Exercise-Induced Adaptive Increase in Rate of Oxidation of β-Hydroxybutyrate by Skeletal Muscle[1] (37406)

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Blood ketone levels increase moderately during strenuous, prolonged exercise and can attain very high levels after cessation of exercise in physically untrained individuals (1–5). In contrast, individuals who have adapted to endurance exercise, such as long distance running or bicycling, maintain low levels of blood ketones during and after strenuous exercise (2–5). The metabolic basis for this interesting difference is not known. To provide information regarding one possible adaptation to endurance exercise which might help protect the trained individual against postexercise ketosis, the rates of oxidation of β-hydroxybutyrate were compared in homogenates of gastrocnemius muscles of sedentary and chronically exercised rats.

Materials and Methods. Male rats of a Wistar strain (specific pathogen-free CFN rats, Carworth Farms) weighing approximately 100 g were divided into three groups. An exercising group was trained by means of a 12-wk long program of treadmill running, described previously (6), at the end of which the rats were running continuously for 2 hr daily, 5 days/wk. The exercising group was provided with food *ad libitum*. A freely eating sedentary group was also provided with food *ad libitum*. A paired-weight sedentary group had their food intake restricted so as to maintain their body weights the same as those of the exercising animals. All the animals were maintained on a diet of Purina chow and water.

Animals were killed by decapitation 24 hr following the last exercise period. Gastrocnemius muscles were homogenized (1:10) in 175 m*M* KCl containing 10 m*M* glutathione and 2 m*M* EDTA, pH 7.4. The rate of oxidation of D-β-hydroxybutyrate-3-^{14}C (New England Nuclear) by whole homogenates of gastrocnemius muscle was assessed by measuring the rate of $^{14}CO_2$ production in a reaction mixture which contained in a final volume of 2 ml: 5 m*M* $MgCl_2$; 107.5 m*M* KCl; 20 m*M* potassium phosphate buffer; 0.078 m*M* cytochrome *c*; 2 m*M* EDTA; 2 m*M* ADP; 5 m*M* glutathione; 10 m*M* Tris Cl; homogenate equivalent to 100 mg of muscle; and either 0.2 m*M* or 1.0 m*M* D,L-β-hydroxybutyrate; pH 7.4. In addition, each flask contained 0.05 μCi D-β-hydroxybutyrate-3-^{14}C. Reaction mixtures were placed in 25 ml flasks equipped with serum caps and hanging center wells, in a shaking Dubnoff incubator at 30°. The reaction was started by addition of the β-hydroxybutyrate and ADP. After 20 min the reaction was stopped by addition of 0.5 ml of 60% citric acid. The $^{14}CO_2$ was trapped in 0.3 ml of 1 *M* Hyamine hydroxide placed in the hanging center well. Thirty minutes after the addition of citric acid, the center wells were transferred to vials containing 5 ml of Insta-Gel (Packard Instrument Co.) for determination of radioactivity in a Nuclear Chicago liquid scintillation counter.

Citrate synthase activity was measured as described by Srere (7) with the use of 5,5′-

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dithiobis-(2-nitrobenzoic acid).

Results. The rates of β-hydroxybutyrate oxidation and the levels of citrate synthase activity were the same in the paired-weight and the freely eating sedentary animals. The results on rats from these two groups have, therefore, been combined.

It has been shown that the levels of activity of a number of the enzymes of the respiratory chain and citric acid cycle, including citrate synthase, are increased in skeletal muscles of animals that have adapted to endurance exercise (8). Citrate synthase activity was used as an indicator of the magnitude of this effect of exercise-training in the present group of animals. Citrate synthase activity averaged 35.6 ± 3.2 μmoles/min/g wet wt in the gastrocnemius muscles of 7 runners, compared to a value of 19.3 ± 1.1 μmoles/min/g for 7 sedentary controls ($p < 0.001$). This approximately 2-fold increase in citrate synthase in the muscles of the runners, provides evidence for a highly significant training effect.

