Hemoglobin, Erythropoietin and Doping testing

Ola Ronsen MD, PhD
Norwegian Olympic Sports Center
Box 4004, Ullevaal Stadion
0806 Oslo, Norway

Outline

1. Hemoglobin and erythropoietin
2. Pre-race hemoglobin testing
3. Doping control: responsibilities and rights
4. Summary
5. Suggested readings
1. Hemoglobin tests, Erythropoietin and Doping testing

Hemoglobin and erythropoietin

Hemoglobin (Hgb) is a tetramer of four molecules, with the purpose of binding oxygen in the high oxygen environment of the lung and releasing oxygen in the low oxygen environment of the working tissues. Mature red blood cells are almost totally made up of hemoglobin. The total amount of hemoglobin in the circulation is one of the limits to the transport of oxygen to the tissues during heavy exercise. In other words, all other things being equal, increasing circulating hemoglobin will result in improved oxygen delivery to working muscles and subsequently increased oxygen uptake. Thus, a supra-normal hemoglobin concentration is beneficial to performance in endurance sports like cross country skiing. This has been demonstrated in studies where total blood volume and concentrations of Hgb have been artificially increased by blood transfusions or injections of recombinant erythropoietin (rhEPO) in well-trained individuals. Consequently, these methods are considered unethical and have been made illegal in all sports.

Erythropoietin (EPO) is a hormone synthesized in the kidney that stimulates stem cells in the bone marrow to multiply and differentiate toward a red cell line (i.e. reticulocytes and erythrocytes). Under the continued influence of EPO, stem cells mature to the stage of reticulocytes and are then released into the circulation as they gradually mature to red blood cells (erythrocytes). Erythrocytes live for approximately 100 days before they are cleared from the circulation by the reticulo-endothelial system found in the liver and spleen. Under normal sea level conditions, about 1% of red cells are destroyed each day, but the same
number of reticulocytes and new erythrocytes emerge from the bone marrow to replace the old red cells.

Under conditions of hypoxia (i.e. lower than normal number of oxygen molecules in a given volume of air), the kidneys is stimulated to increase its production of EPO, which in turn causes an increased rate of production of reticulocytes in the bone marrow. As the degree of hypoxia increases, the amount of EPO synthesized and released increases in exponential fashion. If hypoxic stimulation is maintained for two to three weeks or more, the total number of red blood cells in the circulation will increase, and thus improving oxygen transport. Provided that total plasma volume is kept constant, this will result in a higher concentration of hemoglobin when measured from an antecubital vein. However, total plasma volume is not constant. It is influenced by hydration status, prior exercise, body position during blood drawing, how long a tourniquet has been applied, and in particular by altitude changes. During exposure to altitude there is an early, rapid reduction in total plasma volume (see fig 2). As the acclimatization process proceeds, it is partly restored -- but not completely to normal levels. Then, after return to sea level, a further increase in plasma volume occurs, often to higher levels than before the altitude camp. If this netto increase in total plasma volume is equal to the increase in the number of circulating Hgb molecules (and red blood cells), the Hgb concentration (grams of Hgb per liter blood) will be unchanged from before to after altitude camp. In other words, if expansion of total plasma volume equals the increase in Hgb content in the blood, the Hgb concentration will stay the same. Nevertheless, a positive effect on performance would be expected since both increased total plasma volume and total number of Hgb molecules/ red blood cells are both positive factors for increased work capacity.
In the late 1980’s rhEPO was developed for treatment of anemia (i.e. low Hgb concentration) caused by kidney failure. Recombinant erythropoietin proved to be very effective in raising the Hgb level in these patients. However, it was soon discovered that rhEPO’s ability to increase Hgb concentrations in anemic patients could also be used for improving Hgb concentrations --and ultimately performance-- in endurance athletes. The molecular structure of rhEPO is almost identical to the natural EPO hormone and leaves the body rapidly -- a half time of 16 hours after subcutaneous administration. Moreover, if used properly, the effects of the drug would last three to four weeks, while the actual drug will have left the body completely within one week of the last injection. This leaves a two to three weeks "open window" where performance is enhanced and there is no drug present. Standard doping control procedures are based on detecting the banned drug in the body of the athlete, thus detection of this substance has been very difficult under the present doping control procedures.

