BLOOD TRANSFUSIONS IN SPORTS. HAZARDS AND SIDE-EFFECTS.

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Introduction

The use of performance enhancing drugs and techniques, by the world's top athletes has been a persistent issue in world sporting events for nearly five decades.

High physical performance is of value for all competitive athletes, especially endurance ones such as long distance runners, swimmers, cyclists, triathletes who need a high amount of circulating Red Blood Cells to achieve the maximum of Oxygen (VO2max) content that is necessary for aerobic metabolism during competition.

Articicially, the enhancement of oxygen transport throughout the body includes blood transfusions as well as specialized high-altitude training techniques, and newly engineered medical substances, and can enable athletes to take advantage of faster and less strenous means of improving endurance and performance.

A blood transfusion is the transfer of blood or blood products from one person (donor) into another person's bloodstream. This is usually done as a life saving maneuver to replace blood cells or blood products lost through severe bleeding, during surgery when blood loss occurs or to increase the blood count in an anemic patient.**[1]**

Used by athletes blood transfusion is prohibited by WADA antidoping code.

The definition of blood doping includes methods or substances administered for non-medical reasons to healthy athletes for improving aerobic performance. It includes all means aimed at producing an increased or more efficient mechanism of oxygen transport and delivery to peripheral tissues and muscles.**[2]**

Blood transfusions have been used in order to augment circulating Red Blood Cell's and consequent total haemoglobin mass that is a key factor for maximal exercise capacity.**[3]**

We shortly review the use of homologous and/or autologous blood transfusions used by high level athletes for doping purposes, the tests that are used to detect cheaters and the hazards and side-effects observed as a result of their misuse by apparent healthy athletes.

Blood Doping

Blood doping is defined by World Anti-Doping Agency (WADA) as the misuse of techniques and/or substances to increase red blood cell count.**[4]**

Increased performance after blood transfusion was first demonstrated more than 60 years ago, but the technique did not create attention until

the early 1970s when it was dubbed 'blood doping' by the media **[5]** and was not made illegal until 1986. Before then it was in vogue among middle-and long-distance runners, notably the Finn Lasse Viren, winner of the 5,000m and 10,000m in the Munich and Montreal Olympics, and was also used by the US cycling team in the 1984 Los Angeles Games **[6]** the only sports national team that confessed to infusing RBC before competition.**[7]**

Blood Transfusions

Blood transfusions used by athletes may be either autologous (athlete's own blood re-infusion) or homologous (another person's compatible blood) and the technique is based on extraction several weeks before competion of an amount of 900ml blood, that is being freezed and re-infused 1-2 weeks before competition.

The use of blood transfusion has suddenly declined after 1980s' following the widespread use of recombinant human erythropoietin among elite endurance athletes but recently was reintroduced in use because of new methods detecting the use of rHuEPO in urinary samples in 2000.**[8]**

Reinfusion of whole blood or packed red blood cells (RBCs) increasing the haemoglobin concentration ([Hb]) above the individual's normal values, increases VO2max and enhances physical performance. During submaximal exercise heart rate and blood lactate concentration ([Hla]) are reduced, while arterial blood pressure remains unchanged despite increased haematocrit (Hct). There is no method available for detecting this type of blood 'doping'.**[9]**

Forms of blood transfusion.

Autologous blood doping is the transfusion of one's own blood, which has been stored (refrigerated or frozen) until needed. For autologous infusions, glycerol freezing techniques are needed to allow prolonged RBC storage without degradation.**[10]**

Homologous blood doping is the transfusion of blood that has been taken from another person with the same blood type. For homologous infusions, refrigeration techniques may be used for short-term storage; however, RBC will progressively degrade and the maximum storage period is about 42 d. Blood storage and handling techniques are important as they influence RBC function and survival rate which contributes to interstudy dose-response variability.**[11]**

