Oxidative Stress Leads to Inhibition of Calcium Transport by Sarcoplasmic Reticulum in Skeletal Muscle[1] (42873)

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Abstract. **Iron administration results in the development of oxidative stress in skeletal muscles, as evidenced by increases in amounts of lipid oxidation fluorescent end products, decreases in vitamin E concentration, and inhibition of calcium transport by sarcoplasmic reticulum. Exhaustive physical loading or hyperoxia, or their combination, does not lead to apparent modification in calcium transport by sarcoplasmic reticulum in skeletal muscle homogenates. However, physical loading or hyperoxia does in fact induce oxidative stress since they magnify the effect of iron loading on the inhibition of calcium transport.** [P.S.E.B.M. 1989, Vol 190]

It is well documented that exhaustive physical loading (prolonged physical exercise) is accompanied by oxidative stress leading to a variety of effects including glutathione depletion and decrease in the amounts of antioxidants (mainly tocopherol), finally resulting in muscle membrane damage (1–4). Pronounced functional and structural changes have been described in muscle mitochondria. These are suggested to be responsible for disturbances in oxidative capacity, calcium transport, and contractility (1–3). In skeletal muscle cells, the bulk of membranes are the sarcoplasmic reticulum membranes whose main function is electromechanical coupling, i.e., the transformation of the nervous signal transmitted to the sarcolemma into a mechanical-chemical response of contractile proteins (5, 6). This function is provided for by alteration of calcium concentration in the sarcoplasm: a rise causing muscle contraction, a decrease causing muscle relaxation. Only few *in vivo* studies on sarcoplasmic reticulum damage under any conditions of oxidative stress have been performed (7, 8).

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In this study we examined calcium transport by sarcoplasmic reticulum in skeletal muscle homogenates from animals subjected to different kinds of oxidative stress, viz., iron loading, physical loading, hyperoxia, and combinations thereof.

Materials and Methods

Wistar male rats (180–200 g) were used. The animals were fasted for 24 hr before the experiment. Oxidative stress in animals was induced by (i) iron loading by intramuscular injection of ferrum Hausmann in the right hind limb (single dose of 500 mg iron/k body wt, 24 hr before the experiment; (ii) exposure to 100% oxygen atmosphere in a hermetic chamber (3 hr); (iii) exhaustive physical loading, achieved by making the rats swim until exhaustion (about 3 hr at water temperature of 30°C). Combinations of stressory treatments were used: iron + hyperoxia, iron + swimming, and hyperoxia + swimming.

The animals were killed by decapitation. Skeletal muscles from both injected and noninjected hind limbs were homogenized in a medium containing 20 m*M* histidine + 100 m*M* NaCl, pH 7.0 (for measurements of calcium transport (9)) or in a medium containing 1.15% ice-cold KCl (for measurements of total iron, vitamin E content, and endogenous fluorescent lipid peroxidation (LPO) products).

Calcium transport was monitored using a calcium selective electrode (Orion 9320) in the medium containing 100 m*M* KCl, 15 m*M* sodium oxalate, 3 m*M* $MgCl_2$, 5 m*M* sodium azide, 20 μM $CaCl_2$, 3 m*M* ATP, skeletal muscle homogenate (50 μg of protein in 1 ml), and 20 m*M* Hepes (pH 7.4 at 35°C). Under the condi-

tions used (addition of sodium azide) calcium transport in skeletal muscle homogenate is effectuated exclusively by sarcoplasmic reticulum (10).

Vitamin E content in muscle homogenate was determined fluorometrically (λ exitation, 296 nm, λ emission, 325 nm; Perkin Elmer 44B spectrofluorometer) after preliminary saponification and extraction with *n*-hexane according to the method of Taylor *et al.* (11).

Total iron concentration was determined according to the method of Brumby and Massey (12) using 1,10-phenantroline. Endogenous fluorescent LPO end products were assayed (13) in chloroform extracts (λ exitation, 365 nm; λ emission, 440 nm). Protein concentration was assayed by Biuret reagent using bovine serum albumin as standard. Ferrum Hausmann (Pharmachim), ATP (Reanal), histidine (Fluka), sodium oxalate, Hepes, NaN_3, NaCl, $CaCl_2$, HNO_3, HCl, 1,10-phenantroline (Merck), *n*-hexane, methanol (Reachim), ascorbate (Reanal), albumin (Sigma), chloroform (Loba Chemie; highly purified) were reagents employed.

