

Nordic Winter Sports, Biological Passport (ABP) and the Legal Challenges

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1. Introduction

Why do athletes blood dope? From a physiological point of view we need to consider which factors limit the endurance exercise capacity. During whole body endurance exercise, performance is limited by the amount of oxygen delivered to the working muscles. The oxygen delivered, is a function of the capacity of the heart to pump blood out into the systemic circulation (cardiac output) and the amount of oxygen in the blood leaving the heart, the arterial blood. Oxygen in the arterial blood is mainly chemically bound to hemoglobin, a molecule contained in the red blood cell, the erythrocyte. The hemoglobin concentration in the blood depends on the total amount of hemoglobin relative to the liquid part of the blood, the blood plasma. An increase in the hemoglobin concentration results in an increase in the amount of oxygen delivered to the working muscles. As long as sufficient amounts of oxygen are available to the muscles, the accumulation of fatigue-inducing metabolites will be postponed.

A recent review by Schmidt and Prommer (2010) nicely illustrated the good correlation between the hemoglobin concentration and the maximal oxygen consumption ($VO_2\max$). Since there is a relatively good correlation between the $VO_2\max$ and performance in endurance exercise, $VO_2\max$ could be used as an index of performance. In the article it was shown that an acute manipulation of the hemoglobin concentration either by blood withdrawal (decrease in hemoglobin) or recombinant erythropoietin administration (increase in hemoglobin) changed the $VO_2\max$ accordingly. They also calculated that a change in hemoglobin mass, the total amount of hemoglobin circulating in the body, by 100 g will result in a change in $VO_2\max$ of 400 mL. Based on these data one could speculate, whether athletes have higher hemoglobin values compared to sedentary people or less trained individuals. The answer is “yes” and “no”. In a recent publication from Jelkmann and Lundby (2011), the hematocrit (relative amount of erythrocytes in the blood) and hemoglobin mass was compared in four different groups of subjects: (1) Moderately trained individuals, (2) trained runners, (3) highly trained cyclists and (4) elite cross-country skiers. It was shown that there was no difference in hematocrit between those groups (Jelkmann/Lundby 2011). Actually, there was a tendency to lower blood values in the most well trained group. Nevertheless, the hemoglobin mass was significantly larger in the well-trained subjects compared to the others.

The physiological explanation has been addressed by Michael Sawka in 2000 (Sawka et al. 2000). In his review, results from different training studies were graphed and changes in blood volume, plasma volume and erythrocyte volume plotted against time. It was evident that the initiation of a training program results in an almost immediate increase in plasma and a delayed increase in erythrocyte volume. These changes result in an enlarged blood volume and since the relative increase in plasma volume exceeds the increase in erythrocyte volume, a hemodilution will occur with a resultant decrease in hematocrit and hemoglobin concentration. Therefore, from a physiological and hematological perspective, elite athletes are considered to have normal or even slightly decreased hematocrits/hemoglobin concentrations.

2. Doping Testing by the International Skiing Federation (FIS)

Although blood manipulation has spread during recent years to different types of sports, it is still considered mainly to take place in sport activities relying on a large oxygen consumption. With cross-country skiing being the most demanding, the likelihood of blood manipulation in elite cross-country skiers is considerable. The first experimental study conducted on skiers showing performance enhancements of blood transfusions, were performed in 1987 by a Swedish research group, who found a 5% decrease in time to ski a given distance three as well as fourteen days after the reinfusion of autologous blood (Berglund/Hemmingson 1987). The publication of these results has probably increased the likelihood of blood manipulation in cross-country skiing.

Therefore, the Medical Committee of FIS decided to start testing for heterologous blood doping in 1988, and in 1989 the first samples were collected during the World Championships. By taking blood tests and storing the results, FIS was also able to follow changes in blood values over time on an individual basis, and could thereby detect suspicious deviations in blood values. In 1989, the mean hemoglobin concentration in male skiers was 8 grams/deciliter (g/dL) lower than in a normal reference population (Videman et al. 2000). This is in accordance with the physiological response to exercise training described previously.

