

A Review of Erythropoietin Abuse: An Analysis of Effectiveness and Safety in Exercise

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Abstract

Many athletes have been abusing performance enhancing substances for centuries to gain an advantage over their competitors. More recently, athletes such as cyclists, distance runners and cross-country skiers have been utilizing recombinant human erythropoietin (RhEPO) to improve their endurance performance. In clinical practice, RhEPO is used to treat anemia associated with various disorders by increasing hemoglobin (Hb) and hematocrit (Hct) levels. Endurance athletes hope to use this effect in order to increase their VO_2 max, which may potentially translate into better athletic outcomes. In exercise studies RhEPO use has shown to increase Hb, Hct, VO_2 max, and time to exhaustion (TTE). Despite these potential benefits, RhEPO use has been associated with serious cardiovascular events and even death. This review will provide an in-depth analysis of RhEPO's effectiveness on exercise performance from various exercise studies, discuss its safety profile, and also analyze detection methods.

Introduction

Athletes have been utilizing performance-enhancing substances dating back to the early Olympians of 776 BC [1]. In more recent decades, blood doping and abuse of erythropoietin stimulating agents (ESA) have become more commonplace among endurance athletes. Blood doping, which involves blood transfusions, refers to the infusion of his or her own (autologous) or matched (homologous) red blood cells (RBC) to increase hemoglobin (Hb), hematocrit (Hct), and oxygen transport [2]. Blood doping became heavily abused in endurance athletes such as cyclists, distance runners, triathletes, and cross-country skiers in the 1970s and 1980s [3]. However, blood doping has its drawbacks primarily infection risks and storage issues [3]. In 1989, recombinant human erythropoietin (RhEPO) was approved by the Food and Drug Administration (FDA) and has been used clinically for the treatment of anemia associated with chronic renal failure, chemotherapy, HIV, and surgical procedures [4]. Endogenous erythropoietin is a 34-39 kDa glycoprotein which stimulates erythroid proliferation, synthesized in the kidney [5]. Currently, there are two ESAs approved for use in the United States: epoetin alfa and darbepoetin. Both ESAs act similarly to endogenous erythropoietin by stimulating erythroid progenitor's cells to produce red blood cells [4]. As one might expect, abuse of RhEPO became more popular because it does not require transfusions, infection risk is decreased, and storage requirements are less complicated.

Endurance athletes abuse RhEPO to increase their Hb and Hct levels, resulting in enhanced oxygen carrying capacity. As a result, administration of exogenous RhEPO is thought to augment maximal oxygen uptake (VO_2 max). VO_2 max, which is a marker of aerobic power is the maximal oxygen uptake that can be used at the cellular level for the entire body [6]. Higher VO_2 max levels have been shown to improve endurance performance [7-9]. In addition to enhanced oxygen carrying capacity, RhEPO has demonstrated anabolic effects such as an increase in total body weight [10]. Despite these potential endurance benefits, there are known complications with RhEPO use. Serious adverse effects may consist of polycythemia, myocardial infarction, stroke, hypertension, congestive heart failure, seizure, embolism, and death [9,11-15]. The mechanism behind many of these complications are possibly related to miscalculation of RhEPO doses as well as increased levels of Hb and Hct. Dehydration during an endurance event coupled with hyper viscosity and elevated blood pressure due to elevated Hb and Hct levels may attribute to the potentially severe adverse events [11-15]. As a result of the potential athletic advantage and harm, the World Anti-Doping Agency (WADA) has added RhEPO or any analog to its list of prohibited substances. Other anti-doping organizations

such as the International Olympic Committee (IOC), United States Olympic Committee (USOC), National Collegiate Athletic Association (NCAA), Union Cycliste Internationale (UCI) have also implemented WADA's code [16]. In this review, we will illustrate the effectiveness and safety of erythropoietin in exercise settings.

Methods

A comprehensive MEDLINE search (1966 – March 2012) for English language reports of clinical trials in human subjects was performed. The following search terms were used: erythropoietin stimulating agents, erythropoietin, darbepoetin, abuse, exercise, aerobic, sprinting, sports endurance, athletes, volume expanders, effectiveness, side effects, adverse effects, and VO_2 max.

Results

Effectiveness

Multiple studies examined the use of RhEPO on its effect in exercise performance. The following studies below will describe the effectiveness of RhEPO on Hb, Hct, VO_2 max, and time to exhaustion (TTE) in various exercise tests (Table 1).