A protocol to deter and detect the use of rhEPO was tested out in the 2000 Olympic Games in Sydney, Australia. It consisted of both a blood and a urine test. In addition to the Hgb level, the blood test indicates the presence of accelerated erythropoiesis, normal erythropoiesis or inhibited erythropoiesis. The blood test does this by measuring the size and number of reticulocytes, the level of EPO in blood and the level of soluble transferrin receptor (sTr) in blood. The urine test can differentiate between rhEPO and natural EPO because rhEPO is made in cell-cultures that use different sugars compared to natural EPO from human kidney cells. Since rhEPO and natural EPO is coated with different sugars on their surface, the molecules have different electric charges and can therefore be detected separately.
Pre-race hemoglobin testing

Since reliable methods for direct detection of rhEPO in athletes was difficult to establish, an alternative approach of testing for the effect of the drug, (i.e. high concentrations of Hgb) was pursued by the International Ski Federation (FIS). Before the 1996/1997-ski season, FIS became the first international federation to introduce Hgb limits for athletes entering a competition. This was done after having measured Hgb-concentrations in blood collected during regular post race doping test over a 7-year period from 1989-1996. These data showed gradually increasing mean Hgb-values in both male and female cross-country skiers, but the increasing extreme values found in a few of the skiers raised the most concern and suspicion. Since several incidents of sudden death in cyclists with supra-physiological red cell mass and hemoglobin values had been documented during the same period, actions had to be taken in order to prevent the same development in cross-country skiing.

While recognizing that they were not able to detect all use of rhEPO, FIS was now able to limit the extent of the cheating and also therefore the medical risk involved. These risks include intravascular thrombosis, which can lead to the potentially fatal complications of strokes, heart attacks and pulmonary emboli. In 1996 the first limits were set at 185 g/l in men and 165 g/l in women based on population norms plus 3 standard deviations. However, as of the season 2000/2001, the limits have been lowered to 175 g/l and 160 g/l for men and women, respectively. The two main reasons for introducing Hgb limits for race participation were: 1) to protect the health of the athlete and 2) to minimize the extent of unfair advantage gained by blood doping methods.

BOX:
FIS/IOC actions to prevent and detect blood doping
1. 1989: FIS decision to take post race blood test for measurements of Hgb and detection of heterologous blood transfusions
2. 1996: FIS decision to take pre-race Hgb measurements and exclude men with Hgb>185 g/l and women with Hgb>165 g/l from participation in the race
3. 2000: FIS decision to reduce the pre-race limits of Hgb for men to 175 g/l and for women to 160 g/l
4. 2000: IOC decision to introduce a urine test for rhEPO and a modified blood test for Hgb, reticulocytes and transferrin receptors in the Sidney Olympics

As with any testing procedure, knowledge was gained as the pre-race Hgb test was used and subsequent refinements were made. The initial problems concerning athlete selection criteria, timing of the announcements, proper facilities for testing and standardization of the procedures for blood drawing and Hgb analysis have been taken care of. Today, Hgb levels are sampled by random selection of athletes in the two hours prior to the race. They are measured from a venous blood draw and analysed on a Hemocue photometer in the presence of the athlete. There has been some controversy over the limits for Hgb concentrations used by FIS. The first limits of 185 g/l and 165 g/l were relatively high Hgb concentrations for endurance athletes, thus possibly leaving "room for cheating" below the Hgb concentration limits. However, after lowering the limits to 175 g/l for men and 160 g/l for women, there may be a problem of excluding athletes with genetically high levels of Hgb, particularly if they are training and competing at altitudes of 1500-2000 m. Another concern regarding Hgb testing has been the conditions under which the blood is drawn. Total plasma volume, as mentioned above, is not completely stable and a change in plasma volume will alter the Hgb concentration in the blood. If the athlete recently ascended to altitude has had an infection, is particularly nervous or aggravated, or if the athlete is not seated or laid down properly before
the blood test, the measured hemoglobin concentration may be artificially high. Also, if an unethical team doctor recognizes that one athlete have too high Hgb concentrations prior to the official testing, it is possible to ”dilute” the hemoglobin concentration by intravenous administering of saline. To avoid any problems with the pre-race Hgb testing the athlete should be properly advised about correct preparations and procedures as suggested in the text box

