**Athletes can avoid doping sanctions by using low dose rHuEPO regimens**

Elite sport is marred by the illicit use of recombinant human erythropoietin (rHuEPO), which some athletes use to boost red cell mass and thereby endurance performance.[1] The increase in performance imparted by rHuEPO use dwarfs the improvement an athlete could expect to achieve using permissible means (eg. altitude training), therefore to protect the notion of a 'level playing field' sport authorities have invested considerable effort over many years to deter athletes from using rHuEPO.

The 2000 Sydney Olympic Games marked the first occasion when authorities were able to detect the presence of rHuEPO,[2] and several dozen athletes have since been sanctioned for rHuEPO use. However in recent years there have been persistent rumours that callous drug cheats have learnt to evade detection by carefully titrating their dosage regimen in order to keep the percentage of basic isoforms indicative of rHuEPO use below the 80% threshold that must be exceeded in order to declare a sample positive.

It is vital for stakeholders to know whether existing deterrent strategies have been circumvented by cynical pharmacological intercession. Therefore we sought to simulate a so-called 'microdose' rHuEPO regimen to establish whether it was possible to titrate rHuEPO dosages to the point where an athlete could continue to reap the illicit benefit of doping with negligible risk of failing doping controls.

Two well-trained male subjects (28 yo, 74 kg, 176.5 cm, regional level triathlete; 31 yo, 62 kg, 170 cm, national level cross-country skier) gave informed consent to participate in the study which was reviewed and approved by the Regional Ethics Committee. Initially red cell production was rapidly accelerated in both subjects using high doses of rHuEPO (~260 IU/kg injections on days 0, 2, 4, 7, 9 and 11) in conjunction with a single intravenous iron treatment (100 mg), with the goal to elevate haematocrit (Hct) to approximately 50%. Over the next three weeks, injections were given every 2-3 days (injections on days 15, 17, 19, 22, 24, 26, 29, 31 and 33) and dosages were adjusted by a pharmacologist guided only by basic haematological information (blood and reticulocyte counts, no urine profiles were provided). Subject 1 was typically given 13.5 IU/kg injections, whilst subject 2 usually received smaller doses of 6.6 IU/kg. Urine samples were collected three times per day during the microdose phase (7-9h, 11-13h, 19-21h), and analysed for the presence of rHuEPO at the French national antidoping laboratory (Laboratoire National de Dépistage du Dopage, Paris).

As expected high dose rHuEPO treatment rapidly elevated Hct within a two week period (43.7% to 51.6%; 42.6% to 51.8%, subjects 1 and 2 respectively). We found that it was possible to maintain elevated Hct values using microdoses of rHuEPO. After three weeks of the microdose regimen Hcts were still 50.6% and 48.4% (51.5% and 48.1% one week after all injections ceased). During the microdose phase reticulocyte percentages ranged in value from 0.8-1.2% and 0.38-1.1% for the respective subjects.

Urine samples collected more than 24 hours after the most recent microdose injection typically fell below the threshold required to declare a sample positive for the presence of

rHuEPO (Figure 1). Certainly the window of detection was reduced to something less than two days. In some cases samples collected as little as 12-18 hours after a microdose of 6.6 IU/kg (subject 2) did not show a sufficient abundance of rHuEPO bands to have declared a positive result. It is noteworthy that our pharmacologist was able to quickly devise an effective microdose regimen utilising limited feedback and with few prior attempts.

Interestingly isoelectric profiles showed the re-appearance of natural erythropoietin during the microdose phase. This is in contrast to the existing paradigm which holds that natural erythropoietin production is suppressed when the red cell mass has been increased beyond the homeostatic set point. The implications of this remain unclear, however it can be speculated that were an athlete to receive microdoses of rHuEPO for an extended period (>2-4 weeks), it is conceivable that endogenous bands of erythropoietin would be of sufficient magnitude to further reduce the effective window of detection of the test for rHuEPO.

Our results show that it is possible for athletes to maintain illicit rHuEPO doping even during multiday endurance events when competitors are tested at the end of each day's competition (ie every 24 hours). Rogue athletes could similarly thwart out-of-competition testing by evading doping control officers for just a few hours immediately after receiving an rHuEPO injection. This study provides compelling evidence that antidoping authorities must revisit current testing strategies if they wish to eradicate rHuEPO doping in sport.

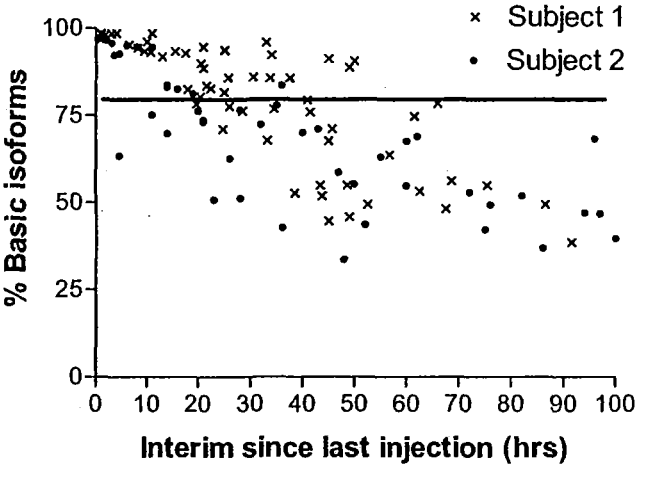


Figure 1. The percentage of basic isoforms in urine samples collected at various intervals after each of the nine injections of 6-20 IU/kg rHuEPO. Injections were given over a three week period during which time both subjects maintained an elevated haematocrit of approximately 50%. Antidoping regulations stipulate that samples are only considered positive for rHuEPO use if the percentage of basic isoforms exceeds 80% (as indicated by the horizontal line).

1. Ashenden M. Contemporary issues in the fight against blood doping in sport. Haematologica 2004;89:901-3.
2. Lasne F. Double-blotting: a solution to the problem of non-specific binding of secondary antibodies in immunoblotting procedures. Journal of Immunological Methods 2001;253:125-31.