Effect of L-carnitine and stimulated lipolysis on muscle substrates in the exercising rat

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Summary. To test the effect of L-carnitine on glycogen sparing when fat oxidation is increased, 100 mg/kg/d were given to rats orally for 3 days, resulting in 1.8-fold higher muscle carnitine levels. Even when FFA were raised by heparin-stimulated lipolysis, the rate of glycogen degradation was not reduced during exercise.
Key words. Rat; carnitine; lipolysis; exercise; glycogen; triglycerides; muscle.

Exercise stimulates the utilization of glycogen and triglycerides stored as local reserves in muscle [1, 2]. The rate of utilization is modified by the availability of alternative blood-borne substrates, notably adipose tissue FA (fatty acids)[3], whose release into the blood depends on the intensity and duration of the exercise. During moderately heavy or strenuous exercise, not all of the FFA taken up by muscle is oxidized immediately[4]. It is of interest that after depletion of muscle glycogen, a high exercise intensity can no longer be maintained in spite of a seemingly adequate supply of lipid substrates. It is possible that intracellular regulators within the muscle control the maximal rate of lipid oxidation during exercise, but these remain to be identified. Carnitine is the essential carrier for activated long chain fatty acids across the mitochondrial membrane[5, 6]. In addition, as an acceptor of acyl groups from acylCoA, it increases the availability of free CoA, thereby promoting the flux of substrates through the tricarboxylic acid cycle[7]. Muscle tissue is unable to synthesize carnitine and therefore depends on an exogenous supply. In humans, muscle carnitine has been reported to be depressed by exercise[8].

The present work investigates the potential of carnitine supplements to alter the depletion of muscle substrates in the exercising rat, when FFA have been artificially raised several-fold in plasma.

Material and methods

Male Sprague-Dawley rats, fed a semi-purified diet[9] were trained to run on a motor-driven treadmill (Quinton) for 3 weeks. They were attributed to 4 treatment groups. On the last day (mean weight 288 g), half the rats of each group were killed as resting controls at time H. The other half underwent forced exercise for 30 min (20 m/min), starting from H. The 4 groups were characterized as follows. CARN rats were given 100 mg/kg/day of L-carnitine p.o. for the last 3 days, including one dose at time H − 1. This treatment was designed to saturate tissue carnitine levels. OIL rats were tube-fed with 1 ml maize oil at H − 3 h, then injected with heparin (200 I.U.) at H − 20 min; this procedure is known to increase the concentration of circulating FFA several-fold[10]. CARN + OIL rats were treated additively as described. CONT rats were untreated. At sacrifice, blood glucose, FFA, glycerol and free carnitine[11], liver glycogen, muscle (soleus and deep, red quadriceps) glycogen, triglycerides, total carnitine and free carnitine (FC) were determined as described elsewhere[9]. Acylcarnitine (AC) was taken as the difference between total carnitine and FC. The data were evaluated using one-way analysis of variance and Duncan's multiple range test. $p > 0.05$ = NS.

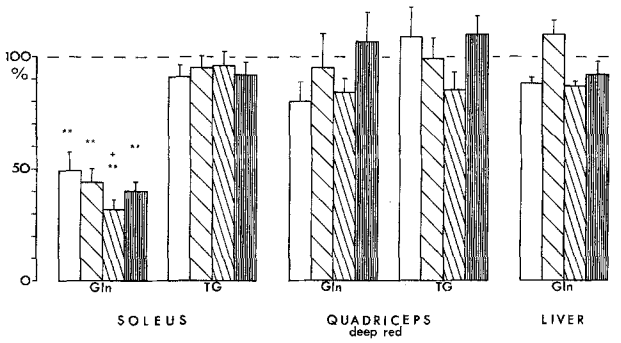
Results and discussion

Treatment with a bolus of oil and heparin raised plasma FFA concentrations 4–8-fold prior to exercise (table). The concentrations were still twice as high at the end of the exercise (not shown), when endogenous lipolysis secondary to exercise had begun to develop in the controls. Therefore, the availability of circulating lipid substrates was markedly improved. Feeding carnitine for 3 days resulted in a 5-fold rise of plasma carnitine levels at the onset of exercise. Free carnitine in the soleus increased from 290 ± 28 (SEM) to 524 ± 37 nmol/g wet tissue ($p < 0.01$), but acylcarnitine was unchanged (from 297 ± 32 to 327 ± 27, NS), as was seen previously after a longer supplementation protocol[9]. In contrast, carnitine status in the quadriceps was unaffected (FC from 686 ± 31 to 781 ± 47, NS; AC from 171 ± 25 to 141 ± 54 nmol/g, NS).

