ASH Education Program Book
asheducationbook.hematologylibrary.org

doi: 10.1182/asheducation-2013.1.627
ASH Education Book December 6, 2013 vol. 2013 no. 1 627-631

Blood manipulation: current challenges from an anti-doping perspective

Jakob Mørkeberg[1]
+ Author Affiliations

Abstract

The delivery of oxygen is the limiting factor during whole-body endurance exercise in well-trained individuals, so manipulating the amount of hemoglobin in the blood results in changes in endurance exercise capacity. Athletes began using novel erythropoiesis-stimulating agents well before they were approved for medical use. Older manipulation practices, such as autologous blood transfusions or the administration of first-generation recombinant human erythropoietins, are still widely abused due to challenges in their detection. More recent performance enhancement maneuvers include efforts to mask doping and to induce increased endogenous erythropoietin expression. Confessions by athletes have revealed an ongoing yet extremely sophisticated modus operandi when manipulating the blood. In this review, weaknesses in detection methods and sample collection procedures are scrutinized and strategies developed to circumvent the test system discussed.

Introduction

According to the World Anti-Doping Agency (WADA), blood manipulation is described as the reintroduction of blood or blood products, the artificial enhancement of oxygen transportation, and any form of intravascular manipulation of the blood or its components.[1] With this broad description, one could argue that blood manipulation also covers doping methods such as the administration of recombinant human erythropoietin (rHuEPO) and analogs, the use of diuretics and other masking agents such as plasma volume expanders, and, conceivably, the use of biologic response modifiers to alter gene expression profiles.[2] In this review, methods and substances from different sections of the WADA guidelines are described, all of which are used with the same objective: to gain an unfair competitive advantage by increasing the exercise capacity through the manipulation of the composition of the blood and its components. It is important to stress that every blood manipulation practice described here has side effects that could be life-threatening. For example, increasing the viscosity of the blood leads to an increased risk of several deadly diseases such as heart disease, stroke, and cerebral and pulmonary embolism. I strongly discourage any attempts to replicate any of the methods or procedures described here.

For several decades, athletes attempting to manipulate their blood to gain a competitive advantage have done so without risk of repercussions. Lack of detection methods and insufficient testing programs by anti-doping authorities have made blood manipulation widespread in endurance sports. In the 1990s, when rHuEPO first became commercially available, no detection method had been developed. Certain sports federations introduced a so-called “health rule” under which athletes with blood values above a certain threshold were banned from competition until their blood values had returned to levels below the threshold.[3] The International Cycling Federation introduced an upper limit for hematocrit of 50% to reduce the abuse of rHuEPO and thereby the possible health risks of exercising with “thick blood.”[4] It was perceived in some endurance sports that success was not possible without some form of performance enhancement through blood manipulation. Since the

development of WADA in 1999, considerable progress has been made in developing new detection methods and harmonizing the anti-doping system. Nevertheless, recent reports and confessions from numerous endurance athletes have revealed ongoing and highly sophisticated strategies of manipulating the blood.[5⇓⇓-8] Based on the scientific literature and confessions from athletes, the objective of this review is to describe the possible methods and strategies used by blood manipulators today.

Blood transfusions

Athletic performances at the Mexico City Olympic Games in 1968 were likely affected by altitude-impaired oxygen delivery. Subsequently, blood transfusions came to public attention when the Finnish steeplechaser Lasse Viren introduced the method as he won the gold medals in the 5000 and 10 000 endurance runs at the Olympic Games in Münich in 1972.[8,9] After the Olympic Games in Los Angeles in 1984, the International Olympic Committee banned the procedure. Two main procedures for blood transfusions exist, homologous and autologous. Homologous blood transfusions are unlikely to be used today. Before the 2004 Olympic Games in Athens, a robust flow cytometric test based on FACS was developed at the Royal Prince Alfred Hospital in Sydney[10] to detect variances in blood group antigens. Had an athlete received a blood transfusion antigenically distinct from his or her own RBCs, the flow cytometric test was able to differentiate these cell populations. This test is able to detect small populations of mixed cell populations (< 5%) for several weeks.[11] Only a handful of athletes have tested positive for homologous blood transfusions, with the majority erroneously receiving someone else's blood instead of their own.

