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RECENT ADVANCES  
IN DOPING ANALYSIS  
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## **BLOOD SAMPLING IN DOPING CONTROL - WHICH NEW INFORMATION MAY BE OBTAINED ?**

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As the control with traditional doping agents like stimulants and anabolic steroids has become more effective, athletes that want to dope seek for other means to improve performance. Doping control has traditionally relied primarily on detecting banned exogenous substances in urine samples, and therefore an obvious way to go is to apply endogenous substances that cannot be detected in such samples, at least by the methods applied today. Such doping methods include blood doping (intravenously infusing one's own or donated blood into an individual to induce erythrocytaemia) and the misuse of commercially available recombinant peptide hormones like erythropoietin (EPO) and growth hormone (GH) (1-3). This development has led many athletes, team physicians and sport administrators to urge for new methods applicable in doping control, including blood sampling (4).

The International Amateur Athletic Federation (IAAF) decided to include blood sampling in doping control during four World Cup meetings in 1993, to have an effective control against non-autologous blood doping and to gain experience from a wide variety of analyses aiming at detecting the application of GH, EPO and testosterone. In the present report, we present the results from analysing testosterone, haemoglobin and erythropoietin levels in blood samples from 51 athletes tested during these IAAF meetings.

### **Material and methods**

*Study subjects and reference population.* The athletes studied were those that were chosen for doping control during four IAAF meetings in 1993 (Oslo, Zurich, Berlin and Brussels). All samples were taken during doping control immediately after the sport event. Whole blood was

sampled in two 5 ml EDTA-tubes and one serum-tube (A sample) and in one additional 5-ml EDTA-tube (B-sample). The tubes were sealed and placed in sealed A and B containers, and transported to the Hormone Laboratory, Aker Hospital within 48 hours. Upon arrival, the A containers were opened, serum was isolated and frozen until analysis. The B samples were kept at 4° C for the case of an A sample indicating blood doping with donated blood.

Two control groups were used for comparison with the results obtained in the samples from the athletes 1) 110 male sport students willing to declare that they did not use any medication known to influence the parameters studied and 2) 227 healthy blood donors.

*Analytical methods.* Testing for non-autologous blood doping was performed immediately upon sample arrival at the Blood Bank, Ullevål Hospital. For the screening of blood samples by ABO-, Rh (CcDEe)-, K and Jk typing, the gel technique of DiaMed Inc. of Switzerland was applied. Antigen positive and negative cells are distinguished by their ability to pass through a gel which contains agglutinating antibodies to the actual antigen. For the screening by Fy and MNSs typing, the gel technique has proved less reliable for the detection of admixture of small amounts of allogenic blood. For these systems conventional blood grouping in glass vials with centrifugation and microscopic reading by two persons was applied. We measured haemoglobin levels in EDTA-blood.

Serum testosterone was measured with radioimmunoassay (RSL, ICN Biomedicals INC., Costa Mesca, CA, USA).

## **Results**

All athletes tested negative for non-autologous blood doping. Figure 1 shows the distribution of haemoglobin levels in athletes and controls. There was a skewness to the left in the distribution of values measured in the athletes, with a non-significant tendency to lower mean haemoglobin level in male athletes than in the controls.

