The clinical pharmacology of performance enhancement and doping detection in sports

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3

ERYTHROPOIETIN DOPING IN CYCLING: LACK OF EVIDENCE FOR EFFICACY AND A NEGATIVE RISK–BENEFIT

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ABSTRACT

Imagine a medicine that is expected to have very limited effects based upon knowledge of its pharmacology and (patho)physiology and that is studied in the wrong population, with low-quality studies that use a surrogate end-point that relates to the clinical end-point in a partial manner at most. Such a medicine would surely not be recommended. The use of recombinant human erythropoietin (rHuEPO) to enhance performance in cycling is very common. A qualitative systematic review of the available literature was performed to examine the evidence for the ergogenic properties of this drug, which is normally used to treat anaemia in chronic renal failure patients. The results of this literature search show that there is no scientific basis from which to conclude that rHuEPO has performance-enhancing properties in elite cyclists. The reported studies have many shortcomings regarding translation of the results to professional cycling endurance performance. Additionally, the possibly harmful side-effects have not been adequately researched for this population but appear to be worrying, at least. The use of rHuEPO in cycling is rife but scientifically unsupported by evidence, and its use in sports is medical malpractice. What its use would have been, if the involved team physicians had been trained in clinical pharmacology and had investigated this properly, remains a matter of speculation. A single well-controlled trial in athletes in real-life circumstances would give a better indication of the real advantages and risk factors of rHuEPO use, but it would be an oversimplification to suggest that this would eradicate its use.

INTRODUCTION

Sport is big business

The summer of 2012 was an intensive summer of sports. From all these events, it is clear that sports play a very important role in our society as it brings people together, gives pleasure, keeps people healthy and can bring professional athletes fame and honour.

Sport has grown to be so important that large amounts of money are now involved and the will and pressure to win have steadily increased. Cheating has therefore become a threat to all sports, with some sports being more susceptible to it than others. Cheating by use of medicines has understandably taken place outside the realm of clinical pharmacology and evidence based medicine. We question if this is correct, as uncontrolled use of a substance induces risks for the users, irrespective of such a substance being used legally or illegally. In this review we will focus on the use of recombinant human erythropoietin (rHuEPO) in cycling, a sport that has had many reports of cheating, culminating in the last decennia, with many suspicions and suspensions. We will address the question if the current available evidence even warrants the widespread use of this substance. Many of the big champions in cycling have been associated with, or suspended for use of (blood) doping. In the Tour de France of 1998 the entire Festina team, as well as the TVM team, were taken out of the race on suspicion of rHuEPO use. This Tour was later given the name 'Tour du Dopage' and many confessions of systematic doping (i.e. rHuEPO) use throughout the peloton were given. In spite of this, later champions in the Tour de France, Giro d'Italia and Vuelta a España have also been suspended because of proof of blood doping, but the Code of Silence also called 'omerta', was never broken. Seven years after the last of seven consecutive Tour de France wins, one of the most successful road cyclists ever, Lance Armstrong, has been suspended for life by the United States Anti-Doping Agency (USADA) on charges of doping (e.g. rHuEPO) use and trafficking in the biggest doping case ever, backed by confessions of many of his teammates.[1]

Knowledge of both the effects and side effects of rHuEPO in this population is essential, especially with so many misconceptions among the people involved. Firstly, if the effects are not pronounced, the motives for misuse will be less strong. Secondly, even if the effects are pronounced, knowledge of the potentially dangerous side effects needs to be communicated to the cyclists, who are likely to be under severe pressure to use performance enhancing agents, together with the coaches and physicians supervising them.[1]

Physiology of erythropoietin

Erythropoietin (EPO) is a (glyco)protein that is mainly involved in erythropoiesis, the (re-)generation of erythrocytes, or red blood cells. Red blood cells are cells without a nucleus and transport oxygen through the blood. Due to a lack of ability to repair themselves without a nucleus and other cellular machinery, erythrocytes have a life span of approximately 120 days in the circulation and after that need to be replaced.[2] The spleen removes the old erythrocytes (2-3 million every second) and to keep oxygen carrying capacity of the blood at a steady level, constant erythropoiesis is necessary.[2] Erythropoiesis starts in the bone marrow, where red blood cells originate from pluripotent stem cells.[3] These stem cells continuously make identical copies of themselves and in that way create progenitor cells for, among others, erythrocytic cells.[3] These cells go through different stages, one of which is the burst-forming unit-erythroid (BFU-e). This cell type matures into a colony-forming unit-erythroid (CFU-e), which in turn forms the proerythroblast, which divides four times into 16 reticulocytes, later developing into mature red blood cells.[3] The first report of a factor influencing this red blood cell production was by Carnot and Deflandre,[4] who called it "hemopoietine". This factor, now called erythropoietin, is a hormone of 165 amino acids with four glycosylation sites and a molecular weight of 30,400 and a carbohydrate content of 40%.[5,6] Under normal (non-hypoxic) conditions the concentration in blood is relatively constant at 5 pmol/L, essential to stimulate cells in the bone marrow to produce new erythrocytes, compensating for the physiological demise of erythrocytes.[3] This level is equal to ~20 mU/mL when erythropoietin is quantified as 'international units' (IU), assuming a specific activity of 130,000 IU/mg. The cells that are the main target for the hormone are the CFU-e's and proerythroblasts, containing the highest density of erythropoietin receptors (EpoR).[7] The main effect of EPO is on CFU-e's, as it promotes survival of these cells.[8] One of the pathways involved in this process, activated by EPO, is the cell proliferation pathway of Ras/MAP kinase.[9,10] After binding of EPO to its receptor dimerization of two EpoR molecules occurs, starting the intracellular signalling leading to proliferation of CFU-e's.[11,12]

