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

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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.

Sport is big business

The summer of 2012 was an intensive summer of sport. From all these events, it is clear that sport plays a very important role in our society, because 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 ques-

tion whether this is desirable, because uncontrolled use of a substance involves risks for the users, irrespective of such a substance being used legally or illegally.

In this review, we focus on the use of recombinant human erythropoietin (rHuEPO) in cycling, a sport that has had many reports of cheating, culminating in the last decade, with many suspicions and suspensions. We will address the question of whether the currently available evidence justifies the widespread use of this substance. Many of the major 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, was 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 the 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 that transport oxygen through the blood. Owing to a lack of ability to repair themselves without a nucleus and other cellular machinery, erythrocytes have a lifespan 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 the 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. 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, a molecular weight of 30 400 and a carbohydrate content of 40% [5, 6]. In normal (nonhypoxic) conditions, the concentration of EPO in blood is relatively constant at approximately 5 pmol l^{-1}, 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^{-1} when EPO is quantified as 'international units' (IU), assuming a specific activity of 130 000 IU mg^{-1}. The cells that are the main target for the hormone are the CFU-Es and proerythroblasts, containing the highest density of erythropoietin receptors (EpoRs) [7]. The main effect of EPO is on CFU-Es, because 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/mitogen-activated protein kinase [9, 10]. After binding of EPO to its receptor, dimerization of two EpoR molecules occurs, and this starts the intracellular signalling that leads to proliferation of CFU-Es [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 1000-fold in low blood oxygen content, for example in severe anaemia [3]. Production of EPO 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 h before EPO levels start to increase in a linear manner, reflecting the time of signal transduction and hormone synthesis and secretion. Peak EPO concentrations after hypoxia are reached within 48 h, with the 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 rate of erythropoiesis [17].

The proposed oxygen-sensing mechanism that regulates EPO production involves the hypoxia-inducible factor (HIF), a transcription factor [18]. Expression of HIF is seen in cells exposed to hypoxia within 30 min [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, which accumulates in hypoxic conditions as a result of the sequential dephosphorylation of ATP, ADP and AMP. This non-HIF pathway also increases EPO mRNA expression [22]. GATA-2, a transcription factor, 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 h [24], corresponding to the time course of CFU-E differentiation into mature erythrocytes [25]. The estimated half-life ($t_{1/2}$) of endogenous EPO is approximately 5.2 h [26], and mechanisms of clearance are somewhat extraordinary. Clearance of EPO by the liver is, as for many other glycoproteins, rather low, mainly due to the terminal sialic acid

residues, which prevent galactose receptor binding, internalization and degradation in the liver. Indeed, it has been shown that desialated EPO experiences 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.

Recombinant erythropoietin in disease

Given that EPO plays an important role in the regulation of 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 by the US Food and Drug Administration for human use in patients with chronic renal failure in 1989 [22]. These first recombinant forms of EPO (called epoetin alfa, e.g. Eprex®) are identical to endogenous human EPO with regard 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 fairly similar to endogenous EPO (4–9 h) [38], which is also the case for second-generation rHuEPO, epoetin beta (e.g. Neorecormon®) [39]. The same holds true for a later generation of recombinant EPO produced in human cells, epoetin delta (Dynepo®) [40]. Other forms of EPO, darbepoetin 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 h [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 to overcome the insufficient production of EPO resulting from the kidney damage and maintain steady-state erythropoiesis.

. . . and in sport. But does it work?

The treatment immediately attracted the attention of athletes. Given that 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 list of prohibited substances in 1990. The list has now been expanded to all erythropoiesis-stimulating agents [e.g. EPO, darbepoetin, HIF stabilizers, methoxy polyethylene glycol-epoetin beta (CERA) and peginesatide (Hematide®)] [43]. The World Anti-Doping Agency 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 erythropoiesis-stimulating agents increase red blood cell mass in world-class cyclists and does this result in increased stamina and performance? Here, we examine 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 are the maximal oxygen uptake ($\dot{V}_{O_2 max}$), the lactate threshold (LT) and the 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 $\dot{V}_{O_2 max}$ [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.