The exercise load was chosen in such a way that muscle glycogen would not become exhausted but would be in the phase of steep decline, which is thought to be more

Plasma metabolites		CONT	CARN (8)	OIL	CARN + OIL	Pooled SD
Free L-carnitine	μmol/l	25	142**	23	127(8)**	33
Free fatty acids	mmol/l	0.24	0.37	1.90**	1.67**	0.64
Glycerol	mmol/l	0.07	0.09	0.32**	0.28**	0.08
Triglycerides	mmol/l	2.65	1.82**	0.78**	0.70**	0.64
Glucose	mmol/l	8.5	8.7	8.8	8.9	0.6

Effect of oral administration of L-carnitine and/or vegetable oil on the concentration of plasma metabolites prior to exercise (see figure for details of treatment). Means of n = 9 (8 for CARN). **: $p < 0.01$ for the difference with CONT.



Effect of pre-exercise treatments on post-exercise (30-min duration) concentrations of substrates in rat tissues. ☐ control (exercised) = CONT; ▨ maize oil (1 ml p.o.) + heparin (200 I.U. i.p.) = OIL; ▨ L-carnitine (100 mg/kg/day × 3 days, p.o.) = CARN; ▨ combination of OIL and CARN treatments = CARN + OIL. Gln = glycogen, TG = triglycerides. Mean values (+ SEM) are expressed as percent of resting concentration per wet weight; n = 8 or 9. **: $p < 0.01$ for the difference with pre-exercise values; +: $p < 0.05$ for the difference with CONT.

sensitive to differences in treatment. More than half the glycogen content of the soleus muscle (intermediate oxidative) was used up during exercise (fig.). The rate of utilization of soleus glycogen was slightly *faster* in CARN-treated than in CONT animals ($p < 0.05$); we have no explanation of this observation. Glycogen in the quadriceps (high oxidative) was not reduced significantly, and muscle triglycerides also did not change.

A slowing of the rate of muscle glycogen utilization during exercise is potentially beneficial for endurance performance[12]. In rats, this effect has been reported in the presence of elevated FFA by some[10,13], but not all[14] authors. It is thought to be mediated through an inhibition of glycolysis by lipid oxidation products[15]. There are at least 6 steps required for the transport of FFA from the adipocyte to the site in skeletal muscle where they are ultimately oxidized[16]:

1) release from the fat cell; 2) transport via the blood; 3) transport from the capillary across the interstitial space to the muscle cell; 4) transport into the cytoplasm; 5) transfer to the mitochondria, and 6) β-oxidation by the mitochondria.

In the present study neither carnitine alone[9] nor an improved carnitine status, nor both conditions combined exerted any glycogen sparing effect. In addition, there appeared to be no net consumption of intramuscular triglycerides. Energy expenditure being essentially unchanged at equal levels of exercise, our results indicate that circulating FFA were not used to a greater extent after carnitine and/or lipolysis treatment than with no treatment. Therefore, neither step 1, 2 or 5 above would limit fat oxidation in our condition.

Some of the physiological adaptations which are brought about by endurance training, such as development of the capillary bed[17], and preferential proliferation of subsarcolemmal mitochondria located close to the capillary[18], are directed towards reducing diffusion distance, hence facilitating the access of FFA and oxygen to the cell. In fact, it has recently been proposed that FFA diffusion from the blood across the interstitial space to the muscle cell might constitute a major limitation to their utilization in exercise[16]. Our results are compatible with this hypothesis.

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