Production and metabolism

The main EPO producing organ in humans is the kidney,[13,14] where peritubular interstitial cells are the cells governing this production.[15,16] Production of EPO is highly regulated, and it has been shown that baseline EPO levels can increase up to a 1,000-fold in low blood oxygen content, for example in severe anaemia.[3] EPO production is therefore highly dependent on blood oxygen levels, with hypoxia increasing EPO production, irrespective of the cause of reduced tissue oxygen supply.[3] This process takes approximately 1.5-2 hours before EPO levels start increasing linearly, reflecting the time of signal transduction and hormone synthesis and secretion. Peak EPO concentrations after hypoxia are reached within 48 hours, with concentration being dependent on the severity of hypoxia.[3] However, only moderately elevated serum concentrations of EPO seem to be sufficient to maintain an increased erythropoiesis rate.[17] The proposed oxygen-sensing mechanism regulating erythropoietin production involves the hypoxia-inducible factor (HIF), a transcription factor.[18] HIF expression is seen in hypoxia exposed cells within 30 minutes,[19] after which the heterodimeric protein travels to the nucleus to activate the EPO enhancer,[20] inducing EPO transcription. In the presence of oxygen this factor is hydroxylated, suppressing the activity and promoting degradation.[21] Another pathway involved in EPO production is the kinase C pathway, activated through adenosine. This non-HIF transcriptional factor also increases EPOmRNA expression.[22] GATA-2 inhibits the EPO promoter, and is a third regulational pathway of EPO. GATA inhibitors can therefore also enhance EPO production.[23] After hypoxia-induced erythropoietin production a rise in red blood cells and haematocrit (Hct) is seen after 60-70 hours,[24] corresponding to the time course of CFU-e differentiation into mature erythrocytes.[25] This also is in line with the observation that endogenous erythropoietin has an estimated half-life much shorter than that, of approximately 5.2 hours.[26] So the clearance of EPO is, like many other glycoproteins, rather slow. This is mainly due to the terminal sialic acid residues, preventing galactose receptor binding, internalization and degradation in the liver. Indeed, it has been shown that desialated EPO results in rapid hepatic clearance,[27] but this process almost does not occur *in vivo* and therefore plays only a minor role in endogenous EPO clearance.[28] Also renal clearance plays a minor role, as the disappearance rate does not change markedly in the anephric state.[29] The major elimination route for erythropoietin seems to be EpoR mediated uptake and degradation.[30] As Widness *et al*[31] showed, bone marrow ablation after myoablative conditioning led to a decrease in EPO elimination. Similar observations were made in irradiated dogs after hypoxia-plasma,[32] and the opposite was seen in patients with hyperactive marrow due to haemolytic anaemia.[33] This mechanism in turn would indicate that elimination of EPO is related to its affinity to and residence time at the receptor.

Recombinant erythropoietin in disease

As EPO plays an important role in regulating erythropoiesis, a major step in medicine was taken when recombinant EPO was first produced by Lin *et al*[34] and Jacobs *et al*[35] in Chinese hamster ovary cells, later optimised for clinical use in patients with renal anaemia. Trials with the first recombinant human EPO (rHuEPO) showed a correction of anaemia in end-stage renal disease[36] and rHuEPO was approved by the FDA for human use in patients with chronic renal failure in 1989.[22] These first recombinant forms of EPO (called epoietin alfa, e.g. Eprex®) are identical to endogenous human EPO with regards to the amino acid backbone and four glycosylation sites, although some differences in molecular composition of the N-glycans have been found.[37] Half-lives are quite similar to endogenous EPO (4-9 hours),[38] which is also the case for second generation rHuEPO, epoietin beta (e.g. Neorecormon®).[39] The same holds true for a later generation of recombinant EPO produced in human cells, epoietin delta (Dynepo), recently taken off the market in 2009.[40] Other forms of EPO, darbepoietin-alfa (NESP/Aranesp) and Mircera (CERA) have a longer half-life due to differences in amino-acid sequence, hyperglycosylation (NESP; $t_{1/2}$ = 24-26 hours[41]) and incorporation of a large polymer chain (CERA; $t_{1/2}$ = 6 days[42]). All these forms of recombinant human erythropoietin can help patients with chronic renal failure (CRF) to overcome the insufficient production of EPO due to the damaged kidneys and maintain steady-state erythropoiesis.

…and in sport. But does it work?

The treatment immediately also got the attention of athletes. As rHuEPO increases red blood cell mass and exercise capacity in anaemic patients, it might have the same effect in the athlete's body, thereby enhancing performance. With this rationale athletes started using rHuEPO, and the use of rHuEPO was put on the International Olympic Committee's (IOC) list of prohibited substances already in 1990. Now the list has been expanded to all "Erythropoiesis-Stimulating Agents (ESAs) [e.g. erythropoietin (EPO), darbepoetin (dEPO), hypoxia-inducible factor (HIF) stabilisers, methoxy polyethylene glycol-epoietin beta (CERA), peginesatide (Hematide)]".[43] The World Anti-Doping Agency (WADA) defines blood doping as "… the misuse of certain techniques and/or substances to increase one's red blood cell mass, which allows the body to transport more oxygen to muscles and therefore increase stamina and performance."[43] But do rHuEPO and other ESAs actually increase red blood cell mass in world-class cyclists and does this result in increased stamina and performance? First we look into the factors that determine stamina and endurance performance, especially in elite cycling and then the effects of rHuEPO on these parameters are reviewed.

What is endurance performance?

Main determining factors

The main determinants of aerobic endurance performance according to a model by Pate and Kriska[44] are maximal oxygen uptake (VO_{2max}), work economy (C) and the lactate threshold (LT). These three factors are now generally accepted as key factors in endurance performance[45–47] and are supported by findings in different studies on VO_{2max},[48,49] C[47,50,51] and LT.[47,48,50,51] A fourth factor, the lactate turn point (LTP), has also gotten some attention.[52]

