PROJECT REVIEW

Skeletal muscle proteome alterations after long term anabolic steroid abuse

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Specific aims

- Detect and identify unique skeletal muscle proteins affected by long-term usage of banded substance.
- Identify biological markers in muscle and blood that can detect differences between doped and non-doped individuals.

Significance

Detection of anabolic steroids and other banned substances in human blood and urine is only possible for a limited time after intake. However, the effects of such abuse on skeletal muscle morphology can be detected many years after secession of drug administration (Eriksson *et al.*, 2006). The development of a fast and inexpensive method to detect such alterations eliminates the possibility to mask drug abuse, even when the drug is no longer detectable. Abnormal changes in the skeletal muscle proteome may be the only way to detect genetic doping. Supporting a proteomic method development for skeletal muscle can result in a useful tool for future studies regarding genetic doping.

Background

The use of banned substances among athletes is a well-known phenomenon, and the effects on physical performance documented. For example, the myotrophic effects of testosterone have been previously demonstrated (Sinha-Hikim *et al.*, 2003). These effects are further enhanced when drug treatment is combined with strength training. However, the mechanisms associated with testosterone and other anabolic steroids on increased muscle fibre area and strength of the muscle fibres has not been clarified.

Methods

A unique group of individuals having used testosterone and anabolic steroids for > 5 years is available for muscle and blood sampling. Two dimensional differential gel electrophoresis and mass spectrometry will be used to detect and identify proteins. Multivariate statistical methods can identify discriminating proteins between groups. Functional testing can relate performance to protein expression.

Results (Preliminary / Related)

Our laboratory have demonstrate changes in the muscle cell after anabolic steroid abuse more than 10 years after secession of intake. Using 2D proteomics we can detect >2300 individual proteins from muscle tissue, > 80 of these proteins are changed by using banned substances. Screening for relevant markers for doping can now be accomplished.

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Results and Conclusions

Seventeen trained elite strength athletes were recruited through personal contacts. Ten of the athletes were using AAS (Doped) and seven reported that they had never used AAS (Clean). All subjects were active in sports such as weight lifting, strong men competitions and body building at national and international level. Muscle and blood samples were taken and maximal muscle strength tested. DEXA scanning determined body composition. Blood was analysed for a panel of hormones related to muscle and steroid metabolism. Muscle samples were analysed using fluorescent immunohistochemistry and proteomic screening (2D DIGE/MALDI-TOF).

Doped and Clean athletes had higher bone mineral density at all measured sites compared to Controls, and Doped had more lean body mass compare to Clean and Controls. The use of AAS in combination with strength training results in significantly different skeletal muscle gene expression compared to strength training without AAS. The Doped athletes produced significantly lower results in the strength measurement then the Clean athletes despite more muscle mass and similar muscle fiber size and fiber type composition.. By using cluster analyses, there is a clear separation between Doped and Clean athletes based on morphological, hematological and proteomic data. Major findings were that the number of capillaries/fiber and myonuclei/fiber was higher in Doped compared to Clean athletes. The doped group had approximate one more capillary per muscle fiber compared to the clean group. We conclude that the Doped group has a lower capacity to generate force per muscle mass and fiber cross section area, possible due to the long term use of AAS

Results from the present study should be used as a base for further investigations regarding long term (possible permanent) effect of AAS and may also be used to generate indirect test (hormones, metabolites, proteins) to screen athletes for the use of banned substances in blood, muscle and urine. The advantage of this approach compared to direct measurements of banned substances or its metabolites is that masking agents and clearance rates will be ineffective to avoid detection.

The research approach and analytical methods used in the present study can distinguish a AAS doped athlete from a Clean athlete, regardless of substance used, training regimen, age and duration of AAS supplementation.