A blood doping procedure, independent of transfusion type, induces some pronounced physiological changes. A desired effect of blood doping is the increased total red blood cell mass leading to increments in haemoglobin, which after successfully induced blood doping is in the magnitude of at least 10%. In addition, storing of blood leads to a constant decline in RBC count due to the limited life span of the RBC (haemolysis). Thereby, serum iron and bilirubin levels will increase and reach maximal levels within the first day. Haemolysis is more accentuated after storing of blood in a blood bank than in the frozen state.**[12]**

Physiology of Blood Transfusions

Oxygen by inspiration, arrives in alveoli and by passive movement through alveolar-capillary membrane, is conjugated with Haemoglobin contained in Red Blood Cells (RBCs') forming Oxyhaemoglobin (HbO2) that transports O2 and delivers it to working tissues. The CO2 contained in Carboxyhaemoglobin (HbCO2) is eliberated passively in alveoli and consequent in atmosphere by expiration. Blood transfusion by means offers the necessary O2 content in RBCs mass which then is delivered to

working muscles in order to improve endurance performance of athletes during training or competition.**[13]**
Simple clinical indices, such as hematocrit or hemoglobin concentration, are often used to estimate changes in RBC mass. These clinical indices are influenced by independent effects of both RBC mass and plasma volume; so if RBC infusion (or other ESA's administration) would also alter plasma volume, then these indices would provide inaccurate estimates of RBC mass changes.
Several studies have performed blood volume measurements on persons both before and after either RBC infusion.**[14,15]**
For a given person, the more RBC infused the greater the increase in hemoglobin. For example, *Spriet et al.(1986)***[16]** reported that when healthy subjects were infused the RBC product of two blood units, hemoglobin increased by ≈8% and when infusing the RBC product of three blood units, hemoglobin increased by ≈10%. When comparing healthy subjects, the magnitude of the hemoglobin increase is quite variable for a given amount of RBC infused.
The influence of transfused blood on exercise performance is investigated by several scientists by measuring the maximal O2-uptake (VO2max). Investigators report that the relationship between changes in hemoglobin/hematocrit and changes in $\dot{V}O_{2max}$ after blood doping are strong with group analyses, but do not hold well with individual subject analyses.**[17]**
Therefore, it appears that a person's ergogenic response to blood doping might depend on a variety of physiologic factors such as genetics, fitness level, and training state.**[18]**

Synthetic oxygen carriers
A new class of substances might represent the next step of fraudulent improvement of the maximal oxygen uptake: artificial oxygen carriers, such as solutions based on recombinant, bovine or human hemoglobin and perfluorocarbon-emulsions have been shown to improve oxygen delivery to the muscle.**[19]**
Solutions based on human or bovine hemoglobin (HBOC) with commercial names Hemopure, PolyHeme, Hemolink and HemAssist, possess vasoconstrictor properties in addition to their oxygen transport capacity. The impact of vasoconstriction on tissue perfusion and organ function is however not yet fully understood. **[20]**
The purely synthetic perfluorocarbon (PFC) emulsions increase the physically dissolved portion of arterial oxygen content. Due to their particulate nature (emulsion droplets) PFCs may only be infused in low doses to avoid overload and malfunction of phagocytic cells of the reticulo-endothelial system.**[20]**
Oxygen therapeutic products have several advantages compared with packed red blood cells, including a prolonged shelf-life, lack of a cross-matching requirement, and minimal infectious risks or concerns about immunogenicity.**[21]**
Synthetic oxygen carriers appear useful for emergency therapeutic purposes when human blood is not available, the risk of blood infection is high or when there is not enough time to properly cross-match donated blood with a recipient. However, their misuse for doping purposes carries the risk of cardiovascular disease in addition to various serious side effects (e.g., stroke, myocardial infarction, embolism)**[22]**

