The clinical pharmacology of performance enhancement and doping detection in sports

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EFFECTS OF ERYTHROPOIETIN ON CYCLING PERFORMANCE OF WELL TRAINED CYCLISTS: A DOUBLE-BLIND, RANDOMISED, PLACEBO-CONTROLLED TRIAL

LANCET HAEMATOLOGY 2017;4(8):E374–86

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ABSTRACT

Substances that potentially enhance performance (e.g., recombinant human erythropoietin [rHuEPO]) are considered doping and are therefore forbidden in sports; however, the scientific evidence behind doping is frequently weak. We aimed to determine the effects of rHuEPO treatment in well trained cyclists on maximal, submaximal, and race performance and on safety, and to present a model clinical study for doping research on other substances. We did this double-blind, randomised, placebo-controlled trial at the Centre for Human Drug Research in Leiden (Netherlands). We enrolled healthy, well trained but non-professional male cyclists aged 18–50 years and randomly allocated (1:1) them to receive abdominal subcutaneous injections of rHuEPO (epoetin-beta; mean dose 6000 IU per week) or placebo (0.9% NaCl) for 8 weeks. Randomisation was stratified by age groups (18–34 years and 35–50 years), with a code generated by a statistician who was not masked to the study. The primary outcome was exercise performance, measured as maximal power output (P_{max}), maximal oxygen consumption VO_{2max}, and gross efficiency in maximal exercise tests with 25 W increments per 5 min, as lactate threshold and ventilatory threshold 1 (VT1) and 2 (VT2) at submaximal levels during the maximal exercise test, and as mean power, VO_2, and heart rate in the submaximal exercise tests at the highest mean power output for 45 min in a laboratory setting and in a race to the Mont Ventoux (France) summit, using intention-to-treat analyses. The trial is registered with the Dutch Trial Registry (Nederlands Trial Register), number NTR5643. Between March 7, 2016, and April 13, 2016, we randomly assigned 48 participants to the rHuEPO group (n=24) or the placebo group (n=24). Mean haemoglobin concentration (9.6 mmol/L versus 9.0 mmol/L [estimated difference 0.6, 95% CI 0.4 to 0.8]) and maximal power output (351.55 W versus 341.23 W [10.32, 3.47 to 17.17]), and VO_{2max} (60.121 mL/min per kg versus 57.415 mL/min per kg [2.707, 0.911 to 4.503]) in a maximal exercise test were higher in the rHuEPO group compared with the placebo group. Submaximal exercise test parameters mean power output (283.18 W versus 277.28 W [5.90, −0.87 to 12.67]) and VO_2 (50.288 mL/min per kg versus 49.642 mL/min per kg [0.646, −1.307 to 2.600]) at day 46, and Mont Ventoux race times (1 h 40 min 32 s versus 1 h 40 min 15 s [0.3%, −8.3 to 9.6]) did not differ between groups. All adverse events were grade 1–2 and were similar between both groups. No events of grade 3 or worse were observed. Although rHuEPO treatment improved a laboratory test of maximal exercise, the more clinically relevant submaximal exercise test performance and road race performance were not affected. This study shows that clinical studies with doping substances can be done adequately and safely and are relevant in determining effects of alleged performance-enhancing drugs.

INTRODUCTION

Use of drugs that potentially enhance performance (also called doping) is a major problem in many competitive sports, partly shown by the thousands of annual adverse analytical findings.[1] The 2017 prohibited list of drugs is substantial (>300 substances) and open-ended because all compounds that potentially enhance performance could be forbidden.[2] The list is not necessarily based on solid evidence, as shown by the criteria for including substances and methods section on the Prohibited List of the World Anti-Doping Agency (WADA) Code, which states as one of the criteria that there only needs to be "experience" that a substance "has the potential to enhance performance".[3] This criterium is probably driven by the assumption that scientific evidence cannot be obtained in many cases or generation of such evidence is too time-consuming and expensive. Moreover, in the time required to collect the evidence, the substance could be used, leading to unfair situations. Therefore, it is not surprising that the scientific evidence supporting the ban on substances to be used by athletes is scarce. A publicly known example of such a banned drug is recombinant human erythropoietin (rHuEPO), which has been under constant scrutiny by anti-doping authorities since its first alleged use in the late 1980s. Although relatively few athletes have been caught for rHuEPO abuse during their active careers, the attention for this banned substance has spiked recently because many professional cyclists competing in the 1990s and 2000s admitted to having used rHuEPO to improve their cycling performance.

rHuEPO induces erythropoiesis and thereby enhances blood haemoglobin concentrations, and it was assumed that this induction would result in increased muscle delivery of oxygen and hence improved exercise performance. However, the evidence for the performance-enhancing effects of rHuEPO in high-level competitive sports is rather scarce. The evidence constitutes of small, often uncontrolled studies,[4] in arguably unrepresentative populations and is often inappropriately expressed only in exercise parameters that mainly evaluate maximal exercise performance. These tests are often of incremental intensity or at a very high intensity, and therefore lead to exhaustion, usually within 20 min. By contrast, submaximal tests are at intensity levels that can be maintained for a long period of time (>20 min), which is the level at which cyclists perform most of the time. Therefore, both types of tests evaluate different types of performance parameters. Well powered studies on the effects of rHuEPO on submaximal exercise parameters in trained athletes are lacking. Additionally, studies[5] have reported that an increase in haematocrit and a subsequent increased blood viscosity is associated with a marked reduction in muscle oxygen delivery. Furthermore, elite athletes improve their work economy or submaximal performance, not their maximal oxygen consumption, when improving their performance over time, indicating that maximal oxygen consumption might not be a rate-limiting factor.[6] Finally, whether increasing haemoglobin beyond normal values is beneficial is unclear; data in patients with anaemia suggest that the goal of rHuEPO treatment should not be normalising haemoglobin concentrations because this results in an increased incidence of ischaemic stroke.[7] Furthermore, possible sudden deaths of professional cyclists related to rHuEPO were suggested in the late 1980s and early 1990s.

We decided to study rHuEPO as a model doping drug. The aim of this study was to evaluate the effect of rHuEPO in well trained cyclists on maximal and submaximal performance parameters in a laboratory setting and in a real-life road cycle race. Additionally, we evaluated if this trial design would be a practical approach to investigate other doping substances.

METHODS

Study design and participants

We designed a double-blind, randomised, placebo- controlled study of healthy male cyclists between ages 18 years and 50 years. We undertook the study at the Centre for Human Drug Research (CHDR) in Leiden (the Netherlands). Participants were recruited via advertisements, social media, newsletters of cycling clubs, and through the help of national sports associations. Main inclusion criteria were being fluent in Dutch and having a maximum power- to-weight ratio during the maximal exercise test at screening that exceeded 4 W/kg, normal exercise electrocardiogram (ECG), screening haemoglobin between 8.0 mmol/L and 9.8 mmol/L (equivalent to 12.8–15.7 g/dL), screening haematocrit below 48% and not being subject to anti-doping regulation or using medication that could potentially interact with the study drugs or study assessments. After passing a preliminary screening over the telephone, participants underwent a medical screening, followed by a maximal

exercise test to determine peak exercise performance. From the first dose to 3 months after the final dose, participants were not allowed to take part in sports events that were subject to anti-doping regulations. All participants gave written informed consent before any study-related activity.

The study was approved by the Independent Ethics Committee of the Foundation Evaluation of Ethics in Biomedical Research (Stichting Beoordeling Ethiek Biomedisch Onderzoek, Assen, Netherlands). The study is registered in the Dutch Trial Registry (Nederlands Trial Register), number NTR5643. Our study protocol is available online.

Randomisation and masking

Participants were randomly assigned (1:1) to either the rHuEPO group or the placebo group. To reduce potential variability between the groups due to age differences, a stratified randomisation was used with one block of participants aged 18–34 (inclusive) and another of participants aged 35–50 (inclusive). The randomisation code was generated by a statistician who was not masked to the study and was not involved in the execution of the study. Until study closure the treatment codes were only available to this statistician and the Leiden University Medical Centre (LUMC) pharmacy, that distributed the study agents. Participant enrollment was done by a physician who was masked to the study.