Birkeland et al. [17] performed a double-blind, randomized placebo-controlled study with 20 male athletes involved in cycling, triathlon, and cross-country skiing who received either RhEPO (5000 IU three times weekly) versus placebo for four weeks or reached a Hct ≥ 50 %, with a follow-up period of four weeks. Ten subjects were randomly assigned to each group. They analyzed Hct and VO_2 max levels before and after RhEPO through an incremental exercise test via cycle ergometry. The incremental exercise test increased by 50 W every two minutes until exhaustion. After RhEPO administration, Hct levels

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Author	Sample	Treatment	Exercise intervention	Primary end point
Birkeland et al.	20	5000 IU three times weekly	Cycle ergometry	Hct levels increased 19.0% (42.7 to 50.8%; P<0.001) and VO ₂ max increased 7.5% (P=0.001)
Wilkerson et al.	15	150 IU/kg per week	Cycle ergometry	Hb levels increased 7% (15.8 – 16.8 g/dl; P < 0.01) 12% in Hct (43- 49%; P < 0.01) 7% improvement in VO ₂ max (P < 0.05) TTE was extended by 22% (P < 0.01)
Thomsen et al.	8	5000 IU injections (per protocol)	Cycle ergometry	Hb increase by an average of 10.3 % (14.6-15.9 g/dl; P < 0.05) VO ₂ max by 8.6% (P < 0.05). TTE was significantly increased by roughly 54.0% (P < 0.05)

RhEPO: Recombinant human erythropoietin
Hct: Hematocrit
Hb: Hemoglobin
IU: International Units
TTE: Time to exhaustion
Kg: Kilograms

Table 1: Trials with RhEPO versus placebo controlled studies [17,18,19].

and VO₂ max increased 1-day post-treatment and remained elevated 3 weeks post-RhEPO administration (Table 1). The authors concluded that administration of RhEPO resulted in an increase in Hct and VO₂ max in endurance athletes using cycle ergometry.

Wilkerson et al. [18] performed a double-blind, randomized placebo-controlled study evaluating the administration of subcutaneous RhEPO (150 IU/kg per week) versus placebo (sterile saline) for four weeks in a sample of 15 subjects (8 RhEPOs vs. 7 placebos). They assessed the effects of RhEPO on Hb, Hct, VO₂ max, VO₂ peak power output, and TTE during an incremental exercise test via cycle ergometry in young healthy adults. The incremental exercise test transitioned from different work rates (i.e. moderate to heavy to severe over 10-12 minutes). The authors concluded administration of RhEPO resulted in an increase in Hb, Hct, VO₂ max, VO₂ peak power output, and TTE during an incremental cycle ergometry test (Table 1).

Thomsen et al. [19] described an analytical study with 8 unblinded subjects in the RhEPO group (5000 IU injections as follows: weeks 1-2, one injection every second day, week 3, three injections on three consecutive days, and weeks 4-13, one injection every week) versus a blinded control-group (saline) for 13 weeks. The authors investigated the use of RhEPO effect on endurance performance via cycle ergometry. The incremental exercise test increased workload by 40 W every 1.5 minutes until exhaustion. Hb, VO₂ max, and TTE were measured before RhEPO administration to determine baseline characteristics and after 4 and 11 weeks of treatment. After RhEPO administration, there was an increase in Hb, and VO₂ max. In addition, TTE was significantly increased at weeks 4 and 11 respectively. The increase in TTE, which the authors also recognized as a limitation may have been attributed to the study design and is not conclusive due to differing exercise intensities during the course of the study. The authors concluded administration of RhEPO resulted in an increase in VO₂ max and a possible increase in TTE after 4 and 11 weeks via cycle ergometry (Table 1).

Other exercise studies concluded similar effects of RhEPO on Hb, Hct, and VO₂ max in exercising subjects (Table 2) [13,20,21]. Parisotto et al. [20] performed a double-blind trial with 27 recreational athletes who received a combination of RhEPO (50 IU/kg three times weekly) plus intramuscular (IM) iron injections, RhEPO plus oral (PO) iron tablets, or placebo for 25 days followed by a 4 week wash-out period. Hb and VO₂ max were measured either on a treadmill or cycle

ergometer. After four weeks, the (RhEPO + IM) and (RhEPO + PO) groups both observed an increase in Hb and VO₂ max from baseline (Table 2). Lundby et al. [21] performed an unblinded observational study with 8 male recreational athletes treated with RhEPO (5000 IU injections as follows: weeks 1-2, one injection every second day; week 3, three injections on 3 consecutive days, and weeks 4-15, one injection every week) for four months via cycle ergometry. The results showed an increase in Hb and Hct in maximal exercise after RhEPO treatment (Table 2). O₂ delivery and VO₂ max both increased by 300 ml. Balsom et al. [13] performed an unblinded observational study with six subjects who received RhEPO (2000 IU three times weekly) which included fifteen 6-second high-intensity bouts of uphill running (10° gradient) on a treadmill, interspersed with 24 seconds of passive rest before and after the 6 week RhEPO administration. Results showed an increase in Hb and VO₂ max an (Table 2). Whether the enhanced results in controlled exercise environments, as mentioned in the above studies, translate into actual improvements in live competition has yet to be published and will unlikely occur due to ethical issues.