VO_{2MAX} is a prerequisite but not a sole determining factor

VO_{2max}, the maximal oxygen uptake, has traditionally been regarded as the most important measure in endurance performance. According to Fick's Law it is dependent on cardiac output and the arterio-venous oxygen difference. These in turn, are mainly dependent on total blood volume (BV), the main limiting factor of stroke volume, and total body haemoglobin. However, lung diffusing capacity, heart rate, distribution of the blood volume to working skeletal muscles and arterial O_2 extraction contribute to VO_{2max} as well, as reviewed by Joyner *et al*[45] and Bassett *et al*[53] and reported by other researchers.[54,55] Heinicke *et al*[54] demonstrated the relationship between VO_{2max} and BV and total body haemoglobin in endurance disciplines. Training can improve many of the mentioned factors to increase VO_{2max}, such as increasing blood volume,[56] and indeed, VO_{2max} values of champion endurance athletes are 50-100% greater than those observed in normally active healthy young subjects.[45] That an increase in VO_{2max} has a great potential to increase endurance performance was already shown by Buick *et al*[57] and Brien *et al*.[58] After autologous red blood cell reinfusion elevating haemoglobin and haematocrit levels in well-trained runners, running performance was significantly increased. Ekblom *et al*[59] cites another article by Celsing *et al*[60] to show that a haemoglobin increase irrespective of baseline haemoglobin levels will increase maximal aerobic power and therefore performance. However, the last statement in this paper by Ekblom *et al* is at least as important, where the authors warn to extrapolate this finding to the physically fit athlete, as in these subjects other factors than haemoglobin and maximal aerobic power seem to play a role in performance. Later research emphasised this warning, as VO_{2max} was found not to be the only determinant of endurance performance and more emphasis has recently come to the other two factors described by Pate and Kriska. VO_{2max}, although a prerequisite to perform at a high level,[48] has a very limited predictive value for endurance performance within a group of high-performance athletes for example.[61–67] Also, it has been shown that although successful endurance athletes reached a high VO_{2max} after initial years of training, later on they stayed at a plateau in their VO_{2max} but despite that kept improving performance[47,68,69] (note that one of these reports[69] is about Armstrong). Research into training for endurance performance shows the same trend: moderately trained athletes are able to improve VO_{2max} (as well as LT and C) by interval and/or intensive training,[70,71] whereas these training regimens do not improve VO_{2max} in well-trained athletes, but mainly improve the economy and lactate threshold,[50,72] possibly caused by improving buffering capacity.[73]

It is more than the VO_{2MAX}

Correspondingly, it is not VO_{2max}, but power output at submaximal intensities such as the first (VT1) and second (VT2) ventilation threshold, or respiratory compensation point (RCP) that significantly differ between elite (i.e. amateur) and professional cyclists.[64,74] All these findings indicate that other factors than VO_{2max} play an important role in professional and world-class cyclists. For example, when a published model[75] predicting endurance performance is used to predict the 1-hour cycling world record as described by Padilla *et al*,[76] predictions are far from the observed results. Based on the VO_{2max} and body mass of the studied subject, Miguel Indurain, the distance covered in 1 hour would have been 43.645 km predicted by the model, whereas the actual world record was set at 53.040 km/h. Calculating back from this record, the model would predict an impossible VO_{2max} of 10.3 L/min, where ranges for world-class athletes are 5-6 L/min.[67,77,78] This and another model[79] both identify VO_{2max} as the most important determinant for endurance performance and describe the relationship as being proportionally curvilinear, meaning that the better the athlete is trained, a similar increase in VO_{2max} leads to a proportionally smaller increase in performance. This also demonstrates that in world-class athletes, an increase in VO_{2max} will have only limited effect on performance. As the model by Nevill *et al* is not accurate to predict 1-hour performance in world-class cyclists, this suggests other factors than VO_{2max} play important roles in endurance performance at this level of performance.

Lactate Threshold

Therefore we now first take a look at the importance of lactate threshold in endurance athletes. Lactate threshold (LT), similar to the first ventilatory threshold (VT1 or VT), is the intensity of work or VO_2 at which the blood lactate concentration gradually starts to increase.[80] Aerobic enzyme activity is a major determinant of this LT, reflected by a decline in activity during a period of detraining accompanying a reduction in LT.[81] Because LT reflects an onset of anaerobic metabolism and the coinciding metabolic alterations (see a review by Joyner *et al*[45] and Bassett *et al*[53] for more detail on the mechanisms), this in turn determines the fraction of maximal aerobic power that can be sustained for an extended period. Several studies show that the VO_2 at this lactate threshold is highly related to performance, more so than VO_{2max}.[45,47,63–65,67,68,82] Elite cyclist are reported to be able to reach lactate thresholds between 300 and 400 W,[63,77,83] or 70-85% VO_{2max} (3.5-4.7 L/min).[65,67] Lactate threshold therefore reflects a balance between the rate of lactate production in the muscles and the rate of lactate efflux to the blood and clearance from the blood. In this balance another independent factor appears to play a role in endurance performance; difference in performance (time to fatigue) in cyclists with similar VO_{2max} can be explained by $\%VO_{2max}$ at LT, but an additional increase in performance in some athletes seems to be related to a high muscle capillary density.[45,65] A similar correlation between endurance performance and capillary density was found in another study by Coyle *et al*,[67] and Anderson *et al*[84] found that capillary density increases with training. This might indicate these athletes have a higher capacity to remove and recycle muscle fatiguing metabolites allowing muscles to better tolerate lactic acid production and anaerobic metabolism,[85] or maintain/elongate mean transit time of the blood to increase oxygen extraction.[86]

Lactate Turn Point

Furthermore, another factor related to lactate, although less frequently used to measure endurance performance, is the lactate turn point (LTP),[52] or similar measures called respiratory compensation point (RCP),[87] second ventilatory threshold (VT2) or the onset of blood lactate accumulation (OBLA).[88] These factors represent a level of high work intensity at which lactate levels show a sudden and sustained rise and hypocapnic hyperventilation occurs.[63,68] This value is notably high in professional cyclists and an important factor during extreme endurance events.[64,83] A relationship between RCP and endurance performance has been reported,[63,89] with world-class cyclists having values up to 430-505 W,[63,76,83,90] or 90% of VO_{2max}.

