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Skeletal muscle metabolic enzymes are altered by hyperbaric oxygenation treatments

A. G. NELSON, E. G. WOLF, JR., P. O. BRADSHAW, C. M. HEARON, and B. LI

Department of Kinesiology, Louisiana State University, 112 Long Field House, Baton Rouge, LA 70803; and United States Air Force School of Aerospace Medicine, Brooks Air Force Base, TX 78235

Nelson AG, Wolf EG Jr, Bradshaw PO, Hearon CM, Li B. Skeletal muscle metabolic enzymes are altered by hyperbaric oxygenation treatments. Undersea & Hyperbaric Med 1993; 20(3):189–196—To test whether repeated HBO exposures would increase activity of skeletal muscle metabolic enzymes, 27 rabbits (3 groups) were exposed 90 min/day, 5 days/wk to either 100% O_2 at 243 kPa (HBO), 100% O_2 at 101 kPa (HIO), or 21% O_2 at 101 kPa (CON). Four animals per group were killed after 2 wk of treatment, and the remaining five per group were killed after 8 wk of treatment. Soleus, plantaris, and tibialis anterior muscles were removed, and the activities of adenylate kinase, α-glycerophosphate dehydrogenase, and citrate synthase were measured. After 8 wk there was no difference in enzyme activity between groups for either plantaris or tibialis anterior. In the soleus after 8 wk there was no difference between groups in adenylate kinase activity, but α-glycerophosphate dehydrogenase activity was 56% greater ($P < 0.05$) in HBO than in HIO and 50% greater than in CON, and citrate synthase activity in HBO was 24% greater ($P < 0.05$) than that in HIO and 36% greater than that in CON. Inasmuch as the soleus is a postural muscle, these results suggest that long-term HBO treatments can increase enzyme activity in an actively contracting muscle.

citrate synthase, α-glycerophosphate dehydrogenase, adenylate kinase

Hyperbaric oxygenation, the exposure to hyperoxia (i.e., 100% oxygen) at pressures greater than 1 atm, is used as a therapeutic modality for a variety of diseases. Numerous studies have documented that repeated exposures of 30–60 min over a period of several days are beneficial in restoring diseased and damaged tissues (1). In spite of the widespread use of therapeutic hyperbaric oxygenation, it is not without its side effects. One of these side effects is oxygen toxicity, which is tied with the inactivation of many different enzyme systems (2). Among those enzymes that are susceptible to high oxygen concentrations are members of both the tricarboxylic acid cycle (3) and the glycolytic pathway (4). Along with the inactivation of these two energy pathways, Chance and associates (5, 6) have shown that the concentration of NADH can be reduced with hyperbaric oxygenation. Because many of the enzymes in the above metabolic pathways are involved with the reduction of NAD to form

NADH, lower concentrations of NADH further suggest that exposure to an HBO environment could limit the activity of the metabolic pathways.

This inactivation of metabolic enzyme activity could be likened to the condition seen during an intense bout of acute exercise, during which an inability of the metabolic system to maintain a balance between energy production and energy demand can occur, even though the metabolic enzymes may be working at maximum capacity. This mismatch between demand and production could be interpreted as a functional inactivation of metabolic systems. If, however, acute bouts of exercise are repeated over a period of time, increases in the activity of marker enzymes of both the tricarboxylic acid cycle and the glycolytic pathways have been reported (7). This suggested that repeated intermittent exposures to hyperbaric oxygenation might also induce increases in the activity of marker enzymes of the above metabolic pathways. Unfortunately, very little information is available concerning the effects of exposure to HBO on enzyme activity, and the information available does not address the effects of repeated intermittent hyperbaria. Das and Hems (8) reported that 3 wk of continuous exposure to HBO produced increases in glycogen phosphorylase activity in the liver and skeletal muscle of lean mice. Also, Jain (1) recounts an experiment by Belkokurov (9) in which rats with intestinal obstruction were able to reestablish the normal activities of succinate dehydrogenase and cytochrome oxidase in liver and kidney tissue only when corrective surgery was coupled with HBO treatments (nature and length of treatments not specified). In synthesizing the above information, we hypothesized that repeated intermittent exposures to HBO would affect the activity of marker enzymes of skeletal muscle metabolism similar to that of repeated bouts of acute exercise, i.e., enzyme activity will increase. To evaluate this hypothesis, we exposed rabbits to HBO treatment. After either 10 or 40 days of exposures we measured the activity of three enzymes commonly measured in exercise studies as markers of oxidative and glycolytic processes: citrate synthase, α-glycerophosphate dehydrogenase, and adenylate kinase. These enzymes were measured in three separate hindlimb muscles, one slow twitch and two fast twitch. We found that hyperbaric oxygenation can induce increases in enzyme activities, but these increases seem to be specific to the slow-twitch muscle.

