Erythropoietin doping in cycling: Lack of evidence for efficacy and a negative risk–benefit

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Summary

Aim & Methods

Imagine a medicine that is expected to have very limited effects based upon knowledge of pharmacology and (patho)physiology, is studied in the wrong population, with low quality studies that use a surrogate endpoint that relates to the clinical endpoint in a partial manner at most. Such a medicine would surely not be recommended. Recombinant human erythropoietin (rHuEPO) use to enhance performance in cycling is very common. A qualitative systematic review of the available literature was performed to look at the evidence for these ergogenic properties of this drug normally used to treat anaemia in chronic renal failure patients.

Results

The results of this literature search show there is no scientific basis to conclude 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.

Conclusions

rHuEPO use 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 under real life circumstances would give a better indication of the real advantages and risk factors of rHuEPO use, but it would be an oversimplification that this would eradicate its use.

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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 desirable, 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 justifies 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. Additionally, 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

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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 of EPO in blood is relatively constant at approximately 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 EPO 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-Es, 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 and this starts the intracellular signalling leading to proliferation of CFU-E's.[11;12]

Production and metabolism

The kidneys are the main EPO producing organs in humans[13;14], where peritubular interstitial cells govern its production[15;16], which is highly regulated. Baseline EPO levels can increase up to 1,000-fold in low blood oxygen content, for example in severe anaemia.[3] EPO production is highly dependent on blood oxygen tension, with hypoxia increasing EPO production, irrespective of the cause of reduced tissue oxygen supply.[3] There is a latency of 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 EPO 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

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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 which accumulates under hypoxic conditions as a result of the sequential dephosphorylation of ATP, ADP and AMP. This non-HIF pathway also increases EPOmRNA expression.[22] GATA-2 inhibits the EPO promoter, and is a third pathway of EPO regulation. GATA inhibitors can therefore also enhance EPO production.[23] After hypoxia-induced EPO 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]. The estimated half-life of endogenous EPO is approximately 5.2 hours[26] and mechanisms of clearance are somewhat extraordinary. Clearance of EPO by the liver is, like with many other glycoproteins, rather low, 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 pathway is only of minor importance.[28] Renal clearance also plays only a minor role, as the disappearance rate does not change markedly in the anephric state.[29] The major elimination route for EPO seems to be EpoR mediated uptake and degradation[30], and bone marrow ablation after myoablative conditioning led to a decrease in EPO elimination[31]. Similar observations were made in irradiated dogs during altitude exposure[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 EpoR 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 optimized 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

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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).[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 rHuEPO 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 (ESA's) [e.g. erythropoietin (EPO), darbepoetin (dEPO), hypoxia-inducible factor (HIF) stabilizers, methoxy polyethylene glycol-epoetin 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 ESA's 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

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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 are maximal oxygen uptake (VO2max), the lactate threshold (LT) and work economy (C).[44] These three factors are now generally accepted as key factors in endurance performance[45-47] and are supported by findings in different studies on VO2max[48;49], LT [47;48;50;51] and C [47;50;51]. A fourth factor, the lactate turn point (LTP), has also received some attention[52]. Here we consider briefly each factor in turn.

VO$_2$max is a prerequisite but not a sole determining factor

VO$_2$max, 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 (tHb). However, lung diffusing capacity, heart rate, distribution of the blood volume to working skeletal muscles and arterial O$_2$ extraction contribute to VO$_2$max 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$_2$max and BV and tHb in endurance disciplines. Training can improve many of the mentioned factors to increase VO$_2$max, such as increasing blood volume[56], and indeed, VO$_2$max values of champion endurance athletes are 50-100% greater than those observed in normally active healthy young subjects.[45] That an increase in VO$_2$max 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

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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 against extrapolating this finding to the physically fit athlete, as in these subjects other factors than haemoglobin and maximal aerobic power may play a limiting role in performance. Later research emphasized 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.[61-67] Also, although successful endurance athletes reached a high VO_2max after initial years of training, they subsequently maintain a plateau in their VO_2max but continue to improve further their 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 LT and C[50;72], possibly by improving buffering capacity[73].

It is more than the VO_2max

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 amateur and professional cyclists.[64;74] Thus factors other than VO_2max play an important role in determining performance in professional and world-class cyclists. For example, when a published model[75] for predicting endurance performance is used to predict the 1-hour cycling world record as described by Padilla *et al*[76], its predictions are far from the observed results. Based on the VO_2max and body mass of Miguel Indurain, a professional cyclist, the distance covered in 1 hour predicted by the

model would have been 43.645 km, whereas the actual world record he set was 53.040 km/h. Calculating back from this record, the model would predict an impossible VO_2max of 10.3 l/min, whereas ranges for world-class athletes are 5-6 l/min[67;77;78]. This and another model[79] both rely on 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. The failure of such a model [75] to predict 1-hour performance[76] suggests that factors other than VO_2max are limiting in endurance performance at this level of performance.