The same year a recombinant form of erythropoietin (rhEPO) was approved by the U.S. Food and Drug Administration (FDA). This potent erythropoiesis-stimulating hormone soon became the preferred doping agent in many sports. An indication of the massive abuse of EPO in cross-country skiing is evident by looking at the mean blood values over time in elite skiers. Considering mean values in a large cohort of subject, one would anticipate unchanged levels over time, since these are genetically determined and relatively stable. Nevertheless, the data showed a marked increase in the mean hemoglobin

concentration after 1989, and at the World Championships in 1995 all medal winners had hemoglobin concentrations above 17.5 g/dL, with some individuals having levels of 20 g/dL (Videman et al. 2000). Since no detection method for rhEPO had been developed at that time, other strategies were introduced to reduce the rhEPO abuse. In 1996 a “no start” rule was implemented. If skiers had hemoglobin concentration above the mean + three standard deviations, they were not allowed to compete. This “no start” rule was modified in 1997, so males were not allowed to compete, if they had values above 18.5 g/dL and females 16.5 g/dL. Nevertheless in 1999, approximately 30 male skiers had still values between 17.0 and 18.7 g/dL, with medal winners showing levels above 17.0 g/dL. Although, it seemed that these new thresholds had had an impact, athletes were still doping.

This was confirmed at the World Championships in Lahti, Finland, in 2001. Six Finnish skiers, the so-called “Lahti Six”, were tested positive for hydroxy ethyl starch (HES). HES is a plasma volume expander used to expand the plasma volume. The resulting dilution of blood decreases the hemoglobin concentration/hematocrit. A test for HES was unknown to the public before the championships, and since the test was secretly implemented before the event, it was possible to catch six athletes.

An interesting article about the blood values of the skiers participating in the championship was subsequently published (Stray-Gundersen et al. 2003). In the article, the blood values were linked to the specific ranking of skiers in nine different races. From the data, it became evident that the highest ranked skiers also had the most abnormal blood values, defined as either high or low reticulocyte value, high hemoglobin concentrations and high levels of free hemoglobin.

Based on the scandal of the “Lahti Six” and the abnormal blood values, FIS chose to intensify their testing program. In addition, a test for rhEPO was developed at the same time (Lasne 2000). The test was based on isoelectric focusing (IEF) separation of the EPO isoforms. The intensification in testing and a test for rhEPO resulted in a significant decrease in the mean hemoglobin concentration by approximately 1 g/dL (Mørkeberg et al. 2009). Furthermore, changes in the relative amount of reticulocytes (% reticulocytes) decreased from values indicating supra-physiological bone marrow stimulation, till markedly decreased bone marrow stimulation. The latter occurs when the circulating amount of hemoglobin exceeds normal physiological levels. Then production of erythrocytes decreases through a negative feedback mechanism. It seemed like the skiers had decreased their doping habits or at least modified them.

The EPO test developed in 2000 was able to detect the two types of recombinant EPO present at the time: Alpha and beta. In 2001 a new analogue

was developed: Darbepoetin alpha. This type differed in glycosylation pattern from alpha and beta, with a far more acidic isoelectric point (pI) value than epoetin alpha and beta. It was unknown whether this molecule could be differentiated from endogenous EPO with the IEF method.

Before the Winter Olympic Games in Salt Lake City in 2002 research was conducted to test the trace ability of darbepoetin alpha. It was shown that darbepoetin alpha was easily differentiated from endogenous EPO due to its hyperglycosylated molecular structure compared to endogenous EPO (Catlin et al. 2002). Nevertheless, again the results were kept secret to the public. It was therefore very surprising when FIS announced that three high-profile skiers had been tested positive for darbepoetin alpha during the Olympic Games, since no one knew that a test had been implemented. It was high-profile skiers. They had won nine medals during the games and a total of 38 medals in previous Olympics and World Championships. The blood profile of one of the convicted athletes also showed large deviations in hemoglobin concentration and reticulocyte values before the Games supporting the positive urine sample.