MATERIALS AND METHODS

Animals

All work on animals in this study was performed after the proper authorization and approval was obtained from Brooks AFB animal care committee, and the housing and care of the animals were according to USAF Regulation 169-2, HSD supplement 1. Twenty-seven New Zealand white rabbits were used in this study. Their median weight at the start and end of the study was 2.5 kg. The rabbits were randomly assigned to one of three groups of nine rabbits each.

Hyperbaric oxygenation protocol

Each group of nine rabbits was randomly assigned to one of three treatments: hyperbaric oxygenation (HBO), hyperoxia (HIO), and control (CON). The HBO

protocol simulated the human treatment regimen currently used at the Davis Hyperbaric Medicine Laboratory at Brooks AFB. Specifically, the treatment consisted of 90 min of breathing 100% oxygen at a pressure of 243 kPa (2.4 atm abs). The descent to 243 kPa took 5 min, and the ascent to normobaria after the treatment lasted 15 min. During both the descent and ascent the animals also breathed 100% oxygen. Thus, the animals were in the hyperbaric chamber and exposed to 100% oxygen a total of 110 min each treatment period. Temperature within the hyperbaric chamber was maintained near 22°C by venting a continuous flow of 100% O_2 through the chamber. This venting procedure also prevented the build-up of expired CO_2. The venting flow rate was set at a flow that ensured that the oxygen content of the vented gases, as determined by a polarographic oxygen analyzer, was greater than 95%. All animals were exposed simultaneously by placing them in a rack that contained 10, 16 × 19 × 26-cm compartments. Group 2 (HIO) received treatment similar to the HBO group, except that their exposure was 110 min of 100% O_2 at 101 kPa (1 atm abs). Group 3 (CON) served as the control and was exposed to normobaric atmospheric air (101 kPa, 21% O_2, 0.03% CO_2, 79% N_2). All animals were treated daily, 5 days/wk, with the exposures for each group always performed at the same time of day.

Tissue harvest

Four animals from each group were euthanized after 2 wk of treatments (10 total treatments). The remaining five animals in each group were euthanized after 8 wk of treatments (40 total treatments). Euthanasia and subsequent muscle harvest occurred 12–16 h after the last treatment session (i.e., harvesting began in the morning of the day immediately following the last day of treatment). The animals were given a lethal dose of sodium pentobarbital injected intravenously via an ear vein; the left hindlimb was exposed; and the soleus, plantaris, and tibialis anterior muscles quickly removed. After a muscle was removed, it was weighed and quick frozen in Freon cooled to −80°C. The frozen muscles were then wrapped in foil and stored at −80°C until analyzed.

Tissue analysis

Each muscle was analyzed for three different enzymatic activities. The enzymes measured were citrate synthase, α-glycerophosphate dehydrogenase, and adenylate kinase.

Citrate synthase activity was measured on either 5% (soleus) or 10% (plantaris, tibialis anterior) homogenates following the procedure of Srere (10). The muscle samples were homogenized with a high-speed, stainless steel tissue homogenizer. The homogenizing buffer (pH 7.6) consisted of 175 m*M* KCl, 10 m*M* glutathione (GSH), and 2 m*M* ethylenediamine tetraacetic acid (EDTA). The resulting tissue homogenates were then subjected to a cycle of three to four freeze–thaw cycles to facilitate the breakdown of the mitochondria. The homogenates were diluted 1:50 (vol/vol) with 10 m*M* tris(hydroxymethyl)aminomethane (tris) buffer (pH 8.0). The reaction mixture had a final concentration of 65 m*M* tris, 0.3 m*M* acetyl concanavalin A, 0.1 m*M* 5,5'-dithiobis[2-nitrobenzoic acid] (DTNB), 0.5 m*M* oxaloacetate, plus homogenate.

Cytoplasmic α-glycerophosphate dehydrogenase activity was measured on either 0.25% (plantaris, tibialis anterior) or 1% homogenates following the procedure outlined by Holloszy and Ocsai (11). The homogenizing buffer (pH 7.4) consisted of 175 m*M* KCl, 2 m*M* EDTA, and 10 m*M* tris. The reaction mixture had a final concentration of 80 m*M* tris (pH 7.5), 0.18 NADH, 3 m*M* dihydroxyacetone phosphate (Li salt), plus homogenate.