Lactate Threshold (LT)

We therefore now address the importance of LT in determining the performance of endurance athletes. LT, similar to 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 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[45;53], LT 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 LT is highly related to performance, more so than VO_2max.[45;47;63-65;67;68;82] Elite cyclists are reported to be able to reach LTs between 300 and 400W[63;77;83], or 70-85% VO_2max (3.5-4.7 l/min)[65;67]. LT reflects a balance between the rate of lactate production in the muscles (and hence the rate of lactate influx 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

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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 (LTP)

LTP is a distinct factor related also to lactate[52]. Respiratory compensation point (RCP)[87], second ventilatory threshold (VT2) or the onset of blood lactate accumulation (OBLA)[88] are related measures. These factors represent a level of high work intensity at which lactate concentrations show a sudden and sustained rise and hypocapnic hyperventilation occurs.[63;68] This threshold 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 430-505W[63;76;83;90], or 90% of VO_2max.

Economy (C)

The third main factor contributing to endurance performance is assumed to be completely independent of VO_2max and lactate-related factors and is called work economy or efficiency (C). It is the ratio between work output (speed, power) and oxygen cost. Running economy is commonly defined as the steady-rate VO_2 in millilitres per kilogram per minute at a standard velocity, cycling economy as the caloric expenditure at a given work rate. Several physiological and biomechanical factors 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 altering the mechanical properties of the muscles, and more efficient mechanics leading to less energy wasted on braking forces and excessive vertical oscillation[44]. C is a discriminator of endurance performance independently of VO_2max in runners[48;68;91-93] and cyclists[63;69;78], becoming more

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important than VO$_2$max once a certain level of fitness is reached[63]. A possible explanation for differences in C between individuals 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). C (and hence endurance performance) is 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].

Other factors

Besides these main determinants several other factors were also reported to influence endurance performance. Heart rate for example, shows a rightward shift in its relationship with running speed[68] as a consequence of chronic endurance training, although values corresponding to physiological markers such as LT and VT2 remain stable[68;83]. This could be related to increased cardiac volume due to endurance training[98;99], leading to increased 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 possible 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 have been shown to have extremely low peak blood lactate concentrations, which might indicate a mechanism for their outstanding performances[68;69] (note that one of these reports is about Armstrong[69]).

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Summarizing, 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 C then determines how much speed or power is achieved for a given energy consumption. The relative importance of each of these factors differs at different levels of training. Moderately trained athletes can easily improve all factors, whereas increasing performance in elite athletes is mainly governed by changes in LT, LTP and C. Additional factors including capillary density, heart rate and heart volume, muscle mass and breathing pattern can also influence endurance performance.

Studying the effects of rHuEPO on endurance performance

Search strategy

Several studies have addressed the effects of rHuEPO with regards 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.

Study population mismatch with professional cyclists

Some of the identified studies included "(endurance trained) recreational athletes" or "well trained individuals", others "healthy normal subjects". This raises a problem when interpreting these studies. No standard, such as that proposed by Jeukendrup *et al*[77], has been used to classify the cycling abilities of the subjects, and included subjects vary in baseline endurance performance and fitness level within a study and between studies. The level of training of the subjects is poorly reported, but when trying to classify cycling ability [77] using the scarce information reported, based on maximal power output and VO_2max (absolute and per kg body weight) subjects in these studies would be either "untrained cyclists"

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[104-110] or "trained cyclists"[111-115] or "unclassifiable"[116]. It is, however, clear that in no study reported subjects are at a "competing" level of cycling performance. This highlights a very problematic aspect, which is that the studies do not use well-trained cyclists, still less elite or world-class cyclists, who, would be expected[77] to have VO_2max values above 70 ml/kg/min (5 l/min) and power outputs above 5 W/kg. Figure 1 compares the studied subjects with these reference values. In the only study using subjects with mean power outputs above 5W/kg the mean VO_2max is only ~64 ml/kg/min[111]. The studied subjects may not have reached a plateau in VO_2max, confounding the interpretation as explained above. Cyclists classified as "well-trained" or higher differ in factors contributing to endurance performance from "trained" or "untrained" cyclists.[45;77;117] VO_2 kinetics are very different even between "well-trained" and "world-class" cyclists[63]. Additionally, this classification shows that there are major discrepancies between the groups in training status, which makes comparison difficult. Hopkins *et al*[118] state 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 subelite 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.

