Clin Chem Lab Med 2006;44(12):1395–1402 © 2006 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2006.262

Opinion Paper

Blood transfusions in athletes. Old dogmas, new tricks

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Abstract

Blood doping consists of any illicit means used to increase and optimize oxygen delivery to the muscles and includes blood transfusions, administration of erythropoiesis-stimulating substances, blood substitutes, natural or artificial altitude facilities, and innovative gene therapies. The use of blood transfusion, an extremely straightforward, practical and effective means of increasing an athlete's red blood-cell supply in advance of competition, became rather popular in the 1970s, but it has suddenly declined following the widespread use of recombinant human erythropoietin among elite endurance athletes. Most recently, following implementation of reliable tests to screen for erythropoiesis-stimulating substances, blood transfusions have made a strong resurgence, as attested by several positive doping tests. Doping by blood transfusion can be classified as homologous, where the blood is infused into someone other than the donor, and autologous, where the blood donor and transfusion recipient are the same. The former case produces more clinically relevant side effects, but is easily detectable using current antidoping protocols based on erythrocyte phenotyping by flow cytometry and, eventually, erythrocyte genotyping by DNA testing. Since the donor and recipient blood are identical in autologous blood doping, this is less risky, though much more challenging to detect. Indirect strategies, relying on significant deviations from individual hematological profiles following autologous blood donation and reinfusion, are currently being investigated. For the time being, the storage of athletes' blood samples to allow testing and sanctioning of guilty athletes once a definitive test has been introduced may represent a reliable deterrent policy.
Clin Chem Lab Med 2006;44:1395–402.

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Keywords: antidoping testing; blood doping; blood transfusion; hemoglobin; sports.

Background

Ergogenic aids are not new to athletes. Owing to the fame, honor and economic benefits arising from success in competitions, there is rather a long history of cheating in sports (1). Since the early 1960s it has been recognized that any means to increase and optimize oxygen delivery to the muscles would profoundly enhance an individual's athletic performance, especially for demanding physical exercise. Basically, this process, also known as induced erythrocythemia, blood boosting or, more recently, blood doping, consists of techniques administered to the athlete to ''increase one's red blood cell mass, which allows the body to transport more oxygen to muscles and therefore increase stamina and performance'' (2). Erythrocythemia induced by the infusion of 400–920 mL of packed red blood cells (RBCs) effectively enhances performance capacity in endurance track races, probably due to an increase in oxygen delivery to the working muscles (3, 4). Reinfusion of 750 mL of RBCs substantially increases maximum oxygen consumption, although any performance improvement is not apparently associated with observed changes in the hematological profile (5). It is noteworthy that the maximum oxygen increase per gram change in hemoglobin is fairly comparable following either reinfusion of blood or recombinant human erythropoietin injections and persists for several weeks post-reinfusion, regardless of the return to baseline of the main hematological values (6, 7). Increased performance for 3 h to 14 days following reinfusion of 1350 mL of autologous blood is also evident in cross-country skiers, based on improved race time over a specified distance (8). Significant improvements in treadmill endurance tests have also been reported following induced erythrocythemia (6, 9). Apparently, blood transfusions also work for anaerobic sports, as the extra proton-buffering capacity of infused hemoglobin raises the lactate threshold and contributes to lower lactate generation during submaximal exercise (10). Hence, the great potential to improve athletic performance by blood transfusion is well-established (7).

Originally, blood transfusions were not intended to support athletes in competition, but instead to treat patients with severe forms of acute and chronic anemia. However, they also serve for the former purpose, as clear evidence attests that each method for boosting blood oxygen-carrying capacity improves the

endurance performance (2). There are two main strategies for doping by blood transfusion: autologous, whereby the blood donor and transfusion recipient are the same person, and homologous (allogeneic), whereby blood is transfused into someone other than the donor. The traditional procedure for autologous blood transfusion involves the withdrawal of 1–4 units of blood (1 unit = 450 mL of blood, corresponding to 225 mL of concentrated RBCs at a packed cell volume of 0.75) several weeks before competition to allow re-establishment of the RBC mass to the baseline level. The blood is immediately centrifuged, the plasma components are reinfused and the corpuscular elements (principally RBCs) are refrigerated at 4°C or frozen at –80°C. Stored erythrocytes are then traditionally reinfused into the athlete 1–7 days before a major endurance event (11).

