Can altitude training be differentiated from recombinant human erythropoietin doping: A gene expression study

J. Shurlock1,2, Y. Pitsiladis2, G. Wang2

Introduction

The use of recombinant human erythropoietin (r-HuEPO) is prohibited by the World Anti-Doping Agency (WADA), due to its performance enhancing effects. Current testing methods are shown to lack sensitivity1 and be susceptible to fluctuations caused by legal practices, such as altitude training.2 Recently ‘omics’ technologies have been used to demonstrate a ‘molecular signature’ of rHuEPO administration.3 The identification of a robust “molecular signature” of altitude training in endurance-trained athletes will have profound implications for current rHuEPO detection methods such as the application of longitudinal gene expression patterns to assist differentiation of legitimate forms of performance enhancement (e.g. altitude training) from rHuEPO doping.

Methods

20 elite level, endurance athletes (12 male, 8 female) were randomised into an altitude-training group (n=12, age: 23 ± 2 years, weight: 59.20 ± 7.98 kg, height: 175 ± 9 cm) and control group (n=8, age: 23 ± 2 years, weight: 63.61 ± 8.66 kg, height: 176 ± 8 cm). The altitude group trained at altitude in Sierra Nevada, Spain (2320 m) to train (duration: 17 ± 4 days). Blood samples were taken for haematological and gene expression analysis at baseline, during, and post-48hr, 1 week, 2 weeks and 4 weeks-post altitude exposure. Gene expression analysis was carried out using Affymetrix QuantiGene Plex Assay.

Results

Comparison was made between the group mean differences for haematological parameters, using pairwise independent t-test. Statistical significance was determined as Bonferroni corrected p < 0.05. Differential expression analysis was carried out using the “limma” function implemented in the statistical software R (The R Foundation, Vienna, Austria). Fold change was calculated and compared to averaged baseline values, after log2 adjustment. A false discovery rate of 5% was implemented in addition to a 1.5 fold-change threshold.

There were no significant differences between gene expression in the altitude group and the control group, at comparable time points. (FDR adjusted P value >0.97 and >0.60)

Discussion

We were able to successfully use gene expression analysis of whole blood samples to identify genes with some differential regulation during- and post-altitude exposure, demonstrating significant overlap with rHuEPO administration.4 Many of the differentially expressed genes have roles in the structure and/or function of erythrocytes and haemoglobin. This is unsurprising due to the effects of altitude training such as increased haemoglobin concentration and endogenous EPO production.

Discussion

We were able to successfully use gene expression analysis of whole blood samples to identify genes with some differential regulation during- and post-altitude exposure, demonstrating significant overlap with rHuEPO administration.4 Many of the differentially expressed genes have roles in the structure and/or function of erythrocytes and haemoglobin. This is unsurprising due to the effects of altitude training such as increased haemoglobin concentration and endogenous EPO production.

Measured haematological parameters were not significantly altered at any time point, potentially due to the short duration of altitude exposure. This may implicate ‘omics’ technologies as a more sensitive measure of parameters such as Ret%. Additional altitude studies with focus on a live-high, train-low protocol and repeat exposure are required. An untargeted gene expression analysis is currently underway in an attempt use altered gene function to differentiate rHuEPO from altitude exposure.

Clinical impact

This study serves as proof-of-principle and has the potential to inform personalised medicine. This includes identifying and managing individual responses to altitude and those who fall ill at altitude.

Acknowledgments: The authors would like to thank the World Anti-Doping Agency for providing financial support for the study.

References

4. pools.