Maximal oxygen uptake is a prerequisite but not a sole determining factor

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 arteriovenous oxygen difference. These, in turn, are mainly dependent on total blood volume, which is 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 $\dot{V}_{O_2 max}$ as well, as reviewed by Joyner and Coyle [45] and Bassett and Howley [53] and reported by other researchers [54, 55]. Heinicke *et al.* [54] demonstrated the relationship between $\dot{V}_{O_2 max}$ and blood volume and total body haemoglobin in endurance disciplines. Training can improve many of the mentioned factors to increase $\dot{V}_{O_2 max}$, such as increasing blood volume [56], and indeed, the $\dot{V}_{O_2 max}$ values of champion endurance athletes are 50–100% greater than those observed in normally active healthy young subjects [45]. That an increase in $\dot{V}_{O_2 max}$ has a great potential to increase endurance performance was already shown by Buick *et al.* [57] and Brien and Simon [58].

After autologous red blood cell reinfusion to elevate haemoglobin and haematocrit levels in well-trained runners, running performance was significantly increased. Ekblom [59] cites another article by Celsing *et al.* [60] to show that an increase in haemoglobin, irrespective of baseline haemoglobin levels, will increase maximal aerobic power and therefore performance. However, the last statement in this paper by Ekblom is at least as important, where the author warns against extrapolating this finding to the physically fit athlete, because in these subjects factors other than haemoglobin and maximal aerobic power may play a limiting role in performance. Later research emphasized this warning, because $\dot{V}_{O_2\,max}$ 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 [44]. The $\dot{V}_{O_2\,max}$, 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 $\dot{V}_{O_2\,max}$ after initial years of training, they subsequently maintain a plateau in their $\dot{V}_{O_2\,max}$ but continue to improve their performance further [47, 68, 69] (note that one of these reports [69] is about Lance Armstrong). Research into training for endurance performance shows the same trend; moderately trained athletes are able to improve $\dot{V}_{O_2\,max}$ (as well as LT and *C*) by interval and/or intensive training [70, 71], whereas these training regimens do not improve $\dot{V}_{O_2\,max}$ in well-trained athletes, but mainly improve the LT and *C* [50, 72], possibly by improving buffering capacity [73].

It is more than the $\dot{V}_{O_2\,max}$

It is not $\dot{V}_{O_2\,max}$, but power output at submaximal intensities, such as the first (VT1 or VT) and second ventilation threshold (VT2), or the respiratory compensation point (RCP) that differ significantly between elite amateur and professional cyclists [64, 74]. Thus, factors other than $\dot{V}_{O_2\,max}$ 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 h cycling world record, as described by Padilla *et al.* [76], its predictions are far from the observed results. Based on the $\dot{V}_{O_2\,max}$ and body mass of Miguel Indurain, a professional cyclist, the distance covered in 1 h predicted by the model would have been 43.645 km, whereas the world record he set was 53.040 km h^{-1}. Calculating back from this record, the model would predict an impossible $\dot{V}_{O_2\,max}$ of 10.3 l min^{-1}, whereas ranges for world-class athletes are 5–6 l min^{-1} [67, 77, 78]. This and another model [79] both rely on $\dot{V}_{O_2\,max}$ 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 $\dot{V}_{O_2\,max}$ leads to a proportionally smaller increase in performance. This also demonstrates that in world-class athletes, an increase in $\dot{V}_{O_2\,max}$ will have only limited effect on performance. The failure of such a model [75] to predict 1 h performance [76] suggests that factors other than $\dot{V}_{O_2\,max}$ are limiting in endurance performance at this level of performance.

