 3	136 $\pm$ 3*	138 $\pm$ 2*	137 $\pm$ 2*	137 $\pm$ 2*	141 $\pm$ 5*	121 $\pm$ 6	115 $\pm$ 3	116 $\pm$ 3
Diastolic blood pressure, <i>mmHg</i>	81 $\pm$ 1	85 $\pm$ 3	81 $\pm$ 2	81 $\pm$ 3	81 $\pm$ 2	80 $\pm$ 2	81 $\pm$ 2	73 $\pm$ 2	79 $\pm$ 3
$\text{VO}_2$ , <i>ml/(kg·min)</i>	5 $\pm$ 0	18 $\pm$ 1*	18 $\pm$ 1*	18 $\pm$ 1*	18 $\pm$ 0*	18 $\pm$ 0*	6 $\pm$ 0	5 $\pm$ 1	5 $\pm$ 0
$\text{VO}_2$ %	11 $\pm$ 1	39 $\pm$ 3*	39 $\pm$ 4*	39 $\pm$ 4*	38 $\pm$ 3*	39 $\pm$ 3*	12 $\pm$ 1	12 $\pm$ 2	10 $\pm$ 1
VE, <i>liters/min</i>	11 $\pm$ 1	34 $\pm$ 3*	34 $\pm$ 3*	34 $\pm$ 3*	35 $\pm$ 3*	36 $\pm$ 2*	14 $\pm$ 1	13 $\pm$ 1	11 $\pm$ 1
RER, %	75 $\pm$ 4	84 $\pm$ 3*	85 $\pm$ 2*	83 $\pm$ 2*	86 $\pm$ 4*	88 $\pm$ 3*	77 $\pm$ 3	73 $\pm$ 2	79 $\pm$ 5
Plasma lactate concentration, <i>mM</i>	0.9 $\pm$ 0.2	0.8 $\pm$ 0.2	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1	—
Plasma $\beta$ -hydroxybutyrate concentration, $\mu\text{M}$	70 $\pm$ 30	50 $\pm$ 20	60 $\pm$ 30	70 $\pm$ 40	70 $\pm$ 40	70 $\pm$ 40	100 $\pm$ 40	110 $\pm$ 40	70 $\pm$ 30

Measurement	High intensity								
	Rest	Exercise					Recovery		
		5	10	15	20	30	10	30	60
Heart rate, <i>beats/min</i>	68 $\pm$ 6	149 $\pm$ 5*	166 $\pm$ 5*	170 $\pm$ 5*	169 $\pm$ 3*	177 $\pm$ 3*	108 $\pm$ 5*	83 $\pm$ 5	79 $\pm$ 6
Systolic blood pressure, <i>mmHg</i>	124 $\pm$ 2	162 $\pm$ 8*	162 $\pm$ 3*	162 $\pm$ 5*	169 $\pm$ 3*	165 $\pm$ 5*	115 $\pm$ 5	116 $\pm$ 4	110 $\pm$ 3*
Diastolic blood pressure, <i>mmHg</i>	82 $\pm$ 2	78 $\pm$ 5	80 $\pm$ 2	72 $\pm$ 2*	68 $\pm$ 3*	69 $\pm$ 3*	82 $\pm$ 2	76 $\pm$ 2*	78 $\pm$ 2*
$\text{VO}_2$ , <i>ml/(kg·min)</i>	5 $\pm$ 0	37 $\pm$ 1*	41 $\pm$ 2*	40 $\pm$ 2*	40 $\pm$ 2*	40 $\pm$ 2*	6 $\pm$ 0*	5 $\pm$ 0	5 $\pm$ 0
$\text{VO}_2$ %	11 $\pm$ 1	71 $\pm$ 14*	89 $\pm$ 3*	87 $\pm$ 3*	87 $\pm$ 2*	87 $\pm$ 2*	14 $\pm$ 1*	12 $\pm$ 1	12 $\pm$ 1
VE, <i>liters/min</i>	11 $\pm$ 1	97 $\pm$ 10*	109 $\pm$ 11*	108 $\pm$ 13*	112 $\pm$ 11*	118 $\pm$ 12*	15 $\pm$ 2*	11 $\pm$ 1	12 $\pm$ 1
RER, %	77 $\pm$ 3	106 $\pm$ 4*	101 $\pm$ 2*	98 $\pm$ 1*	97 $\pm$ 1*	97 $\pm$ 1*	70 $\pm$ 2*	67 $\pm$ 2*	73 $\pm$ 3
Plasma lactate concentration, <i>mM</i>	0.7 $\pm$ 0.1	3.0 $\pm$ 0.5*	4.4 $\pm$ 0.5*	4.8 $\pm$ 0.4*	5.4 $\pm$ 0.6*	5.8 $\pm$ 0.5*	3.5 $\pm$ 0.5*	1.8 $\pm$ 0.3*	—
Plasma $\beta$ -hydroxybutyrate concentration, $\mu\text{M}$	80 $\pm$ 30	70 $\pm$ 20	60 $\pm$ 20	50 $\pm$ 10	60 $\pm$ 10	80 $\pm$ 10	130 $\pm$ 30	90 $\pm$ 40	70 $\pm$ 20