Procedures

Participants received weekly abdominal subcutaneous injections of epoetin-beta (NeoRecormon, Roche, Basel, Switzerland) or saline (0.9% NaCl) for 8 weeks. Target haemoglobin in the rHuEPO group was a 10–15% increase compared with the baseline haemoglobin concentration. Haemoglobin was measured with the HemoCue Hb 201+ analyser (Radiometer Benelux BV, Zoetermeer, Netherlands) and haematocrit with the Haematokrit 200 centrifuge (Hettich Benelux BV, Geldermalsen, Netherlands) before each dose administration and measurements were only available to personnel who were not masked to the study. All haematology samples were collected after participants were seated with their feet on the floor for at least 10 min. All participants in the rHuEPO group received 5000 IU per injection for the first four rHuEPO injections. If the haemoglobin concentration was below the target range, a physician who was not masked or related to the study modulated the dose to 6000 IU, 8000 IU, or 10 000 IU in the subsequent 4 weeks to reach the target range. When haemoglobin was in the target range during the treatment period, rHuEPO dose was adjusted to 2000 IU. For safety reasons, a placebo injection was administered if the haemoglobin concentration exceeded the upper limit of the haemoglobin range or if the haematocrit concentration was equal to or exceeded 52% (dose decision tree, Figure 1). Doses were given in maximum 1 mL injections and distributed over two syringes if this volume was exceeded.

The different doses were prepared by a technician not masked to the study from multidose vials containing a lyophilisate of 50 000 IU epoetin-beta and 10 mL solvent for solution for injection. rHuEPO and placebo were visually indistinguishable (both colourless solutions) and dose changes (changes in injected volume) were also randomly assigned to placebo participants by the statistician and pharmacy before the start of the study.

During the treatment period, all participants also received open-label daily oral doses of 200 mg ferrous fumarate (Pharmachemie BV, Haarlem, Netherlands) and 50 mg ascorbic acid (Pharmachemie BV), and received standard instructions about concomitant food intake. Intake of these supplements was recorded daily by the participant in a diary.

Participants were instructed to maintain their usual training programme throughout the study. The racing bikes of participants were equipped with a Single Leg Power Meter SGY-PM910H2 (Pioneer Europe, Antwerpen, Belgium) with Shimano Ultegra 6800 crank (Shimano, Osaka, Japan) to log training data on the bicycle during the entire study. Data of bicycle trainings were uploaded to the dedicated database Cyclo-Sphere. Additionally, participants recorded all exercise activity in a diary, including other sports or cycling done without the power meter.

Exercise tests were done on a Monark LC4r ergometer (COSMED, Rome, Italy). Gas exchange was measured by Quark CPET system (COSMED), with breath-by-breath sampling technology and integrated heart rate measurement, with one of two wireless heart rate straps (COSMED and Polar, Kempele, Finland). Data were collected on a dedicated computer with the Omnia Metabolic Modules software (COSMED). Before each test, the gas analysers and flow meter were calibrated. Maximal exercise tests were done during screening, at baseline (up to 14 days before

first dose), and during the treatment period at days 11, 25, 39, and 53 (participants could deviate by 1 day) after the first dose administration. Submaximal exercise tests were done at baseline (up to 14 days before first dose and at least three days after the baseline maximal test) and at 46 days (participants could deviate by 1 day) after the first dose administration.

For both the maximal and submaximal exercise tests, the start of the protocol dictated 1 min rest without pedalling, followed by a 2 min warm up at a pedalling workload of 75 W. In the maximal exercise protocol, a ramp test was done where the pedalling workload was increased after the warm up to 175 W, and increased by an additional 25 W every 5 min. Cadence had to be maintained between 70 rpm and 90 rpm. Exhaustion was reached when cadence could not be maintained above 70 rpm or when a participant terminated the test. Subsequently, a 3-min recovery with a pedalling workload of 50 W was initiated. Between 4 min 15 s and 4 min 45 s into each step and immediately after termination of the exercise test (at peak pedalling workload), blood was collected from an intravenous cannula in the right forearm to measure blood lactate concentrations with a Lactate Pro 2 meter (Arkray, Kyoto, Japan). The screening maximal exercise test was similar, except that lactate was not measured (blood was not collected) and an exercise ECG was monitored and recorded with a 12-lead ECG system (COSMED or Labtech Ltd, Debrecen, Hungary).

In the submaximal exercise protocol, pedalling workload was set at 80% of the maximal power reached during the baseline maximal exercise test. Participants were instructed to produce the highest mean power output during a 45-min period, attempting to mimic competitive cycling time trials. Participants could adjust the power on the bike by indicating with hand gestures to increase or decrease in power by steps of 10 W. Cadence had to be maintained between 70 rpm and 90 rpm and the test was stopped after 45 min, followed by 3 min recovery at 50 W. Blood was collected from an intravenous cannula at 10 min, 30 min, and 45 min to measure blood lactate concentrations.

Approximately 12 days (range 10–16) after the last dose participants competitively climbed Mont Ventoux (Vaucluse département, France) in an open course via Bédoin (France), bridging an altitude of 1610 m over 21.5 km, resulting in an average gradient of 7.5%. The race was preceded by a stage of 110 km in Provence (France; total elevation gain 1524 m) that was completed collectively (i.e., all participants finished the course to the foot of the Mont Ventoux in a closed pack). Before the 110 km stage and at the top of Mont Ventoux, blood was collected and all participants, including their bicycles, were weighed. After the race, participants were asked by personnel (masked to the study) whether they thought they had been treated with rHuEPO or placebo during the treatment period.

Vital signs were measured regularly and adverse events documented during every visit. Additionally, before and regularly during the treatment period blood samples were taken in which haematology, coagulation, and endothelial function markers were measured. A broad range of markers was measured to evaluate potential risk of rHuEPO treatment in well trained cyclists.

All data were stored in a clinical trial database (Promasys, Omnicomm Inc, Fort Lauderdale, FL, USA) and checked for accuracy and completeness. A masked data review was done before code-breaking and analysis, according to a standard procedure at our unit.

Outcomes

The primary outcome was exercise performance, under both maximal and submaximal conditions, as assessed with multiple measures. For the maximal exercise test, the primary outcome was measured as maximal power output (P_{max}), maximal oxygen consumption VO_{2max}, and gross efficiency. P_{max} was calculated with the following formula:

$$P_{max} = [\text{power of the last completed step}] + \left[\frac{\text{time (s) in the subsequent step} \times 25\text{W}}{300\text{ s}}\right]$$

The breath-by-breath dataset was averaged in epochs of 30 s and the VO_{2max} was determined. Gross efficiency was calculated by the following formulas:

$$\text{Gross efficiency} = \frac{\text{power}}{\text{energy expenditure}} \times 100$$

where energy expenditure was calculated at the last level when the respiratory quotient was less than 1.0 and the power step had lasted longer than 180 s using the formula:

$$\text{Energy expenditure} = [((3.869 \times VO_2) + (1.195 \times VCO_2)) \times \frac{4.186}{60}]$$

For the submaximal levels during the maximal exercise test, the primary outcomes were measured as the lactate threshold, determined with the modified Dmax method, and ventilatory threshold 1 (VT1) and 2 (VT2),[8] for which assessments were done by two masked staff members (JH and PG), in consensus.

We obtained secondary outcomes from the maximal exercise test (e.g., heart rate, respiratory min volume [VE], volume of CO_2 expired [VCO_2]) from the last completed 30 s average before the recovery phase. We calculated the mean power, VO_2, and heart rate primary outcomes during the 45-min submaximal exercise test on the basis of the breath-by-breath dataset. We calculated cycling economy, a primary outcome, as described previously using the following formula:

$$\text{Cycling economy} = \frac{\text{mean power}}{\text{mean } VO_2\text{(L/min)}}$$

Secondary outcomes were lactate concentrations measured at 10 min, 30 min, and 45 min. In the Mont Ventoux race, secondary outcomes were race time, average efficiency, and average power. Secondary safety outcomes were blood pressure, heart rate, adverse events, and coagulation and endothelial function markers. Additional measurements of skin blood flow and diagnostic aspects of detection of rHuEPO use were done and these will be published separately.

Statistical analysis

No studies have been published that allowed a formal power calculation based on enhancement of submaximal performance by rHuEPO in trained cyclists. Therefore, we based the power calculation on the increase in VO_{2max} observed in a previous study[9] with moderately trained participants (3.8 mL/min per kg). We assumed the effect in well trained participants would be smaller; therefore, the power calculation was done on a VO_{2max} increase of 1.7 mL/min per kg. To detect a difference of 1.7 mL/min per kg with a power of 80%, a sample size of 22 was needed, assuming that the common SD is 1.95, using a two-tailed t test with a 0.05 two-sided significance level. When taking into account a 10% attrition rate, 24 participants were required in both groups.

