

# Effects of Endurance Training on Mitochondrial Nitric Oxide Synthase Protein Concentrations in Skeletal Muscle

Andy Sershon

Carroll College, 100 N. East Ave., Waukesha, WI 53186

## Abstract

In this study, endurance training of rats was used to analyze its effects in the production and activity of an enzyme known as mitochondrial nitric oxide synthase. Found within the mitochondria, this enzyme is believed to be influenced by the production of cytochrome c oxidase through endurance training. Hypothyroidism in humans has shown an increase in mitochondrial nitric oxide synthase expression through decreased protein concentrations of cytochrome c oxidase, explaining the symptoms of decreased endurance capability associated with the hormone deficiency.

## Introduction

Endurance exercise training is currently viewed as a way to improve health and muscular strength. Cytochrome C oxidase is an enzyme involved in regulating endurance exercise training by being able to stimulate the production of ATP through oxidative phosphorylation in the mitochondria. Moderate exercise produces a beneficial effect on mitochondrial function by increasing cytochrome oxidase activity (Navarro et al., 2004; Delp et al., 1997; Gavin et al., 2000).

Current evidence shows that nitric oxide (NO) plays a major role in inhibiting the production of cytochrome c oxidase (Carreras et al., 2001; Ellering et al., 2002; Talayan et al., 1996). NO concentrations are controlled by the enzyme mitochondrial nitric oxide synthase (mtNOS) within the inner wall of mitochondria. Decreases in endurance produced by hypothyroidism have been linked to decreased cytochrome c oxidase activity produced by increasing concentrations of both mtNOS and NO production inside the mitochondria (Carreras et al., 2001).

Therefore, this study is aimed at comparing the enzyme levels of both mtNOS and cytochrome oxidase from an exercised and sedentary group of rats. The hypothesis of the experiment is that exercise training will decrease the amount of mtNOS protein concentrations, leading to a decrease in NO production and resulting in an increase in endurance.

## Methods

### Endurance Exercise Training

- A motorized treadmill was used to run rats on 5 days a week for 4-5 weeks at a speed of 30meters/min at a 10 degree incline.

### Mitochondrial Isolation

- The vastus intermedius muscle was isolated and removed from each hind leg.
- Tissue samples were cut into small pieces and suspended in a homogenizing buffer.
- A tissue grinder sufficiently lysed the tissue cells.
- Differential Centrifugation
- Mitochondrial pellet was successfully isolated and suspended in a supporting buffer.

### Cytochrome C Oxidase Assay

- Ferricytochrome c oxidase was oxidized by cytochrome c oxidase to determine enzyme activity.
- Spectrophotometer read absorbance levels of cytochrome c oxidase.



Table 2.

Rat	Exercise	Weight of Muscle Sample (grams)
C-1	0	.22 .2
C-2	0	.21 .21
C-3	0	.22 .19
E-1	4 weeks	.22 .21
E-2	5 weeks	.23 .2
E-3	5 weeks	.24 .22

Table 1.

Control		Experimental	
Absorbance	Units (millimoles/ml.)	Absorbance	Units (millimoles/ml.)
.148 (blank)		.151 (blank)	60.4
.16	69.1	.162	60.4
.172	131.9	.17	99.9
.164	96.7	.168	93.4

$$\text{Units/mL} = \left( \frac{\Delta \text{absorbance}}{\Delta \text{min}} \dots \frac{\Delta \text{min}}{\text{min}} \right) \times (6.0 \times 10^3) \times 1.1 \quad (\text{Equation 1})$$

(0.05 pl) x 21.84

## Results

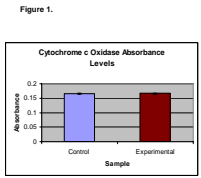
The experimental test group of Wistar rats ran on a motorized treadmill five days a week for four and five weeks. After endurance training was completed, the vastus intermedius muscle was extracted from each rat. Mitochondrial isolation of each tissue sample was achieved through the use of a dounce homogenization centrifugation. Figure 1 represents the absorbance levels of cytochrome c oxidase at 550nm in buffer suspension of the mitochondria between the experimental and control group. Calculations in determining activity of the sample were included in Table 1 describing that one milliliter will oxidize 1.0 μmole of ferricytochrome c per minute at pH 7.0 at 25°C. Table 2 represents the amount of tissue extracted from each rat following post-exercised training. These weights were used in determining overall units for cytochrome c oxidase activity.

## Conclusion

- Mitochondria were isolated successfully from rat muscle tissue using a dounce homogenization differential centrifugation.
- The mitochondria isolated using homogenization had quantifiable cytochrome c oxidase activity (Table 1).
- Cytochrome c oxidase activity of the exercised group was not statistically significant compared to the sedentary group.
- The results went against the expected outcome that exercise training would promote an increase in cytochrome c oxidase activity.
- The lack of significance may indicate an inefficient training procedure using the motorized treadmill or an ineffective method of isolation suspension storage.
- mtNOS concentrations were not analyzed in this study.

## References

- Carreras, M.C., Peralta, J.G., Converso, D.P., et al. Modulation of liver mitochondrial NOS is implicated in thyroid-dependent regulation of O2 uptake. *American Journal of Physiology: Heart and Circulation Physiology* 281: H2282-H2288, 2001.
- Delp, M.D. and Laughlin, M.H. Time course of enhanced endothelium-mediated dilation in aorta of trained rats. *Medicine and Science in Sports and Exercise* 29: 1454-1461, 1997.
- Gavin, T.P., Spector, D.A., Wagner, H., et al. Nitric oxide synthase inhibition attenuates the skeletal muscle VEGF mRNA response to exercise. *Journal of Applied Physiology* 85: 1152-1158, 2000.
- Navarro, A., Gomez, C., López-Cepero, J.M., et al. Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. *Journal of Physiology: Regulatory, Integrative and Comparative Physiology* 286: R505-R511, 2004.



## Acknowledgments

I would first like to thank Dr. John Bennett for all of his guidance during this project. Special thanks to Frank Mahler and Katie Palan for the pilot study that started the research. I would also like to thank Lynn Peterson in providing access to the animal room.