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Research Article

# EFFECT OF ORAL IRON SUPPLEMENTATION AND ENDURANCE TRAINING ON CYTOCHROME C OXIDASE ACTIVITY IN RAT SOLEUS MUSCLE

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**ABSTRACT**

Cytochrome c oxidase, a copper and iron - containing enzyme in mitochondrial respiratory chain, plays an important role in oxidative phosphorylation. Its function as complex IV of respiratory chain is to receive an electron from each of four cytochrome c molecules, and transfer them to one oxygen molecule, converting molecular oxygen to two molecules of water. In present study, activity of cytochrome c oxidase as a key enzyme in cell respiration was measured in soleus muscle of four groups of rats. The first group was considered as control group and received no treatment. The second group received oral iron 4 mg/kg daily. The third group subjected to endurance treadmill exercise and the fourth group subjected to endurance treadmill exercise and received oral iron 4 mg/kg daily iron. The results showed that iron in combination with exercise significantly increased cytochrome c oxidase activity of soleus muscles as compared to third group.

**Keywords:** Cytochrome C oxidase activity, Iron oral supplementation, Endurance training, Soleus muscle.

## INTRODUCTION

Cytochrome C oxidase (COX, EC 1.9.3.1) as complex IV in mitochondrial respiratory chain plays an important role in oxidative phosphorylation[1, 2, 3]. It is the last enzyme in the respiratory electron transport chain of mitochondria (or bacteria) located in the mitochondrial (or bacterial) membrane. It receives an electron from each of four cytochrome c molecules, and transfers them to one oxygen molecule, converting molecular oxygen to two molecules of water. In this process, it binds four protons from the inner aqueous phase to make water, and in addition translocates four protons across the membrane, helping to establish a transmembrane difference of proton electrochemical potential that the ATP synthase then uses to synthesize ATP[3].

This complex is a large integral membrane protein composed of several metal prosthetic sites and 13 protein subunits in mammals. In mammals, ten subunits are nuclear in origin, and three are synthesized in the mitochondria. The complex contains two iron-containing hemes, a cytochrome a and cytochrome $a_3$, and two copper centers, the $Cu_A$ and $Cu_B$ centers. In fact, the cytochrome $a_3$ and $Cu_B$ form a binuclear center that is the site of oxygen reduction. Cytochrome c reduced by the preceding component of the respiratory chain (cytochrome bc1 complex, complex III) docks near the $Cu_A$ binuclear center, passing an electron to it and being oxidized back to cytochrome c containing $Fe^{+3}$. The reduced $Cu_A$ binuclear center now passes an electron to cytochrome a, which in turn passes an electron to the cytochrome $a_3$- $Cu_B$ binuclear center. The two metal ions in this binuclear center are 4.5 Å apart and coordinate a hydroxide ion in the fully oxidized state[4].

Iron as a transition metal and trace element is essential to nearly all known organisms. In cells, iron is generally stored in the centre of metalloproteins, because "free" iron -which binds non-specifically to many cellular components - can catalyse production of toxic free radicals. In animals, plants, and fungi, iron is often incorporated into the heme complex. Heme is an essential component of cytochrome proteins, which mediate redox reactions, and of oxygen carrier proteins such as hemoglobin, myoglobin, and leghemoglobin. Inorganic iron also contributes to redox reactions in the iron-sulfur clusters of many enzymes, such as nitrogenase (involved in the synthesis of ammonia from nitrogen and hydrogen) and hydrogenase. Non-heme iron proteins include the enzymes methane monooxygenase (oxidizes methane to methanol), ribonucleotide reductase (reduces ribose to deoxyribose; DNA biosynthesis), hemerythrins (oxygen transport and fixation in marine invertebrates) and purple acid phosphatase (hydrolysis of phosphate esters). Iron-containing enzymes such as catalase, usually containing heme prosthetic groups, participate in catalysis of oxidation reactions in biology, and in transport of a number of soluble gases[5].