Adenylate kinase activity was measured on 1% homogenates following the procedure of Bergmeyer (12). The homogenizing buffer (pH 7.6) consisted of 100 m*M* triethanolamine (TEA), 0.5% bovine serum albumin (BSA), and 5 m*M* 2-mercaptoethanol. The reaction mixture had a final concentration of 71.4 m*M* TEA, 0.36% BSA, 3.6 m*M* 2-mercaptoethanol, 1.4 m*M* AMP, 1.2 m*M* ATP, 0.39 m*M* phospho(enol)pyruvate, 1.2 m*M* $MgSO_4$, 142 m*M* KCl, 0.2 m*M* NADH, 20 U pyruvate kinase, 55 U lactate dehydrogenase, plus homogenate.

Statistics

A two-way (treatments × time) analysis of variance was used to compare the differences between groups for each separate muscle and enzyme activity. When a significant *F*-ratio ($P < 0.5$) was found, a Student-Neuman-Kuel's test was used to establish intergroup differences.

RESULTS

Adenylate kinase

The mean activity of adenylate kinase at 2 and 8 wk for each treatment group for each of the three muscles is presented in Table 1. In each muscle there was no difference among treatment groups in adenylate kinase activity at either the 2- or 8-wk period. There was also no significant difference between the 2- and 8-wk means for each treatment.

Table 1: Adenylate Kinase Activity[a]

Treatment	HBO	HIO	CON
Plantaris			
2 wk	444 ± 80	469 ± 74	385 ± 59
8 wk	466 ± 18	478 ± 80	439 ± 67
Tibialis anterior			
2 wk	396 ± 28	471 ± 46	429 ± 16
8 wk	454 ± 58	470 ± 71	420 ± 49
Soleus			
2 wk	196 ± 13	245 ± 39	203 ± 18
8 wk	198 ± 27	214 ± 39	206 ± 16

[a]Values are expressed as mean ($\mu mol \cdot g^{-1} \cdot min^{-1}$) ± SD.

α-Glycerophosphate dehydrogenase

The mean activities for this enzyme are presented in Table 2. In the plantaris and tibialis anterior there was no difference in α-glycerophosphate dehydrogenase activity among treatments after either 2 or 8 wk of treatment. Moreover, neither of the treatments nor the control showed a significant within-treatment difference in α-glycerophosphate dehydrogenase activity between the 2- and 8-wk groups. In the soleus, however, the response to the treatments was different. The α-glycerophosphate dehydrogenase activity of the 8-wk HBO group was 30% greater ($P < 0.05$) than the α-glycerophosphate dehydrogenase activity of the 2-wk HBO group. The 8-wk CON and HIO groups on the other hand had α-glycerophosphate dehydrogenase activities similar to their 2-wk counterparts. The α-glycerophosphate dehydrogenase activity of the 8-wk HBO group was also significantly greater ($P < 0.05$) than the mean activities of both the CON and HIO 8-wk groups. The enzyme activity of HBO was 56% greater than the activity of HIO and 50% greater than the activity of CON.

Citrate synthase

The response of citrate synthase activity to the experimental treatments was similar to the response of α-glycerophosphate dehydrogenase activity (Table 3). In the plantaris and tibialis anterior there was no difference in citrate synthase activity among treatments after either 2 or 8 wk of exposure. Also, none of the treatments showed a significant within-group difference in citrate synthase activity between the 2- and 8-wk groups. In the soleus, however, response to the treatments was different. The citrate synthase activity of the 8-wk HBO group was 17% greater ($P < 0.05$) than the citrate synthase activity of the 2-wk HBO group. The 8-wk CON and HIO groups on the other hand had citrate synthase activities similar to their 2-wk counterparts. The mean citrate synthase activity of the 8-wk HBO group was also significantly greater ($P < 0.05$) than the mean activities of both the CON and HIO 8-wk groups. In this instance, enzyme activity of HBO was 24% greater than that of HIO and 36% greater than that of CON.

