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ADDITIVE EFFECT OF ERYTHROPOIETIN USE ON EXERCISE-INDUCED ENDOTHELIAL ACTIVATION AND HYPERCOAGULABILITY IN ATHLETES

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ABSTRACT

Recombinant human erythropoietin (rHuEPO) is known to increase thrombotic risk in patients and might have similar effects in athletes abusing the drug. rHuEPO is prohibited by anti-doping legislation, but this risk has not been investigated thoroughly. This analysis was designed to evaluate whether rHuEPO impacts haemostatic profile in trained subjects, and whether the combination with exercise affects exercise induced alterations in haemostasis. This double-blind, randomised, placebo-controlled trial enrolled healthy, well trained male cyclists aged 18–50 years. Subjects were randomly allocated (1:1) to receive subcutaneous injections of rHuEPO (epoetin-beta; mean dose 6000 IU per week) or placebo (0.9% NaCl) for 8 weeks. Subjects performed five maximal exercise tests and a race to the Mont Ventoux (France) summit, coagulation and endothelial markers were measured at rest and directly after each exercise effort. rHuEPO increased P-selectin (+7.8% (1.5-14.5), p=0.02) and E-selectin (+8.6% (2.0-15.7), p=0.01) levels at rest. Maximal exercise tests significantly influenced all measured coagulation and endothelial markers, and in the rHuEPO group maximal exercise tests led to 15.3% ((7.0%-24.3%), p=0.0004) higher E-selectin and 32.1% ((4.6%-66.8%), p=0.02) higher PF4 levels compared to the placebo group. In conclusion, rHuEPO influenced endothelial function in trained cyclists as shown by changes in E- and P-selectin indicating enhanced endothelial activation and/or platelet reactivity. Exercise itself induces hypercoagulability, and the combination of rHuEPO and exercise might increase this thrombotic risk by increasing E-selectin and PF4 more than by either factor alone.

INTRODUCTION

Recombinant human erythropoietin (rHuEPO) is on the World Anti-Doping Agency's Prohibited List as it is considered to possess performance enhancing properties and represents a potential health risk to athletes.[1] Effects on actual performance, however, are not indisputably proven in athletes.[2] Moreover, in a recent study we found that although rHuEPO treatment improved laboratory tests of maximal exercise, more relevant endurance performance variables such as time trial and road race performance remained unchanged.[3] Similarly, although there is evidence that rHuEPO increases thrombogenicity and risk of stroke in patients,[4] it is unclear whether athletes abusing rHuEPO could also display increased thrombotic risk. It would be informative to understand whether rHuEPO affects mechanisms involved in coagulation and endothelial function in athletes, especially given that exercise itself is known to impact the haemostatic profile.[5] Although exercise is generally accepted to be beneficial for health, cases of thrombotic events induced by exercise have been reported. This is likely due to the effects of exercise on the haemostatic profile, which seem to be correlated with exercise intensity.[5] Intense exercise, often performed by athletes, in combination with increased erythropoiesis and subsequent haemoconcentration after rHuEPO use might increase the risk for thrombotic events. Additionally, potential direct effects of rHuEPO on pathways involved in coagulation and endothelial function could add to this risk. In the current analysis as part of the same study[3] we investigated the effects of rHuEPO, exercise and the combined effects of rHuEPO and exercise in trained cyclists on markers that are associated with thrombotic risk.

MATERIALS AND METHODS

Study design and participants

The study design was previously described.[3] Briefly, forty-eight healthy male cyclists between 18 and 50 years participated in a double-blind, randomised, placebo controlled, parallel, single centre study. Among the inclusion criteria were Hb concentration between 8.0 and 9.8 mmol/L (equivalent to 12.8 – 15.7 g/dL, within the normal range for this population) and Ht level below 48% at screening and

not using medication that could potentially interact with the study drugs or study assessments. All participants gave written informed consent prior to 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, NL, and is registered under study number NTR5643 in the Dutch Trial Registry (Nederlands Trial Register, NTR).

Randomisation and masking

Participants were randomly assigned in a balanced manner to either rHuEPO or placebo treatment. A stratified randomisation block was used with participants aged 18-34 (inclusive) and aged 35-50 (inclusive) to reduce variability between groups due to age differences.

