AGHADI, Artur, DYBAŁA, Ewelina, CUBER, Iwona, MAZUREK, Magdalena & BIAŁOWĄS, Edyta. Erythropoietin as banned substance in professional sports: effects on maximal aerobic capacity, endurance and detection methods - a review. Journal of Education, Health and Sport. 2023;13(3):331-336. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2023.13.03.042
https://apcz.umk.pl/JEHS/article/view/42251
https://zenodo.org/record/7641712

The journal has had 40 points in Ministry of Education and Science of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of December 21, 2021. No. 32343.
Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical Culture Sciences (Field of Medical sciences and health sciences); Health Sciences (Field of Medical Sciences and Health Sciences).
Punkty Ministerialne z 2019 - aktualny rok 40 punktów. Załącznik do komunikatu Ministra Edukacji i Nauki z dnia 21 grudnia 2021 r. Lp. 32343. Posiada Unikatowy Identyfikator Czasopisma: 201159.
Przypisane dyscypliny naukowe: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 23.01.2023. Revised: 26.01.2023. Accepted: 14.02.2023.

Erythropoietin as banned substance in professional sports: effects on maximal aerobic capacity, endurance and detection methods - a review

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Abstract

Introduction and purpose: Erythropoietin (Epo) and its analogs are used as performance-enhancing substances and are considered doping and are therefore forbidden in sports, however, the scientific evidence behind doping is frequently weak. We aimed to determine the mechanisms of erythropoietin on endurance including maximal aerobic capacity and to present detection methods.

State of knowledge: Erythropoietin is a glycoprotein cytokine. During cellular hypoxia, Epo is secreted mainly by the kidneys, it stimulates bone marrow production of red blood cells. To compensate for normal red cell turnover, low levels of Epo are constantly secreted in sufficient quantities. In addition to anemia and chronic lung disease, cellular hypoxia can result in elevated levels of Epo. VO_2 max is the peak volume of oxygen that a person can consume during exercise. Essential for breathing, oxygen is inhaled by the lungs and converted into energy, which then fuels human cells and expels carbon dioxide in the exhaled breath. This allows the body to manage more efficiently aerobic workouts that involve a large amount of oxygen intake, such as running, swimming or other cardio exercises.

Conclusions: Current data from PubMed indicate the relationship between Epo overuse and increased endurance in professional sports. The rate of VO2 max is an important determinant of aerobic power in sports because VO2 max correlates with the Hb concentration. That is one of the most important reasons to pay attention to the effects of Epo and detection methods.

Keywords: erythropoietin, maximal aerobic capacity, detection methods.

INTRODUCTION AND PURPOSE

Epo has long been recognized as a prohibited substance by the World Anti-Doping Agency(WADA) and the International Olympic Committee (IOC). In 2004 it was incorporated into the list of banned substances and methods issued by the IOC, even prior to this being added to the World Anti-Doping Agency's Prohibited List. This performance enhancer is often abused in endurance sports as it increases red blood cell mass allowing the body to transport more oxygen to the muscles, increasing stamina and performance. Studies have demonstrated that Epo can increase maximal oxygen consumption and time to exhaustion.This review aims to provide an overview of the current knowledge including the relationship between endurance and Epo overuse. We will also describe various detection methods in current usage.

RESULTS:

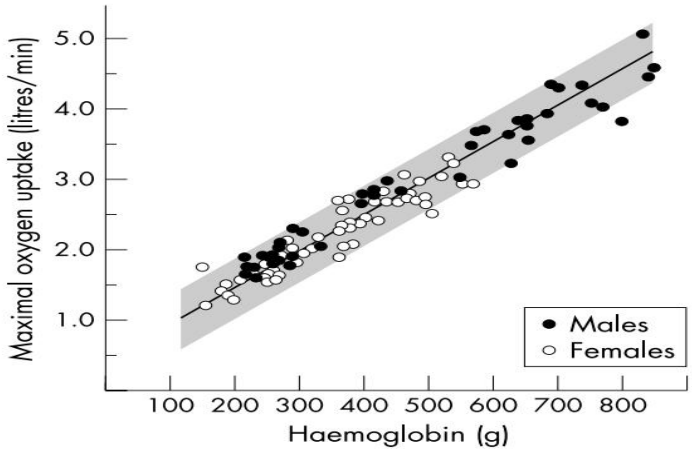
Erythropoietin

Erythropoietin (Epo) is a glycoprotein that is involved in the production of red blood cells. It has several glycans attached to it, which are important for its function and stability. The N-glycans are particularly important for its secretion, receptor binding, and activity in the body. Epo can be produced in the body naturally and can also be produced using recombinant methods. Red blood cells are produced from hematopoietic stem cells, and the expression of the Epo receptor (Epo-R) is increased as the cells mature. Epo binding to its receptor activates intracellular signaling pathways, which leads to the production of more red blood cells. The action of Epo is terminated when the Epo-R-complex is internalized by the target cells and the receptor is dephosphorylated by a protein called SHP-1. Reticulocytes and mature erythrocytes do not have the Epo-R.Iron is an essential mineral for human health. It is primarily incorporated in the heme structures of hemoglobin (Hb) which is the protein that carries oxygen in the blood. The daily loss of iron in healthy individuals is counterbalanced by the absorption of dietary iron. Iron is carried in the blood plasma by the protein transferrin, which delivers it to cells via specific transferrin receptors (TfRs). The levels of transferrin saturation and soluble TfR and ferritin in the blood can provide information on the rate of erythropoiesis (red blood cell production) and the body's iron status. Iron homeostasis is regulated by the hormone hepcidin, which binds to the cell surface iron export protein ferroportin, causing its internalization and degradation. This process increases in conditions of iron overload, hypoxia, or inflammation. Recently, another regulator of iron homeostasis has been identified, the glycoprotein erythroferrone, which is produced by erythrocytic progenitors in response to erythropoietin (Epo) to increase iron absorption and mobilization from stores. The administration of recombinant human Epo (rhEpo) causes a decrease in hepcidin production within 24 hours.[1] Epo is primarily produced in the liver and kidney, with the latter being responsible for about 80% of production following birth. Studies have suggested that the proximal tubule of the kidney is the main location for Epo production, as it closely matches oxygen consumption. In the liver, Epo is produced both in hepatocytes and in interstitial cells known as Ito presinusoidal cells. The production of Epo is upregulated by hypoxia, and it stimulates erythropoiesis in a dose-dependent manner. This led to the idea of a negative feedback loop, similar to the regulation of blood glucose by insulin and of peripheral endocrine hormones by pituitary tropins. Hypoxia increases Epo production in the kidney, which then circulates in the plasma and binds to receptors on erythroid progenitor cells, promoting their viability, proliferation, and differentiation, and increasing red blood cell mass. This, in turn, increases the oxygen-carrying capacity of the blood and enhances tissue oxygen tension, thus completing the feedback loop and suppressing further expression of Epo[2,3,4,5].

Maximal aerobic capacity (VO2 max)

VO2 max is considered to be one of the best indicators of cardiovascular fitness and endurance. During intense exercise, the body demands more oxygen to generate energy for muscle contractions. The VO2 max test measures the maximum amount of oxygen that an individual can consume, transport and utilize during exercise. A higher VO2 max value indicates that the body is able to use oxygen more efficiently, which in turn allows for greater endurance during physical activity. VO2 max can be improved by regular exercise and training, particularly by incorporating both endurance and interval training into your workout routine. The intensity and duration of exercise should be gradually increased over time. However, genetics also plays a role in determining an individual's VO2 max. People with naturally high VO2 max values tend to have larger hearts, more efficient lungs, and higher red blood cell counts, which allows them to transport and utilize oxygen more efficiently. VO2 max is calculated as the highest rate of oxygen uptake recorded during the test, typically expressed in milliliters of oxygen per kilogram of body weight per minute (ml/kg/min). VO2 max values can vary widely among individuals, but generally, a value of 40-50 mL/kg/min is in most cases considered excellent, while values below 30 mL/kg/min are in most cases considered very low. It is also important to note that VO2 max

can decrease with age, so it is important to continue regular exercise and training to maintain cardiovascular fitness[7]. The most accurate method for measuring VO2 max involves performing a graded exercise test on a treadmill or stationary bike while wearing a mask or mouthpiece to collect respiratory gas samples. The test starts at a low intensity and gradually increases in intensity until the individual can no longer continue. During the test, the subject's heart rate, rating of perceived exertion, and oxygen and carbon dioxide levels are measured and recorded. The test is usually performed by a trained exercise specialist and interpreted by a doctor or sports physician, and can provide valuable information about an individual's aerobic fitness level and the effectiveness of their training program.