Table 2: α-Glycerophosphate Dehydrogenase Activity[a]

Treatment	HBO	HIO	CON
Plantaris			
2 wk	51.5 ± 5.0	62.0 ± 7.1	54.3 ± 7.3
8 wk	62.7 ± 7.6	59.5 ± 10.1	58.3 ± 11.2
Tibialis anterior			
2 wk	50.3 ± 7.8	56.2 ± 8.5	50.1 ± 9.2
8 wk	59.6 ± 3.6	56.1 ± 8.1	58.8 ± 11.3
Soleus			
2 wk	3.0 ± 0.6	3.3 ± 0.3	2.6 ± 0.5
8 wk	4.3 ± 0.8[b]	2.8 ± 0.7	3.1 ± 0.8

[a]Values are expressed as mean ($\mu mol \cdot g^{-1} \cdot min^{-1}$) ± SD. [b]Denotes a mean significantly greater ($P < 0.05$) than all other means for a specific muscle.

Table 3: Citrate Synthase Activity[a]

Treatment	HBO	HIO	CON
Plantaris			
2 wk	13.9 ± 3.2	10.8 ± 1.6	13.5 ± 3.5
8 wk	13.1 ± 0.8	14.6 ± 2.2	13.9 ± 3.2
Tibialis anterior			
2 wk	18.5 ± 2.0	24.1 ± 4.9	20.2 ± 1.7
8 wk	22.7 ± 3.0	23.8 ± 3.3	22.6 ± 4.0
Soleus			
2 wk	23.5 ± 0.7	22.2 ± 3.0	22.4 ± 3.2
8 wk	27.6 ± 0.8[b]	23.6 ± 3.5	22.1 ± 4.0

[a]Values are expressed as mean ($\mu mol \cdot g^{-1} \cdot min^{-1}$) ± SD. [b]Denotes a mean significantly greater ($P < 0.05$) than all other means for a particular muscle.

DISCUSSION

Metabolic enzyme activity in skeletal muscle is plastic and can be increased or decreased by varying conditions. For instance, Holloszy and associates (13) showed that a strenuous exercise training regimen can cause as much as a twofold increase in selected citric acid cycle enzymes. During a strenuous exercise bout there can be a reduced availability of readily available energy stores (e.g., glycogen, ATP), creating a mismatch between energy demand and energy production. Booth and Thomason (14) suggest that a deficit between energy demand and production could serve as a signal for increasing metabolic enzyme production. As stated previously, a single exposure to HBO inactivates metabolic enzymes, and this inactivation could, in turn, create a mismatch between energy demand and production similar to that seen in exercise training. From these observations, we wondered if repeated intermittent exposures to HBO would produce enzyme inactivations of sufficient cumulative magnitude to cause an increase in skeletal muscle metabolic enzyme activity. The results of our experiment suggest that repeated HBO treatments can indeed induce a modest increase in enzyme activity in selected skeletal muscles.

The exact mechanism behind the observed increases in enzyme activity (Tables 2 and 3) cannot be ascertained from this study. However, two points should be considered. First, the increases in enzyme activity were only seen in the soleus muscle. The soleus muscle in the rabbit is a slow-twitch oxidative muscle. In contrast, the plantaris and tibialis anterior are fast-twitch muscles with both glycolytic and oxidative–glycolytic muscle fibers. Also, in mammalian hindlimbs, slow-twitch muscles are mainly postural muscles whereas fast-twitch muscles are predominately locomotory muscles (15–19). Thus, the soleus differs from the other two muscles in both its biochemistry and function. Therefore, the susceptibility of the soleus to the HBO treatments probably is due to an interplay between these two factors. Second, of the three enzymes analyzed, the two enzymes that demonstrated an increase in activity, citrate synthase and α-glycerophosphate dehydrogenase, have not been identified as enzymes that are inhibited by high oxygen concentrations (2). Citrate synthase and α-glycerophosphate dehydrogenase, however, are directly linked to

multienzymatic pathways, and both are used as markers of activity of their respective metabolic pathways. Haugaard (20) has pointed out that enzyme inhibition can occur at several steps in the glycolytic and citric acid cycle pathways. Thus, both enzymes could experience intermittent inhibition indirectly through either upstream or downstream intermittent inhibition of other enzymes in the pathway. Moreover, exercise training studies (7, 13, 21) have shown that a stimulus that increases the activity of individual enzymes can influence the activity of other enzymes in the same pathway. Therefore, obtaining increases in these two enzymes actually helps strengthen the hypothesis that repeated enzyme inactivations from repeated intermittent exposure to HBO provide a stimulus to induce increases in enzyme activity.

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