

Detection of Autologous Blood Transfusions in Cross-Country Skiers

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Abstract

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Transfusion of autologous blood (blood doping) has been used by athletes to improve performance in sports events. This practice has been banned by the International Ski Federation (FIS) and the International Olympic Committee (IOC). So far, no reliable method for detection of blood doping has been available. In the present study, a group of six elite cross-country skiers, who were phlebotomized and retransfused with 1350 ml of blood 4 weeks later, was compared with a control group ($n = 7$) in whom no blood doping was performed. The blood was stored at $+4^{\circ}\text{C}$ for 4 weeks. Hemoglobin increased by 7.9% from the prephlebotomy level and by 14% from the preinfusion level. The reinfusion of blood caused a 60% reduction ($P < 0.001$) in serum erythropoietin in 24 h and a sharp increase ($P < 0.05$) in serum iron and bilirubin after a test race performed on the day of reinfusion. It is therefore concluded that a combination of measurements of hemoglobin and bilirubin, iron, and erythropoietin in serum could detect 50% of the blood-doped athletes by a single test sample during the 1st week after reinfusion. If two test samples were used, an increase in Hb of more than 5%, and a decrease in serum erythropoietin by more than 50%, would be discussed in 50% of the blood-doped athletes throughout the first 2 weeks after reinfusion and without implicating any of the controls.

Key words: blood doping, blood boosting, hemoglobin, hemolysis, iron, bilirubin, erythropoietin, physical exercise

Introduction

The intent of autologous reinfusion of blood ("blood doping" or "blood boosting") is to increase maximal oxygen uptake by increasing the oxygen transport capacity of the blood thereby improving performance. Provided the Hb volume is sufficiently increased by this procedure, performance in endurance events can be significantly improved (1, 7). This procedure has been banned by the International Ski Federation (FIS) and International Olympic Committee (IOC) despite the fact that so far no analytical method has been devised for unequivocal detection. In the present study, autologous reinfusion of blood was performed after storage in a refrigerator for 1 month. The purpose was to compare a group of blood-doped skiers with a control group of non-doped skiers in connection with reinfusion and physical exercise to look for reliable methods of disclosing blood doping.

Material and Methods

Test Subjects

Twelve male and one female well-trained cross-country skiers, aged 19-35 years, participated in the study. They trained approx. 500 h/year and were just below the national elite. Six of the males were blood-doped and the other subjects served as controls. The skiers consumed the same type of mixed diet and continued their ordinary training program throughout the study.

Blood Letting and Reinfusion

Six of the subjects let blood on two occasions with 1-week interval. Two units (900 ml) were withdrawn the first and one unit (450 ml) the second time. Immediately after the first phlebotomy, the subjects were given an infusion of physiologic saline to minimize symptoms of hypovolemia. The blood was stored in a refrigerator ($+4^{\circ}\text{C}$) until reinfusion 4 weeks after the last phlebotomy (8). The control group took part in a "blind" procedure with blood-letting and immediate reinfusion on the first two occasions and slow reinfusion of physiologic saline on the third occasion. By mistake, one unit of blood was let on the first occasion in two control subjects. For both groups, all procedures were performed with blindfolds, thus making the study "single blind" and minimizing the influence of psychological factors. From the first phlebotomy until 2 days before reinfusion, all participants were given an oral iron preparation (Fe-sulphate 100 mg 2 times daily).

Test Race

A test race was performed on an approximately 15-km race course (race time 45-50 min) 3 h after reinfusion of blood. (For more details see ref. 1.)

Sampling

Blood samples for analysis were taken before blood-letting, immediately before and immediately after reinfusion of blood, approx. 5 h after reinfusion (= approx. 1 h after a test race), 1 day, 2 days, 1 week, 2 weeks, and 4 weeks after reinfusion. All blood samples from the 1st day after reinfusion and thereafter were taken in a fasting state after a night's rest.

Hematological Variables

Hemoglobin concentration (Hb) and erythrocyte volume fraction (EVF) were analyzed in a Hemolog (12, 14).

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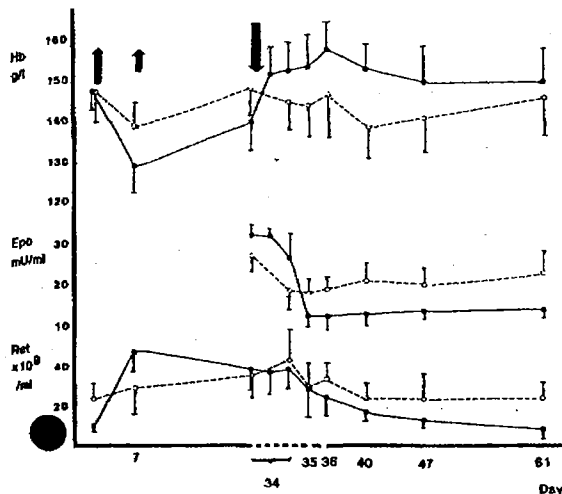


Fig. 1 Time course of Hb, erythropoietin, and reticulocytes in the blood-doped group (solid line) following the removal and reinfusion of 1350 ml autologous blood and in a control group (dotted line). † indicates phlebotomy, ‡ indicates reinfusion of blood.