rHuEPO 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 (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

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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 expectation of a positive treatment-effect which is potentially of major influence on exercise tests performed in the studies, whose outcome depends on perseverance and hence motivation. 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 effects on haematological parameters albeit with a suggestion of dose-related effect. Reticulocyte numbers approximately doubled in the lower doses[109;110] and tripled in the higher doses[114;116], and declined to below baseline approximately 7-14 days after rHuEPO treatment ceased[109;110;114;116]. EPO concentrations also drop below baseline after rHuEPO treatment is stopped.[109;116] Increases of 4.6%-17.4%, and 8.3%-19% are reported for haemoglobin concentration [Hb] and haematocrit (Hct) respectively (Table 1), with no obvious differences between athletes with different training statuses. These levels are reported to return to baseline within one month after cessation of treatment.[109] An increase in Hct 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]. 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 cardiac work. Decreased plasma volume during exercise exaggerates increased Hct[120], as may

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dehydration, hyperthermia and altitude, so it is not obvious what effects a rise in Hct will have in professional cyclists. rHuEPO treatment not only increases [Hb] and Hct, but at the same time decreases plasma volume, thereby resulting in almost no effect on, or a slight decrease in BV[123]. rHuEPO could therefore counteract the plasma volume expansion of endurance training[56]. Nevertheless, 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 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, namely 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 estimated by a time-to-exhaustion test of 22%[106] and 54.3%[105] in untrained subjects and a smaller increase of 9.4% (versus 1.5% in placebo-treated subjects)[115] and 16.6%[112], in trained subjects. Importantly this surrogate parameter (time-to-exhaustion) 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.

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Additionally, time to exhaustion protocols like the ones used here are subject to high variability and poor reproducibility[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 distribute their power during a race. This combined with (team) tactics, the terrain and the effects of drag force mean that cyclists only work a small amount of time at their peak intensities, or even above intensities where lactate accumulation occurs. Investigations in world-class cyclists show that during 3-wk 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) are stable during a training year in 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 still has attracted the most attention when looking at rHuEPO effects. Some studies that

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evaluated 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, economy (C), was only measured by one group[105] and did not change after rHuEPO treatment. This would be expected from the non-haematological, bio-mechanical factors that determine C as discussed previously. There is, however, some evidence that prolonged exposure to

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rHuEPO in healthy subjects may induce changes in the human skeletal muscle with an increase in the relative amount of the slow myosin light chain (MLC) (Type I fibres) and decreased fast MLC (Type II) fibres, possibly leading to improved C.[134]More evidence is needed to draw conclusions about effects of rHuEPO on C. Especially when Lance Armstrong, accused of having the biggest doping (e.g. rHuEPO) network in the history of sports, was reported to have 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 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 of physical well-being and reported a positive effect on perceived physical condition and strength[113] which on the basis of evidence are unlikely to be related to an increased muscular mass or improved vascularisation. In a publication[136] from the same study performed by Thomsen *et al*[105] no effects of prolonged rHuEPO treatment on capillarization or muscle fibre hypertrophy in healthy volunteers were reported. Although this is contrasted by an animal study showing that overexpression of EPO resulted in 14% higher muscle volume and 25% increase in muscle vascularisation, even these effects did not translate to increased muscle force or stamina.[135] .

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

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paracrine/autocrine system to mediate the protection of tissues subjected to (metabolic) stress.[141] However, these effects have been not been confirmed in properly designed clinical trials. In fact, most clinical trials focusing on postulated 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, acute kidney injury and surgery [142], compatible with a pro-coagulant state and / or an augmentation of acute inflammation[143]. The data therefore do not suggest substantial beneficial effects on recovery of muscle injury during exercise.

Thus, except on VO_2max, 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

Because 1) most of the research with rHuEPO on endurance performance has focused on a parameter for maximal exercise, VO_2max, 2) the factors that make professional and world-class cyclists unique are not VO_2max, but LT, RCP and C, 3) endurance performance in professional cycling such as in time trials is best correlated with submaximal exercise factors (e.g. LT, VT1, OBLA, RCP), 4) only small parts of professional cycling races are cycled at severe or maximal intensities (above OBLA/RCP) and 5) the characteristics of the study populations differed from the population suspected of rHuEPO abuse, it cannot be concluded that rHuEPO use enhances performance in professional or elite cyclists.