Procedures

Treatment

Treatments were as described previously.[3] Briefly, participants received weekly abdominal subcutaneous injections of Epoetin-beta (NeoRecormon, Roche, Basel, CH) prepared from multidose vials or saline (0.9% NaCl) for 8 weeks. Hb and Ht were measured before each dose and only available to dedicated un-blinded personnel. The target Hb in the rHuEPO group was an increase of 10-15% compared to the baseline Hb concentration, measured with the HemoCue Hb 201+ analyser (Radiometer Benelux b.v., Zoetermeer, NL). In the first four weeks, participants received a 5000 IU dose per injection, after which it could be increased to 6000 IU, 8000 IU or 10 000 IU to reach the target range. rHuEPO dose was 2000 IU if Hb was in the target range. For safety reasons, participants received a placebo injection if the Hb was above the target range, or if Ht was equal to or exceeded 52%, measured by the Hematokrit 200 (Hettich Benelux b.v., Geldermalsen, NL). rHuEPO and placebo were visually indistinguishable (both colourless solutions) and dosage changes (changes in injected volume) were also randomly assigned to placebo participants.

All participants also took open-label daily oral doses of 200 mg ferrous fumarate and 50 mg ascorbic acid (both from Pharmachemie b.v., Haarlem, NL) during the study to make sure subjects were not iron deficient during the study.

Maximal graded exercise test

Maximal graded exercise tests were performed on a Monark LC4r ergometer (COSMED, Rome, IT) during screening, at baseline (up to 14 days before first dose) and during the treatment period at 11, 25, 39 and 53 days (±1 day) after the first dose administration. The protocol started with 1-minute rest without pedalling, followed by two minutes warm-up at 75 Watts. The pedalling resistance was then increased to 175 Watts, and 25 watts every five minutes. Cadence had to be maintained between 70 and 90 rpm. The test stopped when cadence could not be maintained above 70 rpm or when a participant terminated the test. Participants then pedalled at 50 Watts during a 3-minute recovery. Blood for coagulation and endothelial markers was drawn from an intravenous cannula in the right forearm before and directly after the exercise tests.

Mont Ventoux race

Approximately 12 days after the last dose participants took part in a road stage of 110 km in the French Provence (total elevation gain 1524 m), after which they competitively climbed the Mont Ventoux (Vaucluse département, F) in an open course via Bédoin, F, bridging an altitude of 1610 m over 21.5 km. Blood for coagulation and endothelial markers was drawn before the 110 km stage and at the top of the Mont Ventoux.

Coagulation and endothelial markers

Markers for coagulation were determined in one of two collection tubes: prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer and fibrinogen were determined in plasma retrieved from venous blood collected in a 3.2% citrate tube of 2.7 mL, which was processed (spinning at 2000 g for 20 minutes) within 30 minutes from collection and stored at ≤ -70 °C. Beta thromboglobulin (beta-TG), prothrombin fragment 1+2 (F1+2), Factor VIII (FVIII), platelet factor 4 (PF4) and Thrombin:Antithrombin (TAT) were determined in plasma retrieved from venous blood collected in a Coagulation Sodium Citrate (CTAD) tube of 3.5 mL, which was pre-cooled in ice-water, and placed back on ice before processing (spinning at 2000 g for 20 minutes at 4 °C, brake off) within 30 minutes of collection. Plasma was then pipetted into a pre-cooled tube and spun again for 20 minutes at 2000 g and 4 °C, brake off. Plasma was then again pipetted into a pre-cooled tube, mixed, and subdivided in aliquots which were snap frozen with dry ice and methanol for 15 minutes

and stored at ≤ -70 °C. For the samples collected just before and directly after the Mont Ventoux race, centrifugation of CTAD tubes was performed at room temperature. Endothelial function markers E-selectin, intercellular adhesion molecule 1 (iCAM), P-selectin, thrombomodulin, vascular cell adhesion molecule 1 (vCAM) and Von Willebrand Factor antigen (vWF) were measured from the same CTAD tube.