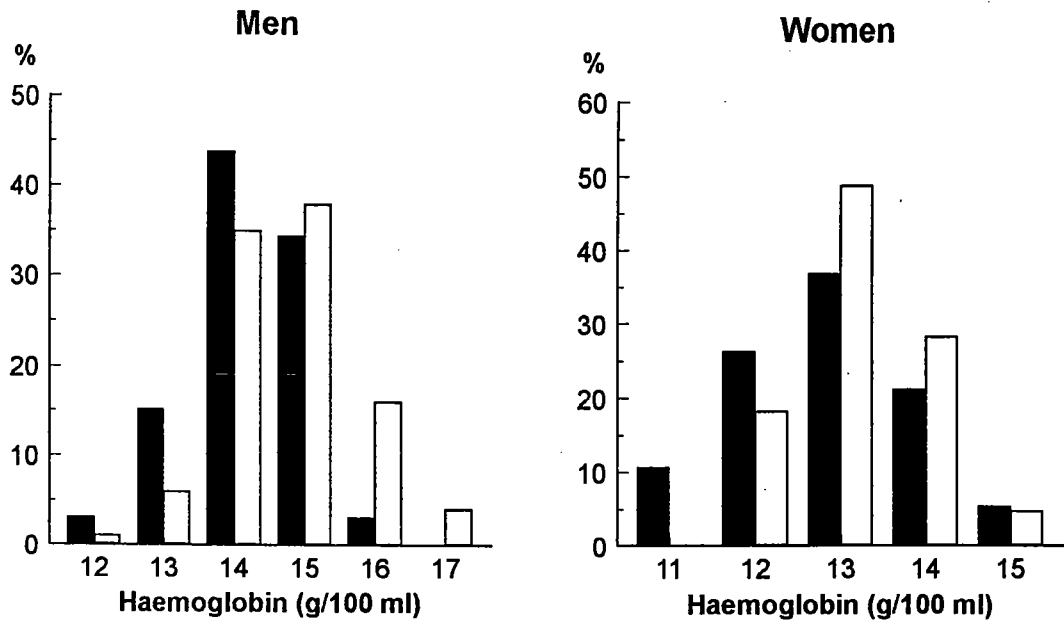


Fig.1. Distribution of haemoglobin levels in athletes (n=51) and controls (n=227)

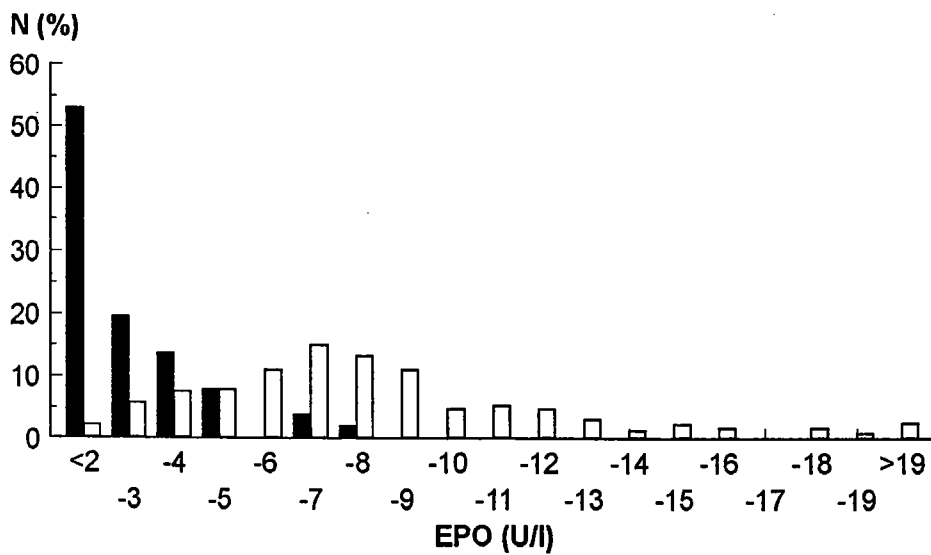


Figure 2. Distribution of erythropoietin levels in athletes (n=51) and controls (n=227)

Figure 2 shows the distribution of EPO in athletes and controls. The distribution was markedly skewed to the left in athletes, and 27 of 51 had EPO levels below the detection limit for the assay.

Figure 3 shows the distribution of serum testosterone in male athletes and controls. The distribution of testosterone values in men was highly skewed to the left as compared to the values obtained in the 110 healthy male sport students of similar age, and half of the athletes had values lower than the lower reference limit (8 nmol/l).