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

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abilities as close as possible to those of professional cyclists and under conditions closely resembling racing conditions and the required performance duration. It can be argued that putting the treatment on the prohibited list falsely implies a proven beneficial effect on performance in professional cycling and unintentionally stimulates its abuse[144], although it should also be recognized that there is no convincing evidence that any drug works in this context.

rHuEPO adverse effects 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 adverse effects. A significant rise in systolic blood pressure (SBP) at rest or during submaximal exercise[112;129] was reported. The number of subjects and treatment times in the presented studies are too small to detect rare adverse events. Larger studies, namely patient studies, must be consulted for this, although it must be kept in mind that results of these studies do not per se translate to well-trained athletes. One patient study 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]. 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 previously described rise in blood pressure and enhanced coagulation[150], endothelial activation and platelet reactivity[151] and inflammation[152] after rHuEPO treatment have been evoked to explain these thrombotic events. Acute exercise on itself also enhances coagulation[153], although this is less pronounced in trained than in untrained subjects. Because plasma volume and blood volume are reduced in acute exercise, Hct is increased[120]. This is even more pronounced in dehydrated and hyperthermic exercise conditions[154;155]. This combination of factors

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might increase the risk of thrombotic events in endurance performance athletes using rHuEPO. Increased Hct may lower cerebral blood flow and limit oxygen supply to the brain, predisposing to cerebral infarction.[156] 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. High Hct also predisposes to heart failure, myocardial infarction, seizures and pulmonary embolism.[158;159] Another association is hypertensive posterior encephalopathy.[160] Red cell aplasia, a rare side-effect mainly linked to anti-erythropoietin antibody formation due to Eprex® use[161] is another dire adverse effect of rHuEPO treatment. The improper handling and storage of rHuEPO preparations associated with illicit use in sport might be expected to increase the risks of this and possibly also of other immunogenic complications[162;163]. Finally, rHuEPO use may promote tumour growth and angiogenesis in tumours, although this is contested[164].

To summarize: published case reports have linked adverse effects to rHuEPO use in cyclists. Patient-studies indicate that rHuEPO has several cardiovascular effects, raising the risk of thrombotic events, encephalopathy and other complications. These risks might plausibly be higher in cyclists because the circumstances in this sport could compound these risks. Also, secrecy due to illicit use in sports might lead to bad handling and storage of the rHuEPO, plausibly elevating the risks of side-effects such as red cell aplasia.

Cyclists and rHuEPO: a risky choice to what advantage?

As the case of the United States Anti Doping Agency *versus* Armstrong proves again, rHuEPO has been used by many professional (including champion) cyclists. As it increases Hct, it is thought to enhance performance in professional cycling and has been put on the list of prohibited substances of the International Olympic Committee. Because 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 in professional or even well-trained cyclists. 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 a scientific basis for performance enhancing properties, nor possible harmful side-effects have been provided for athletes or trainees.

The situation with 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 under real life circumstances would give a better indication of the real advantages and risk factors of rHuEPO use, but it would be an oversimplification that this would eradicate its use, even if no benefit was 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 would 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 was small these efforts could be directed elsewhere.