The antidoping fight
Historically the term ''blood doping'' was changed to ''Enhancement of Oxygen Transfer'' in 2004 and defined as the use of autologous,

homologous or heterologous or red blood products of any origin, other than for medical treatment.
Blood transfusion in sports was forbidden by the International Olympic Committee (IOC) after the 1984 Olympics, despite the fact that no methods had been devised for unequivocal detection.
Prohibited techniques were first introduced as such in 1990 and defined as "blood doping and techniques."
Erythropoietin (EPO) was added as a banned substance in 1992, and blood plasma expanding products (e.g. HES) and artificial oxygen carriers were included in 2000.
In 2002 any sort of blood transfusion was prohibited as well as all erythropoiesis-stimulating proteins.
In general, use of products that enhance the uptake, transport, or delivery of oxygen is prohibited.
One of the most significant achievements in the fight against doping in sport to date has been the drafting, acceptance and implementation of a harmonized set of anti-doping rules, the World Anti-Doping Code (Code). Since it entered into force on January 1, 2004, the Code has proven to be a very powerful and effective tool in the harmonization of anti-doping efforts worldwide.**[23]**
Conform to 2011 Prohibited List, Prohibited Methods, Blood transfusions are prohibited according to section M1. ENHANCEMENT OF OXYGEN TRANSFER, of the list and is refereed as *''Blood doping, including the use of autologous, homologous or heterologous blood or red blood cell products of any origin''*. In the same section is also considered as doping and hence is prohibited, *''Artificially enhancing the uptake, transport or delivery of oxygen, including, but not limited to, perfluorochemicals, efaproxiral (RSR13) and modified haemoglobin products (e.g. haemoglobin-based blood substitutes, microencapsulated haemoglobin products), excluding supplemental oxygen''.***[24]**

Forms of transfusions that can be detected
Homologous blood doping (transfusion of compatible blood taken from another person) is detectable by flow cytometry**[25]** (the test was implemented at the 2004 Summer Olympic Games in Athens), using current antidoping protocols based on erythrocyte phenotyping and, eventually, erythrocyte genotyping by DNA testing.**[26]**
Autologous blood doping (transfusion of one's own blood) cannot be detected at present by direct measures. The implementation of indirect markers of blood doping stored in an Athlete's Biological Passport provides a powerful means to deter any form of blood transfusion.**[27]**
The donor of red cells stored for autologous re-transfusion can be identified by comparative blood group-typing and other immunological markers. WADA is funding research projects aimed at developing a test for autologous transfusions. In order to further improve detection of abnormal blood profiles, WADA is leading the development of a strategy against doping in sport called the ''Athlete Passport'', which is based on following athlete's biological variables over time. The objective of this strategy, which will be added to other anti-doping strategies including "traditional" testing, is to detect abnormal variations of determined biological variables in order to better target testing and/or sanction those found with abnormal variations.**[28]**
Conform to a recently report in the newspaper 'The Guardian' , by Shane Stokes, on September 15, 2011**[29]**''Autologous blood transfusion test could be in place for 2012 London Olympics. Scientists have been working for years on methods to detect so-called autologous blood transfusions, which many cyclists and other endurance sportspeople have used for

decades to gain a clear, but illegal, advantage''. Until now only homologous transfusions have been detectable. Tyler Hamilton, Santiago Perez, Alexandre Vinokourov and Andrei Kashechkin are four riders who have been given long bans after testing positive for this. As Professor David Cowan explained at the British Science Festival in Bradford, ''a method has been developed to pinpoint if autologous doping has been performed. It involves studying the cells for signs of ageing''.