The facts

There is a long history of blood doping, conventionally originating with the anecdote of athletes being encouraged to drink reindeer blood to achieve extraordinary performances (12). Although the earliest proof of improved sport performances after blood transfusions was provided in 1947, the first evidence of blood doping came later, in 1972, when a controlled experiment clearly showed a considerable increase in performance of athletes undergoing autologous transfusion of packed RBCs from an earlier venesection (13). Since then, there are consistent records, extensively reviewed by Leigh-Smith, of athletes experimenting with blood transfusions who achieved incredible success in competitions (11). Besides the first anecdotal reports, this technique became fairly popular during the 1980s and was widely used by distance runners, cyclists, and skiers, particularly during the 1980 and 1984 Olympics. Although no reliable test had been devised for unequivocal detection, the International Olympic Committee (IOC) officially banned blood doping after the 1984 Olympics. In the same year, the USA Olympic Committee declared that seven cyclists, including four medallists, out of 24 athletes of the national team who participated in the Olympic Games, used transfusions (14). Since the mid-1980s, several other techniques for enhancing blood oxygen-carrying capacity were developed, including various erythropoiesis-stimulating substances (recombinant erythropoietin and analogues), blood substitutes, natural or artificial altitude facilities, and innovative gene therapies and molecules targeted to enhance the endogenous erythropoietic response (2). Regardless of their biological and side effects, some of these techniques were banned, although others are still licit. From a practical perspective, exogenous erythropoietin administration became fairly popular among professional endurance athletes owing to several advantages over blood transfusions, which include no logistically challenging practices (blood withdrawal, storage, and reinfusion), no decay in performance or training after a period of blood withdrawal, and limited detection within antidoping controls (15). Years later, following the implementation of reliable strategies for detecting doping with recombinant erythropoietin and analogues, blood transfusions, which had fallen out of favor, made a strong comeback. In March 2002 at the Salt Lake City Olympics, the IOC investigated the discovery of discarded blood transfusion equipment at the quarters of the Austrian cross-country skiers. Following DNA testing, two Nordic skiers (who had been placed in the 40s, and not the Austrian team's three medallists) were disqualified and had their results cancelled (11). For the same reason, some professional cyclists, one of whom nearly died after being injected with poorly stored blood, were found guilty and suspended in 2004 (12).

In a recent investigation of samples obtained as part of routine International Ski Federation blood-testing procedures in participants at the World Ski Championships, abnormal hematological profiles, defined as those deviating from the 1989 Nordic Ski World Championships and the IOC Erythropoietin 2000 project data set, were identified in 36% of the skiers tested and finishing within the top 50 places in the competitions. In addition, 50% of medal winners and 33% of those finishing from 4th to 10th place had highly abnormal hematological profiles. In contrast, only 3% of skiers finishing from 41st to 50th place had highly abnormal values (16). Although these data cannot be immediately associated with blood doping practices, including blood transfusions, and it is very unlikely that blood doping would be less common in other endurance sports, the present situation is highly suggestive of a phenomenon that is not being controlled by the ongoing antidoping testing program. In fact, it has been hypothesized that a combination of blood transfusion and recombinant human erythropoietin administration could also be used by such athletes. The drawing of blood for appropriate storage induces natural stimulation of the bone marrow by a complex mechanism that involves the hypoxia inducible factor (HIF) pathway. At the same time, administration of recombinant human erythropoietin at low, subtherapeutic doses increases bone marrow production. Recombinant human erythropoietin can be subcutaneously administered several weeks before competition or during resting periods, allowing the collection of up to 3–5 units of blood in a month, with minimal changes in hemoglobin concentrations, as clearly demonstrated by preoperative medicine. Blood reinfusion then increases the hemoglobin concentration, which is already raised by the exogenous hormone. At the time of competition, athletes finally show up with increased hemoglobin, but virtually undetectable erythropoietin in urine using the official electrofocusing technique (17). The success of the old technique of blood transfusion in hemodoping is also linked to the disappointing results of new technologies for inducing bone marrow to release RBC cohorts and increase hemoglobin. The intermittent hypobaric hypoxia induced by specific facilities such as tents

