INCREASED ACETYL CARNITINE IN RAT SKELETAL MUSCLE AS A RESULT OF HIGH-INTENSITY SHORT-DURATION EXERCISE

Implications in the control of pyruvate dehydrogenase activity

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1. Introduction

Several functions have been proposed for carnitine (β-hydroxy-γ-trimethylammonium butyrate) such as:

(i) The oxidation of fatty acids in tissues by serving as a carrier of 'activated' fatty acids across the mitochondrial inner membrane to the site of β-oxidation [1];

(ii) A 'buffer' for acetyl-coenzyme A in spermatozoa by forming acetyl carnitine and coenzyme A, via acetyl-CoA:carnitine *O*-acetyl-transferase (EC 2.3.1.7) (CAT) [2];

(iii) A factor in branched chain amino acid oxidation in skeletal muscle for removal as branched chain acyl-CoA esters out of mitochondria to other tissues for further oxidation [3].

The high activity of carnitine transacetylase in mitochondria from various tissues is highly correlated with the necessity of carnitine esters for fatty acid oxidation [4]. This enzyme is absent in both the bee and fly species which utilize only carbohydrates and highly active in the locust which utilizes fatty acids during flight [5,6]. However, in the blowfly flight muscle, which is rich in carnitine but deficient in fatty acid oxidase, carnitine is used in the metabolism of pyruvate especially during transition from rest to rapid contraction [7].

Exercise increases pyruvate generation by stimulating glucose transport into the mammalian muscle cell as well as by enhancing glycogenolysis and glycolysis, however, this does not by itself account for the large increase in pyruvate oxidation [8]. Many physiological modulators of pyruvate dehydrogenase (EC 1.2.4.1) (PDH) which affect the transition from PDH_b (inactive) to PDH_a (active) and which may affect the activity of PDH_a per se have been elucidated [9–15]. However the role of CAT and changes in acetyl carnitine/acetyl-CoA ratios and their possible function in control of pyruvate dehydrogenase in exercising skeletal muscle has not been postulated.

Here we demonstrate that high-intensity short-duration exercise in rats significantly increases the proportion of acetyl carnitine relative to total acyl carnitines while significantly lowering acetyl-CoA. These data indicate that in the transition from rest to rapid contraction the mammalian skeletal muscle utilizes carnitine to activate PDH via CAT by decreasing the ratio of acetyl-CoA to CoA.

2. Materials and methods

Rats (Sprague-Dawley strain, 140–160 g, males) were fed Purina rat chow, ad libitum, for several days prior to the experiment. Rats (untrained) were assigned randomly to two experimental groups:

(i) Control or resting – which were decapitated immediately upon removal from the cage, and

(ii) High-intensity short-duration exercise – which were decapitated immediately following 5 min sustained swimming.

Blood was collected and tissues immediately removed and pulverized between plates pre-cooled with liquid nitrogen. Samples of tissues were stored

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Table 1
The effect of high-intensity short-duration exercise on carnitine and acetyl-CoA concentrations in the rat

	Carnitine													
	Blood plasma		Heart		Liver		Kidney		Hind limb skeletal muscle					
	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Acyl	Acetyl	Acetyl-CoA	Acetyl carnitine / Acetyl-CoA
	(nmol/ml)				(nmol/g)						(nmol/g)			
Resting														
(6)	57	62	755	466	183	135	386	286	540	367	173	71	29.5	2.65
SD	4	4	92	120	46	35	70	41	52	87	107	21	7.6	1.22
Swimming														
(7)	54	53	739	427	193	130	376	283	510	306	204	159	15.8	15.62
SD	7	5	174	131	37	39	64	67	33	70	62	23	9.2	11.20
$P <$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.001	0.01	0.02

Numbers in parentheses denote number of animals

in liquid nitrogen until assayed. All extracts were prepared as in [16]. Free and total carnitine [17], acetyl carnitine and acetyl-CoA [18] were assayed. All chemicals and enzymes were of the highest purity commercially available.