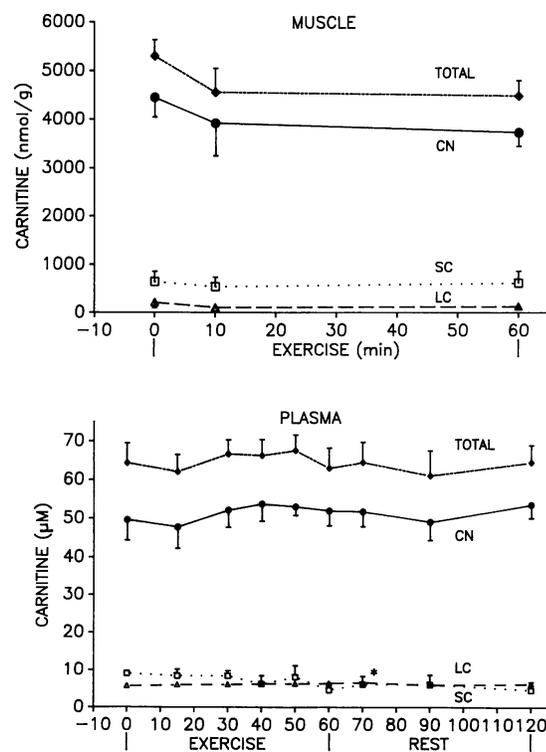
Subjects were studied at low-intensity and high-intensity work loads as described in the text. Within groups, \* indicates  $P < 0.05$  compared with resting value using analysis of variance.

diastolic blood pressure, or the plasma concentrations of lactate or  $\beta$ -hydroxybutyrate. All values remained constant between 10 and 60 min of exercise except for a small, 2-liter/min increase in VE at 60 min of exercise.

With low-intensity exercise, there was no change in the muscle content of free carnitine ( $4,450 \pm 410$  nmol/g at rest and  $3,730 \pm 280$  nmol/g at the end of exercise, Fig. 1). Short-chain acylcarnitine content was  $640 \pm 220$  nmol/g at rest and  $620 \pm 250$  nmol/g after 60 min of exercise. Similarly, long-chain acylcarnitine and total carnitine content did not change with exercise.

In plasma with low-intensity exercise, long-chain acylcarnitine concentration increased from  $5.7 \pm 0.5$   $\mu$ M at rest to  $6.6 \pm 0.5$   $\mu$ M at 10 min of recovery (Fig. 1). The plasma concentrations of free carnitine, short-chain acylcarnitine, and total carnitine remained unchanged. The urine excretion of free carnitine and short-chain acylcarnitine did not change after 60 min of exercise or 60 min of recovery (Table III).

**High-intensity exercise state.** High-intensity exercise was characterized by greater increases in heart rate, systolic blood pressure,  $VO_2$ , VE, and RER than observed with low-intensity exercise (Table II). The blood lactate concentration was  $0.7 \pm 0.1$  mM at rest, increased after 5 min of exercise to  $3.0 \pm 0.5$  mM, and continued to rise to a concentration of  $5.8 \pm 0.5$  mM after 30 min of exercise. As previously described, several other parameters appeared not to reach steady state



**Figure 1.** Carnitine metabolism during low-intensity exercise. In the upper graph, the muscle content of free carnitine (CN), short-chain acylcarnitine (SC), long-chain acylcarnitine (LC), and total carnitine (TOTAL) was measured at rest, and after 10 and 60 min of exercise. In the lower graph, the plasma concentrations of free carnitine and acylcarnitines were measured at rest, and at multiple time points during exercise and recovery. Data are mean  $\pm$  SE and \* $P < 0.05$  compared with the resting value.