and rooms to simulate altitudes of up to 5500 m is an effective artificial technique to boost erythropoiesis, and is even more effective than natural exposure to the hypoxic environment of high altitudes (>2800 m). This technique increases erythropoietin serum concentrations in trained athletes, although the main hematological parameters do not significantly differ from those of similarly trained athletes exposed to natural hypobaric hypoxia (18). The phenomenon of neocytolysis regulates sudden changes in erythropoietin concentrations in the body, inducing apoptosis of young RBCs (19). The reintroduction of old, but still efficient RBCs, cannot substantially influence the downregulation of RBC mass when it is excessive. Hence, neocytolysis can explain some of the potential pitfalls of autologous transfusion when the hemoglobin increase is lower than expected and serum bilirubin concomitantly increases. This may be due not to improper storage of the blood (20), but rather to excessive introduction of RBCs, stimulating a downregulation of erythrocyte release.

The very recent suspension of several professional road cyclists from the 2006 Tour de France could represent the tip of the iceberg, with more than 200 athletes in different sports disciplines implicated in an international doping probe including blood transfusions and exogenous hormone administration (21). In an apartment building in Madrid occupied by a doctor, Spanish police discovered clandestine equipment for international performance enhancement, seizing more than 200 450-mL blood bags, along with records and several other doping substances, which allowed investigators to finally match code names of athletes with their highly detailed doping records. This sophisticated pan-European doping ring either treated athletes locally or arranged the transport of stored blood through a system of couriers to athletes at race sites (22). Hence, based on the riders named in this one investigation, the problem is endemic and requires an urgent solution. The question arises as to how many identical hidden organizations are still operating worldwide.

Testing strategies

The ramifications of doping are not limited to top-class athletes who may feel compelled to risk their health for fame and money, but also extend to amateur athletes eager to exhibit superiority in the athletic field. Owing to difficulties in actually proving the intent to cheat, the World Anti-Doping Agency (WADA) enforces a principle of strict liability for positive test results for banned substances. Antidoping laws generally exist to provide a safe and fair environment for participation in sports. They encompass a broad, continuously updated panel of laboratory tests for the most recent list of banned substances, which includes traditional as well as promising new drugs and techniques that may find actual applications in doping athletes (23). Blood testing in athletes was originally introduced for medical reasons and to limit the misuse of drugs and other banned substances employed to artificially enhance the oxygen-carrying capacity of blood (24). Originally, the only means to test for doping by blood transfusion was the adoption of arbitrary thresholds for hematocrit and/or hemoglobin. Blood doping practices were suspected when blood tests showed hemoglobin values exceeding 175 g/L for men and 155 g/L for women (International Ski Federation), and hematocrit values above 0.50 for men and 0.47 for women, with reticulocytes >2% (International Cycling Union) (25). Athletes with random values exceeding such limits were prevented from racing in official competitions. Nevertheless, such a questionable strategy involved several drawbacks, including the difficult interpretation of several hematological parameters because of wide inter-individual variability, the possible occurrence of false-positive results that would have penalized clean athletes with naturally increased values, and the possibility to arbitrarily expand or titrate the RBC mass up to the allowable threshold (16). These pitfalls have persuaded the scientific community to develop more suitable and sophisticated strategies. A reliable plan to test for homologous blood transfusions was implemented for the 2004 Summer Olympic Games in Athens and the WADA is funding research projects to develop a test for autologous transfusions.

