

PROJECT REVIEW

“The Effects of Factors such as Exercise and Disease on the Distribution of Urinary Erythropoietin Isoforms”

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The purpose of this project is to determine the variability of the natural isoform pattern of EPO in a group of subjects and determine the extent to which the isoform distribution can be influenced by external factors such as vigorous exercise and disease. Such information is needed to support the urinary test for human recombinant EPO.

At present the detection of doping with human recombinant erythropoietin (EPO) relies on the detection of abnormal blood parameters such as those reported by Parisotto (Parisotto et al 2001) coupled with the presence of recombinant human EPO in a corresponding urine sample. The urine test developed by LNDD uses isoelectric focussing and a patented double blotting technique (Lasne 2001) to separate the EPO isoforms into a series of bands. A positive cannot be declared unless the urine has bands which correspond to those found in human recombinant EPO. Data collected so far indicate that normal urinary EPQ has isoforms which are more acidic than those found in human recombinant EPO although there is some overlap (Lasne and De Ceaurriz 2000). A positive is declared if the percentage of basic isoforms is greater than 80%. This value was statistically determined on the basis of the range of values found in a relatively small number (a few hundred) of normal subjects.

Whilst some data has been obtained showing that the distribution of urinary EPO isoforms is not significantly affected by external factors such as altitude there has been no large systematic study on factors such as acute, extreme and long term exercise, and disease. It is only a matter of time before the technical aspects of the urinary EPO test are legally challenged and hence it is essential that data be collected and published in advance to establish whether extreme exercise or disease can alter the pattern of isoforms present so that they more closely resemble those found in recombinant EPO. Such data will be essential to support the urinary EPO test if it is ever to be used without a blood sample to confirm doping with recombinant EPQ.

The effects of factors such as exercise and disease on the distribution of urinary erythropoietin isoforms.

Results and Conclusions

This project was undertaken to determine if there were any significant changes on the distribution of urinary erythropoietin (EPO) isoforms induced by exercise or disease. It is important to know if such changes do occur as the current test for detecting doping with recombinant EPO depends on the fact that the EPO isoform distribution in the urine of those who have been administered EPO is more basic than the distribution found naturally. In an attempt to determine whether exercise can induce a change to more basic isoforms, subjects were studied who underwent a range of exercise regimes ranging from a short duration (10 minute) exercise test to exhaustion, through a full marathon of approximately three hours, to a 100 km cross country run with a typical duration of over 24 hours. In all cases urine samples were collected in order to measure both the concentration of urinary EPO and the distribution of isoforms.

The results show that the concentration of EPO in urine is not affected by any of the levels of exercise. It was also found that the concentration of EPO in urine is highly variable and for some individuals can vary by more than a factor of four from one collection to the next. For most levels of exercise up to including a full marathon the variation in distribution of urinary EPO isoforms was small and within the range of normal variability found for individual subjects. However it appears that the extreme long duration exercise can produce a small but significant increase in the percent basic isoforms found in the urine. It is not known whether this increase relates to changes in EPO production or changes in EPO excretion. The magnitude of the change was not sufficient to require changes in the criteria currently used to assess whether a urine sample contains recombinant EPO.

The samples from the subjects who were part of the disease study were all suffering from anaemia resulting from severe kidney disease. It was hoped to determine if the EPO excreted from such subjects was different in isoform distribution possibly due to a greater contribution from the liver. Unfortunately it was not possible to draw any conclusions from this aspect of the project because the current method was found to be unsuitable for such urine samples owing to their high protein content.

Publications (including in press or submitted) and poster presentations

- 1) K. Nelson et al., The effects of exercise on urinary erythropoietin levels and isoform distribution. In: Schänzer W, Geyer H, Gotzmann A, Mareck U, editors. Recent Advances in Doping Analysis (10) Köln: Sport und Buch Strauss, 2002:169-177.