The alternative to homologous blood transfusions are autologous blood transfusions. In contrast to homologous blood transfusions, detection of autologous blood transfusions has been challenging. Since the 1980s, several attempts have been made to develop a test for autologous blood transfusions. Changes in erythropoiesis-sensitive blood parameters[12] such as the serum erythropoietin concentration, the percentage of reticulocytes (%ret), and the hemoglobin concentration (Hb), increased urinary levels of plasticizers leaked from the blood collection bags during storage,[13] or erythrocyte membrane lesions caused by storage[14] have been studied. Unfortunately, each has failed to provide the anti-doping community with adequately sensitive test that could be used with a sufficient window of detection. Changes in Hb and %ret after transfusion may be difficult to distinguish from normal physiologic variations.[15] At baseline, a well-trained endurance athlete may have an expanded total blood volume of 8 L and a hematocrit of 45%, such that transfusion of a single unit may only make up ~ 5% of the total RBC pool in circulation. In addition, the almost immediate posttransfusion removal of as much as 25% of the transfused RBCs from circulation limits the potential window of detection of RBCs with storage lesions.[16] With no reliable test for autologous blood transfusions, this blood manipulation method remains very attractive today.

Detailed reports from the Spanish doping investigation, nicknamed “Operacion Puerto” revealed the systematic use of autologous blood transfusions by several athletes.[17] Hundreds of frozen blood units from professional athletes, along with calendars with reinfusion dates, were discovered by police. Blood was typically withdrawn 4 to 5 weeks before important competitions using conventional refrigerated storage procedures and preservative solutions, and then reinfused a few days before either one-day competitions or during multiday competitions into the donor's circulation. During multiday competitions, blood transfusions were used to stabilize hemoglobin levels, which tend to decrease during repeated periods of exhausting exercise due to plasma volume expansion.[18,19]

Cryopreservation is a more sophisticated storage alternative but requires additional handling procedures during freezing and thawing. With careful thawing, the 24-hour post-reinfusion recovery of RBCs is in the realm of 85%.[20] This storage procedure extends the potential pretransfusion period, making the timing of withdrawal less critical. Although the method is far more complicated and expensive than conventional refrigerated storage, there is evidence that cryopreservation of blood bags has been performed.[17] An investigation revealed that a top Danish cyclist, Michael Rasmussen, together with other professional endurance athletes, purchased an automated Haemonetics ACP 215 device used to glycerolize, freeze, thaw, and wash RBCs. He withdrew blood while serving a 2-year ban from competition from July 2007 to July 2009 and stored the RBCs frozen with the aim of reinfusing the blood at the 2009 Tour de France.[21] Due to the logistical challenges of withdrawing blood at the right time before competition, storing the blood at the correct temperatures, and reinfusing the blood close to competitions or during stage racing, it is anticipated that this strategy for autologous blood transfusions would be performed by a relatively limited number of top athletes who can afford this rather expensive procedure.

rHuEPO administration and masking strategies

Another well-known procedure is the administration of rHuEPO, which was prohibited by the International Olympic Committee in 1990. At that time, there was no test available to detect exogenous erythropoietin. Due to its potency, rHuEPO thus became the most popular doping agent in endurance sports. A direct detection method for rHuEPO was developed by Lasne and de Ceaurriz in 2000.[22] With the use of isoelectric focusing patterning, it was possible to separate rHuEPO molecules from endogenous EPO (uEPO) due to differences in glycosylation. The first-generation compounds, rHuEPO alfa and beta (also called epoetin alfa and epoetin beta), are less acidic than uEPO[23⇓-25] and therefore will show a more "basic" band distribution when rHuEPO alfa and beta have been administered compared with a sample containing uEPO only. In addition, when rHuEPO is administered, the endogenous EPO production decreases, resulting in a lack of "endogenous bands."[26]