This data is showing the relation between total body hemoglobin and maximal aerobic capacity for 94 subjects, age 7–30 years.[6]

Epo in sports

Physical exercise can affect erythropoiesis, the process of producing red blood cells, in a number of ways. The plasma level of Epo, a hormone that regulates erythropoiesis, is not usually affected by single bouts of strenuous exercise at sea level. This is because the oxygen sensor that controls Epo synthesis is not located in skeletal muscles or the heart, but in the kidneys. Typically, there are no major differences in basal red blood cell count, hematocrit, blood hemoglobin concentration, and mean corpuscular hemoglobin concentration in athletes compared to non-athletes, but hemoglobin mass is increased in elite endurance athletes. In contrast, sportsmen, particularly those practicing endurance sports, may present with relatively low hemoglobin concentrations and hematocrit values. This is known as "sports anemia" and is usually a pseudoanemia due to an enlarged plasma volume. If there is an increased rate of intravascular hemolysis caused by compression, the red blood cell lifespan will be reduced[8].Erythropoietin (EPO) is a hormone that stimulates the production of red blood cells. Recombinant human erythropoietin (rHuEPO) is a version of this hormone produced using biotechnology methods. This has made it possible for it to be widely used as a therapeutic treatment, but it has also been misused in sports as a performance-enhancing drug. Pharmaceutical companies are currently developing alternative methods of stimulating erythropoiesis, which could also be used for doping[9,10].Recombinant human erythropoietin (rHuEpo) is a substance that has been shown to have an ergogenic effect, particularly in endurance events. Studies have shown that it improves hematological parameters, VO2max, and power output at different intensities, and can increase sub-maximal more than maximal aerobic parameters, which is important for endurance events that are performed at sub-maximal intensities. The cognitive effects of rHuEpo, such as reducing recovery time between workouts, and the ability to improve psychological resources, are also important factors to consider. However, it should be noted that rHuEpo has been misused by athletes, and detection of its use can be difficult. Direct methods include isoelectric focusing, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and membrane-assisted isoform immunoassay. Indirect methods include the hematological module of the Athlete Biological Passport, which calculates an abnormal blood profile score (ABPS) for each athlete. The last frontier of detection is represented by omics, the analysis of gene expression which may be altered by rHuEpo administration. It's also important to consider that rHuEpo may be misused in combination with other performance-enhancing drugs, and the interactions between them should be evaluated.[11,12,13,14].Empirical trials have shown consistent and positive effects of rHuEpo (recombinant human erythropoietin) on VO2max, with an improvement of 6-8% when moderate doses are administered[15]. However, further research is needed to clarify the effects of rHuEpo on top-class athletes and sub-maximal performance, as well as the non-erythropoietic effects, interactions with other substances, and the impact of

genetics on responsiveness to rHuEpo administration. Additionally, more rigorous methodological approaches are needed in future studies.

Adverse effects:
Doping is banned to create a level playing field for athletes and protect them from potentially harmful substances. Studies on rHuEPO in healthy or trained subjects have not focused on adverse effects. The small number of subjects and treatment times in these studies make it difficult to detect rare side effects. Larger patient studies should be consulted for this information, but it's important to keep in mind that their results may not apply to well-trained athletes. One patient study was stopped early due to an increased incidence of blood clots in rHuEPO-treated metastatic breast cancer patients. Other studies and meta-analyses showed similar trends in different patient groups treated with rHuEPO. These studies used doses four times higher than those used in endurance performance studies in healthy subjects. The increase in blood viscosity, blood pressure, and coagulation, as well as platelet reactivity and inflammation after rHuEPO treatment may explain these blood clots. Acute exercise can also increase coagulation, especially in untrained subjects. The combination of reduced plasma volume and increased Hct in acute exercise may increase the risk of blood clots in endurance athletes using rHuEPO. Increased Hct can also lower cerebral blood flow and increase the risk of heart failure, myocardial infarction, seizures, and pulmonary embolism. Red cell aplasia is another rare side effect of rHuEPO treatment. The illicit use of rHuEPO in sports could increase the risk of immunogenic complications. Finally, there is a debate over whether rHuEPO use may promote tumor growth and angiogenesis[16,17].