Screening for homologous blood transfusions

The plasma membrane of RBCs expresses a wide series of blood group antigens that are actually complex oligosaccharides that differ mainly in their biochemical structure. In addition to the traditional AB antigen cluster, RBCs are characterized by the rhesus (Rh) polypeptides and the associated glycoprotein RhAG. This blood group system is the most polymorphic of the human blood groups, including at least 45 independent antigens (26). Owing to the complex combination of AB and Rh antigens on the RBC surface, hematopoiesis in the bone marrow produces erythrocytes characterized by a genetically determined, virtually unique antigen pattern. The antidoping method recently implemented by WADA is based on the quantification of antigenically distinct donor and recipient erythrocytes by flow cytometry, using standard blood-bank antisera combined with a fluorescent-labeled secondary antibody directed against human immunoglobulin. This strategy allows the detection of small populations (<5%) of cells that are antigenically distinct from an individual's own RBCs with a degree of sensitivity, provided there is at least one antigen mismatch between the donor and recipient (27, 28). The test is based on phenotyping for several RBC antigens, including C, c, E, e, K, k, Fya, Fyb, Jka, Jkb, S and s. The chance of detecting two identical samples by this screening strategy is reportedly less than 1:500. Hence, flow cytometry appears

to be a suitable approach for detecting mixed antigenic populations in patients transfused with at least one unit of homologous blood. In contrast to doping with recombinant human erythropoietin, the window of detection is not a major drawback. Transfused erythrocytes remain in the circulation for up to 120 days in sedentary individuals and for 60–90 days in elite athletes, because RBCs typically display shorter survival in physically active individuals. However, this test may fail to detect doping in individuals who are blood group chimeras, RBCs coated with molecules such as immunoglobulins, and transfusions with major antigen-matched blood, as the RBC-surface antigen patterns of the donor and recipient may be indistinguishable (29).

The identification of a second RBC population in persons who are blood group chimeras may indicate either a transient (homologous RBC transfusion) or static (life-long chimera) phenomenon. In such a case, serial testing easily allows discrimination between these two situations (30). In other cases, enlargement of the antigen panel by Rh50 glycoprotein, CD47, glycophorin B, Duffy, LW glycoprotein, and Band 3 testing may represent a reliable solution (31). Since the molecular basis of RBC antigens has become clear during the last decade, genotyping is now widespread in transfusion and laboratory medicine. Therefore, an alternative strategy that may soon become faster and cheaper is to screen blood for these antigens using genomic DNA and the associated single-nucleotide polymorphisms (SNPs). These techniques, which have already found practical applications in other branches of clinical medicine such as feto-maternal incompatibility, autoimmune anemias, organ transplantation and criminology (29), would allow the clear distinction of homologous donor from recipient blood. Many genetic tests based on real-time PCR technology, such as PCR-RFLP, SSP-PCR and Taqman-PCR, have been proposed for evaluating RHCE and RHD patterns, showing a high concordance rate with classic serologic assays (32, 33). In addition, high-throughput techniques are being developed to genotype the whole donor cohort for all clinically relevant RBC antigens, including D, C/c, E, S/s, K/k, Kp(a/b), Fy(a/b), FY0 (-33 promoter silencing polymorphism), Jk(a/b), Di(a/b) and human PLT antigen (HPA)-1a/1b (34). Innovative and less labor-intensive microarray technologies based on bead array tests may also be suitable for the antidoping context, as they have the potential to genotype hundreds of samples per day, providing results within 36 h from the receipt of the specimens (35, 36). Nevertheless, DNA analysis displays limited diagnostic efficiency in populations of athletes characterized by a higher prevalence of non-expressed RHD. In addition, it is important to mention that PCR-based assays are prone to different types of errors than those observed with the traditional phenotyping techniques, such as contamination with amplified products and the identification of particular antigen genotypes that are not necessarily expressed on the RBC membrane (37).

Screening for autologous blood transfusions

Unfortunately, the current antidoping armamentarium for detecting autologous blood transfusions is much more limited and relies principally on indirect testing strategies based on the identification of significant deviations from the individual hematological profile following either blood donation or reinfusion.