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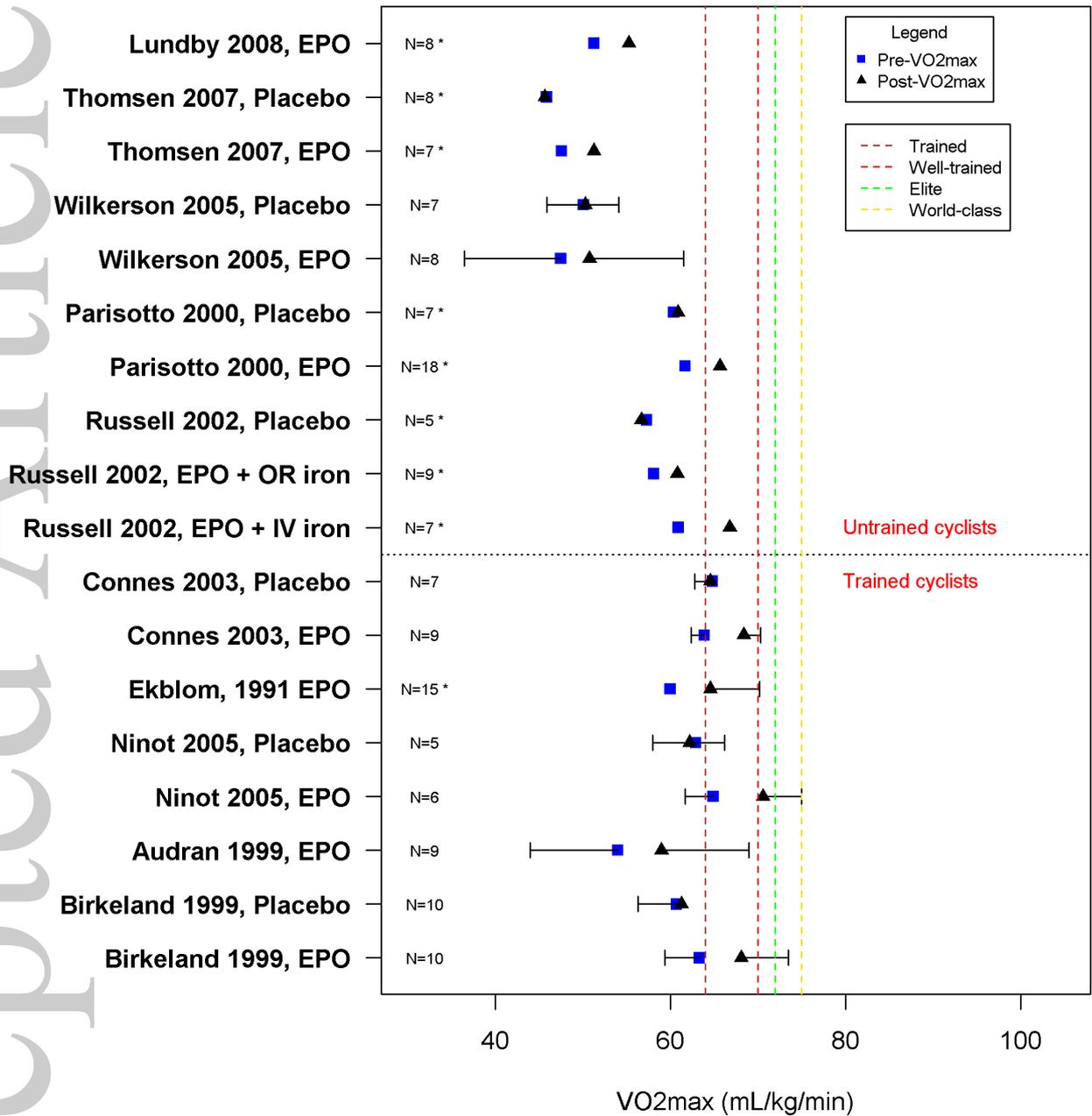
Legends to tables and figures

Table 1   Overview, characteristics and outcomes of the studies looking at the effects of rHuEPO on endurance performance in subjects other than patients. All effects are calculated based on the highest difference found in the parameter when multiple measuring time points were reported.

Figure 1 VO_2max before (■) and after (▲) 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 l/min, which has been converted to ml/kg/min by dividing this value by mean weight of the group for comparison purposes (No SD for these studies because of this conversion). Studies above the horizontal line were using subjects classified as untrained, below the horizontal line classified as trained cyclists. Vertical lines represent minimum values of VO_2max for different classifications of cyclists as suggested by Jeukendrup *et al*.

Study	Type of subjects	Study set-up	Product	Dosing	Max. Hb increase (%)	Max. Hct increase (%)	Max. VO2max 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	-

Overview studies effect EPO on VO2max

Legend
- ■ Pre-VO2max
- ▲ Post-VO2max

- Trained
- Well-trained
- Elite
- World-class

Study	N
Lundby 2008, EPO	N=8 *
Thomsen 2007, Placebo	N=8 *
Thomsen 2007, EPO	N=7 *
Wilkerson 2005, Placebo	N=7
Wilkerson 2005, EPO	N=8
Parisotto 2000, Placebo	N=7 *
Parisotto 2000, EPO	N=18 *
Russell 2002, Placebo	N=5 *
Russell 2002, EPO + OR iron	N=9 *
Russell 2002, EPO + IV iron	N=7 *
Connes 2003, Placebo	N=7
Connes 2003, EPO	N=9
Ekblom, 1991 EPO	N=15 *
Ninot 2005, Placebo	N=5
Ninot 2005, EPO	N=6
Audran 1999, EPO	N=9
Birkeland 1999, Placebo	N=10
Birkeland 1999, EPO	N=10

Untrained cyclists

Trained cyclists

VO2max (mL/kg/min)

BJCP review Figure 1.GIF