Lactate threshold

We therefore now address the importance of LT in determining the performance of endurance athletes. Lactate threshold, similar to VT1, is the intensity of work or oxygen uptake ($\dot{V}_{O_2}$) 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]. Given that 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 $\dot{V}_{O_2}$ at this LT is highly related to performance, more so than $\dot{V}_{O_2\,max}$ [45, 47, 63–65, 67, 68, 82]. Elite cyclists are reported to be able to reach LTs between 300 and 400 W [63, 77, 83], or 70–85% $\dot{V}_{O_2\,max}$ (3.5–4.7 l min^{-1}) [65, 67]. The LT reflects a balance between the rate of lactate production in the muscles (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; the difference in performance (time to fatigue) in cyclists with similar values of $\dot{V}_{O_2\,max}$ can be explained by %$\dot{V}_{O_2\,max}$ 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 and Henriksson [84] found that capillary density increases with training. This might indicate that these athletes have a higher capacity to remove and recycle muscle-fatiguing metabolites, thereby allowing muscles to tolerate lactic acid production and anaerobic metabolism better [85], or to maintain/elongate mean transit time of the blood to increase oxygen extraction [86].

Lactate turn point

Lactate turn point (LTP) is a distinct factor that is also related to lactate [52]. The RCP [87], 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 is 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 $\dot{V}_{O_2\,max}$.

Economy

The third main factor contributing to endurance performance is assumed to be completely independent of $\dot{V}_{O_2\,max}$ and lactate-related factors and is called work economy or efficiency (C). It is the ratio between work output (speed or power) and oxygen cost. Running economy is commonly defined as the steady-rate $\dot{V}_{O_2}$ 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 being wasted on braking forces and excessive vertical oscillation [44]. The work efficiency is a discriminator of endurance performance independently of $\dot{V}_{O_2\,max}$ in runners [48, 68, 91–93] and cyclists [63, 69, 78], becoming more important than $\dot{V}_{O_2\,max}$ 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). The work efficiency (hence, endurance performance) is related to the percentage of type I fibres [67, 94, 95]. Training can induce changes from type IIb to IIa and from type IIa to type I in animals [96], and possibly in humans [67, 97].

Other factors

Besides these main determinants, several other factors have also been 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 an increase in cardiac volume due to endurance training [98, 99], leading to an increase in stroke volume and allowing a reduced heart rate for the same cardiac output. Breathing pattern is another factor that influences cycling performance, because professional cyclists have been reported to lack a tachypnoeic shift at high workloads, possibly indicating a more efficient use of their respiratory muscles [100]. Also, the quantity of muscle mass recruited for sustained power production can influence performance, because 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 endur-ance 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 Lance Armstrong [69]).

In summary, endurance performance mainly depends on an athlete's $\dot{V}_{O_2\,max}$, LT, LTP and C; $\dot{V}_{O_2\,max}$ 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 level of 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 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.

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 $\dot{V}_{O_2\,max}$ (absolute and per kilogram of body weight), subjects in these studies would be either 'untrained cyclists' [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
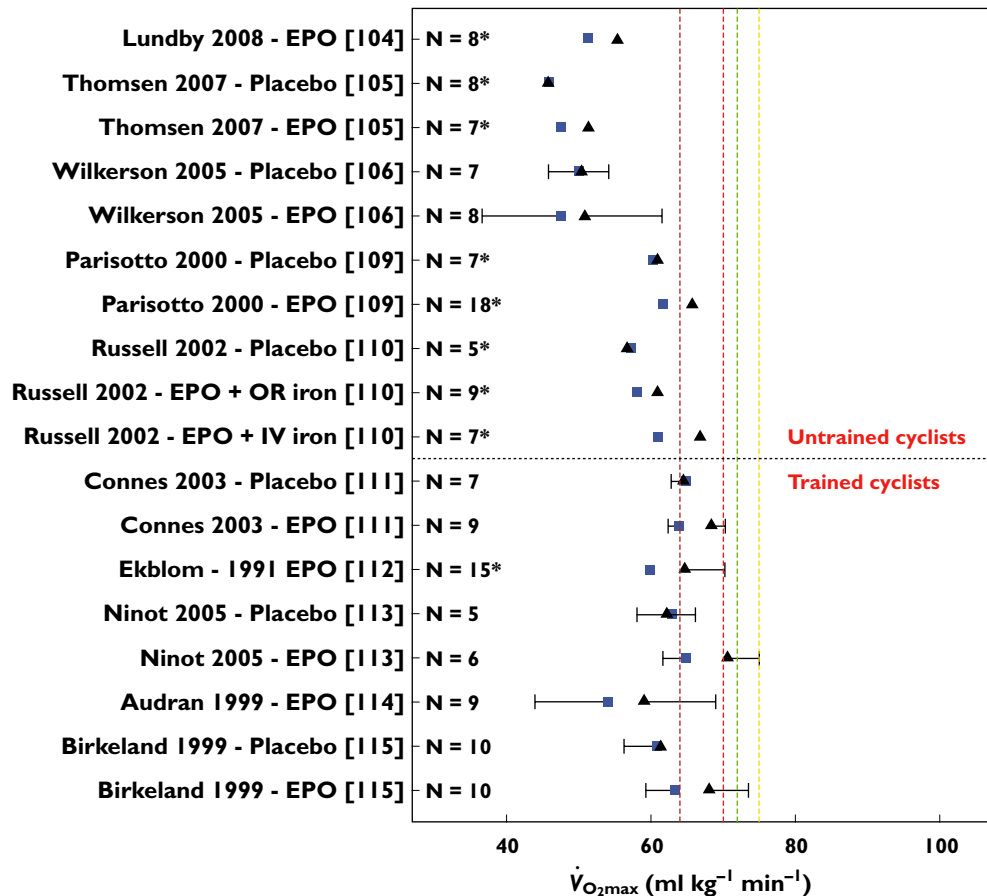
Figure 1