These markers were determined as follows: Activated partial thromboplastin time, Prothrombin time and Fibrinogen were determined using the STA-Evolution Coagulation analyser (Diagnostica Stago C1-030, Theale, GB), D-dimer was determined using the STA-r MAX coagulation analyser (Diagnostica Stago, Theale, GB). Enzyme-linked immunosorbent assay (ELISA) was used for the quantitative detection of: Human CXCL7 (for Beta Thromboglobulin) PicoKine™ ELISA Kit (Boster Biological Technology, Pleasanton CA, USA, Catalog # EK0729), Human sE-Selectin PicoKine™ ELISA Kit (Boster Biological Technology, Pleasanton CA, USA, Catalog # EK0501), Human P-Selectin PicoKine™ ELISA Kit (Boster Biological Technology, Pleasanton CA, USA, Catalog # EK0505), Human PF4/CXCL4 PicoKine™ ELISA Kit (Boster Biological Technology, Pleasanton CA, USA, Catalog # EK0726), human thrombomodulin PicoKine™ ELISA Kit (Boster Biological Technology, Pleasanton CA, USA, Catalog # EK0917). vWF was measured with an in-house developed validated ELISA assay. The vWF ELISA was calibrated against the WHO standard plasma for vWF and has a total assay CV of 8%. Prothrombin activation F1+2 and TAT levels were determined using the Enzygnost (monoclonal) ELISA kit (Siemens Healthcare Diagnostics, Siemens, NL). All assays were performed according to the manufacturers' protocol.

FVIII activity was measured by the one-stage clotting assay on a Siemens BCS-XP analyser (Siemens Healthcare Diagnostics) with the use of commercial reagents (Siemens Actin FSL, Siemens Healthcare Diagnostics) with calibration against a normal reference plasma (SHP, Siemens Healthcare) calibrated against the WHO standard plasma for factor FVIII. iCAM and vCAM were measured using MesoScale Discovery's V-PLEX kits.

Statistical analysis

Participants with at least one available measurement were included in the analyses. For rHuEPO effects, pre-exercise data were analysed with a mixed model analysis of variance with treatment, time and treatment by time as fixed factors, participants as random factor and the pre-value as covariate for treatment effects. For exercise effects, the analysis was performed on the placebo group, with a mixed model analysis of variance, time as fixed factor, participants as random factor and the pre-value as covariate. For combined rHuEPO and exercise effects, the analysis was performed on the post-exercise measures with a mixed model analysis of variance with treatment, time and treatment by time as fixed factors, participants as random factor and the pre-value as covariate for treatment effects. The contrast that is calculated within the models is placebo *versus* rHuEPO, or before *versus* after exercise.

Results of statistical models are reported as estimated means (EM) at the different time points per intervention and estimates of the difference between treatments over the whole time period, including 95% confidence intervals (in percentage for log-transformed parameters) and the p-value of the contrasts.

When 95% confidence intervals are presented, they reflect the estimated difference between the two groups, with a significance level of p<0.050. All calculations were performed using SAS for windows V9.4 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

rHuEPO effects

A total of 48 participants were included in the analyses (24 in the rHuEPO group and 24 in the placebo group). One participant withdrew after the fourth dose administration, all other 47 subjects completed the study. Participants were trained cyclist, confirmed by their average baseline maximal power output values of 335.07 W (SD 33.40) and VO_{2max} of 55.63 mL/min/kg (SD 4.80), with average recorded cycling activity of 4.9 h and 5.9 h for rHuEPO and placebo groups respectively. rHuEPO treatment had clear effects on several variables in rest as we have partly reported previously.[3] Average Hb (+12%) and Ht (+16%) increased upon rHuEPO treatment and was significantly elevated compared to placebo with 0.6 mmol/L (0.44-0.77, p=<0.0001) (equivalent to 0.97 g/dL) and 3.3% (2.5-4.1, p=<0.0001), respectively. In contrast, platelet count was not affected by rHuEPO treatment. rHuEPO significantly increased resting levels of P-selectin (+7.8% (1.5-14.5), p=0.02) and E-selectin (+8.6%

(2.0-15.7), p=0.01) compared to placebo, but iCAM and vCAM were not significantly altered by rHuEPO treatment, see Table 1. aPTT and coagulation markers TAT and D-dimer remained unchanged, as did specific platelet activation markers PF4 and beta-TG.