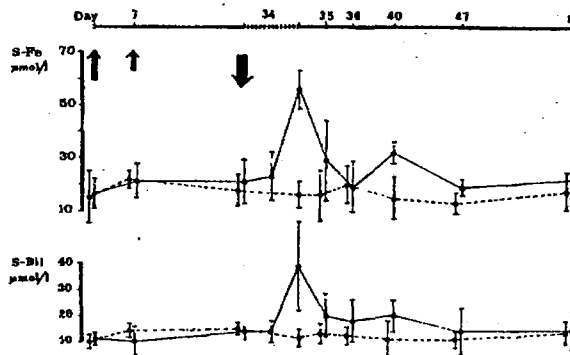


Fig. 2 Time course of s-bilirubin and s-Fe in the blood-doped group (solid line) following the removal and reinfusion of 1350 ml of autologous blood and in a control group (dotted line). † indicates phlebotomy and ‡ indicates reinfusion of blood.

Serum levels of iron (Fe) (9) and bilirubin (bil) (15) were analyzed with an autoanalyzer. Reticulocytes, after staining, were counted manually in a chamber.

Serum Erythropoietin

Serum concentrations of erythropoietin (Epo) were measured by a radioimmunoassay method according to principles described by Birgegard et al. (2). Since then, further improvement has been achieved with the help of an antiserum with higher avidity which can be used in the assay in a dilution of $1:10^6$. A correlation with the polycythemic mouse assay was very close ($r = 0.94$). The lower limit of detection for diluted standard was 4 mU/ml, and the standard curve was linear over the range from 5 to 150 mU/ml. The sensitivity and the specificity in the low range was improved compared with our previous assay, and a population of 26 normal, healthy individuals showed a mean of 12.6 mU/ml \pm 3.95 (SD). The 2nd International Reference Preparation (IRP) for Epo was used as a standard. Pure Epo provided by the National Heart, Lung and Blood Institute was used for labeling. S-Epo was normally distributed in patients and controls.

Results

There was no significant difference in Hb levels in the two groups before phlebotomy (147 ± 7.6 and 148.4 ± 5.1 g/l mean \pm SD, respectively). In the test group, Hb decreased to a nadir of 128.8 ± 8.3 g/l after the second phlebotomy and still had not reached the prephlebotomy level at the time of reinfusion 4 weeks later when the mean Hb was 139.8 ± 8.0 g/l. In the control group, the Hb level was essentially the same initially and on the date of the test

race (Fig. 1). In the blood-doped group, the maximal Hb and EVF was reached 2 days after the reinfusion (158.6 ± 5.3 g/l and $47.0\% \pm 1.4\%$, mean \pm SD, respectively). The increase in Hb and EVF as compared with the prephlebotomy value was 7.9% and 5.9%, respectively.

S-Fe and s-bil both increased significantly ($P < 0.05$) in the blood-doped subjects on the day of reinfusion from 21.3 ± 9.2 $\mu\text{mol/l}$ and 14.3 ± 4.0 $\mu\text{mol/l}$ (mean \pm SD) to a maximum 5 h after reinfusion of 56 ± 7.4 $\mu\text{mol/l}$ and 39.5 $\mu\text{mol/l}$, respectively. These latter values of s-Fe and s-bil were also significantly higher ($P < 0.05$) than those in the control group (15.7 ± 4.8 $\mu\text{mol/l}$ and 11.5 $\mu\text{mol/l}$, respectively) on the same occasion. From 1 day after the reinfusion and onward, no significant differences were detected in these variables (Fig. 2).

Epo levels were significantly higher in the test group than in a normal population (31.1 ± 3.1 mU/ml vs 12.6 ± 3.95 mU/ml; $P < 0.001$). Five hours after reinfusion (immediately after the test race), s-Epo was 26 ± 5.2 mU/ml, which is significantly lower than before, and 24 h after reinfusion, the s-Epo had decreased by 60% to 12.2 ± 2.6 mU/ml ($P < 0.001$) and stayed at this level for the rest of the study (4 weeks) (Fig. 1).