The mean power output per kg of 11 male professional cyclists during a 20 min constant-load test at 80% VO_{2max}, which was determined in a maximal exercise test with a 25 W/min ramp protocol, was 5.2 W/kg (SD 0.2).[10] With a sample size of 22 per treatment group, a difference of 0.172 W/kg could be detected in this population with a power of 80%. This difference would mean that a professional cyclist weighing 75 kg would go from an average of 390 W at 80% VO_{2max} to 402.9 W. With available calculators this power difference, for an athlete on a 9 kg racing bike, in racing position ('drops') at 25°C on a wind-still, flat terrain of 40 km, would produce a speed increase of approximately 0.5 km/h (from 43.80 km/h to 44.32 km/h), which is a relevant difference in cycling. On a mountain climb, that same increase in power would lead to an even larger relative increase in speed, expanding the effect on uphill race time - e.g., a decrease of approximately 2 min on a climb like Mont Ventoux.

To evaluate effects on performance for each variable we selected a suitable statistical model and undertook intention-to-treat analysis. Participants were included in all analyses of outcomes for which they had at least one measurement. Repeatedly measured data were analysed with a mixed model analysis of variance with treatment, time, and treatment by time as fixed factors, participants as a random factor and, if available, the (average) prevalue as covariate. These included parameters of maximal exercise test, haematology, coagulation, endothelial function, and vital signs measurements. Different timepoints for each variable are indicated in the tables.

We compared single measured data with an analysis of variance with factor treatment, and, if available, prevalue as covariate. We analysed parameters of the submaximal exercise test in this way.

We analysed the racing times with a parametric model for failure time (accelerated failure time regression model) with right censored values, to account for participants who did not reach the top of Mont Ventoux. The model for the response variable consists of a linear effect composed of the covariates and a random disturbance term. The covariates are treatment and prevalue. We log-transformed the time to arrival before analysis and the chosen distribution was normal. We chose the P_{max}/kg pretreatment as the prevalue to correct for possible differences in baseline performance.

The contrast that we calculated within the models was placebo versus rHuEPO. We report results of statistical models as estimated means at the different timepoints per group and estimates of the difference over the whole time period, including 95% CI (% for log-transformed parameters) and the p value of the contrasts.

We used a regression model to evaluate the association between haematological parameters and performance. We analysed the association between haemoglobin and haematocrit concentrations and several maximal and submaximal exercise variables with a mixed model regression, with treatment as covariate, a random participant intercept and slope, and an unstructured variance and covariance structure if feasible; a variance components variance and covariance structure otherwise. For the submaximal exercise test variables, there was only one after baseline measurement. We calculated the regression of time in race and haemoglobin and haematocrit concentrations at the time of the race with a regression model without random factors.

To evaluate the association between maximal, submaximal exercise tests and race performance, we calculated Spearman correlations for the maximal and submaximal exercise variables per kg as measured in the test closest to the race and power per kg and time in the race.

When 95% CIs are presented they reflect the estimated difference between the two treatment groups. Significance level was set at $p<0.050$. We did all calculations with SAS version 9.4.

RESULTS

Between March 7, 2016, and April 13, 2016, we enrolled 48 participants and had one reserve participant. The study took place for all participants simultaneously between April and June, 2016, with a follow-up before the end of August, 2016. One participant withdrew after the first dose administration and was replaced by the reserve participant, and another participant withdrew after the fourth dose administration. Both withdrawals were due to personal reasons and not related to the study treatment or medical concerns. In total, 48 participants were included in the analyses, with 24 in the rHuEPO group and 24 in the placebo group (Figure 2). Baseline characteristics were similar between treatment groups (Table 1). Table 2 has a more detailed breakdown of excluded participants before the screening visit. As no effect of the stratification variable age was observed on the results, all analyses were done disregarding this factor. All participants were living at sea level and did not spend any substantial amount of time at (simulated) high altitude. Furthermore, average cumulative cycle training duration per week recorded with the Pioneer equipment (4.9 h for rHuEPO versus 5.9 h for placebo, [estimated difference –16.5%, 95% CI of estimated difference –36.3 to 9.5]) and average training distance and power per week (186.2 km versus 202.0 km [–15.8, –63.5 to 31.8] and 202.1 W versus 205.3 W [–3.2, –25.5 to 19.2]) did not differ among treatment groups, nor did other training activities recorded in the diary (average 1.2 h [SD 0.9] per week for rHuEPO and 1.5 h [1.2] per week for placebo).

Participants in the rHuEPO group received eight doses during the study. Mean rHuEPO dose was 5000 IU per participant per week during the first 4 weeks of the study and 7000 IU in the subsequent 4 weeks. On five occasions a placebo injection was administered to participants exceeding 15% of haemoglobin increase compared with baseline or that had a haematocrit concentration that exceeded 52% (Table 3). The average administered rHuEPO dose was 48 000 IU (6000 IU/week), resulting in an average 12% increase in haemoglobin concentration up to a mean of 10.2 mmol/L and a 16% increase in haematocrit to 50%, whereas haemoglobin and haematocrit concentrations in the placebo group remained relatively stable during the study (Table 4). Diaries showed that participants had taken their supplements as instructed throughout the study period.

Haemoglobin concentrations and haematocrit were higher in the rHuEPO group compared with the placebo group over the treatment period (9.6 mmol/L versus 9.0 mmol/L [estimated difference 0.6, 95% CI 0.4–0.8] and 47.6% versus 44.3% [3.3, 2.5–4.1], respectively; Figure 3, Table 4). In the rHuEPO group, median haemoglobin concentration at baseline was 9.0 mmol/L (range 8.1–10.2) and median peak haemoglobin concentration was 10.1 mmol/L (range 9.0–11.5). The mean increase in haemoglobin concentration at the prerace measurement was 12%; in total, 14 (61%) of 23 participants completing treatment with rHuEPO achieved an increase of more than 10%. Participants who did not achieve the target haemoglobin range all received 5000 IU of rHuEPO in the first four doses of the study and a mean of 8000 IU in the latter four doses of the study.

Analysis of the effects on maximal exercise test variables showed that at baseline the mean maximal power output per kg was 4.37 W/kg (SD 0.365) in the

rHuEPO group compared with 4.36 W/kg (0.223) in the placebo group and mean absolute maximal power output was similar in both groups (335.14 W [34.46] in the rHuEPO group and 335.00 W [33.04] for the placebo group; Table 5). During the course of the study, maximal power output per kg did not differ between the rHuEPO group compared with the placebo group (4.61 W/kg versus 4.50 W/kg [estimated difference 0.11, 95% CI −0.00 to 0.22]; Table 5). However, absolute maximal power output did show a significant increase in the rHuEPO group compared with the placebo group over the entire treatment period (351.55 W versus 341.23 W [10.32, 3.47 to 17.17]; Table 5), which reached significance at the exercise test at 25 days (p=0.0073; data not shown). A similar significant increase in the rHuEPO group was seen in VO_{2max}, VO_2 at VT1 and VT2, and power at VT1 (Figure 4). rHuEPO treatment increased VO_{2max} by 10% compared with baseline, and the placebo group also improved by 4%. This results in a net improvement of about 5% over placebo. Similar effects were found on maximal power output, with an increase of about 4% for rHuEPO treatment compared with placebo. There was no indication that rHuEPO treatment had a stronger effect on maximal power output in the highest performing participants (Figure 5). Gross efficiency, lactate threshold, maximal heart rate, or any of the other respiratory parameters did not differ between groups (Table 5).

Analysis of the effects on the submaximal exercise test showed that mean power output during the study did not differ between groups either in absolute terms (283.18 W for rHuEPO versus 277.28 W for placebo [estimated difference 5.90, 95% CI −0.87 to 12.67]) or per kg (3.72 W/kg for rHuEPO versus 3.66 W/kg for placebo [0.06,−0.04 to 0.16]; Table 6). Mean VO_2 per kg (50.288 mL/min per kg for rHuEPO versus 49.642 mL/min per kg for placebo [0.646, −1.307 to 2.600]), cycling economy, mean heart rate, and lactate levels at 10 min, 30 min, and 45 min were similar between treatment groups (Table 6).