_____________________________________________________

BOX

Advice to the athlete undergoing hemoglobin testing:

1) Be well hydrated several hours prior to testing.
2) Be well rested physically and mentally.
3) Bring your own physician or other personell familiar with the testing.
4) Remove any tight fitting clothing around the arm for venepuncture.
5) If possible, lay down for 5 minutes before your blood is drawn.
6) Make sure that an adequate sample (3-5mls) is collected in a purple top tube.
7) If the first value is abnormal, make the staff repeat the assay from the same tube.
8) Have the staff recalibrate the Hemocue in front of you.
9) Rest in a supine position for 10 minutes before the next sample.
10) Always have your own medical staff present for the second test.

_____________________________________________________

Recently a protocol to deter and detect the use of rhEPO was tested out in the 2000 Olympic Games in Sydney, Australia. It consisted of both a blood and a urine test. In addition to the Hgb level, the blood test indicates the presence of accelerated erythropoiesis, normal erythropoiesis or inhibited erythropoiesis. The blood test does this by measuring the size and
number of reticulocytes, the level of EPO in blood and the level of soluble transferrin receptor (sTr) in blood. The urine test can differentiate between rhEPO and natural EPO because rhEPO is made in cell-cultures that use different sugars compared to natural EPO from human kidney cells. Since rhEPO and natural EPO is coated with different sugars on their surface, the molecules have different electric charges and can therefore be detected separately.

Doping control: responsibilities and rights

It must be emphasized that all relevant information from FIS and National sports federations about all aspects of doping -- i.e. medications and methods used to artificially increase performance -- should be given to the athletes and coaching staff by the team physician. This includes the procedures for regular doping control and the responsibilities of the athlete to perform this control in a correct way. Signing the initial notification and appearing at the doping control office within the time limit of 1 h is very important. The athlete must also be informed that if refusing to provide a sample -- no matter possible disputes over the procedures of the control – it is considered equal to a positive test. However, the athletes should also be informed about their rights during the doping control, including correct ID’s of the doping officials, provision of facilities with proper privacy, approved and sealed equipment for the samples, etc. It is not possible to give a detailed description of how a regular doping control is supposed to be carried out, but the athlete and accompanying person should pay close attention to the numbers on the A and B urine or blood containers. Review all the information that the officials are putting down on the doping control form and be careful to list all medications and nutritional supplements that have been used during the last week prior to the testing. New athletes on the team should always have a knowledgeable physician accompanying them to international doping controls. If possible, all other athletes
should also be accompanied by a health staff member to ensure that all procedures are being carried out correctly. There may be some discrepancies between rules of national ski federations, FIS, and IOC, regarding the regulation of some types of medications as well as some of the procedures/equipment used in the post race doping control. Thus, it is important both for the team physician and the athletes to be aware of such differences and know which competitions are regulated by which organization.