The control group also had a significantly higher mean Epo level than the normal population, 26 ± 3.7 mU/ml ($P < 0.001$). This decreased after the test race to 18 ± 4.5 mU/ml ($P < 0.005$), the level it stayed at for the following 4 weeks, which was still significantly higher than in the blood-doped group ($P < 0.001$). Reticulocytes increased after phlebotomy from a mean of 10×10^9 /l to about 40×10^9 /l, but fell after reinfusion to the initial level after about 1 week. In the control group, there was no significant change in reticulocyte levels.

Table 1 Number of subjects who on different occasions throughout the study fulfilled the following criteria: hemoglobin (Hb) > 120 g/l, s-Fe > 33 μ mol/l and/or s-bilirubin (bil) > 30 μ mol/l and s-erythropoietin (Epo) < 15 mU/ml.

		Before reinfusion	Immediately after race	after reinfusion				
				1 day	2 days	1 week	2 weeks	4 weeks
Hb > 120 g/l	BD C		All subjects					
S-Fe > 33 μ mol/l and/or S-bil > 30 μ mol/l	BD C	0 0	6/6 0	3/6 0	2/6 0	3/6 0	1/6 0	1/6 0
S-Epo < 15 mU/ml	BD C	0 0	0 1/7	6/6 1/7	6/6 1/7	6/6 2/7	5/6 1/7	6/6 1/7
No of subjects fulfilling all criteria	BD C	0 0	0 0	3/6 0	2/6 0	3/6 0	1/6 0	1/6 0

BD = blood-doped group; C = control group.

Table 2 Number of subjects who on different occasions throughout the study, in comparison with control values, had an increase in Hb > 5% and a decrease in s-erythropoietin (Epo) > 50%.

Difference in comparison with prephlebotomy (Hb) and preinfusion (Epo) values		Immediately after race	after reinfusion				
			1 day	2 days	1 week	2 weeks	4 weeks
Hb > 5%	BD C	3/6 1/7	3/6 1/7	4/6 1/7	3/6 0	3/6 0	2/6 0
Epo < -50%	BD C	0 1/7	6/6 1/7	6/6 0	5/6 0	6/6 1/7	5/6 0
No of subjects fulfilling both criteria	BD C	0 0	3/6 0	4/6 0	3/6 0	3/6 0	2/6 0

BD = blood-doped group; C = control group.

Based on one test sample only, an algorithm comprising a combination of a Hb above the lower normal limit (Hb > 120 g/l), s-Fe > 33 μ mol/l and/or s-bil > 30 μ mol/l and s-Epo < 15 mU/ml were fulfilled by 3/6 (50%) blood-doped subjects and none of the controls throughout the 1st week after reinfusion (Table 1).

With a "two-test sample" procedure, an algorithm based on changes in Hb and Epo could be constructed. An increase in Hb > 5% and decrease of Epo > 50% between the tests were fulfilled by 3/6 (50%) and 2/6 (33%) blood-doped subjects throughout the first 2 weeks and 4 weeks, respectively. None of the controls were so implicated (Table 2).

Discussion

Previous studies have shown that an increase in Hb or EVF of > 5% is necessary to improve performance (7). In our study, Hb and EVF increased in comparison with prephlebotomy values and reached a maximum 2 days after reinfusion (7.9% and 5.9%, respectively). Two weeks after the reinfusion, Hb was still increased by 2.3% whereas EVF

was essentially the same as prephlebotomy. The blood doping in our study was "effective" and resulted in improvement of performance in the blood-doped group during at least the first 2 weeks (1).

It is well established that refrigerator storing of blood leads to a constant decline in red blood cells (RBC) due to the limited life span of the erythrocytes (10). Thus, there is a progressive breakdown of RBC prior to reinfusion, and additional RBC will break up shortly after reinfusion due to increased fragility.

The pronounced increase in s-Fe and s-bil in the blood-doped group occurred only after the test race (test sample taken 5 h after reinfusion). There was no significant difference before and after the reinfusion of blood alone. This would seem to indicate that the exercise per se caused hemolysis. However, there was no increase in these variables in the control group. Therefore, it must be assumed that the increase in s-Fe and s-bil was connected with the reinfused blood. Possibly a fairly large number of RBC were fragile due to storage damage and were destroyed during the first hours in the circulation. Since we have no control group

who rested after reinfusion, the role of exercise per se cannot be determined. The turnover of plasma iron is rapid and approximately 10 times the plasma pool each 24 h (5), and the capacity of the liver to take up and metabolize bilirubin is considerable. It is therefore not surprising that we can find no significant difference between the two groups for these variables the day after reinfusion.

Regulation of RBC formation is mediated through the glycoprotein hormone erythropoietin produced by the kidneys (4). Anemia and anoxia causes an increase in Epo production (3), whereas polycythemia vera (2) and hypertransfusion (6) depresses it. The biological Epo assay using hypertransfused mice is based on a down-regulation of Epo production with hypertransfusion. It was therefore suspected that blood doping could affect s-Epo levels. With the use of a sensitive radioimmunoassay, we found that blood doping reduced the s-Epo levels by 62%, to the same level as the mean for a normal population, 1 day after hypertransfusion.