Abuse of the second-generation rHuEPO darbepoetin alfa, which has a longer half-life than the first-generation rHuEPOs, was evident at the Winter Olympics in 2002 in Salt Lake City, when 3 of the most successful cross-country skiers at the time tested positive. The third-generation rHuEPO continuous erythropoiesis receptor activator (CERA) was approved in Europe in 2007.[27] CERA has the same molecular structure as previous rHuEPO drugs except that it is connected to a chemical called polyethylene glycol. This so-called pegylation induces a high hydrodynamic volume that hinders its glomerular filtration. Therefore, in contrast to the isoelectric focusing detection method for first- and second-generation rHuEPOs, which is based on urine samples, CERA is detected in the blood.[28] CERA has the longest half-life of all Food and Drug Administration-approved erythropoiesis-stimulating agents (130-140 hours)[27] and, although it would have to be administered less frequently than contemporary rHuEPOs, the prolonged period in the circulation increases the period of detection. Therefore, CERA is a less attractive option for doping purposes today.

Recent admissions by athletes indicate that first-generation rHuEPOs have regained popularity for blood manipulation because of their shorter half-lives and therefore limited detection periods. Advanced administration strategies are used together with various masking schemes. The detection period of these first-generation rHuEPOs depends on the amount administered and the route of administration. There is a large interindividual variability in the dosage of rHuEPO required to increase Hb. In a recent study, subjects were administered 50 IU/kg of epoetin beta IV twice weekly

for 3 weeks, followed by 2 microdosage injections of 10 IU/kg body weight.[29] This administration scheme increased the Hb from 0.6 to 1.8 g/dL on the individual level. When the target Hb has been reached, administration is titrated through microdosing to maintain the supraphysiological Hb and a physiologically normal amount of reticulocytes in the circulation.

The challenges of blood manipulation have led to the development of the Athletes Biological Passport (ABP).[30] The ABP is an indirect detection method relying on longitudinal monitoring of certain blood parameters on the individual level. The Hb and the %ret are the two most important parameters in the ABP. The main aim for blood manipulators today is to achieve a supraphysiological hemoglobin mass while maintaining Hb values and %ret at relatively stable levels. Because the Hb is dependent on plasma volume and because considerable fluctuations in plasma volume occur over time, the ABP allows for some degree of variation in the Hb. Significant fluctuations in erythropoiesis-sensitive parameters mandate a more thorough investigation of the blood profile by an expert panel. The overall aim for cheating athletes is to navigate within the lower and upper limits of their "passport." Since 2009, it has been possible to sanction athletes for 2 years based on abnormal blood profiles alone. The ABP is also used for targeted collection of urine samples for the direct detection of rHuEPO.

A typical rHuEPO doping regime would consist of 2 administration phases. The first phase takes place several weeks before the competition and is focused on increasing hemoglobin mass. During this phase, athletes are at a higher risk of testing positive and therefore are more likely to travel to distant places with short notice to avoid testers. In addition to rhuEPO administration, another strategy is to train at higher altitude or use hypoxic devices such as altitude tents or masks, which reduce the inspired oxygen and hence stimulates erythropoiesis. These "procedures" are still allowed by sports federations and could therefore be used as an explanation for an increased hemoglobin level achieved by rHuEPO administration. In addition to its intended effect of increased hemoglobin, altitude exposure increases the endogenous EPO production and therefore diminishes the ratio between exogenous and endogenous EPO during rHuEPO administration, and thus the sensitivity of the direct EPO test.

