EPO and blood doping – oxygen above all?

What is Erythropoietin or EPO and how does it work?

EPO is a hormone mainly produced in the kidney if there is a lack of oxygen (hypoxia) in the body. EPO keeps tissue oxygen delivery within a narrow range by controlling the number of the red blood cells, called erythrocytes, circulating in the blood. In a normal individual, any loss of red blood cells, such as by bleeding, decreases the delivery of oxygen to the tissues. This is a signal for the kidneys to secrete EPO into the blood, which is then carried to the bone marrow. More erythrocytes are produced which deliver more oxygen to the tissues. This increased oxygen delivery is now the signal to reduce the EPO production so that the normal steady-state number of red blood cells is maintained. Normally, an overproduction of erythrocytes does not occur even after the most severe loss.

What are the dangers of using EPO?

The use of EPO increases the number of circulating red cells in the blood. Depending on the dose used, the blood becomes more viscous (thicker), its flow capacities is reduced and the load on the heart increased. This, in combination with the exercise-induced slow heart rate and low blood pressure of endurance athletes, might lead to critical situations where the risk of blood clots is increased. In addition, further thickening of the blood might occur during heavy exercise at high temperatures when the body is dehydrated. Therefore, the misuse of EPO carries a clear risk for fatalities like stroke or heart attack.

Detecting rhEPO abuse in sport

Recombinant EPO (rhEPO) became available in Europe in 1987. Due to its ability to increase the oxygen transport of the blood, rhEPO has been used illicitly in endurance sports. Therefore, the IOC Medical Commission decided to ban this drug in 1990, even though all forms of blood doping had already been banned since 1984. Since then, different methods to detect this form of doping have been developed.

Direct methods

The direct detection of rhEPO in blood or urine has the advantage of identifying the drug itself, but also the disadvantage of being expensive, of low sensitivity and delicate to perform.
Endogenous which means produced within the body EPO and rhEPO are slightly different in their molecular structure. One direct test used the different charges of the sugar structures to separate the endogenous from the exogenous (produced outside the body) EPO forms. This technique is very reliable in urine and in blood as long as the samples are collected within 24 hours of the last rhEPO injection. Unfortunately, this method is limited. If the treatment was more than three days prior testing, only half, and after seven days none of the users can be identified.

![Figure 1: Anti-doping urine analysis demonstrating the presence of rhEPO in urine (see lane 4). 1. rhEPO standard. 2. Positive urine (control) 3. Negative urine (control) 4. Sample declared positive. 5. Darbopoietin.](image)

A novel test was published a few months before the Sydney Summer Olympic Games 2000. It separates the exogenous isoforms of rhEPO because they are less acidic than endogenous EPO. This test can also separate different types of rhEPO (see Figure 1).

**Indirect methods**

So called secondary blood markers to detect EPO abuse are reported to be able to determine rhEPO injections performed more than a week prior to testing. They also are supposed to detect all kinds of substances which stimulate the formation of blood. Furthermore,
it was hoped that secondary blood markers could eventually be used to detect athletes who have ceased using rhEPO or other stimulators of red blood cell production.

Despite these advantages, most of the tests have considerable shortcomings. The lack of sensitivity or specificity of some secondary markers encouraged scientists to put them together in a multiple-marker model. Different mathematical models were developed to identify sportsmen under rhEPO treatment (called the ON-model), and of those who took rhEPO in recent days (called the OFF-model). During the Sydney 2000 Olympic Games, the ON-model was used as a screening test to determine which urine samples had to be collected to perform the direct urinary test.

**Targeting abusers**

In the same year, the Laboratoire Suisse d’Analyse du Dopage (LAD) demonstrated that some of the secondary blood markers could be used as part of a screening test, but were not definitive for anti-doping purposes. Their blood screening test was based on the determination of the haematocrit, the haemoglobin and the reticulocyte (immature red blood cells) count and was introduced during the 2001 cycling season at the Tour des Flandres. Since then, more and more sport federations have introduced the screening test, because it quickly demonstrated its ability to detect rhEPO abusers. With time, it has been shown to be even more efficient in the follow-up of athletes. Variations above normal were excellent indicators of blood manipulation.

**Abnormal blood profiles**

In the 1970s, blood transfusion was a common practice for enhancing oxygen transport by increasing red blood cell mass. This method of doping virtually disappeared with the arrival of rhEPO on the market at the end of the 1980s because the hormone is much easier to store, use and is cheaper.

The launch of the direct detection measures of rhEPO in urine samples has resulted in an unwanted side-effect: a return to the “ancient” method of blood transfusion. The regular follow-up of blood parameters showed that some athletes demonstrated abnormal values although rhEPO could not be detected in their urine.

In summer 2004, the LAD introduced new blood doping tests in blood that were able to prove whether these abnormal blood parameters were due to blood transfusions. Federations that have introduced blood testing now have a powerful tool to follow all athletes potentially abusing rhEPO or blood transfusion. This assumes that these federations focus their tests on those
demonstrating abnormal blood profiles. This targeting aid also enables them to determine the prevalence of these doping methods before any validated test is on the market.

Conclusion

It is likely that all cases of blood doping and rhEPO abuse will be identified in the near future. The necessity to take blood samples in order to screen and test for this type of doping has become obvious for sports authorities, but there is still a need to improve the targeting of blood manipulation. The regular follow-up of certain blood parameters is certainly one of the solutions. New biochemical investigations should lead to improved direct detection of this method of doping, too.