Homeostatic volume replacement can allow the safe collection of twice the normal amount of RBCs in a standard donation. Studies in a small number of donors have demonstrated that a temporary decrease in RBC mass is well tolerated when donors give twice the usual amount (170–225 mL) of erythrocytes in a standard 405–495-mL donation, causing no detectable symptoms of reduced oxygen-carrying capacity, as confirmed by negligible changes in heart rate or systolic and diastolic blood pressure (38). Nevertheless, some physiologic adjustments of the hematopoietic response to the acutely reduced RBC mass can be recorded. These include a typical erythropoietin response (serum erythropoietin increases four-fold within 1 day, declining exponentially thereafter), changes in erythrocyte and hemoglobin synthesis (reticulocyte count increases rapidly by 2.4-fold after 7 days, remaining elevated for another 7 days, whereas hemoglobin values remain reduced on average by ~15% for 2 weeks) and changes in iron metabolism (serum ferritin sharply decreases, while the soluble transferrin receptor concentration increases by 60% by day 14). Blood donation of 450 mL from healthy blood donors also leads to an increase in hypochromic reticulocytes from day 1 or 2, peaking on day 9 and reaching a maximum increase of 178%. Hypochromic erythrocytes and soluble transferrin receptor increase within the same period, whilst transferrin saturation decreases. The reticulocyte count increases substantially from the first day, with a maximum increment of 55% (38–40). Reinfusion of 1350 mL of blood generally leads to hemoglobin increases of 8% from the pre-phlebotomy level and of 14% from the pre-infusion level, with simultaneous reduction in serum erythropoietin in 24 h and a sharp increase in serum iron and bilirubin (41). Blood reinfusion also significantly increases both hemoglobin and ferritin concentrations by up to 18% and 68%, respectively. Such changes are associated with a stable and marked decline, approaching 50% of the baseline concentration, of serum erythropoietin (20). Levels of reticulocytes and soluble transferrin receptor also decline progressively from day 7 to day 21 following transfusion (40).

Owing to high individuality, the use of absolute reference ranges for hematological parameters is not really useful for monitoring athletes. However, as the analytical and intra-individual biological variability of most hematological parameters are both contained, the definition of a type of ''hematological passport'' would allow a longitudinal comparison of data for individual patients, accomplished with major transferability among clinical and antidoping laboratories

(42). So far, this appears suitable for detecting a variety of blood doping practices, and would also be acceptable in practice, considering that the individuality index of most hematological parameters (CV_i/CV_g, ratio between intra- and inter-individual biological variability) is always <0.6 (43). The current availability of fully automated hematological systems can easily provide the traditional parameters of the hematological profile, along with a wide range of additional parameters for reticulocytes and erythrocytes, increasing the potential use of laboratory testing in clinical and sports medicine (44). Repeated evaluation over a period of time of several of these parameters, including hemoglobin, hematocrit, reticulocyte count and indexes, would define a highly specific hematological profile, which is supposed to remain relatively stable over time (45). Thus, samples collected during competition for routine blood screens could provide a cost-effective and convenient source of passport data (46). At least five sequential determinations should be obtained to define a reliable subject-specific reference range; substantial variations from the baseline, or any value exceeding the allowable variation, could highlight either pathologies or unfair practices, in both cases providing a good reason for an athlete's withdrawal from competition (47). Same major drawbacks of this approach, including collecting samples at altitude (48), sample manipulation or the use of plasma expanders (49) and the type of instrument used to measure reticulocytes (50), can be prevented by implementation of standardized analytical protocols, exclusion of values obtained from samples collected at altitude, specific instrument calibration (50, 51), complementary analyses, and doping tests (49).