The second rHuEPO administration phase is performed closer to or during competition. Until recently, is was anticipated that rHuEPO was used out of competition when preparing for races only, but recent admissions by athletes have revealed the use of rHuEPO also in competition.[7] Here, the restriction in the timing of sample collection is used to the advantage of the doping athlete. In general, doping control officers are not allowed to collect samples from 11:00 PM until 6:00 AM. Injecting a microdose of first-generation rHuEPOs in the evening shortly after 11:00 PM could theoretically be undetectable the following morning at 6:00 AM, when testers are allowed to collect samples again. The mode of administration may also affect the success of this method. A direct comparison of the first-generation rHuEPOs, alfa and beta, has shown that the terminal elimination half-life after subcutaneous administration is 24 hours for beta but 19 hours for alfa.[31] Injecting rHuEPO IV decreases the half-life to 9 and 7 hours for beta and alfa, respectively.[31]

When rHuEPO is administered in competition, it is anticipated that athletes use very small dosages of epoetin alfa administered IV in the evening, probably followed by considerable water intake. Such hyperhydration-masking schemes is intended to increase the urine flow, dilute the urine, and thereby reduce the amount of EPO in the collected sample. The Swiss cyclist Thomas Frei tested positive for rHuEPO in 2010. At a press conference just after testing positive, he acknowledged that the only reason that he failed the test was that he forgot to drink 1 L of water as he usually did after administering

microdosages of rHuEPO.[8]

In addition to the masking strategy of hyperhydrating, IV infusion of physiological solutions with or without addition of polysaccharides (eg, dextran or hydroxyethyl starch solutions) is not only used to dilute the target analytes but also to decrease the levels of hematological markers measured in the blood passport. Another, in principal, very effective masking strategy would be the administration of supramolecular structures such as phospholipid liposomes with the aim of reducing the free concentration of circulating peptides, polypeptides, and proteins that are analytical targets in direct and indirect detection strategies.[32] Research is being conducted currently to develop test methods for this practice.[8,33]

Microdosing regimes in competition are also used to mask the decrease in reticulocytes occurring as a result of autologous blood transfusions. During strenuous multiday competitions, a normal physiological response is plasma volume expansion, resulting in a decrease in Hb of ~ 15%.[8,18] An athlete with a normal Hb of 14.5 g/dL ends up with an Hb of 12.3 g/dL at the end of a Grand Tour. Nevertheless, the transfusion of one bag of blood increases the Hb by ~ 0.8 g/dL.[12] With 3 transfusions during a Grand Tour, a cyclist would experience relatively stable Hb throughout the race. The telltale sign would be a suppressed %ret because the BM stimulation ceases after transfusions.[34] Using microdosages of rHuEPO after transfusions would provide a sufficient stimulus to the BM to increase the %ret toward normal levels.

Copy epoetins and hypoxia-inducible factor stabilizers

The expiration of the patent for epoetin in Europe in 2004 allowed the manufacturing of biosimilar EPOs. A biosimilar product is a copy version of an already authorized biological medical product with demonstrated similarity in psychochemical characteristics, efficacy, and safety based on a comprehensive comparability exercise.[35] In contrast to biosimilars, copy epoetin products are manufactured, and therefore have not undergone the same strict comparative development program against a reference product. In general, these are less expensive and more accessible through web-based distributors. These agents pose a real threat to clean competition. More than 80 different copy epoetins have been compounded.[36] Every novel copy EPO could differ in structure and chemical properties from conventional and biosimilar EPOs such that the direct EPO test may be insensitive to these agents.[37] An alternative test method using SDS-PAGE gel electrophoresis, which differentiates EPO molecules based on the molecular masses, enables the identification of some of the copy epoetins, but it is unlikely that all of these copies can be identified with conventional detection methods.