The athletes should be taught to check with the team physician before any medication or supplement, prescribed or over-the-counter, is taken. No athlete should carry medication across national borders without approval and knowledge of the team physician. Finally, it is important to stress the fact that ultimately, it is the athletes that are responsible for whatever they chose to take either as medications or nutritional supplements. The athletes should know all the relevant doping rules and regulation in their sport and abide by them.
Summary

1. Hemoglobin (Hgb) is a molecule situated on the red blood cells that binds oxygen in the lung and releases it to the working tissues

2. Erythropoietin (EPO) is a hormone synthesized in the kidney that stimulates stem cells in the bone marrow to multiply and differentiate into reticulocytes and erythrocytes

3. Under continuous hypoxia at altitude, production of EPO is elevated, which results in increased number of red blood cells and thus Hgb molecules in the circulation

4. Since reliable methods for direct detection of rhEPO in athletes was difficult to establish, an alternative approach of testing for the effect of the drug, (i.e. high concentrations of Hgb) was pursued by the International Ski Federation (FIS).

5. The two main reasons for introducing Hgb limits for race participation were: 1) to protect the health of the athlete and 2) to minimize the extent of unfair advantage gained by blood doping methods

6. As of the season 2000/2001, the upper limits for participation in FIS Nordic races have been set at a Hgb concentration of 175 g/l for men and 160 g/l for women

7. To avoid problems with the pre-race Hgb testing the athlete should be properly advised about correct preparations and procedures for the testing (see text box)

8. Relevant information from FIS and National sports federations about all aspects of doping -- i.e. medications and methods used to artificially increase performance -- should be given to the athletes and coaching staff by the team physician

9. All new athletes on the team should be accompanied by an experienced team physician to international doping controls.

10. The athlete, nevertheless, should know the responsibilities and the rights connected to the procedures of FIS doping controls
11. Ultimately, it is the athlete that is responsible for the intake of all medications and nutritional supplements. The athletes should know all the relevant doping rules and regulation in their sport and abide by them.
**Suggested readings:**


With increasing altitude, the oxygen content in the air is reduced (hypoxia) and subsequently the oxygen concentration (PO$_2$) of the blood decreases to below normal (hypoxemia). This lower than normal PO$_2$ is sensed by the kidney and erythropoietin (EPO) producing cells increases their secretion of EPO into the blood circulation. When EPO reaches the bone marrow, it stimulates the production of reticulocytes (immature red blood cells without hemoglobin molecules) and their maturation into hemoglobin-containing and oxygen-carrying erythrocytes, a process called erythropoiesis. After 8-12 days these cells (1-2% as reticulocytes and 98-99% as erythrocytes) exit the bone marrow and start to appear in the general blood circulation.

At sea level a male cross-country skier may have ca 5,5 l of blood circulating through his arteries, veins and heart (= total blood volume). The blood is made up of many red blood cells (RBC), a few white blood cells, and plasma (liquid of proteins, fatty acids and sugars). RBC makes up ca 45 % and plasma ca 55% of the total blood volume. After 30-40 days of high altitude (ca 3000m) exposure, the increased output of new red blood cells from the bone marrow will result in elevated numbers of RBC in the circulation. The increased number of RBC will also draw more liquid to the circulation (by osmosis) and increase the plasma content. Both of this changes contribute to an increased total blood volume of 6,0 L, which contributes to improved the blood supply to working muscle, -- a valuable compensation for the low oxygen content at high altitudes.

People that live permanently at low, medium or high altitudes (1000-5000m altitude) have increasingly elevated number of red blood cells and thereby hemoglobin concentrations.
is a natural compensatory change because of the decreasing oxygen availability at increasing altitudes. Athletes that live and train at these altitudes will reach acclimatization after 10-12 weeks stay, but their work capacity will still not be the same as at sea level. At altitude above ca 5000-6000 m the blood becomes so saturated with red blood cells (“thick soup”) that risk of cardiovascular insults starts increasing drastically.

Doping controls serves the dual purpose of deterring athletes from getting involved in illegal performance enhancing activities as well as detecting ”cheaters”. It is important to teach the athlete how proper routines should be carried out, both on the behalf of the controllers and the athlete. This will reduce the risk of procedure problems with the samples, --that be blood or urine.