To summarize, the dosing ranges of RhEPO administration in the exercise studies ranged from 6,000 to 20,000 IU/week for approximately 7 weeks and consistently demonstrated increases in VO₂ max by 6-9% in the exercising subjects. As such, RhEPO appears to have potential in improving endurance performance.

Safety

We will discuss the safety profile of RhEPO in patients being treated with anemia as well as the exercise studies mentioned above. In the anemia studies, common side effects included fever, pruritus, arthralgia, cough, and congestion. Serious adverse events included polycythemia, myocardial infarction, stroke, hypertension, congestive heart failure, seizure, embolism, and death [9,13-15]. The incidence of these serious adverse events in anemic patients increased as the Hb levels increased above 13.0 g/dl [14,15].

The following exercise studies looked at RhEPO effects on blood pressure. Lundby et al. [21] reported that RhEPO treatment for four months resulted in an increase in resting systolic (135.1 +/- 2.2 to 143.8 +/- 4.3 mmHg), diastolic (76.2 +/- 1.2 to 80.1 +/- 1.2 mmHg), and mean blood pressures (96.7 +/- 1.3 to 102.0 +/- 2.1 mmHg) (P < 0.05). In Parisotto et al. [20] one subject withdrew from the study due to the

Author	Sample	Treatment	Exercise intervention	Primary end point
Parisotto et al.	27	50 IU/kg three times weekly plus intramuscular iron injections or oral iron tablets	Treadmill or cycle ergometer	(RhEPO + IM) and (RhEPO + PO) groups both observed an increase in Hb (6.9 +/- 0.6% and 12.0 +/- 0.7%, respectively) and VO ₂ max (6.3 +/- 1.8% and 6.9 +/- 1.1 %, respectively)
Lundby et al.	8	5000 IU injections (per protocol)	Cycle ergometry	Hb and Hct increased 12% and 12%, Respectively
Balsom et al.	6	2000 IU three times weekly	Treadmill	10.5% increase in Hb and 8% in VO ₂ max

RhEPO: Recombinant human erythropoietin
Hct: Hematocrit
Hb: Hemoglobin
IU: International Units
TTE: Time to exhaustion
Kg: Kilograms
IM: intramuscular
PO: oral

Table 2: Non-placebo RhEPO controlled studies [13, 20, 21].

development of mild hypertension (undefined by the authors) after RhEPO administration. On the other hand, Berglund and Ekblom [22] reported no statistical change in resting systolic (125 +/- 10.8 to 125 +/- 11.4 mmHg) and diastolic (64 +/- 5.7 to 67 +/- 8.7 mmHg) blood pressures after RhEPO administration and placebo (20 IU/kg of RhEPO 3 times a week for 6 weeks and 7 received 20 IU/kg RhEPO 3 times a week for 4 weeks, followed by 40 IU/kg for the next 3 weeks). With regards to sub maximal exercise with cycle ergometry at 200 W, there was an increase in systolic (177 +/- 14.2 mmHg to 191 +/- 19.5 mmHg (P < 0.01) blood pressure. Aside from blood pressure, the various exercise studies did not analyze other safety markers or adverse effects with regards to RhEPO. Aerobic exercise has been shown to reduce resting systolic and diastolic blood pressure in adults [23,24]. Despite this, several participants in the RhEPO study group demonstrated a slight increase in resting systolic blood pressure. However, further research is needed to determine the true effect of RhEPO on blood pressure response among exercisers.

The potential danger of RhEPO abuse in athletes cannot be overlooked. In a case report described by Lage et al. [25] a 26 year-old professional cyclist presented with a 2-month history of headaches that had increased in previous weeks during a cycling competition. An MRI of the brain revealed occlusion of the superior sagittal and right transverse sinuses. He was diagnosed with cerebral sinus thrombosis and admitted using RhEPO 200 IU every 2 days for the past 3 months as well as growth hormone. However, the diagnosis of thrombosis cannot be solely attributed to RhEPO alone but related to a doping cocktail. Additionally, 18 European professional cyclists have died with a possible link to RhEPO abuse [26]. The combination of increased Hct (levels above the normal range), thicker blood viscosity, and dehydration may place athletes at an even greater risk of adverse events with RhEPO use compared to anemic patients.

Detecting methods

The use of RhEPO in endurance athletes is difficult to detect. In addition to the micro-doses of RhEPO that are used, the chemical structure and pharmacodynamics are similar between exogenous and endogenous erythropoietin. However, RhEPO is produced in Chinese hamster cells and have hyposulfated sugar moieties, making it identifiable [27,28]. The IOC, WADA, UCI and various organizations prohibit the use of RhEPO and currently test athletes. In addition, the UCI also analyzes Hct and has specified cutoff levels: 50% (men) and

47% (woman) [20]. In 2009, four cyclists tested positive for RhEPO use and were sanctioned with 2-year bans from competition [29].