A total of 44 participants took part in the Mont Ventoux race, with 21 (48%) in the rHuEPO group. Two participants were unable to attend due to other engagements, and one participant was experiencing gastrointestinal complaints. Weather conditions on Mont Ventoux (afternoon of June 19, 2016) in Bedoin were around 20°C and 40 km/h northern wind, and at the top were around 5°C and 85 km/h northern wind, without precipitation. Out of the 44 participants, four (9%) did not complete the race due to exhaustion (n=2 placebo and n=2 rHuEPO). The mean time of the Mont Ventoux race did not differ among treatment groups (1 h 40 min 32 s for the rHuEPO group versus 1 h 40 min 15 s for the placebo group [estimated difference 0.3%, 95% CI −8.3 to 9.6]), nor did mean pedalling power during the race (3.03 W/kg for rHuEPO versus 3.09 W/kg for placebo [−1.7%, −11.0 to 8.6]; Table 7).

To evaluate whether participants could notice the effects of rHuEPO, all 47 participants that completed the study were asked whether they thought they had received rHuEPO or placebo during the study period. Overall, 27 (57%) of 47 participants correctly indicated their treatment. Out of the participants treated with rHuEPO, only nine (39%) of 23 thought they had received rHuEPO. Six (25%) of 24 participants treated with placebo thought this as well.

Evaluation of the association between haematological parameters and performance revealed a relation between haemoglobin and all maximal and submaximal exercise parameters, including maximal power output per kg, which showed a significant estimated slope in the rHuEPO group (slope 0.27 [95% CI 0.16–0.37]), but not the placebo group (Figure 6). Haemoglobin or haematocrit concentrations were not associated with Mont Ventoux race time.

The correlation between exercise test parameters and race performance was highest for mean power output per kg during the submaximal exercise test, but only partly predicted race time (correlation of −0.63), while power output per kg measured during the race appeared to be more predictive of race time (−0.79). The strongest correlation between a maximal exercise test parameter and average power output during the submaximal exercise test was with maximal power output (0.76). However, parameters from the maximal exercise test a week before the Mont Ventoux race and the uphill race times only showed a moderate correlation at best (spearman correlations range −0.36 to −0.58).

Safety evaluation of the rHuEPO treatment in the well trained cyclists revealed that weight and vital signs, such as heart rate and blood pressure, were similar between treatment groups (Table 8, Table 9). All observed adverse events were mild to moderate (grade 1–2), and the nature and incidence were similar in both groups (Table 10). No events of grade 3 or worse were observed. Of all coagulation and endothelial function markers measured, when compared with the placebo group, rHuEPO increased only E-selectin by 8.6% (95% CI 2.0–15.7) and P-selectin by 7.8% (1.5–14.5; Table 11).

DISCUSSION

The present study of rHuEPO treatment in well trained cyclists compared with those treated with a placebo showed that rHuEPO improved exercise performance during a maximal exercise test. By contrast, no improvement in exercise performance was observed in the submaximal test, nor in a real-life road cycle race. These outcomes indicate that the amount of performance increment in a maximal test might not be immediately translated to a real-life situation. Although no difference in adverse events was observed, the endothelial function markers E-selectin and P-selectin significantly increased in the rHuEPO group compared with the placebo group, potentially increasing the risk of thrombosis. The question remains whether these results can be generalised to the actual population of athletes doping with rHuEPO.

Average weekly training duration in this study was substantially less compared with professional cyclists (who train >20 h and >700 km per week), which was inevitable because participants were not allowed to be subject to anti-doping regulation and were therefore essentially amateur cyclists. Baseline values of maximal power output for our maximal exercise test were on average 335.07 W (SD 33.40) and of VO_{2max} were 55.63 mL/min per kg (SD 4.80) for both treatment groups. Elite cyclists have reported values of about 429 W and 73 mL/min per kg measured with a short exercise protocol, which would indicate that our participants were not comparable to this level of cyclist either.[6] However, because it takes 3–4 min for the body to acclimatise to a given workload and for lactate to be measured accurately,[11] and because longer protocols can be more sensitive to performance changes,[12] we selected a long exercise protocol with 5 min per power step rather than the 1–2 min protocols often used in exercise physiology. Shorter protocols overestimate maximal exercise parameters, including maximal power output up to 20% and VO_{2max} up to 7% compared with longer protocols.[8,13] Although our participants were not professional cyclists, based on the maximal exercise test they have similarity with the elite cyclists tested in a 3-min exercise protocol (similar values for maximal power output of 349 W and VO_{2max} of 60 mL/min per kg)[14] and elite triathletes in a 3-min protocol.[13] This result shows that our participants were well trained cyclists, and at least closely approached the level of elite cyclists. Based on VO_{2max} they are comparable to participants in previous studies[9,15,16] investigating the effects of rHuEPO on cycling performance with the highest exercise values (63–65 mL/min per kg) determined with short exercise protocols. Moreover, based on maximal power output our participants are better trained cyclists than participants reported in previous studies[9,17–21] with short protocols (from 311 W to 402 W).

The average administered rHuEPO dose (6000 IU/week) in our study is difficult to compare with previous studies[9,15–18,20–23] on the effects of rHuEPO in cyclists, which range from 4500 IU/week to 26 000 IU/week over a period of 4–12 weeks. Despite widely varying dose regimens these studies reported similar increases in haemoglobin concentration and haematocrit, without a clear dose-response association. This absence of a clear dose-response is likely to be due to a ceiling effect at the higher doses. The dose in our study is also consistent with known practices in professional cycling.[24]

The net improvement of about 5% for VO_{2max} when rHuEPO treatment was compared with the placebo was in line with previous studies.[9,15–23,25–28] Similar effects were found on maximal power output, with an increase compared with placebo of about 4%, which is also in line with the few previously reported effects,[9,17–21] although the effect there was seemingly slightly larger (6–13%).

Submaximal exercise parameters mimic the physical exertion of real-life cycling races more closely. Extracted from the maximal exercise test, VT1 and VT2 show an increase compared with placebo. However, these parameters do not directly reflect submaximal exercise as exerted during endurance performance. In a 45-min submaximal exercise test, the increases in mean power and VO_2 for the rHuEPO group were small and did not differ compared with placebo in this adequately powered study.

Results of other studies[17–19] found remarkable increases in reported submaximal tests, namely constant-load time-to-exhaustion tests, of 22–70%. These trials used short (between 3 min and 20 min) tests that, similar to the maximal exercise test, lead to exhaustion and therefore are less representative of real-life cycling. Our submaximal test was designed to closely mimic a road time trial of 45 min and in line with that was not intended to lead to exhaustion. Additionally, participants in the rHuEPO group in two of these trials were aware that they were treated with rHuEPO.[18,19] The third trial, a double- blind, placebo-controlled trial by Annaheim and colleagues,[17] did not find any effects of rHuEPO on VO_2 during this test similar to our finding. Our results could reflect a previous finding[29] that the best predictor of time trial performance in highly trained athletes is muscle oxidative capacity, in

contrast to the best predictor of maximal power output, which is oxygen delivery. The absence of a clear effect on average power in the submaximal test does not support an effect of rHuEPO on oxidative capacity.

The goal of using rHuEPO in professional sports is to improve performance during road races, not in maximal exercise tests. Participants therefore took part in a race designed to mimic a professional road race at Mont Ventoux about 12 days after the last dose of the treatment period, which also tested the validity of our laboratory exercise tests as biomarkers of real cycling performance. The two treatment groups did not differ in race time or mean power output, thereby raising doubt about the predictive value of the increase in maximal exercise test parameters by rHuEPO for performance in a road race. This outcome is further supported by the fact that rHuEPO treatment did not show an appreciable effect on a submaximal exercise test in the laboratory. Previous investigators[30,31] have suggested a strong predictive power of parameters obtained during laboratory maximal exercise tests such as maximal power output or power at VT for endurance capacity in a laboratory time trial (correlations of 0.80–0.91), which is similar to our findings (0.76). However, parameters from the maximal exercise test a week before the Mont Ventoux race and the uphill race times only showed a moderate correlation at best. This correlation could explain the discrepancy between the effect of rHuEPO treatment on maximal exercise test parameters and the absence of an effect on race time; many extra variables affect performance in real life. In line with this result, participants poorly predicted whether they had received rHuEPO or placebo during the study. Although the participants were not professional cyclists and might be slightly less sensitive to changes in performance, this result does seem to invalidate the claim of many professional cyclist that they could feel the effect of the rHuEPO treatment.