Another test is currently being finalised by other scientists, namely ''the detection of plasticizers'' in blood. This is however an indirect method of tracing transfusions, as it looks for residue from the blood bags which are often used to store the extracted liquid. The RNA test is potentially far more powerful as it would detect transfusions even if the athlete avoided using bags containing plasticizers. Boosting oxygen transport via transfusions and other methods is regarded as the biggest single advantage cheating athletes can gain. The effects of the new test would therefore be hugely beneficial for sport. Cowan also stated that other tests should be ready in time for London 2012, including one to determine if substances such as nandrolone came from illegal external sources''.**[29]** Attempts may also be made to increase the O2-capacity of the blood by use of hemoglobin-based O2 carriers (HBOCs). However, HBOCs can be detected by electrophoretic methods**[30]** or size-exclusion HPLC.**[31]**

WADA's Athlete Biological Passport Operating Guidelines were approved by WADA's Executive Committee on December 1, 2009, and took effect immediately. "Athlete Biologic Passport Operating Guidelines," are based on the monitoring of several parameters for mature red blood cells and reticulocytes. Blood doping may be assumed, when these parameters change in a nonphysiologic way. The fundamental principle of the Athlete Biological Passport is based on the monitoring of an athlete's biological variables over time to facilitate indirect detection of doping on a longitudinal basis, rather than on the traditional direct detection of doping.

Hematologists should be familiar with blood doping practices as they may play an important role in evaluating blood profiles of athletes with respect to manipulations, as contrasted with the established diagnosis of clinical disorders and genetic variations.**[32]**

Hazards and Side-Effects

Like the other forms of blood doping, transfusions have serious medical consequences even in healthy subjects as athletes.

Acute transfusion reactions present as adverse signs or symptoms during or within 24 hours of a blood transfusion. The most frequent reactions are fever, chills, pruritus, or urticaria, which typically resolve promptly without specific treatment or complications.

Nonhemolytic febrile transfusion reactions are usually caused by cytokines from leukocytes in transfused red cell or platelet components, causing fever, chills, or rigors. A nonhemolytic transfusion reaction is a diagnosis of exclusion, because hemolytic and septic reactions can present similarly.

Allergic reactions typically present with rash, urticaria, or pruritus and are indistinguishable on examination from most food or drug allergies. Allergic reactions are IgE mediated. These reactions are usually attributed to hypersensitivity to soluble allergens found in the transfused blood component. [**33]**

Other signs occurring in temporal relationship, with a blood transfusion, such as severe shortness of breath, red urine, high fever,

or loss of consciousness may be the first indication of a more severe potentially fatal reaction. Transfusion reactions require immediate recognition, laboratory investigation, and clinical management. If a transfusion reaction is suspected during blood administration, the safest practice is to stop the transfusion and keep the intravenous line open with 0.9% sodium chloride (normal saline).

Acute transfusion reactions are typically classified into the following entities: a) Transfusion-related acute lung injury (TRALI), and b) Circulatory(volume) overload.**[34]**

a) Transfusion-related acute lung injury (TRALI) has 2 proposed pathophysiologic mechanisms: the antibody hypothesis and the neutrophil priming hypothesis.**[35]**

The antibody hypothesis**[36]** states that a human leukocyte antigen (HLA class I, HLA class II) or human neutrophil antigen (HNA) antibody in the transfused component reacts with neutrophil antigens in the recipient (ie, when antileukocyte antibodies are transfused passively in a plasma-containing blood component).

However, transfusions of blood components containing neutrophil antibodies may cause a wide range of reactions, including leukopenia, that do not meet the definition of TRALI.**[37,38]**

The neutrophil priming hypothesis does not require antigen-antibody interactions and occurs in patients with clinical conditions that predispose to neutrophil priming and endothelial activation such as infection, surgery, or inflammation. Bioactive substances in the transfused component activate the primed, sequestered neutrophils, and pulmonary endothelial damage occurs.

Both proposed pathophysiologic mechanisms lead to pulmonary edeme, in the absence of circulatory overload.

