CALIFORNIA STATE SCIENCE FAIR
2007 PROJECT SUMMARY

Name(s)
Kanan K. Sindhu

Project Number
S0423

Project Title
Testosterone Depletion and Mitochondrial Damage in Skeletal Muscle: A Mechanistic Study

Abstract

Objectives/Goals
The objective was to study whether testosterone depletion would cause oxidative stress and mitochondrial damage.

Methods/Materials
Methods: Protein levels were determined and western blotting was carried out, which consisted of separation of the proteins on SDS-Acrylamide gels, blocking, reacting with the primary antibodies, washing, reacting with the secondary antibodies, reacting with the HRP conjugated substrate, developing and fixing of the film, and analysis by densitometer.
Materials: Gastrocnemius muscle samples from male mice, Tris-HCl, homogenizer, Refrigerated Centrifuge, Lysis buffer, Bio-Rad kit, polyacrylamide gels, PVDF membranes, 5% dry milk , T-TBS, primary antibodies, secondary antibodies , enhanced chemiluminescent reagent, photographic films, laser densitometer, developer

Results
The protein expression of the gp91phox subunit of NADPH oxidase was markedly induced in the castrated samples, an indicator of oxidative stress. Supplementation with physiological testosterone levels resulted in a significant decrease of the protein. Supplementation with supraphysiological doses of testosterone had no effect on the protein compared to the castrated group. Mitochondrial damage was assessed by monitoring COX (Cytochrome C Oxidase) in the post-mitochondrial fractions. COX is present on the inner mitochondrial membranes and due to mitochondrial damage, would leak into the cytosol. The enzyme protein was significantly higher in the cytosolic fractions of the castrated samples as compared with the controls. Supplementation with physiological doses of testosterone prevented COX from leaking into the post-mitochondrial fractions, suggesting that it prevented mitochondrial damage whereas supplementation with supraphysiological testosterone levels failed to ameliorate mitochondrial damage.

Conclusions/Discussion
Testosterone depletion by castration resulted in a marked induction of the superoxide radical producing enzyme, NADPH Oxidase, and mitochondrial damage as assessed by the release of COX. Supplementation with physiological doses of testosterone ameliorates NADPH Oxidase and mitochondrial damage. These results point to potential implications of androgen therapy in elderly, hypogonadal, human immunodeficiency virus-infected and diabetic men. Treatment with supraphysiological levels of testosterone also caused mitochondrial damage and oxidative stress.

Summary Statement
Testosterone depletion causes oxidative stress and mitochondrial damage.

Help Received
Used lab equipment and samples at Charles Drew University under the supervision of Dr. Ram Sindhu