In addition, different pharmacological and genetic approaches may be able to simulate the effects of hypoxia at the cellular level to increase expression of hypoxia-inducible genes such as hypoxia-inducible factor (HIF). HIF will eventually lead to an increase in the production of EPO and, subsequently, HB or RBC mass. Modulation of the hypoxia-induced genes could be achieved by inhibiting HIF-prolyl hydroxylase, a key regulatory enzyme of HIF. Administering drugs that inhibit HIF-prolyl hydroxylase enzymes changes the stability of the HIF-alfa subunit and thus the activation and expression of hypoxia-responsiveness genes.[27] Selective activation of the erythropoietic cascade has the potential to induce the full complement of factors necessary for erythropoiesis including EPO, EPO-R, and iron-regulatory genes. Of particular concern are the small molecules such as clinically used iron chelators and small molecules currently in phase 2 or 3 studies. Currently, tests do not exist to detect hypoxia-induced gene “doping” with pharmacological and/or genetic strategies. Pharmaceutical companies already offer

HIF-prolyl hydroxylase-inhibitory reagents and it is likely that these drugs are already used by athletes.

One particular substance that is relatively cheap and easy to get hold of is cobalt salt tablets containing cobalt (II) ions (Co^{2+}). This nonpeptide compound stabilizes the HIF transcription factors and increases EPO gene expression.[38] In addition, cobalt may increase the expression of other HIF-dependent genes (eg, genes encoding proteins involved in angiogenisis, iron metabolism, and glucose transport).[39] Measurements of the amount of cobalt within the erythrocyte[40] representing the irreversible uptake of free Co^{2+} or the modeling whole-body and urinary levels of cobalt after high dosage administration[41] have been proposed as detection methods. Future tests will probably rely on advanced biological passport approaches such as molecular signatures of hypoxia, which would detect all previous and future forms of hypoxia-induced gene doping. Because these drugs are in pill form, it is likely to attract unscrupulous athletes who are looking for a simple oral alternative to rHuEPO injections.

Although cobalt in only moderately toxic on single-dose exposure, chronic cobalt exposure can lead to severe side effects. Regular intake of high cobalt salt doses comes with a real risk of organ injury such as thyroid dysfunction, cardiotoxicity, and heart failure. In addition, HIFs can affect several other genes apart from the EPO gene, some of which might have tumor-growth-promoting potential.[42] Therefore, using this substance could pose a real health risk to the athlete.

Conclusion

Recent admissions by athletes have provided insights into doping methods and drugs that have been used in the past, but new blood manipulation strategies are developed constantly and may reach the athletes before they are even approved for medical use. The introduction of the biological passport and the collaboration between WADA and the pharmaceutical industry to identify and transfer information of drugs in development are two important steps in the right direction. It would seem that, at present, the testers always are one step behind the cheaters. Hopefully, the testers are about to catch up.

Disclosures

Conflict-of-interest disclosure: The author declares no competing financial interests. Off-label drug use: None disclosed.

Correspondence

Jakob Mørkeberg, Metabolic Mass Spectrometry Facility, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen O, Denmark; Phone: 45-51943994; Fax: +45 43262546; e-mail: jakobmoerkeberg@hotmail.com.

References

1. The World Anti-Doping Agency The World Anti-Doping Code. The 2013 Prohibited List International Standard. http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-list/2013/WADA-Prohibited-List-2013-EN.pdf. Accessed May 15, 2013.
2. Semenza GL (2009) Regulation of vascularization by hypoxia-inducible factor 1. Ann N Y Acad Sci 1177:2-8. CrossRef Medline Google Scholar
3. Videman T, Leroux I, Hemmingsson P, et al. (2000) Changes in hemoglobin values in elite cross-country skiers from 1987-1999. Scand J Med Sci Sports 10:98-102. CrossRef Medline Google Scholar
4. Robinson N, Schattenberg L, Zorzoli M, Mangin P, and Saugy M (2005) Haematological analysis conducted at the departure of the Tour de France 2001. Int J Sports Med 26:200-207. CrossRef Medline Google Scholar
5. L'Equipe Interview with former pro cyclist Bernhard Kohl. http://www2.lequipe.fr/redirect-v6/homes/Cyclisme/breves2009/20090608_232214_la-confession-de-bernhard-kohl.html. Accessed May 15, 2013.
6. ESPN Interview with former Tour de France winner Floyd Landis http://sports.espn.go.com/oly/cycling/news/story?id=5203604.