Economy

The last important factor contributing to endurance performance is assumed to be completely independent of the previously mentioned factors, and is called work economy or efficiency (C). It is referred to as the ratio between work output (speed, power) and oxygen cost. Running economy is commonly defined as the steady-rate VO_2 in millilitres per minute per kilogram at a standard velocity, cycling economy as the caloric expenditure at a given work rate. A number of physiological and biomechanical factors seem to influence C in trained or elite athletes. These include metabolic adaptations within the muscle such as increased mitochondria and oxidative enzymes, the ability of the muscles to store and release elastic energy by increasing the stiffness of the muscles, and more efficient mechanics leading to less energy wasted on braking forces and excessive vertical oscillation.[44] Work economy has been shown to be a discriminator of endurance performance independently of VO_{2max} in runners[48,68,91–93] and cyclists,[63,69,78] becoming more important than VO_{2max} once a certain level of fitness is reached.[63] A possible explanation for differences in economy is the composition of the working muscles, where higher economy implies an improved efficiency of ATP turnover within muscle fibres during contraction.[94] Different muscle fibre types have different efficiencies; type I fibres (slow twitch) are most efficient, then type IIa fibres are recruited and lastly type IIb fibres (fast twitch). Several observations indicate work economy and endurance performance are related to the percentage type I fibres.[67,94,95] Training can induce changes from type IIb to IIa, and type IIa to type I in animals,[96] and possibly in humans,[67,97] supporting the finding that training can improve work economy, C.

Other factors

Besides these main determinants several other factors were also reported to be influencing endurance performance. Heart rate for example, although values corresponding to physiological markers such as LT and VT2 remain stable,[68,83] shows a rightward shift in its relationship with running speed[68] with chronic endurance training. This could be related to enlargement of the heart volume due to endurance training,[98,99] increasing stroke volume and allowing a reduced heart rate for the same cardiac output. Breathing pattern is another factor influencing cycling performance, as professional cyclists have been reported to lack a tachypnoeic shift at high workloads, indicating a more efficient use of their respiratory muscles.[100] Also the quantity of muscle mass recruited for sustained power production can influence performance, as elite cyclists can use 20-25% more muscle mass in endurance tests, therefore reducing the stress and power production per fibre.[65,101] Additionally, peak power output has been shown to be a predictor of performance in a time trial[102] and power to weight ratios contribute to climbing performance in cycling.[103] Lastly, two world-class endurance performance athletes showed to have an extremely low peak blood lactate concentration, which might indicate a mechanism for their outstanding performances[68,69] (note that one of these reports is about Armstrong[69]).

In summary, endurance performance mainly depends on an athlete's VO_{2max}, LT, LTP and C; VO_{2max} and LT/LTP interact to determine how long a rate of aerobic and anaerobic metabolism can be sustained and economy then determines how much speed or power can be achieved at a given amount of energy consumption. However, the contribution of each of these factors differs between different levels of training. Moderately trained athletes can easily improve all factors, whereas increasing performance in elite athletes mainly seems to be governed by changes in LT, LTP and C. Additional factors, including capillary density, heart rate and heart volume, muscle mass and breathing pattern can influence endurance performance.

METHODS

Studying the effects of rHuEPO on endurance performance

Search strategy

Several studies have addressed the effects of rHuEPO with regard to endurance performance in subjects other than patients. A literature search was conducted in PubMed to identify these papers, using combinations of the key words 'erythropoietin', 'athletic performance', 'physical endurance', 'doping in sports' and 'athletes' for the primary search. Literature references in key papers were examined manually to identify additional papers. We did not attempt to derive quantitative systematic conclusions from a meta-analysis; therefore, this could be termed a qualitative systematic review.

RESULTS

Study population mismatch with professional cyclists

There are quite some studies looking at the effects of rHuEPO with regards to endurance performance in subjects other than patients. Some studies included "(endurance trained) recreational athletes" or "well trained individuals", others "healthy normal subjects". This brings already the first problem when interpreting the observations and results in these studies. As no standard has been used to classify the cycling abilities of the subjects, such as proposed by Jeukendrup *et al*,[77] subjects vary in baseline endurance performance and fitness level within a study and between studies. The level of training of the used subjects is poorly reported, but when trying to use the methods for cycling classification from Jeukendrup *et al*[77] on the scarce information reported, based on maximal power output and VO_{2max} (absolute and per kg body weight) subjects would be placed either in untrained cyclists (or healthy recreationally active subjects)[104–110] or trained cyclists.[111–115] Based on the reported information subjects in one study could not be classified.[116] Although these maximal parameters are not optimal to distinguish between top-level cyclists (as discussed above), it is clear that the studied subjects are not at all at a competing level of cycling performance. Moreover, this points out a very problematic aspect, which is that the studies do not use well-trained cyclists, let alone (for obvious reasons) elite or world-class cyclists, who, according to Jeukendrup *et al* would have VO_{2max} values above 70 mL/min/kg (5 L/min) and power outputs above 5 W/kg (See also Figure 1 to compare the studied subjects with these reference values). The only study using subjects with power outputs above this threshold with ~5.7 W/kg is by Connes *et al*,[111] but on the other hand their VO_{2max} is only ~64 mL/min/kg. As has been described for endurance athletes earlier in this review, it could well be that the studied subjects did not reach a plateau in VO_{2max} yet, which will prove an important flaw for the interpretation of the results of the studies. It is also well known that cyclists classified as well-trained or higher have different physiological characteristics in the factors comprising endurance performance than trained or untrained cyclists.[45,77,117] Lucia *et al*[63] showed for example, that the VO_2 kinetics are very different even between well-trained cyclists and world-class cyclists. Additionally, this classification shows there are major discrepancies between the groups in training status, which makes comparison difficult. As is the case for all research, including research on performance enhancement, Hopkins *et al*[118] stated that "the results of a research study apply with reasonable certainty only to populations that have similar characteristics to the sample under study. Elite athletes almost certainly have genetic endowment, training history, and training programs that differ from those of sub-elite athletes. A treatment may therefore produce different effects on performance in these two groups. It follows that the subjects in a study have to be elite athletes for the results to apply convincingly to elite athletes." Therefore it cannot be assumed that effects found in these rHuEPO studies on healthy untrained or trained individuals automatically apply to well-trained, elite and world-class cyclists.