**Table III.** Urine Carnitine Excretion at Rest and after Exercise and Recovery

Carnitine	Urine carnitine concentration/ creatinine concentration		
	Rest	Peak	Post
	$\times 10^3$		
Free			
LO	11.5 $\pm$ 5.0	16.2 $\pm$ 5.4	14.3 $\pm$ 4.8
HI	7.7 $\pm$ 2.2	8.9 $\pm$ 2.6	6.5 $\pm$ 2.7
Short-chain acyl			
LO	11.5 $\pm$ 1.6	10.7 $\pm$ 1.7	11.7 $\pm$ 1.9
HI	9.5 $\pm$ 0.7	10.2 $\pm$ 1.0	8.9 $\pm$ 0.7
Total acid-soluble			
LO	23.0 $\pm$ 6.5	26.9 $\pm$ 6.5	26.0 $\pm$ 4.7
HI	17.3 $\pm$ 2.8	19.1 $\pm$ 2.4	15.4 $\pm$ 3.1

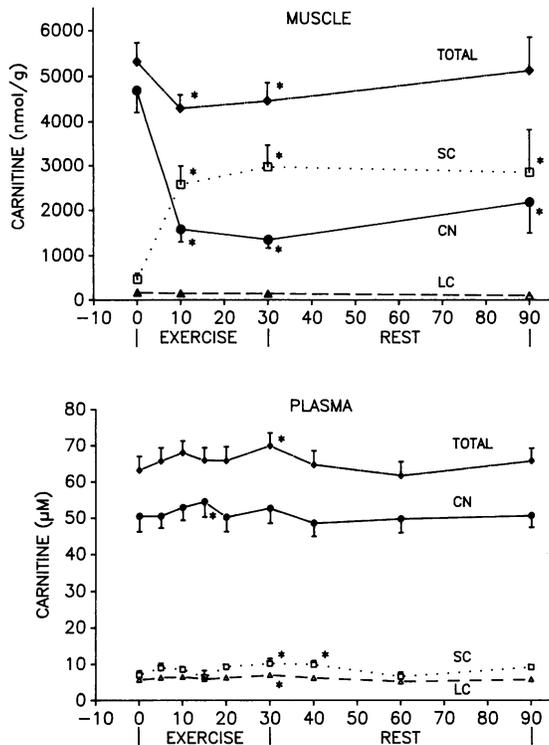
Urine carnitine concentration (micromolar) was normalized to urine creatinine concentration (millimolar) to express the excretion rate of carnitine. "Peak" refers to samples obtained after 60 min of low-intensity exercise (LO) or 30 min of high-intensity exercise (HI), and "Post" to samples obtained after 60 min of recovery. Total acid-soluble is the sum of free and short-chain acylcarnitine. There were no changes in the excretion of free carnitine or acylcarnitines during exercise or recovery.

during high-intensity exercise (6). At 3 min of high-intensity exercise,  $VO_2$  was  $34.9 \pm 1.6$  ml/(kg  $\cdot$  min), then increased to  $41.0 \pm 2.0$  ml/(kg  $\cdot$  min) at 10 min, and remained constant during the remainder of exercise. At 5 min of exercise, heart rate was 81% of maximal heart rate determined from the initial maximal exercise test. However, at 30 min of exercise it had risen to 97% of the maximal exercise heart rate. The plasma concentration of  $\beta$ -hydroxybutyrate did not increase during high-intensity exercise or recovery.

After only 10 min of high-intensity exercise, muscle short-chain acylcarnitine content increased from a resting value of  $470 \pm 140$  nmol/g to  $2,570 \pm 420$  nmol/g, whereas free carnitine content decreased from  $4,690 \pm 490$  nmol/g at rest to  $1,580 \pm 280$  (Fig. 2). At the 10-min time point, muscle long-chain acylcarnitine content was unchanged, but muscle total carnitine content decreased from  $5,320 \pm 420$  nmol/g at rest to  $4,290 \pm 300$ . After completing 30 min of high-intensity exercise, muscle short-chain acylcarnitine content was  $2,970 \pm 500$  nmol/g and muscle free carnitine content was  $1,350 \pm 190$  nmol/g. Skeletal muscle total carnitine did not change between 10 and 30 min of high-intensity exercise ( $4,460 \pm 400$  nmol/g after 30 min), but remained less than resting values.

After 1 h of recovery, skeletal muscle total carnitine content had returned to baseline values, but short-chain acylcarnitine content remained elevated at  $2,850 \pm 990$  nmol/g and free carnitine content decreased to  $2,180 \pm 690$  nmol/g. Thus, high-intensity exercise was characterized by a rapid change in carnitine metabolism that partially persisted through 60 min of recovery.