Accessed May 15, 2013.

7. United States Anti-Doping Agency USADA Report on Lance Armstrong http://d3epuodzu3wuis.cloudfront.net/ReasonedDecision.pdf. Accessed May 15, 2013.
8. Velonation Thomas Frei used micro-doses and water to avoid EPO detection. http://www.velonation.com/News/ID/2048/Thomas-Frei-used-micro-doses-and-water-to-avoid-EPO-detection.aspx. Accessed May 2, 2013.
9. Leigh-Smith S (2004) Blood boosting. Br J Sports Med 38:99–101. Abstract/FREE Full Text
10. Nelson M, Penn H, Sharpe K, and Ashenden M (2003) Proof of homologous blood transfusion through quantification of blood group antigens. Haematologica 88:1284–1295. Abstract/FREE Full Text
11. Giraud S, Robinson N, Mangin P, and Saugy M (2009) Scientific and forensic standards for homologous blood transfusion anti-doping analyses. Forensic Sci Int 179(1):23–33. CrossRef Medline Google Scholar
12. Berglund B, Birgegard G, Wide L, and Ribstedt B (1989) Effects of blood transfusions on some hematological variables in endurance athletes. Med Sci Sports Exerc 21:637–642. Medline Google Scholar
13. Monfort N, Ventura R, Platen P, et al. (2012) Plasticizers excreted in urine: indication of autologous blood transfusion in sports. Transfusion 52:647–657. CrossRef Google Scholar
14. Marrocco C, Balletta V, D'alessandro A, Alves G, and Zolla L (2012) Red blood cell populations and membrane levels of peroxiredoxin 2 as candidate biomarkers to reveal blood doping. Blood Transfus 10(suppl 2):s71–s77. Medline Google Scholar
15. Markeberg I, Sharpe K, Belhage B, et al. (2011) Detecting autologous blood transfusions: a comparison of three passport approaches and four blood markers. Scand J Med Sci Sports 21:235–243. CrossRef Medline Google Scholar
16. Luten M, Roerdinkholder-Stoelwinder B, Schaap NP, et al. (2008) Survival of red blood cells after transfusion: a comparison between red cells concentrates of different storage periods. Transfusion 48:1478–1485. CrossRef Google Scholar
17. El Pais Operación Puerto http://www.elpais.com/articulo/deportes/transfusiones/dolares/Tyler/Hamilton/elppordep/20060626elpepidep_6/Tes.2006. Accessed May 3, 2013.
18. Markeberg IS, Belhage B, and Damsgaard R (2009) Changes in blood values in elite cyclist. Int J Sports Med 30:130–138. CrossRef Medline Google Scholar
19. Hess JR (2009) An update on solutions for red cell storage. Vox Sang 91:13–19. CrossRef Medline Google Scholar
20. Valeri CR, Pivacek LE, Gray AD, et al. (1998) The safety and therapeutic effectiveness of human red cells stored at −80 degrees C for as long as 21 years. Transfusion 29:429–437. CrossRef Google Scholar
21. Brian Askvig Nu indrømmer Kvillingen kab af blodcentrifuge http://ekstrabladet.dk/sport/cykling/article1933256.ece. Accessed May 6, 2013.
22. Lasne F and de Ceaurriz J (2000) Recombinant erythropoietin in urine. Nature 405(6787):635. Medline Google Scholar
23. Lasne F and de Ceaurriz J (2000) Recombinant erythropoietin in urine. Nature 405(6787):635. Medline Google Scholar
24. Lasne F, Martin L, Crepin N, and de Ceaurriz J (2002) Detection of isoelectric profiles of erythropoietin in urine: differentiation of natural and administered recombinant hormones. Anal Biochem 311:119–126. CrossRef Medline Google Scholar
25. Catlin DH, Breidbach A, Elliott S, and Glaus J (2002) Comparison of the isoelectric focusing patterns of darbepoetin alfa, recombinant human erythropoietin, and endogenous erythropoietin from human urine. Clin Chem 48:2057–2059. FREE Full Text
26. Breidbach A, Catlin DH, Green GA, et al. (2003) Detection of recombinant human erythropoietin in urine by isoelectric focusing. Clin Chem 49:901–907. Abstract/FREE Full Text
27. Jelkmann W (2012) Biosimilar recombinant human erythropoietins ("epoetins") and future erythropoiesis-stimulating treatments. Expert Opin Biol Ther 12:581–592. CrossRef Medline Google Scholar
28. Reichel C (2012) SARCOSYL-PAGE: a new electrophoretic method for the separation and immunological detection of PEGylated proteins. Methods Mol Biol 869:65–79. CrossRef Medline Google Scholar
29. Markeberg I, Sharpe K, Karstoft K, and Ashenden MJ Detection of microdoses of rhEPO with the MAIA test. Scand J Med Sci Sports. Published online ahead of print January 24, 2013. doi:10.1111/sms.12049.
30. The World Anti-Doping Agency (2012) The World Anti-Doping Code WADA_ABP_OperatingGuidelines_version_3.0, Accessed May 6, 2013.
31. Halstenson CE, Macres M, Katz SA, et al. (1991) Comparative pharmacokinetics and pharmacodynamics of epoetin alfa and epoetin beta.