At the time of the first maximal exercise test, which was 11 days after the start of the treatment, the effects in the maximal exercise tests were already clearly visible. Only one other study[17] has investigated such early effects on exercise parameters, and their findings appear to confirm our findings. The magnitude of the increase at 11 days in the rHuEPO group was similar to the increase achieved in the placebo group during the entire study period. This early effect was unexpected because at this time the increase in haemoglobin concentration in the rHuEPO group was small, which followed the described time course of the effects on haemoglobin.[32]

Although our data show a correlation between haemoglobin concentration and performance in the maximal and submaximal exercise tests, this association is only true for the rHuEPO group; an association is absent in the placebo group, despite a similar range of haemoglobin concentrations. These findings indicate that there could be other mechanisms for the effect of rHuEPO on exercise tests, like via 2,3-bisphosphoglycerate or the monocarboxylate transporter.[33,34]

We did not observe a difference in incidence or severity of adverse events between the rHuEPO and placebo groups, nor did we detect any clinical signs of thrombosis, effects on blood pressure, coagulation factors, or most of the endothelial function markers. However, we did find a significant rise in E-selectin and P-selectin compared with the placebo group, which are cell adhesion molecules that play a crucial part in thrombogenesis and inflammation.[35,36] This rise might explain the observed increased thrombogenicity and increased risk of stroke after rHuEPO treatment in patients.[7] As the incidence of events such as stroke is relatively low in patients (2.6%) and will be even lower in healthy athletes,[7] our study did not have the power to detect such an increased risk. However, given the increase in endothelial function markers observed in this study, and with widespread and uncontrolled use of rHuEPO among athletes, it is not unlikely that in this population the risk of cardiovascular events also increased. Our study design has several inevitable limitations. Because of WADA regulations, it is currently impossible to do intervention studies with banned substances in professional cyclists. The question remains whether our data can be applied to professional cyclists. However, in our participants there was no indication that rHuEPO treatment had a stronger effect on maximal power output in the highest performing participants.

The size of our study might have generated insufficient statistical power to detect a difference on the road race. However, for an effect of doping to be relevant for cyclists, it should have a clear effect on time trial or race performance. The absence of an effect of rHuEPO treatment on both a 45-min submaximal exercise test and a road race indicates that the effect is at best very small, and disappears in all other variability that is present during such an event.

Finally, there have been suggestions in the literature that the effect of rHuEPO treatment is not, or only partly, mediated by the increase in red blood cell mass. Suggestions for such pleiotropic effects range from effects on the speed of recovery after exercise, direct effects on skeletal muscle, improved lipolysis, psychological

effects on fatigue or motivation, and effects on the immune system. In this case, the design of the tests or timing related to the administration of rHuEPO might have been suboptimal. For example, our race was not a multi-day event, such as the Tour de France, and therefore could not show potential effects of rHuEPO that only occur in such a setting. However, to be of clinical relevance, many of the other effects would have to show an effect on time trial or road race performance, which was not observed in our study.

In the clinical practice of nephrology and oncology in which rHuEPO is used therapeutically, a well recognised optimal dose exists, which results in lower than normal haemoglobin concentrations. When haemoglobin is restored to normal concentrations, mortality increases.[7] In these studies and studies with blood transfusion, such effects only become clear after a medicine is tested in circumstances that resemble the clinical situation. Our study shows that testing in a clinical situation is not different for drugs intended to enhance sport performance. Although we did not find clinical signs of adverse effects of rHuEPO treatment, the observed rise in endothelial function markers might indicate an increased thrombogenicity. Moreover, effects on relevant performance measures were small, largely disappeared in the submaximal test, and were undetectable in a real-life cycling race.

In summary, we showed that it is possible to test potential doping substances in well controlled clinical trials and that results are much less pronounced than claimed in popular literature and accounts. More clinical research like this study will provide the evidence base for the prohibited list and might lead to more focused attention and adequate information to athletes and their medical staff. Overall, the results of our study showed that rHuEPO treatment enhanced performance in well trained cyclists in a laboratory-based maximal exercise test leading to exhaustion, but did not improve submaximal exercise test or road race performance.

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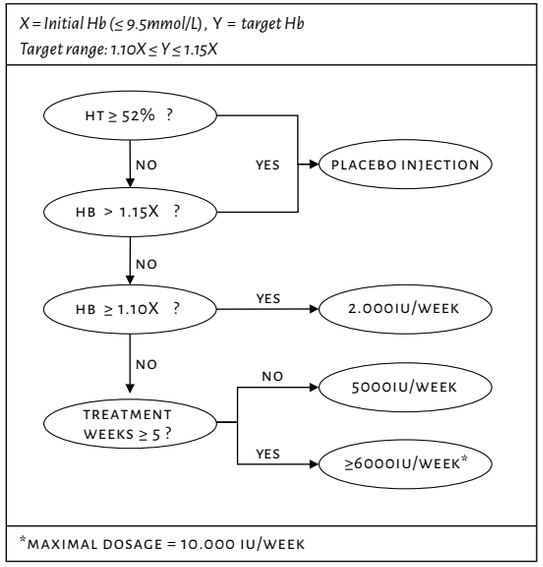
FIGURE 1 DECISION TREE FOR DOSING Decision tree for dosing schedule that was applied before every administration of NeoRecormon/placebo during the 8 week treatment period.



Ht: haematocrit, Hb: haemoglobin, IU: International Units.

FIGURE 2 TRIAL PROFILE rHuEPO, recombinant human erythropoietin.

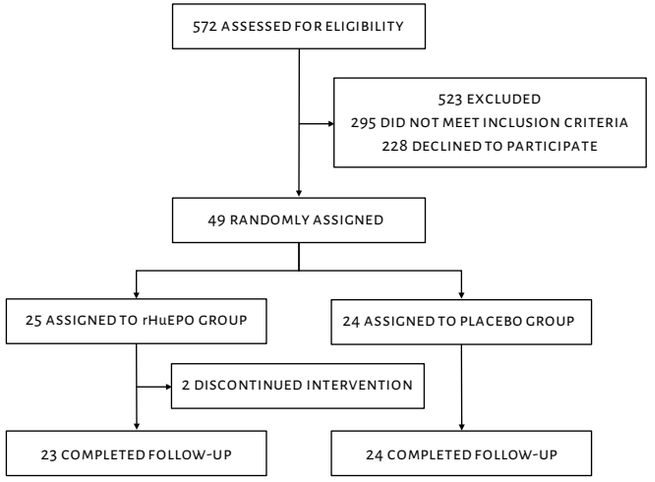


FIGURE 3 MEAN HAEMOGLOBIN CONCENTRATIONS DURING STUDY p<0.0001. rHuEPO, recombinant human erythropoietin

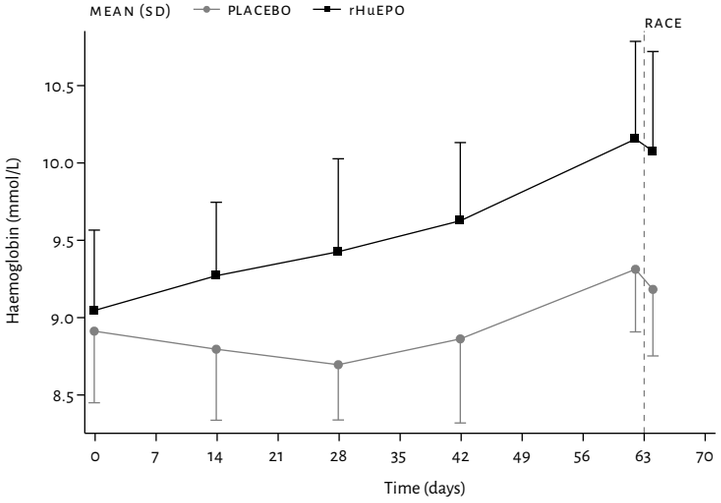


FIGURE 4 MAXIMAL POWER OUTPUT AND POWER AT VT1 DURING THE STUDY Mean maximal power output (p=0.055) and mean power output at VT1 (p=0.0100). P_{max}, maximal power output; rHuEPO, recombinant human erythropoietin; VT1, ventilatory threshold 1.