The plasma free carnitine concentration was increased after 15 min of high-intensity exercise, but was unchanged from resting values during the remainder of exercise and recovery (Fig. 2). The plasma short-chain acylcarnitine concentration increased from  $7.0 \pm 1.2$   $\mu$ M at rest to  $10.2 \pm 1.4$  at 30



**Figure 2.** Carnitine metabolism during high-intensity exercise. In the upper graph, the muscle content of free carnitine (CN), short-chain acylcarnitine (SC), long-chain acylcarnitine (LC), and total carnitine (TOTAL) was measured at rest, and after 10 and 30 min of exercise, and after 60 min of recovery. In the lower graph, the plasma concentrations of free carnitine and acylcarnitines were measured at rest, and at multiple time points during exercise and recovery. Data are mean $\pm$ SE and \* $P < 0.05$  compared with resting values.

min of exercise, and remained elevated ( $9.9 \pm 1.1 \mu\text{M}$ ) 10 min into the recovery period, before returning to baseline levels at 30 min of recovery. The plasma long-chain acylcarnitine concentration was increased from  $5.7 \pm 0.4 \mu\text{M}$  at rest to  $7.0 \pm 0.5$  at 30 min of exercise, and the plasma total carnitine concentration increased from  $63.2 \pm 3.9 \mu\text{M}$  at rest to  $69.9 \pm 3.6$  at 30 min of exercise. The urinary excretion rates of free carnitine and short-chain acylcarnitine did not change from baseline values after 30 min of exercise, or 60 min of recovery (Table III).

## Discussion

Skeletal muscle metabolism varies fundamentally as a function of work load (6, 9). The high-intensity exercise state was associated with a rapid change in muscle carnitine metabolism characterized by a redistribution from free to short-chain acylcarnitines, and a decrease in total carnitine content. In contrast, 60 min of low-intensity exercise was not associated with a change in muscle carnitine metabolism. In plasma, the changes in carnitine metabolism with high-intensity exercise were small and occurred only after 30 min of exercise. No changes were observed in urine free carnitine or acylcarnitine excretion rates with exercise. Thus, skeletal muscle carnitine content was dramatically altered only with high-intensity exercise. These changes in skeletal muscle carnitine metabolism could not be inferred on the basis of measurements in the plasma or urine compartments.

In the current studies, the low- and high-intensity exercise protocols were well defined, based on each individual's lactate threshold, and represented distinctly different states of skeletal muscle metabolism (1–7). Low-intensity exercise was easily maintained for 60 min, without a change in blood lactate concentration. Substrate utilization during low-intensity exercise (from the RER), was estimated to be 50% carbohydrate and 50% fat (29). High-intensity exercise was maintained for 30 minutes (to the point of near-exhaustion in three subjects), with a progressive increase in blood lactate concentration in all subjects. Substrate utilization was estimated to be 100% carbohydrate during the high-intensity exercise period.

In humans and animals, the ratio of short-chain acylcarnitine content to the total acid soluble carnitine content provides a useful index of carnitine metabolism under varied metabolic states (14, 15, 19, 30). A redistribution of the carnitine pool from free toward acylcarnitines is reflected by an increase in this ratio. With low-intensity exercise, this ratio was not changed in either muscle, plasma, or urine after 60 min of exercise, or in plasma and urine after 60 min of recovery (Table IV). Thus, in none of the three compartments was the low-intensity exercise state associated with a change in carnitine metabolism. In contrast, with high-intensity exercise, the ratio of short-chain acylcarnitine to total acid-soluble carnitine in muscle increased from  $0.10 \pm 0.03$  to  $0.61 \pm 0.07$  after only 10 min of exercise and was  $0.67 \pm 0.07$  at the end of exercise. This indicates that a majority of free carnitine in muscle was rapidly converted to acylcarnitines. Compared with the large changes in muscle after 10 min of exercise, the ratio in plasma increased modestly, and only after 30 min of exercise. Measurements in urine demonstrated no change in carnitine metabolism immediately after high-intensity exercise, in contrast to the measurements in skeletal muscle and plasma.

Previous studies in humans evaluating carnitine metabolism with exercise did not thoroughly assess the differences in metabolic state relative to exercise intensity (16, 17). In these prior studies, subjects performed constant-load exercise at

**Table IV.** Ratio of Short-Chain Acylcarnitine/Total Acid-Soluble Carnitine at Rest, during Exercise, and after Recovery