Circulatory overload occurs when the volume of the transfused blood components and any coincidental infusions cause acute hypervolemia and, typically, acute pulmonary edema.**[39]**

Acute hemolytic transfusion reactions may be either immune-mediated or nonimmune-mediated. Immune-mediated hemolytic transfusion reactions caused by immunoglobulin M (IgM) anti-A, anti-B, or anti-A,B typically result in severe, potentially fatal complement-mediated intravascular hemolysis. Immune-mediated hemolytic reactions caused by IgG, Rh, Kell, Duffy, or other non-ABO antibodies typically result in extravascular sequestration, shortened survival of transfused red cells, and relatively mild clinical reactions.**[40]**

Acute hemolytic transfusion reactions due to immune hemolysis may occur in individuals who have no antibodies detectable by routine laboratory procedures.**[41]**

Experimental evidence supports a central role for cytokines in the pathophysiology of hemolytic transfusion reactions. Tumor necrosis factor appears to be the most commonly identified mediator of intravascular reaction and end-organ injury although other cytokines have been implicated including interleukin (IL)-8, monocyte chemoattractant protein, and IL-1 receptor antagonist.**[42]**

Nonimmune hemolytic transfusion reactions occur when red blood cells (RBCs) are damaged before transfusion, resulting in hemoglobinemia and hemoglobinuria without significant clinical symptoms.**[43]**

Blood-thickening disease is one of the major side effects that is caused due to blood doping. People suffering from this condition may experience the following symptoms: Kidney Stones, Gout, Peptic ulcers, Stomach ulcers, Skin reddening, Enlarged spleen, Blood clots.

Viscosity increases vascular resistance independently of blood vessel diameter and requires more forceful cardiac contractions to circulate

the blood.**[44]** Blood viscosity rises exponentially as hematocrit increases above 30%.**[45]** A hematocrit (RBC density) above 60% slows oxygen delivery. Blood viscosity increases linearly with hematocrit below 60%, but exponentially at 70% or greater. Furthermore, glucose levels in plasma decrease. At very high hematocrits (i.e., clinical polycythemia, hematocrit ≥ 55%) the disadvantages of hyperviscosity might be detrimental to exercise performance and health. For athletes who compete or train while blood doped, such high hematocrits can be achieved through a combination of the extra RBC and dehydration, which lowers plasma volume.

The sluggish blood flow associated with very high hematocrit is believed to increase the risks of thromboembolic events such as stroke or myocardial infarction. It can also cause venous stasis in small vessels and perhaps thrombosis, which may contribute to deep venous thrombosis and pulmonary embolism. It was reported by *Pandolf KB, et al.,(1995)***[46]** that blood doping did not increase fibrinolytic activity in healthy subjects exposed to high altitude, despite having hematocrits of 52% and 55% at rest and after maximal exercise, respectively. Whole blood transfusions carry measurable risks due to immunogenicity and the transmission of blood-borne infectious diseases.**[47]**

The risk associated with receiving one unit of appropriately screened and tested RBC from a homologous transfusion is estimated to be about 1 in 200,000 for hepatitis B, and between 1 and 3 in 10,000 for hepatitis C.**[48]**

Other risks from stored blood include major transfusion reactions from blood type incompatibility on the basis of clerical error, minor transfusion reactions including fever and body aches, transfusion-related acute lung injury, and bacterial infection.**[49]**

The complications of blood doping can be informed by the symptoms seen in a rare blood-thickening disease called polycythemia vera: blood clots, enlarged spleen, skin reddening, stomach or peptic ulcers, gout, and kidney stones.

Conclusions

The intent of blood doping is to increase maximal aerobic power by increasing the capacity of blood to carry oxygen. This procedure from an ethical point of view, conform to our position is unethical, unfair, and exposes the athlete to unwarranted and potentially serious, even fatal, health risks.

Homologous blood transfusion (blood doping) can be detectable by laboratory tests, since 2000, but autologous blood doping indeed, is not detectable until now and several tests are in validation state. There is a hope that a new test will be ready for the 2012 London Olympics.

WADA and related scientists believe that the big breakthrough in detecting autologous transfusions is likeley to come with the introduction of the "athlete's passport", by wich the athlete will become his own point of reference.

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