

PROJECT REVIEW

“Identification of Novel Serum Biomarkers for Erythropoietin Abuse: A Proteomic Approach”

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Already in 1990, rHuEpo was banned by the American Medical Association and the International Olympic Committee (IOC). However, the abuse of rHuEpo continues and a sensitive and robust detection test is needed. The current method directly measures urinary rHuEpo, and is based on differences in glycosylation between rHuEpo and endogenous Epo. However, this method is expensive and has low sensitivity.

Protein profiling, or proteomics, provides a novel and powerful method for characterising the complete catalogue of proteins expressed in a biological system. The aim of the current project is to study the impact of more prolonged rHuEpo exposure on serum proteomics in healthy human subjects, in order to detect novel biomarkers for Epo abuse.

The study will have a randomised double-blinded design. A total of 4 experimental groups will be included in the study; 1. rHuEpo treatment, 2. rHuEpo + exercise, 3. Placebo, 4. Placebo + exercise. A total of 12 healthy inactive male subjects will be included in each group. The treatment period will be 12 weeks followed by a 3 week washout period. rHuEpo will be administered s.c. at a dose of 5000 IU (~60IU/kg) every second day for the first 2 weeks, on three consecutive days during week 3, and once weekly during weeks 4-12. All subjects will be supplemented by 100 mg iron orally/week. The exercise will consist of 1½h biking at 60-75% of VO₂max 3 times per week.

Proteomics will be performed on serum samples collected before treatment and on days 16, 84 (end of treatment) and 105 (end of washout period). It is the hope that the current study will identify novel biomarkers that are specific for rHuEpo treatment, and not affected by exercise itself, that can be used in future anti doping test.

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Result and Conclusion

Aim: Implementation of the biological passport, with a longitudinal comparison of the individual athlete's hematological levels, has been a favorable addition to the existing direct test for detection of erythropoietin (Epo) abuse. However, the biological passport does not guarantee a doping free sport, and further improvements in order to increase both the sensitivity and specificity are needed. In the current study, a proteomic approach was used to identify novel serum biomarkers for the detection of Epo abuse.

Methods: Thirty-six healthy young males were randomly assigned to one of the following groups; Sedentary-placebo (n=9), Sedentary-Epo (n=9), Training-placebo (n=10), Training-Epo (n=8). The subjects were treated with placebo or an erythropoietin-stimulating agent (ESA) (Darbepoietin- α , Aranesp, Amgen) subcutaneously once weekly for 10 weeks and further followed during a 3-week washout period. The endurance training consisted of supervised biking 3 times per week for 13 weeks at the highest possible intensity. Serum samples were collected at baseline, after a boosting period (high ESA dose) at the end of week 3, after a maintenance period (lower ESA dose) at week 10, and after a 3-week washout period at week 13. The serum was depleted for the most abundant proteins, such as albumin and IgG. First, the proteins were separated according to their isoelectric point and then according to mass (2D-gel electrophoresis). The identity of proteins that exhibited significantly altered intensity during the study was found by mass spectrometry.

Results: A total of 125 spots were identified in the serum 2DE gels, hereof 80 were observed in all gels. In the current study, isoforms of 6 proteins changed significantly during the intervention and washout period in response to ESA treatment. When comparing all 4 experimental groups, isoforms of 2 proteins (serotransferrin and haptoglobin, respectively) showed a significant response to ESA treatment. Hereof, one spot (G), containing haptoglobin, showed a significant lower intensity in all subjects in the training-Epo group during the ESA treatment period, and an increase in intensity during the washout period. Thus, this isoform of haptoglobin could be a potential new anti-doping marker.

Conclusion: It is possible by a proteomic approach to identify serum protein isoforms that change significantly in response to Epo abuse without being affected by endurance training. Especially spot G seems promising as a novel biomarker for Epo abuse, since the level of this haptoglobin isoform was significantly decreased in all subjects during the treatment period, and increased again in all subjects during the washout period.