	Rest	Low-intensity exercise		
		10 min	60 min	60 min recovery
Muscle	$0.13 \pm 0.04$	$0.14 \pm 0.06$	$0.14 \pm 0.05$	—
Plasma	$0.16 \pm 0.03$	$0.16 \pm 0.04$	$0.08 \pm 0.03$	$0.08 \pm 0.04$
Urine	$0.61 \pm 0.10$	—	$0.48 \pm 0.10$	$0.54 \pm 0.12$
	Rest	High-intensity exercise		
		10 min	30 min	60 min recovery
Muscle	$0.10 \pm 0.03$	$0.61 \pm 0.07^*$	$0.67 \pm 0.07^*$	$0.54 \pm 0.15^*$
Plasma	$0.13 \pm 0.03$	$0.15 \pm 0.02$	$0.17 \pm 0.03^*$	$0.16 \pm 0.02$
Urine	$0.59 \pm 0.05$	—	$0.56 \pm 0.07$	$0.65 \pm 0.07^\ddagger$

The ratio of short-chain acylcarnitine/total acid-soluble carnitine was determined from measurements in muscle, plasma, and urine at rest, during 10 min of exercise, at the completion of exercise, and after 60 min of recovery.

\*  $P < 0.05$  compared with resting measurement.

†  $P < 0.05$  compared with value at 30 min of exercise.

50–56% of their maximal  $\text{VO}_2$ , which may have been metabolically high intensity for some subjects, but low intensity for others (6, 9). The current data suggest that muscle carnitine metabolism does not change in a graded fashion with exercise. Muscle carnitine metabolism changed only when exercise was of sufficient intensity to qualitatively alter muscle substrate metabolism. Consistent with this perspective, short-duration, high-intensity exercise has been shown to increase the skeletal muscle content of acetylcarnitine in humans and animals (31–33), but intermediate exercise intensities, as noted above, have a varied effect on muscle carnitine metabolism (16, 17). In the current study, the specific acyl-moieties incorporated into the skeletal muscle short-chain acylcarnitine pool during high-intensity exercise are unknown, and are currently being characterized.

After 60 min of recovery from high-intensity exercise, total muscle carnitine content returned to baseline values. However, short-chain acylcarnitine content remained elevated, and free carnitine content was only 46% of resting values, reflecting a continued redistribution of the carnitine pool. The finding of a persistent change in muscle carnitine metabolism into recovery is consistent with the observation that in humans, metabolic rate and substrate utilization remain altered for several hours after exercise (34), and may have implications for the performance of repetitive bouts of high-intensity exercise. In contrast to the changes in muscle after 60 min of recovery from high-intensity exercise, the plasma acylcarnitine concentrations, and the ratio of short-chain acylcarnitine to total acid-soluble carnitine had normalized by 30 min of recovery. In urine after 60 min of recovery from high-intensity exercise, the ratio of short-chain acylcarnitine concentration to total acid-soluble carnitine concentration was increased compared with the value at the end of exercise (Table IV). Thus, recovery from high-intensity exercise was associated with alterations in urine carnitine metabolism in humans as suggested by Suzuki et al. (18).

Studies of the arterial–venous differences across exercising muscle in humans have demonstrated that exercise-associated changes in plasma free carnitine and acylcarnitine concentrations could not be accounted for by simple fluxes across the exercising muscle (35). The present studies confirm that plasma poorly reflects exercise-induced changes in muscle metabolism. In animals, alterations in hepatic metabolism can also result in changes in plasma acylcarnitine concentrations (15). Most commonly, enhanced ketogenesis is associated with an elevated plasma short-chain acylcarnitine concentration in humans and animals (15, 36). Plasma  $\beta$ -hydroxybutyrate concentrations provide a crude assessment of the rate of hepatic ketogenesis, and no change in plasma  $\beta$ -hydroxybutyrate concentrations were associated with exercise-induced elevations in plasma acylcarnitine concentrations (Table II). This does not rule out an hepatic source for the increased plasma acylcarnitines with high-intensity exercise, but suggest that it is not a simple reflection of increased rates of ketogenesis.

Carnitine has been suggested as a therapeutic agent for patients with ischemic muscle diseases, including angina and peripheral arterial disease, to clinically improve muscle function and exercise performance (21, 22). The mechanism by which carnitine might improve muscle performance is unclear. The current studies demonstrate that free carnitine levels can fall dramatically with exercise as acylcarnitines are generated. Exercise has been shown to increase plasma acylcarnitine

concentration in patients with peripheral arterial disease (19), and the muscle content of acylcarnitines in patients with chronic renal disease (20). These observations suggest that in animals and possibly in humans, carnitine supplementation may replenish muscle free carnitine content as carnitine is utilized for the formation of acylcarnitines (37). Further studies are in progress to extend the current observations of exercise-induced changes in muscle carnitine metabolism to patients with ischemic diseases. These data are critical in the development of an understanding of the possible use of carnitine as a drug in these settings.

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