Recombinant human erythropoietin dosing

The doses of rHuEPO in all studies vary, but all are subcutaneous injections, most in a similar range of 150 IU/kg per week, see Table 1. Almost all studies used forms of rHuEPO with half-lives similar to endogenous EPO, namely Eprex®,[109–111,113,114,116]

Neorecormon®,[104–107] Recormon®[115] or it was not reported.[112] Only one study used rHuEPO with a longer half-life, NESP.[108] Another problem with evaluating the results of these studies is that only 8 studies[105,106,109–111,113,115,116] out of 13 were placebo controlled. As endurance performance can change significantly due to for example training, it is crucial to control for these effects with a placebo treated group. Moreover, unfortunately only 5[106,109,111,113,115] of these studies were reported to be double-blinded, controlling for any bias due to treatment which is of possible major influence on the exercise tests performed in the studies. As the study using NESP as rHuEPO treatment is not placebo controlled and does not measure any performance parameters during normoxia, it is difficult to draw conclusions about the effects of this form of rHuEPO on endurance performance. Moreover, the newest form of rHuEPO, CERA, to our knowledge has not been studied for effects on endurance performance in athletes yet at all.

Haematological effects of rHuEPO

Although doses differ somewhat across the studies, most studies report similar magnitude of effects in haematological parameters with these doses. There are reports that during rHuEPO administration reticulocyte numbers are increased twofold in the lower doses[109,110] to threefold in the higher doses,[114,116] and drop below baseline approximately 7-14 days after rHuEPO treatment is ceased.[109,110,114,116] EPO concentrations also drop below baseline after rHuEPO treatment is stopped.[109,116] Another effect that is seen in most studies is the rise in [Hb] and Hct. Increases between 4.6% and 17.4%, and 8.3% and 19% are reported for [Hb] and Hct respectively (Table 1), with no obvious differences between training statuses of the athletes. These levels are reported to return to baseline within one month after cessation of treatment.[109] An increase in haematocrit could lead to an increase in oxygen carrying capacity, however, does this enhance performance? Hct is not a good marker of performance, as endurance athletes usually have lower Hct values than untrained subjects due to plasma volume expansion.[119] Additionally, it is a very variable measure and affected by different circumstances.[120] On top of that, increases of Hct cause an increase in viscosity of the blood,[121,122] which might hamper performance due to reductions in blood flow and increased heart muscle work. Considering that during exercise a decrease in plasma volume increases Hct even more,[120] and dehydration, hyperthermia and altitude possibly exaggerate this effect in 3-week races, it is not obvious what the effects of this rise in Hct will have in professional cyclists. Even more so because the rHuEPO treatment not only increases haemoglobin concentration and Hct, but at the same time decreases plasma volume, thereby resulting in almost no effect on, or a slight decrease in, BV.[123] The use of rHuEPO therefore interestingly possibly counteracts the plasma volume expansion of endurance training.[56] Despite all these observations however, the combination of effects seems to increase the performance parameter VO_{2max}, at least in the studied subjects under laboratory conditions.

Effects on VO_{2max}

The most important question in this review is then whether these effects on haematological parameters translate into an effect on performance. The different parameters that determine endurance performance were discussed previously, but unfortunately most studies only examine one of these parameters, being VO_{2max}. In the reported studies this parameter is increased in the rHuEPO treated subjects, with a relatively constant value for all studies, independent of training status of the subjects, between 7% and 9.7% (Table 1). Absolute values of VO_{2max} and treatment effects can be seen in Figure 1. This increase in VO_{2max} has been reported to be accompanied by an increase in power output.[105,106,110,111,114] This, in turn, resulted in an increase in performance a time-to-exhaustion test of 22%[106] and 54.3%[105] in untrained subjects and a smaller increase of 9.4% (and 1.5% in placebo)[115] and 16.6%,[112] in trained subjects. Importantly this surrogate parameter is measured in a test lasting about 20 minutes and leading to exhaustion, quite different from the required ~5 hour performance in a cycling race.

Does it translate to cycling performance?

As mentioned earlier, VO_{2max} is poorly related to cycle performance[64,74] and Lucia *et al*[124] even questions whether VO_{2max} is the limiting factor for maximal endurance performance in some 50% of professional cyclists due to a lack of plateau in VO_{2max} during an exercise to exhaustion test. Additionally, time to exhaustion protocols like the ones used here are subject to high variability and therefore poorly reproducible,[125,126] whereas time trial protocols would give a better performance evaluation,[125] also eliminating the influence of wrongly extrapolating laboratory test setting results to race-events.[118] The use of rHuEPO in these subjects clearly has an effect on VO_{2max}, which might improve performance at peak intensity during severe exercise, although evidence for this is rather “soft”. Apart from the uncertainty whether these same effects can be observed in well-trained or elite cyclists, surprisingly little is known from these studies about effects on submaximal intensities. This might be of major importance when looking at the nature of cycling. Long exercise times during consecutive days with the finish line as a known endpoint (contrary to the “open end” of time to exhaustion tests) makes it crucial for cyclists to dose their power during a race. This combined with (team) tactics, the terrain and the effects of drag force make it that cyclists only work a small amount of time at their peak intensities, or even above intensities where lactate accumulation occurs (i.e. VT2/OBLA/RCP/LTP). Investigations in world-class cyclists show that during 3-week races the subjects’ HR is above such an intensity (HR_{obla}) only 3.6% (119 sec) of the time climbing a “Hors Categorie” climb (hardest climb), even less so during first and second category climbs, 2.6% (45 sec) and 2.5% (22 sec) respectively.[90] Similar low percentages were reported by Lucia *et al*[127] for total race time with HR above the RCP (at 90% VO_{2max}) during the Tour de France or Vuelta a España, 2.7% (149 min) and 3.3% (166 min) respectively. For time trials a difference in time spent with a HR above OBLA was found between different type of time trials, with prologue, short, long and uphill time trials recording 59, 38, 3.5 and 0% for cyclists going all-out.[128] HR values corresponding to physiological markers of performance (e.g. LT, VT2) have been shown to be stable during the course of a training year of professional cyclists.[83]