As accelerated and inhibited erythropoiesis both lead to characteristic changes in several blood parameters, which are supposed to be fairly stable in healthy individuals, it has been supposed that the indirect WADA strategy to test for recombinant human erythropoietin administration would also be useful in screening for autologous blood transfusions. A third-generation approach for this method, which is based on mathematical models or discriminating functions, including several biochemical and hematological markers that predict the administration or withdrawal of erythropoiesis-stimulating substances, has recently been proposed (47). The diagnostic efficiency appears a major advantage of these multiple-variable models, whereby the influence of single-parameter abnormalities is limited, thus decreasing the chance that lifestyle changes or pathologies could produce false-positive results or veil a true positive case. These models originally incorporated combinations of the variables reticulocyte hematocrit, serum erythropoietin, soluble transferrin receptor, hematocrit and percentage macrocytes. The ON model (''current-use model'') is effective in identifying recent use of erythropoiesis-stimulating practices, whereas the OFF model (''recently discontinued model'') reflects depressed erythropoiesis (high hematocrit, low reticulocyte hematocrit and low serum erythropoietin), with diagnostic efficiency for up to several weeks afterwards. The latest version of the mathematical model, which now includes the variance of the parameter hemoglobin, represents an important improvement over the previous algorithm (47). The outputs of this original approach based on thresholds is not excessively influenced by potential variations of laboratory parameters due to chronic exercise (training regimens, environmental habits), even in the presence of abnormal results. Nevertheless, application of these models to detect blood transfusion in athletes has not been validated so far. The recent investigation of Damsgaard et al. (40) demonstrates that the highest OFF-model score during the polycytemic period following an autologous blood transfusion corresponds to a 1:1000 cutoff threshold. In practice, none of the subjects who had undergone an autologous blood transfusion showed a positive OFF-score, nor hemoglobin values higher than the traditional WADA threshold of 170 g/L, making this model virtually ineffective for detecting recently transfused subjects. On the other hand, although evidence indicates that the within-subject biological and seasonal hematocrit variations show a maximal relative change of 15% in sedentary men and 10% in elite athletes, all subjects undergoing autologous blood transfusion exceed this ''normal'' variation, displaying hemoglobin increases ranging from 19% to 39%. Even when the mean hemoglobin concentration is taken as the control value, 90% of subjects exceed the individual upper limit based on a 7.5% addition to the control value (40), an arbitrary limit obtained from the critical hemoglobin difference calculated for the highest biological variability published (52). Notably, changes in other hematological biomarkers (serum erythropoietin, reticulocytes and soluble transferrin receptor) can be recorded at all times, suggesting that the determination of these parameters could be used as supportive evidence of erythropoietic manipulation, with acute hemoglobin increases exceeding 7.5%. Within the limitations of the study, hemoglobin variations exceeding 15% between samples obtained in top-ranked endurance athletes during the anticipated anemic period and shortly before any major competition would hence be suggestive of autologous blood manipulation.

Nevertheless, the crucial element of any approach to screen for blood doping by blood transfusions is the definition of reliable testing protocols and ranges of variability, which would allow authorities to critically analyze changes and assess a reliable ''individual reference state'' independent of all external biological factors of variation. Although there is comprehensive information on the biological variation of hematological parameters in healthy sedentary individuals, less is known about how these parameters may change in athletes. In addition, no definitive reference limits have been acknowledged to adjust single- or multivariable blood tests to the exercise-adapted blood cell system of athletes, who display

rather broad seasonal adaptations that should be carefully taken into consideration in orchestrating testing regulations (53). Basically, competitive athletes display significant differences in hemoglobin, erythrocytes, hematocrit and mean corpuscular volume compared to the sedentary population, whereas other erythrocyte indexes, reticulocyte counts and reticulocyte parameters appear to be less influenced by lifestyle (44, 54). In addition, hematological profiles may vary widely during the competitive season and are highly influenced by training and workload (55, 56). Finally, the substantial impact of extra-analytical variability should always be taken into consideration (57), especially when blood sampling occurs in the race field, rather far from the clinical laboratory, and is carried out by inadequately trained personnel (58). Most recipients of antidoping testing often ignore the possibility of factors other than pathologies or cheating that may contribute to unexpected values, especially those that are considered ''abnormal''. It is essential that everyone who performs antidoping testing or uses its results clearly understands that sports biochemistry and hematology should be strictly linked to knowledge of the principal problems and pitfalls in the extra-analytical phases of various parameters commonly used in monitoring the training, diet, and performances of athletes, to avoid misinterpretation of data and to improve the usefulness of biochemical investigations (59). Although the average analytical variability for most hematological parameters is satisfactory and ideally fulfils the minimal criteria of actual antidoping strategies, the lack of rigorous preanalytical and analytical protocols may occasionally produce unreliable or equivocal results and unjustified complaints against or sanctioning of clean athletes (60–62). Therefore, standardized procedures for subject preparation, specimen collection, handling, manipulation, testing and storage should be implemented and rigorously respected within antidoping control procedures. Test results should be further analyzed, taking into account the sex, ethnic origin and type of sports discipline (44).

