Intramuscular Triglyceride Utilization by Red, White, and Intermediate Skeletal Muscle and Heart During Exhausting Exercise[1] (37081)

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(Introduced by R. E. Shank)

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Oxidation of fat accounts for a large percentage of the energy expended during prolonged exercise (1, 2). Studies of palmitate-^{14}C turnover have shown that only approximately 50% of the fat oxidized during exercise comes from plasma free fatty acids (FFA) (1, 3). This finding led Issekutz and Paul (3) to hypothesize that intramuscular triglyceride stores must supply the remainder of the fat that is oxidized. Direct evidence supporting this hypothesis was recently provided by three studies in which a significant reduction in the concentration of triglycerides in skeletal muscle occurred during exercise (4–6).

In the present study we evaluated the effects of exhausting exercise on triglyceride stores in red, white and intermediate types of skeletal muscle, as well as in the heart, in the context of changes in the concentrations of muscle and liver glycogen and of blood-borne substrates. A major purpose was to obtain information regarding the importance, relative to glycogen, of intramuscular triglycerides as an energy source for the various muscle types during prolonged submaximal exercise to the point of exhaustion.

Materials and Methods. Male rats of a Wistar strain (specific pathogen-free CFN rats, Carworth Farms), weighing 231 ± 4 g, and maintained on a diet of Purina chow and water *ad libitum*, were divided into 2 groups. One group was exhausted by means of swimming and the other was kept sedentary. The swimmers were placed in steel barrels filled to a depth of 44 cm with water maintained at a temperature between 33 and 35°. After 15 min of swimming without a weight, the work load was increased by attaching a weight equivalent to 1% of body weight to each rat tail. Exhaustion was defined as the point at which the animal would no longer right itself when taken out of the water and placed on its back. The exhausted animals and their resting controls were anesthetized with pentobarbital. The quadriceps, and soleus muscles, the liver and the heart were taken. The soleus muscle, in which approximately 96% of the fibers are intermediate and 4% are red (7), was used for studies on the effects of exhausting exercise on triglyceride and glycogen stores in intermediate muscle. The superficial portion of the quadriceps, which consists entirely of white fibers (7), was used for studies of white muscle, while the deepest layer of the quadriceps, which contains approximately 70% red and 30% intermediate fibers (7), was used for studies on red muscle. All tissue samples were frozen with Wollenberger tongs cooled in liquid N_2 (8). A blood sample was drawn from the abdominal aorta. Glycogen was determined by the method of Hassid and Abraham (9). Muscle lipids were extracted as described by Entenman (10). Triglycerides were measured by the method of Kaplan and Lee (11). Plasma FFA were determined using the cobalt method of Novak (12).

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TABLE I. Glycogen Concentrations.[a]

Group	Muscle			Heart
	Red	White	Intermediate	
	Glycogen (mg/g wet wt)			
Control	6.25 ± 0.30 (6)	8.09 ± 0.42 (6)	5.44 ± 0.44 (6)	4.39 ± 0.32 (6)
Exhausted	1.77 ± 0.28[b] (5)	2.55 ± 0.16[b] (5)	1.33 ± 0.17[b] (5)	2.97 ± 0.41[c] (5)

[a] Values are means ± SE; the number of animals per group is given in parentheses.
[b] Exhausted vs control: $p < .001$; [c] $p < .05$.

Blood sugar was measured as described by Slein (13).

Results. Effects of the swim to exhaustion on blood glucose and tissue glycogen levels. The exhausted rats were hypoglycemic, with a mean blood glucose level of 45 ± 2 mg/100 ml for 6 exhausted swimmers compared to a value of 124 ± 6 mg/100 ml for 6 rested controls. Underlying the hypoglycemia was an almost complete depletion of liver glycogen stores, as evidenced by a liver glycogen concentration of 2.0 ± 0.4 mg/g wet wt in 6 exhausted rats compared to 40.9 ± 2.6 mg/g for 6 rested animals. As shown in Table I, glycogen stores were reduced approximately 70% in all 3 types of skeletal muscle fiber in leg muscles of the exhausted swimmers. A smaller (32%), but significant, reduction in glycogen concentration also occurred in the hearts of the rats that swam to exhaustion (Table I).

Effects of the swim to exhaustion on muscle and blood triglyceride levels. As shown in Table II, there was a highly significant reduction, amounting to approximately 70%, in the triglyceride content of red skeletal muscle in response to the swim to exhaustion. A much smaller, but still significant, decrease in triglyceride stores occurred in the intermediate (soleus) muscle fibers, while the concentration of triglycerides in white muscle was unaffected (Table II). Cardiac triglyceride stores showed a 11% depletion that was not statistically significant (Table II). No change took place in liver triglyceride concentration.