Calf muscles, including the soleus are powerful muscles and are vital in walking, running, and dancing. The soleus specifically plays an important role in standing; if not for its constant pull, the body would fall forward[6]. Because of these reasons, it was considered as a source for study of mitochondrial cytochrome c oxidase activity in our experiment.

The present work studied the effect of iron diet supplementation on cytochrome c oxidase activity of rat soleus muscles in exercise.

## MATERIALS AND METHODS

### Materials

KCl, Tris-HCl, $MgSO_4$, EDTA from Merck Chemical Co. (Germany) and ATP-$Na_2$ from Sigma Chemical Co. (USA) were used for separation of muscle mitochondria. Bovine serum albumin, Fouline Cioucalteau reagent, copper(II) sulphate, sodium potassium tartarate, sodium carbonate, and sodium hydroxide from Merck Chemical Co. (Germany) were used for protein determination.

### Animal experiments

This study was performed on 24 male Wistar albino rats (weighing 180-220 g). They were kept in individual cages in a controlled room (temperature, 20–25 °C; humidity, 57 %; exposed to 12 h of daylight). The rats were fed with standard rat food and tap water during experimentation. All experiments on animals were considered carefully to be ethical. The experimental protocol was approved by the Ethic Committee of College of Physical Education and Sports Sciences, University of Tehran.

Following several days of acclimatization, rats were randomly assigned to control and experimental groups. Animals were randomly divided into four groups each comprising six animals and treated for 12 weeks.

The first group (control group) received no treatment. The second group received 4 mg/kg iron[7] as iron sulphate syrup (Exir Pharmaceutical Co., Boroujerd, I.R. Iran) by daily gavage in the afternoon up to the end of experiment. The third group subjected to endurance treadmill exercise and the fourth group subjected to endurance treadmill exercise in combination with 4 mg/kg iron as iron sulphate syrup by daily gavage in the afternoon up to the end of experiment.

The endurance treadmill exercise was done according to the protocols previously described[8, 9, 10]. Briefly, in the first week, animals trained daily for 10 minutes using a treadmill (Sport Science Research Center, Tehran, I.R. Iran) at a speed of 10 m/min and treadmill angle of 0 ° for adaptation with experiment conditions. The duration and speed of treadmill training was gradually increased from the second week (10 min, 10 m/min) to 6th week (60 min, 32 m/min) then kept constant in the rest of experiment (weeks 7-12). From the first week to 6th week of the experiment, angle of treadmill was gradually increased in a slope of 5°/2 weeks until reaching 15° which kept constant.

**Biochemical estimation**

At the end of experiment, soleus muscle mitochondria were isolated as previously described[11]. Briefly, the excised muscle specimen was blotted with filter paper, freed from fat and connective tissue, quickly weighed, and immersed into ice cold 0.15 M KCl. The tissue is cut with scissors into small pieces and rinsed with several portions of 0.15 M KCl. Cutting was continued until a fine mince was obtained. The minced tissue was rinsed with homogenization medium (0.1 M KCl, 0.05 M Tris-HCl buffer, pH 7.4, 0.001 M ATP-$Na_2$, 0.005 M $MgSO_4$, and 0.001 M EDTA) and suspended in about 1 volume of the same. Homogenization was carried out with a hand-operated glass homogenizer for 1-2 minutes. All steps of mitochondrial separation including homogenization were performed at 0-2 °C. The homogenate was diluted with homogenization medium to a volume of 10 times the initial weight of the muscle and centrifuged at 600-650 g for 5-10 minutes. The supernatant fraction was decanted into a new tube and re centrifuged as before. The resulting supernatant was decanted and centrifuged at 14000 g for 10 minutes. The mitochondrial pellets were re suspended in homogenization medium and re centrifuged as above. The washing may be repeated once. The washing medium was discarded, then the surface of the tightly packed pellets was rinsed with 0.15 M KCl. The mitochondrial pellet was finally suspended in 0.15 M KCl to contain 6-10 mg of mitochondrial protein per milliliter of suspension[11].