Conclusions

Shortly after the discovery of blood circulation by the English physician William Harvey in 1628, the first empiric blood transfusion was attempted (63). Since then, blood transfusion technology has substantially evolved, along with the spectrum of clinical applications. It has long been acknowledged that sporting performance can be significantly enhanced by boosting the blood's oxygen-carrying capacity through the use of doping (12). Accordingly, often taking place for a variety of drugs and therapeutic practices, blood transfusion has become an effective means to enhance endurance performance over the last century, making a strong comeback in the early 2000s when reliable strategies to detect doping with erythropoiesis-stimulating substances were implemented (2). In general, testing for banned substances or methods serves two purposes. In theory, it protects the integrity of sports, and, just as importantly, it also prevents athletes from abusing substances that may produce negative and even life-threatening health consequences. Homologous blood transfusions may trigger reactions characterized by fever, urticaria, and anaphylactic shock, and are significantly associated with transmission of blood-borne infectious diseases, including hepatitis, acquired immunodeficiency syndrome, malaria, cytomegalovirus and Creutzfeldt Jakob disease. Occasionally, patients may develop phlebitis, septicemia, bacterial infection, air/clot embolism and transfusion-associated graft-vs.-host disease (TAGVHD), a lethal complication of infusion of non-irradiated cellular blood components into a susceptible recipient (64–66). Consistent evidence shows that a greater than 5% increase in circulating hemoglobin is necessary to improve athletic performance, suggesting that athletes would need to infuse at least one unit of blood to obtain a surreptitious athletic advantage (3–5). The greater the amount of blood transfused, the greater is the expected performance improvement; hence, larger infusions of either homologous or autologous RBCs may be associated with the hyperviscosity syndrome, characterized by increased blood viscosity, decreased cardiac output and blood flow velocity, finally resulting in a reduction in peripheral oxygen delivery (64).

Sports doping is turning to the old school and the trend is clear: out with the new (recombinant human erythropoietin), and in with the old (blood transfusions). Special concern is currently being placed by the WADA and several sport authorities on the use of blood transfusions, as these practices corrupt the fairness of sports, while putting athletes' health in serious jeopardy. Unfortunately, current policies to detect blood doping are mostly ineffective and largely unsuccessful as deterrents, as attested by the high prevalence of athletes still testing positive (67). The major problem lies with being able to implement highly sensitive laboratory tests that can unequivocally detect if an athlete is in fact undergoing blood doping procedures instead of other licit means to enhance RBC mass, such as training at high altitude. The solution is still perplexing and there are no foolproof tests for an athlete who dopes with autologous blood transfusions to date. Autologous blood doping is still a chink in the armor of the antidoping armamentarium; more research and commitment are needed because it is not acceptable that agencies and sports federations that have banned this practice rely on the integrity of the athletes, coaches and their medical support personnel to comply with regulations. The sporting authorities, along with the WADA, should hence develop close cooperation with hematology and laboratory medicine specialists for the development of reliable detection methods to unmask this unfair doping practice and for the implementation of education programs on the associated risks. Blood doping is suddenly becoming a public health concern. Although effective countermeasures are urgently needed, storage of athletes' blood samples for the

time being may represent a reliable preventive strategy, allowing retesting and sanctioning of guilty athletes once a definitive test becomes available.

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Received August 3, 2006, accepted August 28, 2006