Effects of Hypoxia on Oxidative Capacity of Skeletal Muscle in Trained and Untrained Rats[1] (37157)

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(Introduced by H. E. Sauberlich)

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Previous work has shown that exhaustive exercise decreased the oxidation rate of pyruvate, succinate, and palmitate by skeletal muscle mitochondria from trained rats, while the oxidative capacity of skeletal muscle of untrained rats was relatively unaffected (1). During strenuous exercise, the demand for oxygen in the muscle may be greater than can be supplied to the muscle via the cardiovascular system, and an oxygen debt is produced which is accompanied by a lower oxygen tension in the muscle. Since intracellular hypoxia may occur during exercise, a logical extension of our work on the relationships of acute exercise and energy metabolism was to study the effect of hypoxia on the oxidative capacity of the muscle. It would be beneficial to determine if low oxygen tension could possibly be the factor causing changes in enzymatic activity observed with exhaustive exercise.

Methods and Materials. Two experiments were performed with different conditions of training and length of hypoxic exposure. In the first experiment, rats were trained for 9 weeks and exposed to hypoxia for 24 hr. In the second experiment, training lasted for 12 weeks, and the hypoxic exposure was of 6-hr duration.

Experiment 1: Male Carworth rats of the CFN strain that weighed approximately 110 g at the start of the experiment were trained by treadmill[3] running for 9 weeks on the training schedule described by Holloszy (2) except that the last 3 weeks of his schedule were omitted. Both trained and untrained rats were given water and Wayne Lab Blox *ad libitum* with untrained rats allowed to remain sedentary in their cages throughout the experiment.

Six trained and 6 untrained rats were placed in a hypobaric chamber at the end of the training period, and the chamber evacuated at 500 ft/min to a simulated altitude of 25,000 ft. Air leaked through the chamber at a slow rate to replinish utilized oxygen and remove expired carbon dioxide while the temperature was maintained at 22° throughout a 24-hr exposure period. The chamber was repressurized at a rate of 500 ft/min, hypoxic rats were sacrificed immediately by decapitation, the quadriceps-muscle group removed and homogenized for isolation of mitochondria.

Hypoxic rats were allowed food and water *ad libitum* but, as previously reported by Schnackenberg *et al.* (3), hypoxia drastically reduced food consumption. To compensate for the possible effects of food restriction, trained and untrained normoxic rats were pair fed with their hypoxic counterparts and were killed on the day following sacrifice of the hypoxic rats.

Mitochondria were isolated by a modification of the method of Ernster and Nordenbrand (4) as previously described (1). Mitochondrial oxygen uptake rates were determined (30°) in either an oxygraph[4] equipped with a Clark electrode or a YSI[5] model-53

[1] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council.

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[3] Treadmill purchased from Quinton Instrument Company, 3051 44th Ave. West, Seattle, WA 98199.

[4] Gilson Medical Electronics, Middleton, WI.

oxygen monitor. The final concentrations present in the buffer system (pH 7.3) were: 150 m*M* sucrose, 15 m*M* KCl (added with 0.5–1.0 mg mitochondrial protein in 0.1 ml), 25 m*M* Tris–hydrochloride, 6 m*M* K_2HPO_4, 0.3% bovine serum albumin, 0.5 m*M* EDTA, and 0.3 m*M* ADP. The substrates were either pyruvate-malate (8 m*M* each) or succinate (10 m*M*) plus rotenone (0.15 μg/ml).

[1-^{14}C]Palmitate oxidation by 600*g* supernatants of skeletal muscle homogenates was measured by collecting and counting $^{14}CO_2$ produced during the incubation. A 2-ml incubation volume was used containing the following cofactors (pH 7.3): 2.0 m*M* ATP, 0.05 m*M* coenzyme A, 1.0 m*M* dithiothreitol, 0.1 m*M* malate, 1.0 m*M* $MgCl_2$, 0.072 m*M* bovine serum albumin, 0.1 m*M* NAD, 100 m*M* sucrose, 10 m*M* K_2HPO_4, 80 m*M* KCl, 0.1 m*M* EDTA, 1.0 m*M* DL-carnitine and 0.2 m*M* [1-^{14}C]palmitate (1 μCi). The reaction was initiated by adding 0.5 ml of the first 600*g* supernatant obtained during the mitochondrial isolation procedure. Contents of the flasks were gassed with 95% O_2, 5% CO_2, stoppered with a rubber septum stopper containing a polypropylene center cup, and incubated with gentle shaking at 37° for 30 min. Immediately prior to the termination of incubation, 0.2 ml of ethanolamine: methyl cellosolve (1:2) was injected into the hanging centerwell. The reaction was terminated by injecting 0.2 ml of 4.0 *N* H_2SO_4 into the contents of the flask. Flasks were shaken for 60 min to collect evolved $^{14}CO_2$. The centerwell and contents were transferred to a scintillation vial, 15 ml of Bray's scintillation fluid added, and samples counted for 20 min in a liquid scintillation counter with external standardization. Results are expressed as nmoles substrate converted to $^{14}CO_2$/min/g tissue.