Other endurance performance parameters unstudied

For the major part of a race, cyclists therefore exercise well below their VO_{2max} levels, but this parameter surprisingly still has gotten the most attention when looking at rHuEPO effects. Some of the studies that did look at other parameters observed no change in the VO_2-kinetics[105,106,110,129] or VO_2 at submaximal exercise,[112] despite the increased oxygen carrying capacity due to the increase in [Hb] and Hct. This would mean that the oxygen carrying capacity of the blood does not determine VO_2 kinetics, but that this is regulated and limited by factors in the muscles rather than oxygen supply. This would also indicate that there is no change in LT in these subjects resulting from rHuEPO treatment as shown by Wilkerson *et al*,[106] who found no effect on gas exchange threshold (GET), a measure closely related to LT, due to the rHuEPO treatment. However, other researchers did find an increase in VT of 14.3%,[114] although this trial was not placebo controlled, so training and placebo effects cannot be accounted for. Another group[111] using a placebo controlled blinded study also found an increase in VT of 12.6%. No conclusive evidence for effects of rHuEPO on LT/VT is therefore available, with evidence on another important lactate parameter, LTP/OBLA/VT2/RCP, completely absent. It is important to elucidate the effects of rHuEPO on these parameters, as performance in cycling is much better related to these factors.[64,74] Time trial world record performance (1-hour world record) for example, seems to be best correlated to and predicted by the speed or power output at OBLA.[76] Other groups also report that performance in longer time trials is highly correlated to power output at OBLA[130] or power output at LT,[131] or with VO_2 at VT1[132] or LT.[67] In >50km time trials during the Tour the France, performance was correlated with power output at VT1.[133] In these time trials VO_{2max} is not related to performance, which was only demonstrated in shorter time trials (20 min).[131] Lastly, also uphill cycling has been correlated best to power outputs at LT or OBLA.[130] This means that the most determining disciplines for the general classification in stage-races in professional cycling are correlated to submaximal exercise parameters.

In the reviewed rHuEPO studies the last important endurance performance factor, economy, was only measured by one group[105] and did not change after rHuEPO treatment. This would be expected from the non-haematological, biomechanical factors that determine economy as discussed previously. On the other hand, there is some evidence that prolonged exposure to rHuEPO in healthy subjects may induce changes in the human skeletal tissue shown by an increase in the relative amount of the slow myosin light chain (MLC) (Type I fibres) while decreasing the amount of fast MLC (Type II fibres), possibly leading to improved economy.[134] However, more evidence is needed here as well to draw conclusions about effects of rHuEPO on economy. Especially because Lance Armstrong, accused of having the biggest doping (e.g. rHuEPO) network in the history of sports, was reported to have, although in a questionable study, a high muscular efficiency partly contributing to his world-class performance.[69]

Some other parameters, such as blood lactate, end-exercise HR and HR kinetics were investigated and reported as not altered by rHuEPO treatment,[106] although other studies indicate a non-significant drop in blood lactate[110] and HR[110,111] or significant in heart rate,[114] although only at submaximal exercise.[112] A significant drop in blood lactate at rest and 10 min into a time to exhaustion (TTE) test, but not at exhaustion[105] was seen. Blood volume was also not affected.[112,123] One blinded study looked at the effect of rHuEPO on perception on physical self and reported a positive effect on perceived physical condition and strength.[113] Although animals overexpressing EPO had 14% higher muscle volume and a 25% increase in muscle vascularisation, this did not translate to increased muscle force or stamina.[135] Moreover, in healthy males no effects of prolonged rHuEPO treatment on capillarization or muscle fibre hypertrophy were reported in a publication[136] from the same study performed by Thomsen *et al*.[105]

Alternative mechanisms by which EPO works?

It may be argued that focusing on direct endurance measures does not take into account possible mechanisms by which rHuEPO causes better recovery after exercise. rHuEPO may have anti-inflammatory effects and may mitigate ischemia-reperfusion related damage,[137–140] which could potentially improve recovery. It has been suggested that EPO and its receptor function as a paracrine/autocrine system to mediate the protection of tissues subjected to (metabolic) stress.[141] However, these effects have not been confirmed in properly designed clinical trials. In fact, most clinical trials focusing on the tissue protective effects of rHuEPO have shown adverse rather than beneficial effects. Serious untoward effects have also been shown in rHuEPO-treated patients with stroke, myocardial infarction, or acute kidney injury, and in surgical patients (as reviewed by Patel *et al*[142]). These findings appear to be compatible with a pro-coagulant state induced by rHuEPO and possibly also with an augmentation of acute inflammatory reactions by the drug.[143] The data therefore do not suggest substantial effects on recovery of muscle injury during exercise.

Thus, except on $\text{VO}_{2\text{max}}$, no coherent or reproducible findings have been reported for both erythropoietic and non-erythropoietic effects of rHuEPO, rendering the evidence too weak to support any conclusion about effects on performance in professional cyclists.

Lack of scientific evidence

Given that (I) most of the research with rHuEPO on endurance performance has focused on a parameter for maximal exercise, $\text{VO}_{2\text{max}}$, (II) the factors that make professional and world-class cyclists unique are not $\text{VO}_{2\text{max}}$, but LT, RCP and C, (III) endurance performance in professional cycling such as in time trials is best correlated with submaximal exercise factors (e.g. LT, VT1, OBLA, RCP), (IV) only small parts of professional cycling races are cycled at severe or maximal intensities (above OBLA/RCP) and (V) the characteristics of the study populations have been significantly different from that of the population that is suspected of using rHuEPO, it cannot be concluded that rHuEPO use in professional cyclists (or even elite cyclists) will enhance cycling performance.

A more scientific approach needed

Summarizing, the available literature lacks the appropriate information, validity and robustness to conclude that rHuEPO enhances world-class cycling performance. To be able to make such statements, more thorough research needs to be conducted looking at the effects of rHuEPO on submaximal performance parameters and the cycling economy, preferably in a population with cycling performance abilities as close as possible to those of professional cyclists and under conditions closely resembling racing conditions and the required performance duration. As long as the effect on endurance performance in professional cycling is not clear, putting the treatment on the prohibited list falsely implies a proven ergogenic effect, possibly stimulating its abuse,[144] although it should also be recognised that there is no convincing evidence that any drug works in this context.