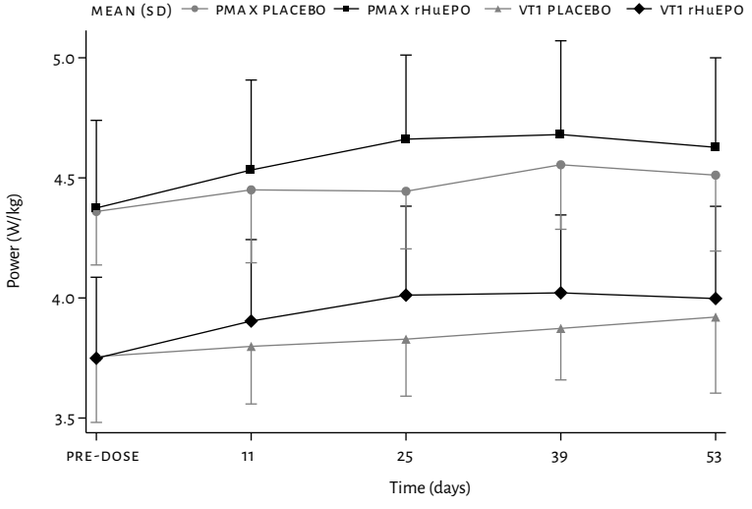


FIGURE 5 CORRELATION BASELINE AND DELTA MAXIMAL POWER AT WEEK 8 This figure displays the correlation between baseline maximal power output and the delta in maximal power output between baseline and week 8 for both treatment groups. Correlation rHuEPO: -0.21; Correlation Placebo: -0.12. rHuEPO: recombinant human erythropoietin; Regr: regression line.

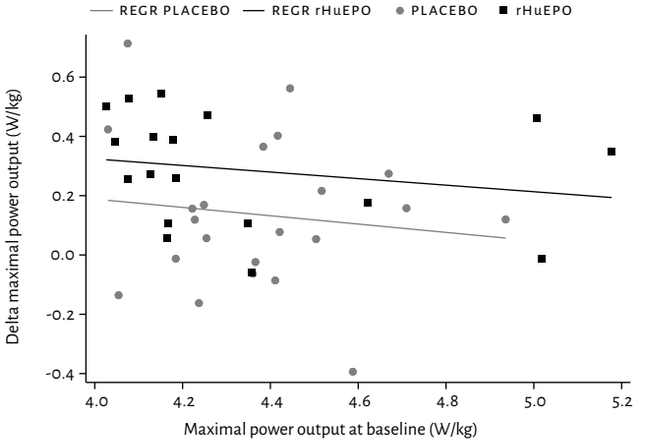
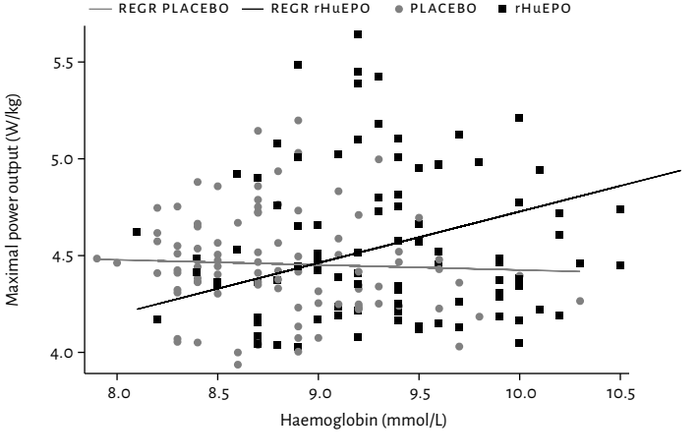


FIGURE 6 REGRESSION HAEMOGLOBIN LEVEL AND MAXIMAL POWER OUTPUT PER KG
This figure displays the regression line in a scatter graph between haemoglobin level and maximal power output per kg for both treatment groups.



*Regression rHuEPO: [P_{max} per kg] = 0.27 [CI 0.16; 0.37] * [Hb] + 2.1;*
*Regression Placebo: [P_{max} per kg] = -0.03 [CI -0.15; 0.10] * [Hb] + 4.7.*
rHuEPO: recombinant human erythropoietin; Regr: regression line; P_{max}: maximal power output.

TABLE 1 BASELINE CHARACTERISTICS Data are median (range) or mean (SD). rHuEPO, recombinant human erythropoietin; VO_{2max}, maximal oxygen consumption.

	Placebo	rHuEPO
n	24	24
Age (years)	33.8 (20.0 - 50.0)	33.5 (22.0 - 48.0)
Weight (kg)	76.9 (8.9)	77.0 (8.9)
Height (cm)	186 (6.7)	186 (7.9)
Hb (mmol/L)	8.9 (0.46)	9.0 (0.52)
Ht (L/L)	0.431 (0.0221)	0.433 (0.0222)
Maximal Power output per kg (W/kg)	4.36 (4.03 - 4.94)	4.37 (4.03 - 5.18)
VO_{2max} (mL/min/kg)	56.0 (4.111)	55.4 (5.132)

TABLE 2 EXCLUDED PARTICIPANTS Reasons for excluding participants from participation

Participant could not be contacted despite multiple attempts	27	
Participant was unwilling to discontinue membership with anti-doping regulation	32	
Study planning made it impossible for participant to participate	76	
Participant considered travel to study site (Leiden, the Netherlands) too far	8	
Participant was not interested in study participation anymore	52	
Participant did not agree with remuneration	3	
Withdrew before screening visit		198
Participant was not a cyclist	13	
Body mass index was too high	5	
Speed on solo cycle tour <30 km/h	76	
Weekly training effort <3 hours	34	
Participant could not communicate in Dutch language	82	
Participants' age exceeded 50	20	
Blood- or plasma donation within 3 months of study participation	4	
Medical history or prohibited medication use	10	
Preliminary screening by telephone		244
Withdrew consent before baseline visit	30	
Insufficient exercise performance (Pmax < 4W/kg)	45	
Hemoglobin > 9.8 mmol/L	5	
Medical history	1	
Failed in- and exclusion criteria		81
Total		**523**

TABLE 3 rHuEPO DOSAGES DURING THE STUDY COURSE Number of participants receiving the corresponding dose. *1 participant dropped out after week 4. IU: international units.

Dosage week	1	2	3	4	5*	6*	7*	8*
Dosage								
0	-				1	2	2	-
2000 IU	-		1	1	4	5	2	2
5000 IU	24	24	23	23	-	-	-	-
6000 IU	-				5	7	3	1
8000 IU	-				9	3	11	3
10000 IU	-				4	6	5	17

TABLE 4 HAEMATOCRIT AND HAEMOGLOBIN LEVELS Raw baseline (and SD) and EM (Estimated Mean) values of haematological parameters at the different time points for both treatment groups, including the estimated differences between the treatment groups. Data analysed with a mixed model analysis of variance with fixed factors treatment, time and treatment by time, random factor participant and the pre-value as covariate.

Parameter	Treatment	Raw baseline	EM Week 2	EM Week 4	EM Week 6	EM Pre Race	Difference between groups
Haemoglobin lab (mmol/L)	Placebo	8.9 (0.5)	8.8	8.7	8.9	9.4	0.60 (0.44, 0.77) p=<0.0001
	rHuEPO	9.0 (0.5)	9.2	9.4	9.6	10.1	
Haematocrit lab (L/L)	Placebo	0.431 (0.022)	0.437	0.436	0.438	0.460	0.0330 (0.0250, 0.0409) p=<0.0001
	rHuEPO	0.433 (0.022)	0.458	0.470	0.474	0.499	

TABLE 5 DIFFERENCE IN EXERCISE PERFORMANCE PARAMETERS AT MAXIMAL EXERCISE TEST BETWEEN EACH TREATMENT GROUP Data are raw baseline (SD) or estimated mean (EM) values. For log-transformed parameters, a back-transformed estimate of the difference in percentage is reported and geometric means for estimated mean. Data were analysed with a mixed model analysis of variance with three fixed factors (treatment, time, and treatment by time), one random factor (participant), and one covariate (pre-value). rHuEPO, recombinant human erythropoietin.