Protein measurements were performed according to the colorimetric method of Lowry[12].

Cytochrome C oxidase specific activity (as units/mg mitochondrial protein) of soleus muscles of rat legs was assayed by Sigma Cytochrome c Oxidase Assay Kit (U.S.A.).

**Statistical analysis**

Statistical differences were determined by one-way analysis of variance (ANOVA) followed by the Tukey multiple comparisons posthoc test on the SPSS statistical package. Differences were regarded as significant at $P < 0.05$.

**RESULTS AND DISCUSSION**

The results have been summarized in figure 1. They show that iron in combination with exercise (4th group) significantly increased cytochrome c oxidase activity of soleus muscles as compared with exercise alone (3rd group) ($P<0.05$). But, Iron and exercise alone did not significantly increase cytochrome c oxidase activity as compared with control group.

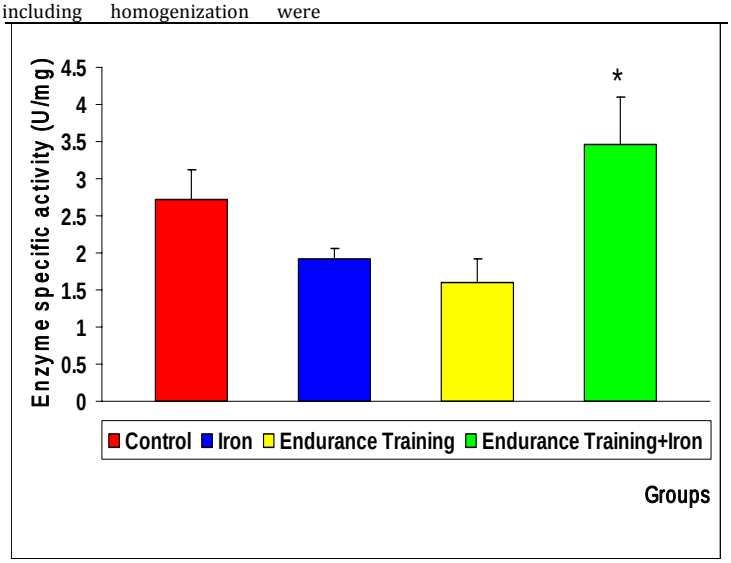


**Fig. 1: Effect of iron and training and specific activity of cytochrome c oxidase in different groups. Values are expressed as Mean ± S.E. of 6 animals in each group. * Significantly different from endurance training group at $P< 0.05$.**

Many enzymes (metalloenzymes) contain metal ions as an integral part of their structure (e.g. copper and iron in cytochrome c oxidase). The function of the metal may be to stabilize tertiary and quaternary protein structure. Removal of the metal ions is accompanied by conformational changes with inactivation of the enzyme[13, 14]. The enzyme can often be reactivated simply by adding the ion to the reaction mixture. Reactivation may take some time, because rearrangement of the polypeptide chains into the active conformation is not instantaneous. The metal ion components of many enzymes appear to play a direct part in catalysis as well as any possible structural role they may fulfill. A metal ion may function in catalysis. For example, by providing an electropositive center in the enzyme with which negatively charged groups in the substrate can form coordinate links[13].

From our results, it seems that iron in combination with exercise could increase cytochrome c oxidase activity of soleus muscles. This may be related to the activatory effect of iron on cytochrome c oxidase which contains iron in its structure. On the other hand, exercise alone could not increase activity of cytochrome c oxidase. This can be related to decreased iron absorption and increased iron excretion[1]. Furthermore, iron alone could not increase activity of cytochrome c oxidase which may be related to excess amount of iron that may have inhibitory effect on cytochrome c oxidase activity[13].

Finally, from our results, it can be concluded that iron and exercise have a synergistic effect on cytochrome c oxidase activity and iron supplementation in exercising individuals may improve cellular oxygen consumption and their endurance during exercise.

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