Maximal oxygen uptake ($\dot{V}_{O_2max}$) 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 $\dot{V}_{O_2max}$ 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 red 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 $\dot{V}_{O_2max}$ for different classifications of cyclists as suggested by Jeukendrup *et al*. [77] (brown dashed line, trained; red dashed line, well trained; green dashed line, elite; and yellow dashed line, world class). ■, pre-$\dot{V}_{O_2max}$; ▲, post-$\dot{V}_{O_2max}$; -----, trained; -----, well-trained; -----, elite; and -----, world class

cyclists, who would be expected [77] to have $\dot{V}_{O_2max}$ values above 70 ml kg^{-1} min^{-1} (5 l min^{-1}) and power outputs above 5 W kg^{-1}. Figure 1 compares the studied subjects with these reference values. In the only study using subjects with mean power outputs above 5 W kg^{-1}, the mean $\dot{V}_{O_2max}$ is only ~64 ml kg min^{-1} [111]. The studied subjects may not have reached a plateau in $\dot{V}_{O_2max}$, 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]. The $\dot{V}_{O_2}$ 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 cer-

tainty 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.

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^{-1} week^{-1} (Table 1). Almost all studies used forms of rHuEPO

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

Study	Types of subjects	Study set-up	Product	Dosing	Max. Hb increase (%)	Max. Hct increase (%)	Max. $\dot{V}_{O_2max}$ increase (%)
Lundby et al. (2008) [104]	Untrained	Uncontrolled	Neorecormon®	5000 IU (~65 IU kg⁻¹) 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⁻¹) on alternating days for 2 weeks, a dose on three consecutive days for 1 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⁻¹ once a week for 4 weeks	7	12	7
Rasmussen et al. (2010) [107]	Untrained	Uncontrolled	Neorecormon®	5000 IU (~60 IU kg⁻¹) on alternating days for 2 weeks, a dose on three consecutive days for 1 week and one dose a week for 12 weeks	NA	12	NA
Lundby & Damsgaard (2006) [108]	Untrained	Uncontrolled	NESP	144 IU kg⁻¹ (0.72 µg kg⁻¹) once a week for 4 weeks	17.4	16.4	NA
Parisotto et al. (2000) [109]	Untrained	Placebo + blinded	Eprex®	50 IU kg⁻¹ three times a week over 25 days (in combination with ~100 mg iron either intramuscularly or orally)	7.4/12	NA	6.3/6.9
Russell et al. (2002) [110]	Untrained	Placebo	Eprex®	50 IU kg⁻¹ three times a week for 3 weeks and 20 IU kg⁻¹ for 5 weeks	NA	15	9.7
Connes et al. (2003) [111]	Trained	Placebo + blinded	Eprex®	50 IU kg⁻¹ three times a week for 4 weeks	9.6	8.3	7
Ekblom & Berglund (1991) [112]	Trained	Uncontrolled	NA	20 IU kg⁻¹ three times a week for 6 weeks (or 4 weeks, and 40 IU kg⁻¹ for the remaining 2 weeks)	NA	11.7	8
Ninot et al. (2006) [113]	Trained	Placebo + blinded	Eprex®	50 IU kg⁻¹ three times a week for 4 weeks, followed by 20 IU kg⁻¹ three times a week for 2 weeks	9.5	10.2	7
Audran et al. (1999) [114]	Trained	Uncontrolled	Eprex®	50 IU kg⁻¹ 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⁻¹ week⁻¹) three times a week	11.2	19	7
Souillard et al. (1996) [116]	Unknown	Placebo	Eprex®	200 IU kg⁻¹ five times in 11 days	4.6	8.9	NA