Adverse effects of rHuEPO in athletes

Apart from creating a level playground for all athletes by banning and trying to prevent doping use, doping is also forbidden to protect the athletes from using possibly harmful substances. The presented rHuEPO studies in healthy or trained subjects do not focus on negative side-effects of the treatment however. What some of these studies did observe is a significant rise in systolic blood pressure (SBP) at submaximal exercise.[112] A second publication[129] from the study described by Thomsen *et al*[105] reported a rise in systolic, diastolic and mean blood pressure at rest or maximal exercise, as well as a rise in systolic and mean blood pressure at submaximal exercise. Although others do not observe a blood pressure rise at rest,[106,114,116] a rise in blood pressure (either at rest or (sub-)maximal exercise) could be a possible threatening side-effect of rHuEPO use in healthy athletes. However, the numbers of subjects and treatment times in the presented studies are too small to detect any (rare) adverse events. To get information on this, larger studies, namely patient studies, must be consulted, although it must be kept in mind that results of these studies do not per se translate to well-trained athletes. One of such patient studies was prematurely discontinued due to increased incidence of thrombotic events in rHuEPO treated metastatic breast cancer patients.[145] Other trials and meta-analyses showed a similar trend in different groups of patients treated with rHuEPO compared to placebo.[146–148] It should be noted however that these studies used ~4 times higher doses of rHuEPO (usually in the range of 40.000 IU or 600 IU/kg per week) compared to the endurance performance studies in healthy subjects. The increased blood viscosity in treated anaemic patients,[122,149] the earlier described rise in blood pressure and enhanced coagulation,[150] endothelial activation and platelet reactivity[151] and inflammation[152] after rHuEPO treatment have been mentioned to be involved in these thrombotic events. On top of these rHuEPO effects, acute exercise also enhances coagulation,[153] although less pronounced in trained than in untrained subjects. And because stroke volume and blood volume are reduced in acute exercise, haematocrit is increased,[120] which is even more pronounced in dehydrated and hyperthermic exercise conditions.[154,155] This combination of factors might increase the risk of thrombotic events in endurance performance athletes using rHuEPO. One of such adverse events could manifest for example after enhanced Hct levels lower cerebral blood flow and therefore oxygen supply to the brain, which in turn might predispose to cerebral infarction.[156] These thrombotic risks are underlined by a case report by Lage *et al*[157] where a professional cyclist presented with cerebral sinus thrombosis, thereafter confessing to 3 months of 2000 IU rHuEPO use every two days, in combination with 15 days of growth hormone and continuous high doses of vitamin A and E. Additionally, high haematocrit values could cause heart failure, myocardial infarction, seizures and pulmonary embolism.[158,159] Another association that has been reported for this treatment, caused by the induced hypertension, is hypertensive posterior encephalopathy.[160] A complication of rHuEPO use in patients that is life-threatening, is the onset of red cell aplasia, a very rare side-effect mainly linked to anti-erythropoietin antibody formation due to Eprex® use.[161] The improper handling and storage in illicit use in sport might enhance the risks of this and other immunogenic complications.[162,163]

And lastly, rHuEPO use has also been connected to promoting tumour growth and angiogenesis in tumours, however Fandrey *et al*[164] report that there is no evidence for such an involvement yet.

Summarizing, as only case reports have been presented on negative effects of rHuEPO use in cyclists, most information available is from patient-studies. These studies indicate that rHuEPO has several cardio-vascular effects, raising the risk of thrombotic events, encephalopathy and possibly some other complications. These risks might be even higher in cycling taking into account the circumstances in this sport which could compound these risks. Also, the needed secrecy in sports might lead to bad handling and storage of the rHuEPO, possibly elevating the risks of side-effects such as red cell aplasia.

CONCLUSION

Cyclists and rHuEPO: a risky choice to what advantage?

As the case of the United States Anti-Doping Agency *versus* Armstrong proves again, recombinant Human EPO has been used by many professional (including champion) cyclists. Given that it increases Hct, it is thought to enhance performance in professional cycling and therefore has been put on the list of prohibited substances of the International Olympic Committee. As rHuEPO is on this list, cyclists caught were breaking the rules and should be punished for doing so. However, this review shows that only very weak scientific evidence exists about the effects of rHuEPO on cycling performance. Sport physicians and cyclists should be informed about the dangers of the use of such a substance, as already proposed by Kuipers about doping in general.[144] Neither scientific basis for performance-enhancing properties, nor possible harmful side-effects have been provided for athletes or trainees.

The situation for rHuEPO use in athletes is analogous to the many forms of non-evidence-based treatments that exist in medical practice and which, by common opinion, should be refuted or confirmed by good clinical trials with real-life endpoints. A single well-controlled trial in athletes during real-life circumstances would give a better indication of the real advantages and risk factors of rHuEPO use, but it would be an oversimplification to suppose that this would eradicate its use, even if no benefit were to be seen with increased biomarkers of risk.

High-quality scientific evidence is always preferable to the current situation, in which athletes risk their career and health with irrational use of a substance. If the size of the athletic benefit could be shown to be large (which, on the basis of the evidence presented in this review, is unlikely), it would support the enormous and apparently largely ineffective efforts currently made to detect and prevent the use of rHuEPO. If the effect were to be small, these efforts could be directed elsewhere.

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FIGURE 1 MAXIMAL OXYGEN UPTAKE BEFORE AND AFTER TREATMENT WITH rHuEPO IN THE DIFFERENT STUDIES PER TREATED GROUP Maximal oxygen uptake (VO_{2max}) before (grey squares) and after (black triangles) treatment with rHuEPO in the different studies per treated group (bars representing SD). N is the number of subjects in each group, with an asterisk indicating that the article reported VO_{2max} values only in litres per minute, which has been converted to millilitres per kilogram per minute by dividing this value by mean weight of the group for comparison purposes (no SD is given for these studies because of this conversion). Studies above the horizontal dotted line were performed using subjects classified as untrained, while below the horizontal line the subjects were classified as trained cyclists. Vertical dashed lines represent minimal values of VO_{2max} for different classifications of cyclists as suggested by Jeukendrup et al.[77] (dashed line, trained; dotted line, well trained; dotdashed line, elite; and longdashed line, world class).

