

PROJECT REVIEW

"Validation of a novel test for autologous blood doping: High altitude, gender and strenuous endurance exercise"

Pr. C. Malm, Dr. I. Granlund, Dr. M. Ericsson, Dr. N. Khoo, PhD, Dr. E. Strahm, Mr. A. Hult, Dr. J. Brundell, Ms. L. Lundgren, Dr. E. Watz, Dr. B. A. Diedrich, Dr. C. Andersson, Dr. P. Matha (Umeå University, ProTest Diagnostics, Doping Control Laboratory, Karolinska University Hospital, Huddinge Hospital, Sweden)

Blood transfusion is the most effective means to increase the number of red blood cells, and enhance athletic performance in endurance events. Due to the complicated procedures with transfusion, the use of Epo has been the dominating method for blood doping, but improved detection technologies have forced cheating athletes again to use autologous blood doping. To date no reliable detection method for autologous blood transfusions exists, and the need for a direct detecting method for autologous blood transfusion is obvious and urgent.

It is known that during storage in the blood bags, RBCs are degraded, the degraded proteins (the "degradome") in circulation will be proportionally higher in doped athlete post-transfusion compared to pre-transfusion or non-transfused athlete.

We have developed an assay/procedure that can discriminate doped from non-doped athletes with a probability of >96%.

Work Plan for current project includes:

A) Verify selected biomarkers/procedure by a repeat autologous blood transfusion study B) Establish the sensitivity and specificity of the procedure to detect autologous blood doping C) Investigate three possible confounding factors: 1) elite endurance training 2) Differences between Males and Females 3) High altitude and low oxygen training

Results and Conclusions

Blood transfusion remains one of the most effective means to increase the number of red blood cells ((RBCs; i.e. hemoglobin mass) in any athlete, and thereby enhance athletic performance in endurance events. The non-medical use of this process is banned by the World Anti-Doping Agency (WADA).

We reported that RBCs change when stored (Malm et al. WADA Grant 08C06CM), and that these changes can be detected by global proteomic

methods, and thus be used to develop a "Doped" profile for autologous blood transfusion.

In the present study, we address challenges attributed to three possible confounding factors

- i) Exercise Training at High Altitude
- ii) High Intensity Exercise Training
- iii) Sex (male or female)

The test subjects analyzed were correctly placed into the respective category, with no false positives or false negative samples using the global proteomics profile modelled using OPLS-DA statistical analysis. The disclosed results support the viability of the test method to accurately detect autologous blood transfusion. The confounding factors (high altitude, exercise intensity or athletes' sex) did not affect the test to detect autologous blood transfusion in the subjects comprised of both elite and recreational athletes. Hematological variables analysis in our hands, did not separate groups investigated as robustly as the proteomic profile analysis test using the same statistical approach. These results support continued protocol development of the test method for enhancement of the current blood passport or athlete biological passport. This work is conducted in collaboration with the doping control laboratory in Huddinge, Sweden. Our team is now focused on reducing the complexity of instrumentation required, amending protocols to existing infrastructure, and expand the scope of the analysis to address possible confounding influence related to genetic variations.