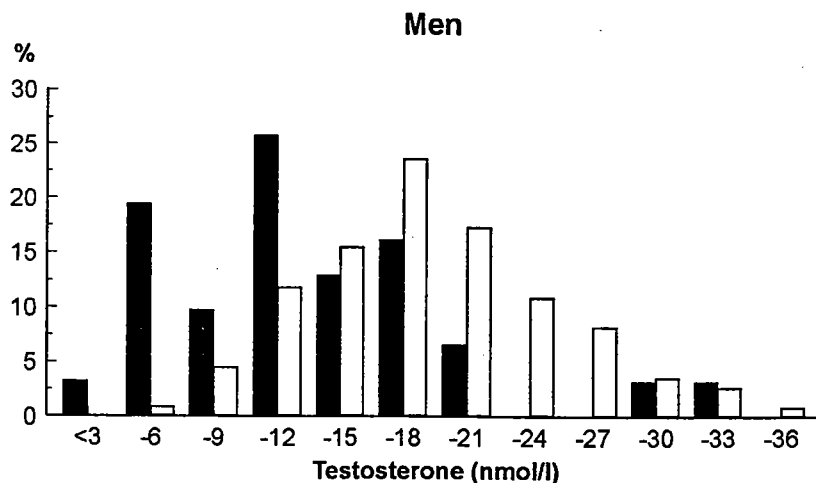


Figure 3. Distribution of s-testosterone levels in male athletes during competition (n=31) and in controls (n=110)

## Discussion

All athletes tested negative for non-autologous blood doping. The combination of antisera used were based upon a previous publication (5), and the expected frequency distribution of blood groups in a European population. However, athletes from all over the world participated in the events that were included in this study, and our findings indicated that the blood group systems that were chosen may not be optimal for detecting blood doping in non-European populations.

The haemoglobin concentration in athletes differed little from what was obtained in the reference population, but both male and female athletes tended to have lower haemoglobin levels than the control subjects. This was somewhat surprising, and should probably indicate that blood doping is not prevalent in athletics. It is accordance with previous reports on low levels of haemoglobin in well trained athletes, often called "sports anaemia", that may be caused by an increase in plasma volume. It may also be due to hemodilution in athletes following drinking after competition and prior to doping control.

As the half-life of EPO in blood after injection is only between 5 and 13 hours, simply measuring EPO concentration in athletes during doping tests is not a reliable method for detection. Almost forty per cent of the athletes had levels of EPO that were below the detection limit of our immunoradiometric assay. We speculate that one reason for these low EPO-levels might be that many of the athletes come from high altitude to participate in the event where the samples were taken, either due to temporary high altitude training or to permanent residence there. This would result in low levels of EPO for several days and probably weeks. However, one cannot rule out, that the suppression of EPO also might be due to artificially induced erythrocytaemia.

The marked lower testosterone values measured in the athletes as compared to controls, is probably due to the timing of the sample taking (8-12 p.m.) and the effect of strenuous exercise.

In conclusion, the analysis of blood samples taken during doping control indicates that blood doping or the misuse of recombinant EPO are not in widespread use by the athletes tested. However, the methods applied in this study may not be sensitive enough to disclose such misuse, and more sensitive and specific methods should be developed for future use.

## REFERENCES

1. Kicman AT, Cowan DA (1992) Peptide hormones and sport: Misuse and detection. *Br Med Bull* 48: 496-517
2. Segura J, de la Torre R, Badia R (1991) Potential use of peptide hormones in sport. In: Reidenberg MM (edt) *The clinical pharmacology of biotechnology products*. Elsevier Science Publishers, pp 257-274
3. Klein HG (1985) Blood transfusion and athletics. *N Engl J Med* 312: 854-856
4. Hemmersbach P, Birkeland KI (eds) *Blood samples in doping control*. On Demand Publishing, Oslo, 1994
5. Videman T, Sistonen P, Stray-Gundersen J, Lereim I. Experiences in blood doping testing at the 1989 World Cross-country Ski Championship in Lahti, Finland. Official Proceedings from the IInd IAF Wolrld Symposium on doping in sport. Monte Carlo, 1989.