3. Doping in Skiing Continues

In order to harmonize the test method for rhEPO, the World Anti-Doping Agency (WADA) together with a group of experts in the field, wrote a technical document (TD) describing the analysis procedures and the positivity criteria. The positivity criteria were described for the identification of the three different kinds of rhEPOs present at that time (epoetin alpha, epoetin beta and darbepoetin alpha). Since then, pharmacological development has afforded the appearance of several new EPO analogues. In a publication from 2009 by Macdougall and Ashenden, it was stated that several new rhEPO products were in development and becoming licensed in Europe, and that in other parts of the world cheaper-production "copy" epoetins were produced (Macdougall/Ashenden 2009). It was estimated that up to 80 such products were sold in countries with less stringent regulatory control of pharmaceutical products. Since the biochemical production process of such rhEPOs differs from each other, the molecular structure of the product also differs. Since the positivity criteria in the WADA TD is based on rhEPO molecules with a certain molecular structure it is highly unlikely that such new rhEPO products fulfill the positivity criteria. In that case, athletes could inject "copycat EPOs" without being tested positive. But since the analysis results from copycat EPO would differ in molecular structure from results obtained with endogenous EPO only, one would know that rhEPO had been used, but it would not be possible to pose a sanction based on the current criteria. By scrutinizing test results from skiers with abnormal blood profiles, it became evident that several athletes had IEF pro-

files that were non-consistent with normal endogenous EPO production, but at the same time non-consistent with the use of epoetin alpha, beta or darbepoetin alpha. Copycat EPO abuse was taking place in skiing.

Therefore, the positivity criteria had to be modified to suit these new challenges. This was done in 2009 and a paragraph in the WADA's new technical document described how profiles non-consistent with endogenous profiles and which did not fulfill the strict criteria defining conventional rhEPOs could be due to the presence of "biosimilars". The new criteria therefore made it possible to detect copycat EPOs.

4. The Biological Passport

Also in 2009, WADA's Executive Committee approved The Biological Passport Operating Guidelines. The principle behind the Athlete Biological Passport (ABP) is based on the monitoring of selected biological variables, which indirectly reveal the effects of doping, as opposed to the traditional direct detection of a prohibited substance. In the evaluation of the biological data, a relatively complex algorithm is used. In this algorithm, not only the athlete's own results are used but also data from a large reference population. The impact of these reference data diminishes as the number of samples from the athlete of interest increases. Due to the structure of the statistical algorithm it treats athletes with genetically different blood values differently. I.e. a person with a genetically high hemoglobin concentration will have a more "suspicious" profile than a person with a normal hemoglobin concentration, when the first blood sample is taken. This is because a high hemoglobin concentration deviates more from the mean hemoglobin concentration of the reference population. Nevertheless, the biological passport concept is much more comprehensive than a statistical algorithm. What the algorithm does is to determine the abnormality of a blood profile. An abnormal profile in itself is not enough to sanction an athlete. First, a panel of experts must evaluate the results. Their role is to exclude the possibility that an abnormal profile could be due to other factors than doping. If the expert panel excludes that other potential factors can explain the profile, the athlete then has the possibility to explain himself in front of the panel. If the explanation is dismissed, the federation has the right to sanction the athlete.

5. Sensitivity of the Blood Passport

Different scientific articles have evaluated the sensitivity of the passport algorithm in "detecting" different kinds of blood manipulation. In one study, the algorithm was able to flag 13% of samples from subjects transfused with one

bag of autologous blood during a period of four weeks after the transfusion (Mørkeberg et al. 2011). In this study, the time points of sample collection were scheduled beforehand and therefore did not replicate a real testing scenario where intelligent target testing today is of considerable importance. When tests are “targeted”, previous sample results are used to collect new samples at time points where the athlete is most likely to dope. Target testing was used in another study by Pottgiesser (Pottgiesser et al. 2011). In this study the sensitivity was higher compared to the previous study, thereby emphasizing the importance of targeting the testing.

In general, it must be recognized that the sensitivity of the passport depends on the magnitude of manipulation/dosage. A higher dosage will induce a larger disturbance in the biological system and therefore change the biological markers to a larger degree than when using smaller dosages. It must be emphasized that the performance-enhancing effects and the health risks probably also will be of a lower magnitude with micro-dosages compared to when standard pharmaceutical dosages are used.

It is true that the biological passport is not perfect in detecting all kinds and degrees of manipulation, but it serves its role by increasing the sensitivity of detection and reduces the performance enhancement and health risks associated with doping.

6. References

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