Parameter	Treatment	Raw baseline	EM Day 11	EM Day 25	EM Day 39	EM Day 53	Difference between groups
Maximal oxygen consumption (L/min)	Placebo	4.298 (0.486)	4.298	4.296	4.450	4.373	0.2237 (0.0824, 0.3650) p=0.0026
	rHuEPO	4.237 (0.466)	4.475	4.536	4.688	4.612	
Max. oxygen consumption per kg (mL/min/kg)	Placebo	55.946 (4.136)	56.392	56.528	58.616	58.122	2.7066 (0.9105, 4.5027) p=0.0041
	rHuEPO	55.322 (5.453)	58.564	59.472	61.611	60.838	
Maximal Power output (W)	Placebo	335.00 (33.039)	339.59	339.97	345.72	339.65	10.315 (3.465, 17.166) p=0.0040
	rHuEPO	335.14 (34.464)	346.19	351.60	354.95	353.46	
Maximal Power output per kg (W/kg)	Placebo	4.36 (0.223)	4.46	4.48	4.56	4.51	0.109 (-0.002, 0.220) p=0.055
	rHuEPO	4.37 (0.365)	4.53	4.60	4.66	4.65	
Lactate threshold Power (W)	Placebo	299.51 (7.671)	298.00	296.23	306.09	298.54	8.493 (-0.609, 17.595) p=0.067
	rHuEPO	290.87 (6.451)	305.65	310.66	311.05	305.47	
Lactate threshold Power per kg (W/kg)	Placebo	3.90 (0.215)	3.92	3.91	4.04	3.97	0.106 (-0.025, 0.238) p=0.11
	rHuEPO	3.84 (0.304)	4.01	4.09	4.12	4.05	
Lactate threshold VO_2 (L/min)	Placebo	4.005 (0.461)	3.948	3.934	4.079	3.963	0.1160 (-0.0165, 0.2485) p=0.084
	rHuEPO	3.855 (0.459)	4.002	4.160	4.209	4.017	
Lactate threshold VO_2 per kg (mL/min/kg)	Placebo	52.159 (3.239)	51.918	51.921	53.885	52.749	1.4375 (-0.4529, 3.3279) p=0.13
	rHuEPO	50.864 (4.476)	52.502	54.863	55.674	53.185	
Ventilatory Threshold 1 VO_2 (L/min)	Placebo	3.863 (0.431)	3.862	3.857	3.916	3.953	0.1781 (0.0481, 0.3081) p=0.0083
	rHuEPO	3.747 (0.434)	3.989	4.057	4.156	4.099	
Ventilatory Threshold 1 VO_2 per kg (mL/min/kg)	Placebo	50.291 (4.056)	50.671	50.772	51.538	52.657	2.0875 (0.3802, 3.7949) p=0.018
	rHuEPO	48.912 (4.974)	52.092	53.261	54.560	54.076	
Ventilatory Threshold 1 Power (W)	Placebo	288.69 (32.883)	290.92	292.28	294.66	294.68	9.701 (2.437, 16.964) p=0.010
	rHuEPO	287.08 (30.562)	299.60	301.72	303.73	306.30	
Ventilatory Threshold 1 Power per kg (W/kg)	Placebo	3.76 (0.274)	3.81	3.84	3.87	3.92	0.119 (0.018, 0.219) p=0.022
	rHuEPO	3.75 (0.337)	3.91	3.96	4.00	4.04	
Ventilatory Threshold 2 VO_2 (L/min)	Placebo	4.077 (0.452)	4.089	4.058	4.145	4.125	0.1621 (0.0021, 0.3221) p=0.047
	rHuEPO	3.985 (0.462)	4.190	4.212	4.343	4.319	
Ventilatory Threshold 2 VO_2 per kg (mL/min/kg)	Placebo	53.097 (4.295)	53.658	53.422	54.577	54.873	1.9471 (-0.1110, 4.0053) p=0.063
	rHuEPO	52.005 (5.114)	54.816	55.279	57.149	57.074	
Ventilatory Threshold 2 Power (W)	Placebo	306.77 (32.450)	311.34	310.80	312.35	311.43	8.423 (-0.855, 17.700) p=0.074
	rHuEPO	306.15 (35.301)	316.76	316.96	320.88	325.01	
Ventilatory Threshold 2 Power per kg (W/kg)	Placebo	3.99 (0.285)	4.08	4.09	4.11	4.14	0.100 (-0.031, 0.230) p=0.13
	rHuEPO	3.99 (0.354)	4.15	4.15	4.23	4.29	

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TABLE 5 *(continuation of previous page)*

Gross Efficiency (%)	Placebo	21.5 (0.792)	21.7	21.8	21.4	21.4	0.4% (-1.6%, 2.5%) p=0.69
	rHuEPO	21.9 (1.096)	21.8	22.0	21.4	21.7	
Heart Rate (BPM)	Placebo	182 (8.78)	182	182	184	181	0.7 (-1.7, 3.1) p=0.56
	rHuEPO	184 (8.17)	183	182	184	182	
Tidal Volume (L)	Placebo	3.029 (0.458)	3.115	3.139	3.162	3.173	0.0288 (-0.1060, 0.1637) p=0.67
	rHuEPO	3.160 (0.340)	3.168	3.177	3.214	3.146	
Respiratory Frequency (1/min)	Placebo	54.3 (8.566)	53.2	52.2	53.8	51.6	0.54 (-2.00, 3.08) p=0.67
	rHuEPO	51.5 (5.887)	52.4	52.7	54.5	53.4	
Respiratory minute ventilation (L/min)	Placebo	161.8 (18.383)	163.2	161.5	167.4	161.7	2.84 (-4.01, 9.69) p=0.41
	rHuEPO	161.3 (21.627)	163.1	164.0	172.9	165.2	
Respiratory Quotient	Placebo	1.07 (0.044)	1.07	1.06	1.06	1.06	-0.003 (-0.022, 0.016) p=0.77
	rHuEPO	1.08 (0.050)	1.07	1.05	1.06	1.06	

TABLE 6 DIFFERENCE IN EXERCISE PERFORMANCE PARAMETERS AT SUBMAXIMAL EXERCISE TEST BETWEEN EACH TREATMENT GROUP Data are raw baseline (SD) or estimated mean (EM) values. For log-transformed parameters, a back-transformed estimate of the difference in percentage is reported, and geometric means for estimated mean. Data were analysed with an analysis of variance with factor treatment, and, if available, pre-value as covariate. rHuEPO, recombinant human erythropoietin.

Parameter	Treatment	Raw baseline	EM Day 46	EM Change from baseline	Difference between groups
Power output submaximal (W)	Placebo	268.00 (27.751)	277.28	7.656	5.898 (-0.872, 12.668) p=0.086
	rHuEPO	270.83 (30.503)	283.18	13.554	
Power output submaximal per kg (W/kg)	Placebo	3.50 (0.199)	3.66	0.135	0.062 (-0.035, 0.159) p=0.20
	rHuEPO	3.53 (0.298)	3.72	0.197	
Average VO_2 submaximal (L/min)	Placebo	3.631 (0.345)	3.758	0.0866	0.0624 (-0.0862, 0.2110) p=0.40
	rHuEPO	3.701 (0.510)	3.821	0.1490	
Average VO_2 submaximal per kg (mL/min/kg)	Placebo	47.594 (3.930)	49.642	1.6189	0.6463 (-1.3074, 2.6000) p=0.51
	rHuEPO	48.180 (4.657)	50.288	2.2652	
Average Heart Rate submaximal (BPM)	Placebo	160 (9.47)	160	-1.0	-0.9 (-4.5, 2.7) p=0.62
	rHuEPO	162 (11.53)	159	-1.9	
Submaximal Lactate 10 min (mmol/L)	Placebo	2.51 (1.090)	2.45	-0.148	-0.301 (-0.864, 0.262) p=0.29
	rHuEPO	2.68 (1.456)	2.15	-0.449	
Submaximal Lactate 30 min (mmol/L)	Placebo	2.78 (1.232)	3.23	0.477	-0.050 (-0.698, 0.598) p=0.88
	rHuEPO	2.67 (1.200)	3.18	0.427	
Submaximal Lactate 45 min (mmol/L)	Placebo	3.33 (1.458)	5.09	1.448	-0.065 (-1.401, 1.271) p=0.92
	rHuEPO	3.80 (1.882)	5.03	1.383	
Cycling economy (W/L/min)	Placebo	73.87 (4.117)	73.86	0.5%	0.2% (-3.0%, 3.5%) p=0.89
	rHuEPO	73.52 (4.429)	74.03	0.7%	

TABLE 7 RESULTS RACE ON MONT VENTOUX EM (Estimated Mean) values of Mont Ventoux race results for both treatment groups, including the estimated differences between the treatment groups. For log transformed parameters a back transformed estimate of the difference in percentage is reported, and geometric means for EM. The racing times are analysed with a parametric model for failure time (accelerated failure time regression model) with right censored values. The model for the response variable consists of a linear effect composed of the covariates and a random disturbance term. The covariates are treatment and pre-value. The time to arrival is log-transformed before analysis and the chosen distribution is normal. The P_{max}/kg pre-treatment value is chosen as pre-value to correct for possible differences in baseline performance.