The protein concentration of the 600*g* supernatant and the mitochondrial suspension was determined using an automated modification of the Lowry method (5).

Experiment 2: Care and treatment of the animals was the same as in Expt 1 except that the following treadmill training regimen was used: The animals were started initially at 0.8 mph for 10 min/day and increased 2–5 min each day so that by the end of 6 weeks they were performing for 2 hr at 1.1 mph 5 days per week. Beginning with the seventh week all trained rats were sprinted for 30 sec at 15 min intervals throughout the 2 hr run. The remainder of the 12-week period they were sprinted at 1.8–2.0 mph for 1 min every 20 min throughout the 2 hr run. This proved to be a very demanding schedule which caused approximately one-third of the trained individuals to be dropped from the experiment.

Five trained and 6 untrained rats were placed in the hypobaric chamber and maintained at 25,000 ft simulated altitude for 6 hr. During this period, neither normoxic nor hypoxic rats had access to food. As soon as the chamber was repressurized, both the hypoxic and the normoxic rats were sacrificed and muscle samples taken as in Expt 1.

Mitochondria were isolated as described previously, and oxygen uptake was measured as in Expt 1 except that pyruvate (15 m*M*) was used as the substrate. Palmitate oxidation by isolated mitochondria was determined by oxygen uptake measurements using the cofactors listed for [1-^{14}C]palmitate oxidation in Expt 1 with 2.0 m*M* palmitate serving as the substrate.

Results and Discussion. The results of Expt 1 are shown in Table I and Table II. The oxidation rates for pyruvate-malate, succinate, and palmitate were relatively unaffected by hypoxia in the untrained rats. However, muscle oxidative capacity was markedly re-

TABLE I. Experiment 1. The Effect of Hypoxia (25,000 ft for 24 hr) on Pyruvate-Malate and Succinate Oxidation by Skeletal Muscle Mitochondria of Trained and Untrained Rats.

	Oxygen uptake (μg atoms/hr/mg protein)	
	Normoxic (Pair fed)	Hypoxic
Pyruvate-Malate		
Untrained	9.94 ± 1.28(6)[a]	10.26 ± 1.03(5)
Trained	9.04 ± 1.06(6)	7.73 ± 1.05(6)
Succinate		
Untrained	11.49 ± 1.12(6)	11.26 ± 1.35(5)
Trained	10.34 ± .81(6)	8.55 ± 1.12(6)

[a] Mean ± SE with number of observations in parentheses.

[5] Yellow Springs Instruments Co., Yellow Springs, OH.

TABLE II. Experiment 1. The Effect of Hypoxia (25,000 ft for 24 hr) on [1-^{14}C] Palmitate Oxidation by Skeletal Muscle Homogenates of Trained and Untrained Rats.

	[1-^{14}C] Palmitate Oxidation (nmoles/min/g tissue)	
	Normoxic (Pair fed)	Hypoxic
Untrained	3.61 ± .36(6)[a]	3.87 ± .74(5)
Trained	3.77 ± .37(6)	2.83 ± .59(6)

[a] Mean, ± SE with number of observations in parentheses.

duced by hypoxia in trained animals with apparent decreases of 15%, 21%, and 33% for pyruvate-malate, succinate, and palmitate oxidation, respectively.

Table III shows the data of Expt 2 and is in agreement with the results found in Expt 1. Hypoxia did not affect oxidative capacity in skeletal muscle of untrained rats to any appreciable extent while, in trained animals, pyruvate oxidation rate demonstrated an apparent decrease of 20% and palmitate oxidation was decreased 50% ($p < 0.01$) due to hypoxia.