Results

Figure 1A shows the typical kinetic curve of calcium transport by sarcoplasmic reticulum vesicles measured by a calcium selective electrode in skeletal muscle homogenates. Addition of calcium to the buffer causes the rapid response of the electrode in the region of 2×10^{-5}–10^{-6} *M* calcium. Subsequent addition of skeletal muscle homogenate to the incubation medium results in calcium uptake, as reflected in a decline of the curve. In controls, the curve declined to base values indicating a complete uptake of added calcium. Figure 1A shows similar calcium transport curves for homogenates of skeletal muscles obtained from animals subjected to hyperoxia or endurance swimming. Although there was some decrease both in the rate of calcium transport and the amount of calcium transported, these differences were insignificant (Table I). Swimming in combination with oxygen treatment also was without significant effect.

When skeletal muscle homogenate from animals treated with iron were examined, it was seen that there were significant decreases in the rate of calcium transport and the amount of calcium transported (Fig. 1B and Table II). This inhibition was less in the noninjected leg than in the injected leg. When the homogenates from the injected leg were examined, it was noted that calcium transport was suppressed, which was reflected by significant decreases of both the rate of calcium transport and the amount of calcium transported (Fig. 1B and Table II). These differences in the effects on calcium transport correspond well to the different amounts of iron found in the injected vs noninjected legs (more than 18-fold, see Table III).

Also, the amounts of vitamin E and LPO products differed in homogenates from the injected vs noninjected legs (Table IV): The amount of vitamin E was

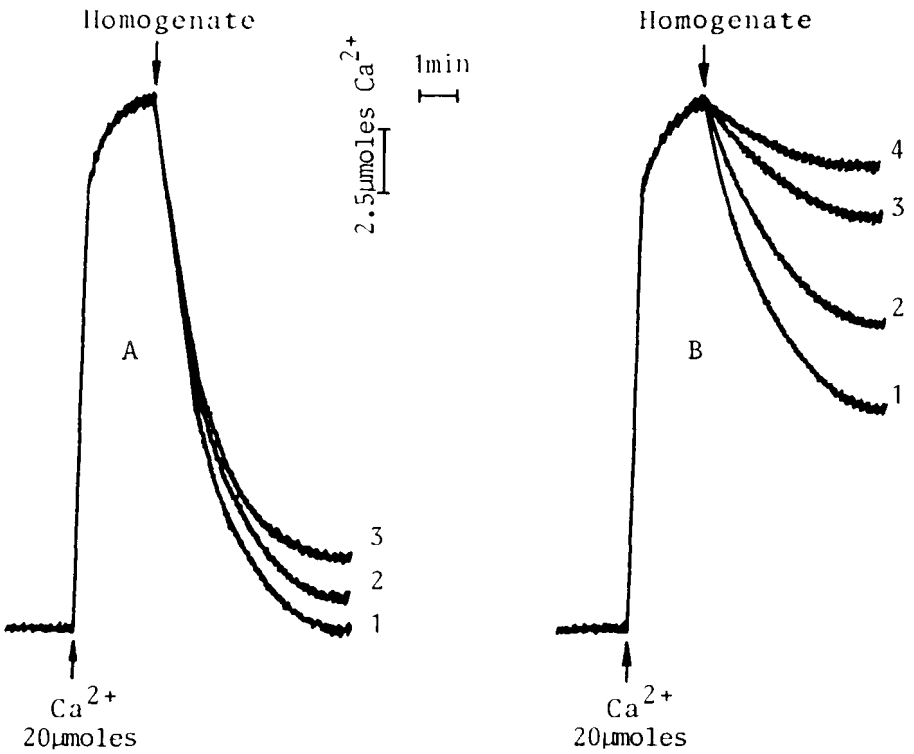

Figure 1. Kinetic curves of calcium transport by sarcoplasmic reticulum in rat skeletal muscle homogenates. (A) 1, control; 2, hyperoxia; and 3, swimming. (B) 1 iron loading (noninjected leg); 2, iron loading (injected leg); 3, iron loading (injected leg) + swimming; and 4, iron loading (injected leg) + hyperoxia.

Table I. Rates of Calcium Transport and Amounts of Calcium Transported by Sarcoplasmic Reticulum in Skeletal Muscle Homogenates from Rats Exposed to Physical Loading or Hyperoxia[a]

Treatment	Rate of calcium transport (μmol Ca^{2+}/min/mg protein)	Amount of calcium transported (μmol Ca^{2+}/mg protein)
Control	5.8 ± 1.8	14.7 ± 5.4
Hyperoxia	5.7 ± 2.5	14.5 ± 6.0
Swimming	4.4 ± 2.3	12.4 ± 4.6
Swimming + hyperoxia	4.2 ± 2.6	20.6 ± 5.9

[a] The number of animals in each group was eight.

