Project Review

"A Gene-Microarray Based Approach to the Detection of Recombinant Human Erythropoietin Doping in Endurance Athletes"

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Living at altitude increases haemoglobin and haematocrit, hence altitude-training is popular among endurance athletes. Since increases in haematocrit can be attained using illicit means like blood-doping or erythropoietin (Epo) use and are potentially hazardous, high haematocrit levels are used to exclude athletes from competition, also without evidence of doping. While sea-level athletes can choose to train at altitude, for others living at altitude has been a way of life for generations (e.g. east-Africans). Recent approaches developed to distinguish the effects of altitude on haematological profiles from those of blood-doping are approximately 20-80% successful. When applying these approaches to the haematological profiles of elite athletes, we found indications of systematic blood-doping or cases of naturally elevated blood markers.

There is therefore an urgent need for these methods to be revised to remove any possibility of athletes being incorrectly banned from competition or, conversely, avoiding sanction due to broad definition of legal limits. In this project we will investigate standard red cell indices and contrast these following Epo administration in athletes not involved in competition. Gene-expression profiles will be assessed using the very latest gene-microarray technology. These results will be used to formulate new methods with improved discriminatory power relative to current detection protocols and in doing so eliminate the possibility of naturally elevated blood markers due to athletes living and/or training at altitude and unidentified doping due to inadequate detection. The use of gene-arrays, validated in this context, may provide gene-expression profiles relevant to other illegitimate approaches to improving oxygen carriage that may have been, or will be in the future, devised.

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Results and Conclusion

The use of recombinant human erythropoietin (rHuEpo) is prohibited by the World Anti-Doping Agency. An OMICS-based longitudinal screening approach has the potential to improve further the performance of current detection methods such as the Athlete Biological Passport. For this project, we successfully used gene expression profiling in whole blood to identify genes that are differentially regulated following rHuEpo administration in Caucasian trained males and Kenyan endurance runners living at sea-level and moderate altitude (~2150 m), respectively. Relative to baseline, the expression of hundreds of genes were found to be altered by rHuEpo. In particular, 15 transcripts were profoundly up-regulated during the 4 weeks of rHuEpo administration and subsequently down-regulated up to 4 weeks post administration in both groups. Importantly, the same pattern was observed in all subjects. Furthermore, 30 transcripts were already differentially expressed two days after the first injection and are therefore promising candidate genes to detect microdose rHuEpo doping. The functions of the discovered genes were mainly related to either the functional or structural properties of the erythrocyte or to the cell cycle and its regulation. In summary, this research project successfully identified the blood “molecular signature” of rHuEpo administration and provided a set of candidate genes with potential to be robust biomarkers of rHuEpo doping. These preliminary results provide the strongest evidence to date that OMICS technologies such as gene expression have the potential to substantially improve and add a new dimension to the current anti-doping methods such the Athlete Biological Passport for rHuEpo detection.

Publications to date:

Jérôme Durussel, Evangelia Daskalaki, Martin Anderson, Tushar Chatterji, Diresibachew H. Wondimu, Neal Padmanabhan, Rajan K. Patel, John D McClure, Yannis P. Pitsiladis. Haemoglobin Mass and Running Time Trial Performance after Recombinant Human Erythropoietin Administration in Trained Men. PLoS One 8(2): e56151. doi: 10.1371/journal.pone.0056151. Epub 2013 Feb 13.