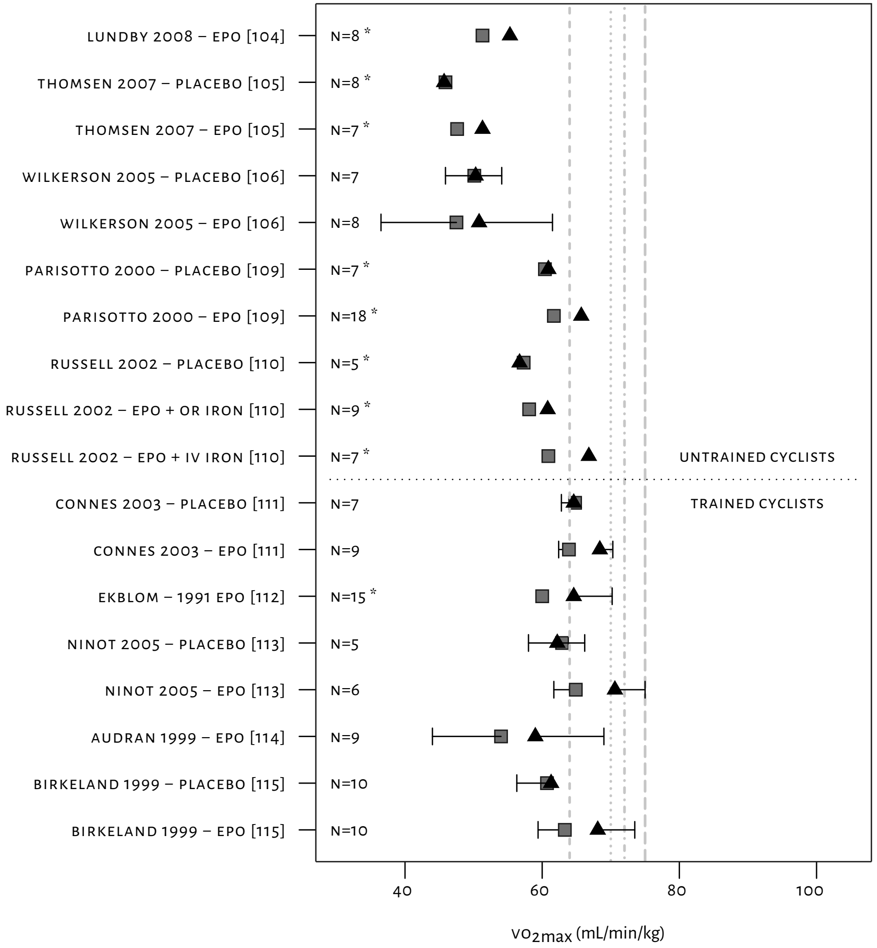

TABLE 1 OVERVIEW OF CHARACTERISTICS AND OUTCOMES OF THE STUDIES INVESTIGATING THE EFFECTS OF RECOMBINANT HUMAN ERYTHROPOIETIN ON ENDURANCE PERFORMANCE IN SUBJECTS OTHER THAN PATIENTS All effects are calculated based on the greatest difference found in the parameter when multiple measuring time points were reported. Abbreviations are as follows: Hb, haemoglobin; Hct, haematocrit; NA, not applicable; NESP, novel erythropoiesis stimulating protein (darbepoietin-alfa); VO_{2max}, maximal oxygen uptake.

Study	Type of subjects	Study set-up	Product	Dosing	Max. Hb increase (%)	Max. Hct increase (%)	Max. VO_{2max} increase (%)
Lundby *et al* 2008[104]	Untrained	Uncontrolled	Neorecormon®	5000 IU (~65 IU/kg) on alternating days for 14 days followed by once a week for 2 weeks	10.2	11.2	7.9
Thomsen *et al* 2007[105]	Untrained	Placebo	Neorecormon®	5000 IU (~60 IU/kg) on alternating days for 2 weeks, a dose on 3 consecutive days for one week and one dose a week for 12 weeks	11.1	10.7	9.1
Wilkerson *et al* 2005[106]	Untrained	Placebo + Blinded	Neorecormon®	150 IU/kg once a week for 4 weeks	7	12	7
Rasmussen *et al* 2010[107]	Untrained	Uncontrolled	Neorecormon®	5000 IU (~60 IU/kg) on alternating days for 2 weeks, a dose on 3 consecutive days for one week and one dose a week for 12 weeks	-	12	-
Lundby *et al* 2006[108]	Untrained	Uncontrolled	NESP	144 IU/kg (0.72 μg/kg) once a week for 4 weeks	17.4	16.4	-
Parisotto *et al* 2000[109]	Untrained	Placebo + Blinded	Eprex®	50 IU/kg three times a week over 25 days (in combination with ~100 mg iron either IM / OR)	7.4 / 12	-	6.3 / 6.9
Russell *et al* 2002[110]	Untrained	Placebo	Eprex®	50 IU/kg three times a week for 3 weeks and 20 IU/kg for 5 weeks	-	15	9.7
Connes *et al* 2003[111]	Trained	Placebo + Blinded	Eprex®	50 IU/kg three times a week for 4 weeks	9.6	8.3	7
Ekblom *et al* 1991[112]	Trained	Uncontrolled	NA	20 IU/kg three times a week for 6 weeks (or 4 weeks, and 40 IU/kg for the remaining 2 weeks)	-	11.7	8
Ninot *et al* 2006[113]	Trained	Placebo + Blinded	Eprex®	50 IU/kg three times a week for 4 weeks, followed by 20 IU/kg three times a week for 2 weeks	9.5	10.2	7
Audran *et al* 1999[114]	Trained	Uncontrolled	Eprex®	50 IU/kg daily for 26 days	9.3	11.5	9.3
Birkeland *et al* 2000[115]	Trained	Placebo + Blinded	Recormon®	5000 IU (181-232 IU/kg/week) three times a week	11.2	19	7
Souillard *et al* 1996[116]	Unknown	Placebo	Eprex®	200 IU/kg 5 times in 11 days	4.6	8.9	-