The fact that the skiers were still slightly anemic compared with their initial Hb levels probably made this decrease more evident. Interestingly, even the control group had a significantly higher Epo throughout the study than a normal population ($P < 0.001$), and a significant decrease ($P < 0.01$) was seen after the test race in both groups. After the doping and the test race, the Epo level remained essentially the same for 4 weeks, the transfused group significantly lower than the controls ($P < 0.001$).

From this study, we conclude that blood doping causes a marked and long-standing reduction in circulating Epo within 24 h. Whether the Epo levels are actually subnormal cannot be determined since at the present time no Epo assay can differentiate between normal and subnormal Epo levels in untreated serum samples. Whether well-trained athletes generally have increased levels of s-Epo needs a larger population than this control group to answer. However, it is quite possible that the increased rate of destruction shown in athletes (11, 13) lowers the Hb levels enough to cause a slight increase in Epo levels. The decrease in Epo after the race in the control group is just barely statistically significant and the group is small ($n = 7$). This finding needs further investigation.

Thus, the present study shows that blood doping with 1350 ml of autologous blood, refrigerator-stored for 4 weeks, causes an increase in Hb levels, a reduction of Epo levels, and increased levels of s-fe and s-bil.

With regard to the detection of blood doping in an individual, several factors must be taken into consideration. Well-trained cross-country skiers may have slightly elevated levels of s-Fe and s-bil without blood doping. In a hitherto unpublished study, we found that 5 of 44 elite skiers had s-Fe above 33 $\mu\text{mol/l}$ and/or s-bil above 30 $\mu\text{mol/l}$, and previous investigators have also found evidence for an accelerated RBC destruction in athletes (11, 13). Furthermore, an increase in s-fe or s-bil occurs in several diseases, some of them compatible with high performance capacity. For these reasons, an increase in s-Fe and/or s-bil alone are not reliable indicators of blood doping.

Even if a fairly substantial increase in Hb level is necessary to improve performance, all our blood-doped skiers had Hb and EVF levels within the commonly accepted normal range. An increase in Hb level alone above the normal range is therefore not a usable criterion for blood doping, especially since Hb may be increased by disease: primary and secondary polycythemia.

A low or normal s-Epo level alone does not indicate blood doping. A marked decrease between two samples is suggestive of blood transfusion, but the slight reduction after exercise in the control group needs further study. Therefore, a combination of tests is necessary. It is possible to design an algorithm based on the combination of a normal Hb, signs of hemolysis, and a low s-Epo that detects about 50% of the blood-doped individuals with a single sample during the 1st week after the race. None of the controls was false positive with this algorithm. In connection with certain sports events, it may be possible to obtain more than one blood sample. Detection of changes in the pertinent variables between two samples (increase in Hb, s-Fe, and s-bil, decrease in s-Epo) strongly indicated blood doping. Our study indicates that an increase in Hb $> 5\%$ and a decrease in s-Epo $> 50\%$ as criteria for blood doping would detect 50%–67% of doped subjects throughout the first 2 weeks after reinfusion without implicating any of the controls.

By this combination of variables, the following diseases can be ruled out. Hemolytic blood disorders that could elevate s-Fe and s-bil give a lowering of Hb and elevation of s-Epo; secondary polycythemia (i.e., due to the hypoxia of high altitude) is associated with a high s-Epo and has no hemolysis; polycythemia vera (PCV) gives both a high Hb and a low s-Epo but no hemolysis. PCV also gives a stable and low s-Epo, but if there is a marked decrease between two test samples, it can be ruled out with even greater certainty.

In conclusion, no single blood test has been found that can detect blood doping with any certainty. However, if a sample taken in the morning the day after a race shows a combination of a Hb in the high upper normal range, a s-Fe $> 33 \mu\text{mol/l}$ and/or s-bil $> 30 \mu\text{mol/l}$ and a s-Epo $< 15 \text{ mU/ml}$, this will detect about 50% of blood-doped athletes. If two serum samples can be obtained, their fulfillment of one of the tests of the algorithm above, or a difference between the two tests of +5% in Hb and -50% in Epo, seems most convincing.

It must be emphasized that a sensitive and reliable RIA for Epo with a well-documented normal range must be used. Furthermore, we want to point out that the algorithm used above applied to this group of athletes and the method of blood doping using refrigerated blood. Further studies are needed in other groups of athletes and with other blood doping methods before blood tests in connection with sports events can be used unequivocally to connect an athlete to blood doping.

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