Most common detection methods:
Isoelectric focusing(IEF)
Firstly method is a separation technique that exploits differences in carbohydrate composition between rHuEPO and endogenous HuEPO. The method involves concentrating the urine sample, separating the EPO molecules present in the urine on an IEF gel with a pH gradient, and visualizing the isoforms using a monoclonal AE7A5 antibody and chemiluminescence. The resulting IEF pattern allows for the detection of rHuEPO and its analogs, darbepoetin alfa, but not epoetin delta, which has an IEF pattern that partially overlaps with that of endogenous HuEPO. However, proteinuria can affect the IEF distribution and lead to an atypical IEF profile[18].

Sarcosyl (SAR)-polyacrylamide gel electrophoresis (PAGE)
Another approach to detect the use of rHuEPO in doping athletes is based on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). SDS-PAGE is a technique that separates proteins based on their molecular weights and was developed in the 1970s. Using mass spectrometry, it was discovered that the molecular weights of rHuEPOs were higher than those of HuEPO due to differences in the glycosylation pattern, which could be detected in urine and/or blood samples using SDS-PAGE. The technique uses two internal standards to calculate the relative mobility values and discriminate the analytes in the specimens. The negative feedback of rHuEPO administration can be detected as the absence of HuEPO bands on the gel, and during washout period, an intense rHuEPO band evolved in a glycoform smear above the HuEPO band[19].

Membrane-assisted isoform immunoassay
This test is also used to detect the use of recombinant human erythropoietin (rHuEPO) in athletes. The assay combines a chromatographic separation of the glycosylated isoforms of EPO with wheat germ agglutinin (WGA), a lectin that binds to specific carbohydrate groups, and a lateral flow immunoassay using anti-EPO carbon black nano strings. The assay is executed on a dipstick and can differentiate endogenous EPO from recombinant EPO based on the affinity of different isoforms with WGA. The assay is faster than existing WADA-accredited methods, such as IEF and SDS/SAR-PAGE, and can screen a batch of 15 samples within 1 hour. However, the test needs further improvement and the results are more difficult to interpret and defend in court than the bands from electrophoresis gel. Despite these limitations, the dipstick test may be an interesting complement to the existing anti-doping tests[20].

Liquid chromatography-tandem mass spectrometry (LC-MS/MS)
LC-MS/MS is a method for the detection of human erythropoietin. The method involves separating erythropoietin from other substances in the sample using liquid chromatography, followed by identification and quantification of erythropoietin using mass spectrometry. In liquid chromatography, a sample is first passed through a column filled with a stationary phase. The components of the sample are separated according to their physical and chemical properties. Erythropoietin is then further purified by mass spectrometry, where it is ionized and split into smaller masses. These masses are then measured and used to identify erythropoietin. Erythropoietin concentrations are determined by comparing the measured mass to a reference standard. The high sensitivity and specificity of this method make it a reliable tool for the detection of human erythropoietin[21].

The Athlete Biological Passport (ABP)

ABP uses changes in an athlete's blood hormone levels, such as erythropoietin (EPO), to detect the use of performance-enhancing drugs (PEDs). EPO is a hormone produced by the kidneys that stimulates the production of red blood cells. When used as a PED, it can improve endurance and performance by increasing the number of red blood cells in the body.The ABP program monitors an athlete's EPO levels over time and compares the results to their individual baseline values. The ABP uses statistical analysis and algorithms to detect abnormal changes in an athlete's EPO levels, which may indicate PED use. If the results of the analysis are deemed suspicious, further testing may be required to confirm the use of PEDs.It's important to note that the ABP is not a single test but a long-term monitoring program that uses multiple blood samples to detect changes in an athlete's EPO levels over time. This approach helps to ensure that any changes in EPO levels are not just due to normal variation but are instead indicative of PED use[22].

CONCLUSIONS

The rate of VO2 max is an important factor when considering aerobic power in sports. This correlates with the hemoglobin concentration and oxygen-carrying capacity of the blood. Furthermore, the body's lactate buffering ability increases with Hb mass. Within accepted limits, higher hemoglobin levels will lead to better performance in aerobic activities. Unfortunate as it is, illegal drug use has also become popular among recreational athletes who don't always have access to professional medical care. Erythropoietic agents are misused in these circumstances with the intention to enhance performance. Sports federations have a powerful tool in the form of blood tests to monitor any potential misuse of rHuEPO or blood transfusion. They should focus their anti-doping efforts on athletes whose blood data is abnormal.

Disclosures: No disclosures

Financial support: No financial support was received.

Conflict of interest: The authors declare no conflict of interest.

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