Currently, there are two main methods of detecting RhEPO: direct and indirect. The direct method uses isoelectric technique based on hyposulfated sugar composition in the urine [30-32]. It is able to detect epoetin-alfa, epoetin-beta, and darbepoetin [27]. This method is accurate and is less invasive than the indirect method [27,30]. However, it is time-consuming, expensive, and the window time for detection is small. Wide et al. [33,34] reports less than 50% of users can declare a positive test once RhEPO use has been stopped for more than 3 days and no samples can be detected after 7 days [33-35].

The indirect method is a blood test that has various parameters: Hct, serum erythropoietin concentration, soluble transferrin receptor level, percentage of reticulocytes, and percentage of macrocytes [30,35]. This method has an ON and OFF model. The ON model identifies RhEPO during or shortly after use and the OFF model is able to identify RhEPO weeks after stopping treatment [30]. The major drawback with the indirect method is the need for blood as well as the need for counter analysis. However, the indirect method is relatively fast and less expensive than the direct method and is an effective screening tool [30,36]. Due to the many challenges and obstacles of detecting RhEPO abuse, WADA has implemented the urine EPO test proposed by Lasne and colleagues as the method of choice to detect urinary RhEPO [33].

A new approach to detecting RhEPO abuse in addition to isoelectric-focusing, double-blotting is with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The use of SDS-PAGE testing has illustrated structural differences between recombinant versus endogenous erythropoietin [37]. The advent of SDS-PAGE has been vital to the anti-doping agencies for detection of RhEPO abuse. Doping athletes have been abusing epoetin-alfa, and epoetin-beta but a newer agent epoetin kappa has been approved in Japan. Current testing techniques with isoelectric focusing polyacrylamide gel electrophoresis (IEF-PAGE) and SDS-PAGE were validated to determine whether they were able to detect epoetin kappa. It has been shown that intravenous (IV) administration with epoetin kappa has a greater clearance than subcutaneous route and detection time with SDS-PAGE is longer than IEF-PAGE (within 24 hours versus 10 hours) [38].

Despite current tests, athletes continue to develop new methods to counter the system. With regards to the Hct cutoffs athletes have

implemented the use of volume expanders to artificially lower their Hb and Hct levels after blood doping or RhEPO use [39]. An example of a volume expander is a colloid solution which consists of albumin, gelatin, dextran, and hydroxyethyl starch. Next, blood doping has regained its popularity due to the difficulty in detection. In 2002, the IOC discovered blood transfusion equipment in several Austrian cross-country skiers' housing [40]. In addition, there were professional cyclists that were found guilty for blood doping in 2004 [41]. In 2006, several cyclists were suspended from the Tour de France due to involvement with blood transfusions and exogenous hormone administration [3]. There have also been reports of the formation of doping rings assisting athletes in storing and administering blood products and other doping substances [42]. Lastly, newer agents to enhance oxygen delivery in the body have been developed. An example is the continuous erythropoietin receptor activator (CERA), a third generation RhEPO that is similar to human erythropoietin [43]. New detection methods are currently being evaluated. High throughput screening methods with enzyme-linked immunosorbent assay (ELISA) are used to detect CERA in serum samples as well as methyl glycine-based anionic surfactant polyacrylamide gel electrophoresis (SARKOSYL-PAGE) [44,45]. In addition, small non-proteins (microRNAs) are currently being evaluated as possible biomarkers to detect CERA [46]. However, there continues to be the ongoing cat and mouse game between the drug dopers and drug testers.

Despite advancement in detection methods, there is a greater concern for abuse of other substances. Currently, there is an investigational product that is able to produce the same effect on oxygen transport similar to RhEPO and lacks the chemical structure related to RhEPO. Peginesatide, a pegylated homodimeric peptide, is currently under Phase-III trial for approval of treatment of anemia associated with chronic kidney disease. As a result, current research studies are evaluating possible ELISA and mass spectrometry to detect peginesatide [47].

Conclusion

In exercise studies RhEPO use has shown to increase Hb, Hct, VO_2 max, and TTE. With the observed increase in VO_2 max and TTE, performance enhancement may be seen in endurance events. This may increase the temptation to abuse RhEPO among athletes, as any slight advantage may be the difference between winning and losing. However, there are potential serious complications with RhEPO use, such as myocardial infarction, stroke, hypertension, congestive heart failure, seizure, embolism, and death. As such, clinicians, athletes, and coaches must be better informed about the potential dangers of RhEPO abuse. Future research should explore the prevalence of RhEPO abuse in athletes.

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