As shown in Table I, a 2- to 3-fold increase in the rate of D-β-hydroxybutyrate oxidation by gastrocnemius muscle homogenates, under conditions of uncontrolled respiration (*i.e.*, in the presence of nonlimiting amounts of ADP and P_i), is another component of the adaptive response of skeletal muscle to endurance exercise. This highly statistically significant increase in D-β-hydroxybutyrate oxidation was evident at both of the physiological concentrations of β-hydroxybutyrate studied.

TABLE I. Effects of the Exercise Program on the Rate of Oxidation of D-β-Hydroxybutyrate-3-^{14}C[a] in Whole Homogenates of Gastrocnemius Muscle.

D,L-β-Hydroxybutyrate concn (m*M*)	Group	D-β-Hydroxybutyrate-3-^{14}C oxidation (nmoles/g/min)
0.2[b]	Sedentary	6 ± 2
	Runners	18 ± 2[d]
1.0[c]	Sedentary	21 ± 3
	Runners	43 ± 4[d]

[a] Expressed as nanomoles of D-β-hydroxybutyrate oxidized per gram of fresh muscle per min. Values are means ± SEM for 7 animals.

[b] Equivalent to 0.1 m*M* D-β-hydroxybutyrate which is the utilizable natural isomer.

[c] Equivalent to 0.5 m*M* D-β-hydroxybutyrate.

[d] Runner versus sedentary, $p < 0.001$.

Discussion. It is well established that ketones can serve as an important fuel in resting muscle (9–11). The role of ketones as an energy source for skeletal muscle during work is less clear. On the one hand, Hagenfeldt and Wahren (12) have reported that, under some circumstances, there may actually be a net production of ketones by the working muscles of the forearm in humans. On the other hand, Johnson and Walton (5) have shown that tolerance to a dose of acetoacetate, as reflected in blood ketone levels, was greater during exercise that at rest, suggesting that ketones are being used as fuel. Along the same lines, it has been shown that a short bout of exercise markedly lowers blood ketone levels in rats made hyperketonemic by a 48-hr fast (13).

One point that does seem clear is that there is a difference in ketone metabolism between physically trained and untrained individuals during and after exercise. Untrained individuals have been shown to undergo a progressive increase in blood ketone levels during prolonged strenuous exercise and to develop a marked postexercise ketosis (1–5). In contrast, trained individuals maintain low blood ketone levels during and after exercise (2–5). It has been suggested that this difference may be due to lower blood free fatty acid (FFA) levels in trained, compared to untrained, individuals during and after exercise, resulting in production of smaller amounts of ketones from FFA by the liver (2). Against this explanation is the considerable evidence that trained individuals usually have higher plasma FFA levels than do untrained during and after exercise (14–16). Another possibility is that utilization of ketones by the muscles during and after exercise is greater in the trained state. In favor of this explanation is the finding that, in the postexercise period, trained individuals have a greater tolerance to an administered acetoacetate load, as reflected in a smaller rise in blood ketone levels, than do untrained.

It has been shown that skeletal muscle adapts to endurance exercise with an increase in the capacity to oxidize various substrates including pyruvate and FFA (17, 18). In this context is seemed of interest to determine whether exercise training also increases the capacity of muscle to oxidize ketones, as is seemed possible that such an increase might, at least in part, explain the trained individual's resistance to exercise-induced ketosis. The new finding, described above, that homogenates of gastrocnemius muscles of physically trained rats oxidize β-hydroxybutyrate 2 to 3 times as rapidly as do those of sedentary control animals is compatible with this interpretation. It does not, of course, prove that the lower blood ketone levels seen in trained individuals are due only to an increased rate of oxidation by skeletal muscle. To clarify this point will require studies of the turnover of ^{14}C-labeled ketones in trained and untrained individuals.

Summary. Homogenates of gastrocnemius muscles from rats trained by means of a 12-wk long program of treadmill running oxidized D-β-hydroxybutyrate-3-^{14}C 2 to 3 times as rapidly as did homogenates of muscles from sedentary animals. This difference was demonstrated at D-β-hydroxybutyrate concentrations of 0.1 and 0.5 m*M*. This adaptation may help to explain why the physically trained individual has a greater resistance to ketosis than the untrained, during and after prolonged exercise.

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