Clin Pharmacol Ther 50:702–712. CrossRef Medline Google Scholar

32. Maruyama K, Mori A, Bhadra S, Subbiah MT, and Huang L (1991) Proteins and peptides bound to long-circulating liposomes. Biochim Biophys Acta 1070(1):246–252. CrossRef Medline Google Scholar

33. The World Anti-Doping Agency WADA Funded Research http://www.wada-ama.org/en/Science-Medicine/Research/Funded-Research-Projects/. Accessed May 8, 2013.

34. Damsgaard R, Munch T, Morkeberg J, Mortensen SB, and Gonzalez-Alonso J (2009) Effects of blood withdrawal and reinfusion on biomarkers of erythropoiesis in humans: Implications for anti-doping strategies. Haematologica 91:1006–1008. Abstract/FREE Full Text

35. Weise M, Bielsky MC, De SK, et al. (2011) Biosimilars: why terminology matters. Nat Biotechnol 29:690–693. Medline Google Scholar

36. Macdougall IC and Ashenden M (2009) Current and upcoming erythropoiesis-stimulating agents, iron products, and other novel anaemia medications. Adv Chronic Kidney Dis 16:117–130. CrossRef Medline Google Scholar

37. The World Anti-Doping Agency WADA Technical Document – TDEPO2013, Accessed May 8, 2013.

38. Goldwasser E, Jacobson LO, Fried W, and PLZAK LF (1959) Studies on erythropoiesis. V. The effect of cobalt on the production of erythropoietin. Blood 13:55–60. Abstract/FREE Full Text

39. Wenger RH, Stiehl DP, and Camenisch G (2005) Integration of oxygen signaling at the consensus HRE. Sci STKE 2005:re12. Abstract/FREE Full Text

40. Simonsen LO, Brown AM, Harbak H, Kristensen BI, and Bennekou P (2011) Cobalt uptake and binding in human red blood cells. Blood Cells Mol Dis 46:266–276. CrossRef Medline Google Scholar

41. Unice KM, Monnot AD, Gaffney SH, et al. (2012) Inorganic cobalt supplementation: prediction of cobalt levels in whole blood and urine using a biokinetic model. Food Chem Toxicol 50:2456–2461. CrossRef Medline Google Scholar

42. Semenza GL (2003) Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3:721–732. CrossRef Medline Google Scholar