Table II. Efficiency of Calcium Transport by Sarcoplasmic Reticulum in Skeletal Muscle Homegenates from Rats Exposed to Iron Loading[a]

Treatment	Efficiency of calcium transport[b] (% of the controls)
Iron loading	32.7 ± 4.3
Iron loading + hyperoxia	12.1 ± 2.4
Iron loading + swimming	15.5 ± 3.8

[a] The number of animals in each group was eight.
[b] Refer to Table I for control rates of calcium transport.

Table III. Total Iron Content in the Skeletal Muscles of Rats[a]

Treatment	Iron content (nmol/g tissue)	
	Noninjected leg	Injected leg
Control	0.08 ± 0.03	—
Iron loading	0.37 ± 0.16	6.77 ± 1.78

[a] The number of animals in each group was eight.

lowered and the concentration of LPO products was increased in the injected leg.

Combined oxidative stresses, i.e., iron + swimming, iron + oxygen, were examined. The disturbances in calcium transport were much greater than when iron was used alone in the injected legs (Fig. 1B and Table II). In the noninjected legs, combinations of iron with other oxidative stress agents did not result in significant differences.

Discussion

The transition metal ion iron plays a key role in the initiation and development of a variety of free radical reactions contributing to oxygen-dependent tissue injury. The mechanisms by which iron overload damages tissue are at present largely speculative, but the evidence indicates that oxidative tissue damage is supported by submicromolar to micromolar levels of nonheme cellular iron, often mobilized from ferritin stores (14, 15). Administration of iron results in the development of oxidative stress in skeletal muscles as evidenced by increases in amounts of LPO fluorescent end products, decreases in vitamin E concentration, and inhibition of calcium transport by sarcoplasmic reticulum. It has been reported that the concentration of vitamin E is reduced in experimental iron overload (16) and in thalassemia major (17). The clinical significance of these observations remains uncertain. There was a correlation between iron tissue content and all parameters of oxidative stress measured, as monitored in controls, noninjected, and injected legs. It is known that accumulation of LPO products in sarcoplasmic reticulum membranes results in concentration-dependent inhibition of calcium transport and of calcium dependent ATPase (4, 7, 8). Thus, it might be suggested that the decreased rate of calcium transport by sarcoplasmic reticulum of iron-loaded animals was due to development of oxidative stress, namely, vitamin E depletion coupled with accumulation of LPO products.

The results of our studies suggest that neither exhaustive physical loading nor hyperoxygenation or their combination lead to apparent modifications in calcium transport by sarcoplasmic reticulum in skeletal muscle homogenates. This may lead one to the conclusion that exhaustive physical loading and hyperoxygenation are not accompanied by the development of oxidative stress. However, this stands in contrast to what has been reported in the literature (1, 3, 4, 18). In this study analysis of our results on combinations of iron loading + physical loading, or + hyperoxygenation, indicates that these conditions (swimming, oxygen) do in fact induce oxidative stress. This is evidenced by the magnification effects of physical loading and hyperoxia on the inhibition of calcium transport seen in iron loading (Table I).

Thus, it might be stated that in addition to effects observed in mitochondria there is damage of sarcoplasmic reticulum resulting in disturbances in muscle contractility under oxidative stress. The data presented also show that under conditions of oxidative stress the requirement for vitamin E (19, 20) should be increased, since physical, oxygen, and iron loadings result in deficiency of antioxidant defensive systems. That is to say, for example, that persons engaged in physical ac-

Table IV. Vitamin E Content and Endogenous LPO Products in Skeletal Muscles of Iron-Loaded Rats[a]

Treatment	Vitamin E content (μg/mg protein)	Fluorescent LPO products (fluorescence intensity, arbitrary units)
Control	0.020 ± 0.005	0.58 ± 0.06
Iron loading (noninjected leg)	0.015 ± 0.003	0.73 ± 0.11
Iron loading (injected leg)	0.008 ± 0.001	1.48 ± 0.38

[a] The number of animals in each group was eight.

tivity or an exercise program may require supplemental vitamin E, especially when the physical activities demand high oxygen turnover (21). Further studies might seek to find application of these findings in physiologic systems, especially endurance exercise.

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