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; NEPS, novel erythropoiesis stimulating protein (darbepoietin-alfa); $\dot{V}_{O_2max}$ maximal oxygen uptake.

with half-lives similar to endogenous EPO, namely Eprex® [109–111, 113, 114, 116] or 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 eight [105, 106, 109–111, 113, 115, 116] of the 13 studies were placebo controlled. As endurance performance can change significantly, for example, due to training, it is crucial to control for these effects with a placebo-treated group. Moreover, unfortunately only five [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 the exercise tests performed in the studies, the outcome of which depends on perseverance, hence on motivation. Given that the study using NESP as rHuEPO treatment was not placebo controlled and did 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 yet been studied for effects on endurance performance in athletes 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 with the lower doses [109, 110] and tripled with the higher doses [114, 116], and declined to below baseline approximately 7–14 days after rHuEPO treatment ceased [109, 110, 114, 116]. Erythropoietin 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 and Hct, respectively (Table 1), with no obvious differences between athletes with different training statuses. These levels are reported to return to baseline within 1 month after cessation of treatment [109].

An increase in Hct could lead to an increase in oxygen-carrying capacity, but does this enhance performance? Haematocrit is not a good marker of performance, because endurance athletes usually have lower Hct values than untrained subjects owing to plasma volume expansion [119]. Additionally, it is a very variable measure and is affected by different circumstances [120]. Increases of Hct cause an increase in viscosity of the blood [121, 122], which might hamper performance owing to reductions in blood flow and increased cardiac work. Decreased plasma volume during exercise exaggerates increased Hct [120], as may dehydration, hyperthermia and altitude, so it is not obvious what effects a rise in Hct will have in professional cyclists. 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, blood volume [123].

Recombinant human erythropoietin could therefore counteract the plasma volume expansion of endurance training [56]. Nevertheless, the combination of effects seems to increase the performance parameter $\dot{V}_{O_2 max}$, at least in the studied subjects in laboratory conditions.

Effects on $\dot{V}_{O_2 max}$

The most important question then is whether these effects on haematological parameters translate into an effect on performance. The different parameters that determine endurance performance have been discussed in the foregoing sections, but unfortunately most studies examine only one of these parameters, namely $\dot{V}_{O_2 max}$. In the reported studies, this parameter is increased in the rHuEPO-treated subjects, with a relatively constant value for all studies, independent of the training status of the subjects, between 7 and 9.7% (Table 1). Absolute values of $\dot{V}_{O_2 max}$ and treatment effects can be seen in Figure 1. This increase in $\dot{V}_{O_2 max}$ 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 (vs. 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 min and leading to exhaustion, entirely different from the required ~5 h performance in a cycling race.

Does it translate to cycling performance?