The results are expressed per gram wet weight of tissue. The water content of the tissues from the two groups was similar. For example, the water content of red muscle averaged 75.2 ± 0.2% in 5 control rats compared to 76.9 ± 0.4% for 5 exhausted animals, while the water content of the liver in 5 rested controls averaged 71.9 ± 1.6% compared to 73.0 ± 1.4% for 5 exhausted animals. As a result the interpretation of the findings is the same when triglyceride and glycogen concentrations are expressed per gram of dry muscle or liver.

A significant, though small, reduction occurred in the plasma triglycerides of the exhausted swimmers, which had an average value of 0.64 ± 0.05 μmoles/ml in 6 animals compared to 0.81 ± 0.05 μmoles/ml in 6 rested animals ($p < 0.05$).

Plasma FFA were significantly elevated in the exhausted swimmers averaging 0.75 μmoles/ml in 6 animals compared to a value of 0.42 μmoles in 6 resting controls ($p < 0.001$).

Discussion. In some experimental situations involving prolonged exercise, particularly when a relatively small proportion of the total muscle mass is involved, the onset of exhaustion correlates well with, and appears to be caused by, the essentially complete depletion of muscle glycogen stores (14). In the present study, in which a large proportion of the animal's muscle mass was involved in the exercise, considerable glycogen (30%) remained in the working muscle. However, liver glycogen stores were essentially completely depleted, resulting in a marked hypoglycemia which may have been the main factor responsible for their exhaustion.

Skeletal muscle in rodents, and a variety of other mammalian species, consists of 3 distinct fiber types. These are the white (low

TABLE II. Triglyceride Concentrations.[a]

Group	Muscle			Heart
	Red	White	Intermediate	
	Triglycerides (μmoles/g wet wt)			
Control	2.31 ± 0.21	1.52 ± 0.12	1.95 ± 0.13	1.79 ± 0.17
Exhausted	0.73 ± 0.06[b]	1.48 ± 0.15	1.46 ± 0.12[c]	1.59 ± 0.11

[a] Values are means ± SE; each value is the mean for 5 animals.
[b] Exhausted vs control: $p < .001$; [c] $p < .05$.

oxidative, high glycolytic, fast twitch), the red (high oxidative, high glycolytic, fast twitch), and the intermediate (intermediate oxidative, low glycolytic, slow twitch) (7, 15). When skeletal muscle is subjected to prolonged intermittent electrical stimulation through the nerve, glycogen stores are depleted much more rapidly in white than in red muscle fibers (16, 17). Intermediate muscle fibers, which have a low glycolytic capacity, undergo glycogen depletion even more slowly than do the red fibers (16, 17). If this information obtained on muscle stimulated to contract *in situ* is applicable to normal exercise, then our finding that glycogen was depleted to roughly the same extent in soleus as in red muscle suggests that the intermediate fibers in the soleus contracted at least as frequently as the red fibers in the quadriceps during the exercise bout. In this context it is rather surprising that the soleus, which is thought to derive its energy primarily from aerobic metabolism, underwent only about one-third as great a depletion of its triglyceride stores as did red muscle. A possible explanation for this finding might be that triglyceride lipase activity is perhaps lower in intermediate than in red fibers during exercise, as a result of either a smaller percentage conversion of inactive to active lipase or a lower concentration of enzyme protein. This possibility remains to be investigated.

The present results demonstrate that, of the three types of skeletal muscle, only the red fibers utilize a large portion (70%) of their intracellular triglyceride store during exhausting exercise. The decrease in triglyceride stores in red muscle was equivalent to roughly 1.3 mg of fatty acids/g of muscle which, if oxidized, would provide approximately 12 calories/g. The concomitant decrease in glycogen in red muscle was 4.5 mg/g muscle, which is equivalent to approximately 18 cal if completely oxidized. Thus intramuscular triglycerides apparently provided approximately two-thirds as much energy as did glycogen in red muscle during prolonged exercise to exhaustion. In contrast, white muscle with a low capacity for fat oxidation, did not appear to utilize its intramuscular triglycerides at all. Heart muscle, which has a high capacity for fatty acid oxidation, underwent a very small, statistically not significant, reduction in its endogenous triglyceride stores. This finding, together with the relatively small decrease in cardiac glycogen is in keeping with the interpretation that the heart's energy needs during prolonged exercise are supplied primarily by blood-borne substrates.

Summary. A bout of swimming to exhaustion resulted in an approximately 70% depletion of triglyceride stores in the red portion of the quadriceps muscle in rats. In contrast intramuscular triglycerides were unchanged in the white portion of quadriceps, and decreased only 25% in soleus muscle which is made up predominantly of intermediate fibers. Cardiac triglycerides underwent a small (11%) and statistically not significant reduction. Glycogen stores were reduced approximately 70% in all 3 types of skeletal muscle, and 32% in the heart. Liver glycogen was almost completely depleted, resulting in hypoglycemia. Assuming complete oxidation of the glycogen and triglycerides that disappeared from red muscle, triglycerides supplied roughly two-thirds as much energy to the working red fibers as did glycogen.

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