The variability in these experiments was slightly greater than anticipated; consequently, the number of rats utilized for these studies was insufficient to always prove significant differences between normoxic and hypoxic rats. However, two separate experiments described in this report led to the same conclusion, *i.e.*, exercise and hypoxia affect the oxidative capacity of skeletal muscle in the same manner based on these data and those published earlier (1). The oxidation rates of pyruvate, succinate, and palmitate are decreased in trained rats but are relatively unaffected in untrained animals. This would seem to lend support to the hypothesis that lowered oxygen tension produced in muscle during exercise caused the changes in oxidative capacity observed with exercise. However, an alternate explanation which appears equally feasible is that the effects of exercise and hypoxia are mediated through a common process. This would imply that intracellular hypoxia may not necessarily be a causative agent during exercise but that both hypoxia and exercise cause another process to occur which results in a diminished oxidative capacity in skeletal muscle of trained rats.

The changes in mitochondrial enzyme activity during exhaustive exercise and hypoxia may occur concomitant with, or be the result of, structural alteration of the mitochondria. Gollnick and King (6, 7) reported that exhaustive exercise caused swelling of skeletal muscle and heart mitochondria while Sulkin and Sulkin (8) found swelling in heart mitochondria of hypoxia exposed rats. This swelling may be the result of membrane phospholipid hydrolysis since added phospholipase A has been shown to reduce mitochondrial respiration and cause mitochondrial swelling (9). In addition, Waite *et al.* (10) have demonstrated that mitochondrial phospholipase A is activated by classical swelling agents such as free fatty acids, and the swelling that results is accompanied by hydrolysis of mitochondrial phospholipids.

The activation of mitochondrial phospholipase by free fatty acids seems to offer a plausible explanation for the greater effect of exhaustion and hypoxia on trained than untrained rats. Issekutz *et al.* (11) have shown that trained animals mobilize and utilize greater quantities of fatty acids during exercise than untrained animals. This suggests that the concentration of free fatty acids in the vicinity of the mitochondria may be greater in trained than untrained rats and could cause a greater activation of phospholipase A. We have no direct evidence at this time

TABLE III. Experiment 2. The Effect of Hypoxia (25,000 ft for 6 hrs) on Pyruvate and Palmitate Oxidation by Skeletal Muscle Mitochondria from Trained and Untrained Rats.

	Oxygen Uptake (μg atoms/hr/mg protein)	
	Normoxic	Hypoxic
Pyruvate		
Untrained	5.62 ± .27(15)[a]	6.16 ± .94(6)
Trained	6.16 ± .44(11)	4.92 ± .66(5)
Palmitate		
Untrained	3.68 ± .26(15)	3.31 ± .57(5)
Trained	3.60 ± .25(11)	1.78 ± .26(4)[b]

[a] Mean, ± SE with number of observations in parentheses.

[b] Hypoxic vs normoxic, $P < 0.01$.

to indicate that fatty acid mobilization is accelerated more in trained than untrained rats during hypoxic exposure. However, trained animals do have greater adipose tissue epinephrine stimulated lipase activity than untrained rats (12) and, conceivably, could respond to the stress of hypoxia by mobilizing more fatty acids than untrained rats.

The proposal that phospholipase activation by fatty acids causes mitochondrial swelling and lowering of oxidative capacity assumes that the effects of exhaustion and exercise are mediated through a common mechanism. However, from the present data we cannot exclude the possibility that cellular hypoxia during exercise causes the decrease in oxidative capacity in trained exhausted rats. Previous investigations have shown trained rats to be adapted to a more aerobic type of metabolism than untrained rats (2, 13–15). Therefore, trained rats may have a greater dependence on oxygen-utilizing reactions for the synthesis of ATP and thus be more sensitive to low oxygen tensions in the muscle than untrained rats.

Summary. Hypoxia reduces the oxidative capacity of muscle mitochondria in a manner similar to that found with exercise when palmitate, pyruvate and succinate were used as substrates. This suggests that low oxygen tension found in the muscle during exercise may cause the effects observed with exercise; or, hypoxia and exercise cause similar effects because they are mediated through a common process.

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