Parameter	Treatment	EM	Difference between groups
Average time in race (hr:m:s)	Placebo	1:40:15	0.3% (-8.3%, 9.6%) p=0.95
	rHuEPO	1:40:32	
Average Efficiency (%)	Placebo	49.8	0.40 (-3.95, 4.76) p=0.85
	rHuEPO	50.2	
Average Power (W)	Placebo	266.37	-3.5% (-13.0%, 7.0%) p=0.48
	rHuEPO	256.93	
Average Power per kg (W/kg)	Placebo	3.09	-1.7% (-11.0%, 8.6%) p=0.73
	rHuEPO	3.03	

TABLE 8 WEIGHT Raw baseline (and SD) and EM (Estimated Mean) values of weight at the different time points for both treatment groups, including the estimated differences between the treatment groups. Data analysed with a mixed model analysis of variance with fixed factors treatment, time and treatment by time, random factor participant and the pre-value as covariate.

Parameter	Treatment	Raw baseline	EM Week 2	EM Week 4	EM Week 6	EM Week 7	EM Week 8	EM Pre race	EM Post race	Difference between groups
Weight (kg)	Placebo	76.94 (8.94)	76.57	76.25	76.12	75.83	75.80	77.43	75.80	0.303 (-0.507, 1.112) p=0.46
	rHuEPO	77.03 (8.99)	76.65	76.61	76.50	76.41	76.27	77.50	75.98	

TABLE 9 VITAL SIGNS Raw baseline (and SD) and EM (Estimated Mean) values of vital signs at the different time points for both treatment groups, including the estimated differences between the treatment groups. Data analysed with a mixed model analysis of variance with fixed factors treatment, time and treatment by time, random factor participant and the pre-value as covariate.

Parameter	Treatment	Raw baseline	EM Week 1	EM Week 2	EM Week 3	EM Week 4	EM Week 5	EM Week 6	EM Week 7	Difference between groups
Systolic blood pressure (mmHg)	Placebo	119 (10)	119	116	117	117	118	115	116	2.3 (-1.1, 5.7) p=0.18
	rHuEPO	120 (11)	122	121	119	118	118	119	118	
Diastolic blood pressure (mmHg)	Placebo	71 (7)	73	74	72	71	72	72	71	0.4 (-2.0, 2.8) p=0.73
	rHuEPO	74 (9)	73	72	74	73	72	71	73	
Heart rate supine (BPM)	Placebo	53 (6)	54	53	52	52	51	53	52	-1.3 (-3.9, 1.4) p=0.34
	rHuEPO	55 (11)	52	50	53	50	50	52	51	

TABLE 10 GRADE 1–2 TREATMENT-EMERGENT ADVERSE EVENTS IN DIFFERENT SYSTEM ORGAN CLASSES Data are number of participants with TEAE (and total number of TEAEs) by System Organ Class and Preferred Term, grouped by intensity and treatment group. TEAEs of grade 1–2 were only reported if they occurred in more than 10% of the subjects. rHuEPO, recombinant human erythropoietin; TEAE, treatment-emergent adverse event.

System organ class	Adverse events	N subjects rHuEPO group grade 1-2	N subjects Placebo group grade 1-2
General disorders and administration site conditions	Fatigue	4 (4)	3 (3)
Immune system disorders	Seasonal allergy	3 (3)	1 (1)
Infections and infestations	Nasopharyngitis	3 (3)	1 (1)
Musculoskeletal and connective tissue disorders	Arthralgia	4 (5)	3 (3)
Musculoskeletal and connective tissue disorders	Myalgia	2 (2)	3 (3)
Musculoskeletal and connective tissue disorders	Pain in extremity	0 (0)	4 (4)
Nervous system disorders	Headache	6 (8)	3 (3)
Skin and subcutaneous tissue disorders	Rash	0 (0)	3 (3)

TABLE 11 COAGULATION AND ENDOTHELIAL FUNCTION MARKERS Raw baseline (and SD) and EM (Estimated Mean) values of coagulation and endothelial function markers at the different time points for both treatment groups, including the estimated differences between the treatment groups. Data analysed with a mixed model analysis of variance with fixed factors treatment, time and treatment by time, random factor participant and the pre-value as covariate.

Parameter	Treatment	Raw baseline	EM Day 11	EM Day 14	EM Day 25	EM Day 28	EM Day 39	EM Day 42	EM Day 53	EM Pre race	Difference between groups
Activated partial Thromboplastin time (s)	Placebo	30.7 (1.4)	30.1	30.1	29.8	30.0	30.4	29.6	30.4	30.0	1.5% (-0.3%, 3.4%) p=0.097
	rHuEPO	29.9 (1.9)	29.8	30.4	30.4	30.4	31.0	30.4	31.1	30.6	
Prothrombin time (s)	Placebo	14.5 (0.6)	14.5	14.3	14.5	14.5	14.6	14.3	14.3	14.2	-0.0% (-1.6%, 1.6%) p=1.00
	rHuEPO	14.4 (0.9)	14.5	14.4	14.7	14.3	14.4	14.4	14.4	14.0	
Fibrinogen (g/L)	Placebo	2.6 (0.3)	2.4	2.5	2.5	2.5	2.4	2.4	2.5	2.5	1.3% (-3.9%, 6.8%) p=0.62
	rHuEPO	2.7 (0.4)	2.5	2.5	2.4	2.6	2.4	2.5	2.5	2.5	
D-dimer (ng/mL)	Placebo	231.6 (145.1)	253.4	222.3	213.1	217.6	186.8	217.1	205.5	251.8	-1.3% (-17.0%, 17.4%) p=0.88
	rHuEPO	258.5 (157.3)	217.1	216.4	242.8	204.9	190.8	218.6	221.4	228.9	
Creatinine phosphokinase (U/L)	Placebo	142 (84)	123	140	126	142	127	137	152	173	-9.4% (-26.9%, 12.2%) p=0.36
	rHuEPO	213 (179)	135	137	119	129	111	139	132	117	
Beta Thromboglobulin (pg/mL)	Placebo	16113 (8283)	26836	11777	22008	14520	15088	16944	15554	23881	11.2% (-10.0%, 37.6%) p=0.32
	rHuEPO	31194 (85294)	26350	13585	27421	14135	19539	16419	19785	25535	
E-selectin (pg/mL)	Placebo	5100 (1978)	4986	4964	5097	5166	4731	4740	4942	4953	8.6% (2.0%, 15.7%) p=0.011
	rHuEPO	5543 (1912)	5446	5117	5475	5270	5568	5251	5477	5387	
Prothrombin fragment1+2 (pmol/L)	Placebo	118.6 (53.4)	169.4	102.9	167.2	108.1	116.4	134.5	122.6	137.1	-0.5% (-15.9%, 17.8%) p=0.95
	rHuEPO	368.2 (1262.8)	160.5	106.1	160.8	109.9	127.5	125.5	126.5	131.1	
Factor VIII (%)	Placebo	129 (37)	119	120	129	129	125	119	117	130	3.7% (-3.1%, 10.9%) p=0.29
	rHuEPO	137 (43)	129	123	136	132	120	125	128	131	
P-selectin (pg/mL)	Placebo	8789 (2112)	9037	8714	8524	8930	8810	8721	8261	8599	7.8% (1.5%, 14.5%) p=0.016
	rHuEPO	9710 (2285)	9010	9120	9248	9590	9846	9267	9173	9789	
PF4 (pg/mL)	Placebo	43019 (46073)	68339	23340	51235	28821	33209	35929	33026	63845	13.1% (-9.3%, 41.1%) p=0.27
	rHuEPO	43988 (85138)	60195	23125	60555	31296	52786	36164	43549	72593	
Thrombine: Antithrombine (ng/mL)	Placebo	2.850 (3.457)	4.304	1.495	3.663	1.575	1.860	2.029	1.795	1.869	4.1% (-20.0%, 35.5%) p=0.76
	rHuEPO	14.737 (63.963)	3.063	1.495	4.358	1.604	2.126	1.932	2.175	2.261	
Thrombomodulin (pg/mL)	Placebo	1383 (1358)	1323	1347	1320	1368	1322	1328	1364	1385	-2.8% (-10.2%, 5.2%) p=0.48
	rHuEPO	1936 (2756)	1252	1283	1218	1317	1319	1315	1387	1377	
Von Willebrand Factor (%)	Placebo	88.434 (27.669)	81.953	86.168	85.932	91.320	86.046	88.634	83.627	90.113	-0.9% (-8.0%, 6.8%) p=0.81
	rHuEPO	100.096 (25.426)	87.897	86.114	82.513	94.138	80.810	86.512	81.131	89.014	