As mentioned earlier, $\dot{V}_{O_2 max}$ is poorly related to cycle performance [64, 74], and Lucia et al. [124] even questions whether $\dot{V}_{O_2 max}$ is the limiting factor for maximal endurance performance in some 50% of professional cyclists, owing to a lack of plateau in $\dot{V}_{O_2 max}$ during an exercise-to-exhaustion test. 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 results from a laboratory test setting to race events [118]. The use of rHuEPO in these subjects clearly has an effect on $\dot{V}_{O_2 max}$, which might improve performance at peak intensity during severe exercise, although evidence for this is rather 'soft'. Apart from the uncertainty concerning 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 end-point (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, means that cyclists work for only 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 week races the subjects' heart rate is above such an intensity (HR_{OBLA}) for only 3.6% (119 s) of the time climbing a 'Hors catégorie' climb (hardest climb), even less so during first and second category climbs, 2.6% (45 s) and 2.5% (22 s), respectively [90]. Similar low percentages were reported by Lucia et al. [127] for total race time with heart rate above the RCP (at 90% $\dot{V}_{O_2 max}$) 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 heart rate above OBLA was found between different types of time trials, with prologue, short, long and uphill time trials recording 59, 38, 3.5 and 0% for cyclists going all-out [128]. Heart rate values corresponding to physiological markers of performance (e.g. LT and VT2) are stable during a training year in professional cyclists [83].

Other endurance performance parameters are unstudied

For the major part of a race, cyclists therefore exercise well below their $\dot{V}_{O_2 max}$ levels, but this parameter has nevertheless attracted the most attention when looking at rHuEPO effects. Some studies that evaluated other parameters found no change in the $\dot{V}_{O_2}$ kinetics [105, 106, 110, 129] or $\dot{V}_{O_2}$ at submaximal exercise [112], despite the increased oxygen-carrying capacity due to the increase in haemoglobin concentration and Hct. This would mean that the oxygen-carrying capacity of the blood does not determine $\dot{V}_{O_2}$ 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, 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], with 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, because performance in cycling is much better related to these factors [64, 74]. Time-trial world-record performance (1 h 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 $\dot{V}_{O_2}$ at VT1 [132] or LT [67]. In >50 km time trials during the Tour the France, performance was correlated with power output at VT1 [133]. In these time trials, $\dot{V}_{O_2 max}$ is not related to performance, which was only demonstrated in shorter time trials (20 min) [131]. Lastly, uphill

cycling also 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 measured by only one group [105] and did not change after rHuEPO treatment. This would be expected from the nonhaematological, biomechanical factors that determine C, as discussed in foregoing sections. There is, however, some evidence that prolonged exposure to rHuEPO in healthy subjects may induce changes in the skeletal muscle, with an increase in the relative amount of the slow myosin light chain (type I fibres) and decreased fast myosin light chain (type II) fibres, possibly leading to an improvement in C [134]. More evidence is needed to draw conclusions about effects of rHuEPO on C, especially given that 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 that partly contributed to his world-class performance [69].

Some other parameters, such as blood lactate, end-exercise heart rate and heart rate kinetics, were investigated and reported not to be altered by rHuEPO treatment [106], although other studies indicate a nonsignificant reduction in in blood lactate [110] and heart rate [110, 111] or a significant reduction in 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 test, but not at exhaustion [105], was seen. Blood volume was also not affected [112, 123]. One blinded study investgated 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, is unlikely to be related to an increased muscular mass or improved vascularization. In a publication [135] 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% greater muscle volume and 25% increase in muscle vascularization, even these effects did not translate to increased muscle force or stamina [136].

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. Recombinant human erythropoietin may have anti-inflammatory effects and may mitigate ischaemia – 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 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 procoagulant 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 $\dot{V}_{O_2 max}$, 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, $\dot{V}_{O_2 max}$; (ii) the factors that make professional and world-class cyclists unique are not $\dot{V}_{O_2 max}$, 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 and 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 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 is needed
In summary, 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 to investigate 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 in 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.

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 adverse effects. A significant rise in systolic blood pressure 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 owing to an 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 with placebo [146–148]. These studies used approximately four times higher doses of rHuEPO (usually in the range of 40 000 IU or 600 IU kg^{-1} week^{-1}) compared with 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 *per se* also enhances coagulation [153], although this is less pronounced in trained than in untrained subjects. Given that 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 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] that describes how a professional cyclist presented with cerebral sinus thrombosis, thereafter confessing to 3 months of 2000 IU rHuEPO use every 2 days, in combination with 15 days of growth hormone and continuous high doses of vitamins 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 adverse 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-based 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 adverse 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. Given that 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. 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 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 end-points. 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.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; and no other relationships or activities that could appear to have influenced the submitted work.

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