3. Results and discussion

The effect of high-intensity short-duration exercise by the rat on carnitine and acetyl-CoA is shown in table 1. There were no significant ($P > 0.05$) changes in total or free carnitine concentrations in blood plasma, heart, liver, kidney, and hind limb skeletal muscle of rats which were exercised for 5 min when compared to non-exercised controls. Acyl carnitine concentrations were not significantly affected by exercise, however, the acetyl carnitine fraction of the acyl carnitines increased 2-fold ($P < 0.001$) in hind limb skeletal muscle upon completion of 5 min high-intensity short-duration exercise with a significant ($P < 0.01$) decline in acetyl-CoA concentration. The acetyl carnitine/acetyl-CoA ratio increases from 2.6 at rest to 15.6 after 5 min high-intensity short-duration swimming. Increases in the concentration of acetyl carnitine in electrically stimulated contracting frog [19] and rat [20] hind limb skeletal muscle have been reported.

A decrease in the ATP/ADP ratio in perfused exercising rat skeletal muscle has been reported [13], however, data from liver [21,22] suggest that this decrease cannot account for the increased activity of pyruvate dehydrogenase. Unless changes within the muscle mitochondria are not reflected by total muscle tissue metabolite concentrations (pyruvate, lactate, citrate, ATP/ADP, creatine phosphate), these effectors may not be important physiological modulators of PDH_a during exercise [13]. During muscular contraction there is an increase in reduced cytoplasmic pyridine nucleotides which reflects the mitochondrial redox state [13] as has been observed in liver [23]. In [13] an initial 11-fold increase in [lactate] at 2 min exercise was observed (in vivo perfusion) with a subsequent decline to only a 4-fold increase above resting concentrations after 15 min exercise. Our data from swimming rats demonstrating increased acetyl carnitine and decreased acetyl-CoA would suggest that this observation [13] could be accounted for by an increased flux of pyruvate through the citric acid cycle.

Long-chain acyl carnitine derivatives (palmitoyl, octanoyl) at μM levels have been shown to cause a significant reduction in pyruvate oxidation in rabbit heart mitochondria whereas it required mM levels of acetyl carnitine to elicit a slight inhibition [24]. Acetyl-CoA/CoASH ratios were elevated 8-fold with acetyl carnitine (4 mM), 2-fold with octanoyl carnitine (0.4 mM), and unchanged with palmitoyl carnitine (0.4 mM) as substrates whereas NADH/NAD^+ ratios were increased 8-fold, >4-fold, and >2-fold, respectively. Calculation of [NADH/NAD^+]/[acetyl-CoA/CoASH] couples using the data in [24] yields the following: no substrate, 5.5; acetyl carnitine, 5.2; octanoyl carnitine, 9.4; palmitoyl carnitine, 15.5. Thus the failure of acetyl carnitine to elicit more than a slight inhibition of PDH would be expected. Long-chain acyl-CoA has also been reported to inhibit CAT [25].

The ability of CAT to reduce the [acetyl-CoA] by forming acetyl carnitine and free CoA would lower the acetyl-CoA/CoASH ratio. Furthermore, the inhibition of α-ketoglutarate oxidation in the presence of pyruvate is relieved by carnitine [26]. These sequential events would favor the conversion of PDH_b into PDH_a resulting in accelerated pyruvate oxidation and ATP production for muscular contraction [7] during high-intensity short-duration exercise. It was proposed [27] that carnitine could act as an acceptor for acetyl-CoA generated by the pyruvate dehydrogenase complex in rat and human skeletal muscle mitochondria. However, the relative success of carnitine in this role would ultimately depend upon the activity of CAT. The possible physiological and/or hormonal regulation of CAT, a key enzyme postulated to control pyruvate dehydrogenase activity indirectly, remains to be elucidated.

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