Exercise effects

Exercise induced significant alterations in haemostatic profile in placebo treated subjects as reflected by increased TAT, D-Dimer, PF4 and beta-TG levels, and decreased aPTT. Endothelial markers E- and P-selectin were also increased by exercise, as well as iCAM and vCAM. All markers were significantly affected by the exercise test, whereas the race only had a significant effect on aPTT, beta-TG, FVIII, P-selectin, PF4, TAT and vWF. The direction of these significant effects was the same for the exercise test and race, although there seem to be differences in magnitude of the effect. See Table 2 for an overview of all changes in markers due to exercise and Figures 1-4 for a graphical representation of these effects for a selection of markers.

Effect of combination of rHuEPO and exercise

The combination of rHuEPO treatment and exercise showed larger changes in several markers compared to exercise alone (in the placebo group). Namely, E-selectin levels post-exercise were 15.3% higher in the rHuEPO treated group than in the placebo group, and 32.1% higher for PF4, see Table 3. For the other markers, there was no difference in post-exercise values between the rHuEPO and placebo groups.

DISCUSSION

Although regular exercise is considered healthy, sporadic cases of exercise-induced thrombotic events, such as ischemic stroke, venous thromboembolism and myocardial infarction have been reported. This is probably related to changes in haemostasis that have been observed after exercise, and especially high intensity exercise, possibly in combination with additional predisposing cardiovascular risk factors.[5] One of such factors could be pharmacological treatment with hematopoietic factors, such as rHuEPO. In anaemic patients rHuEPO was found to increase thrombotic risk, probably in part as a result of haemoconcentration. A meta-analysis of 9353 patients in 57 randomised placebo-controlled trials showed that treatment with erythropoietin was associated with a significantly increased thromboembolic risk [relative risk: 1.67; 95% confidence interval: 1.35 to 2.06].[21] However, whether rHuEPO also increases thrombotic risk in healthy athletes is unknown. Therefore, we investigated the effects of rHuEPO doping use on coagulation and endothelial function markers, both in rest and after intense exercise, in the same study of which we recently published the results on the effects of rHuEPO on cycling performance in trained cyclists.[3] The dose regimen in our study is consistent with known practices in professional cycling.[22] We measured thrombotic risk factors including platelets, clotting factors and endothelium-derived factors and quantified coagulation, platelet activation and endothelial activation after rHuEPO use, after exercise, and when rHuEPO use and exercise were combined.

Evaluating the effects of rHuEPO alone, we found an increase in P-selectin and E-selectin, but no clear effects on platelet and endothelial activation through other markers. Exercise by itself clearly impacted the haemostatic profile of subjects in our study, similar to what has previously been reported,[5] with significant changes in all measured markers in the exercise test. When combining rHuEPO treatment and exercise, the effects observed on E-selectin were additive, but not synergistic. In addition, PF4 was not significantly increased by rHuEPO alone, however when rHuEPO and exercise were combined, the increase in PF4 was larger than for exercise alone.

Clinical relevance

Effects on P-selectin

The clinical relevance of these findings, however, remains uncertain. The estimated mean levels of P-selectin after rHuEPO treatment were 9376 pg/mL, an increase of 8% compared to placebo. The exercise test increased P-selectin by 12.2%, but the combination of exercise and rHuEPO did not lead to a significant increase compared to exercise alone. Nevertheless, rHuEPO increased P-selectin levels consistently over the treatment period and to a similar magnitude as the exercise test does. What the impact is of this increase, however, is not clear, also because absolute levels of

P-selectin seem difficult to compare between studies. It is known that measured levels of P-selectin are dependent on the type of sampling tube used. Collection should be done in citrated plasma to avoid platelet activation in the tube leading to artificially elevated P-selectin levels.[23] However, even when using citrated plasma collection, levels of P-selectin can vary widely between studies (from on average approximately 20000 pg/mL to 242000 pg/mL in healthy volunteers),[23–26] which might be partly due to differences between assays used.[27] So how do the relative increases observed in our study relate to increased risk for clinically relevant thrombotic events? Little is known about the predictive value of P-selectin levels. One study in patients with first unprovoked venous thromboembolism however, showed that patients with higher P-selectin levels during the follow-up had a higher risk of recurrence, with 14% higher levels in patients that would experience recurrence.[28] In our study, increases in P-selectin levels were only slightly smaller after rHuEPO treatment and after the exercise test, and more than double after the race (increase of 34.1%). Increases in P-selectin have also been observed after a mild *in vivo* lipopolysaccharide (LPS) challenge (up to 42% increases).[25] In addition, it is known that P-selectin levels are increased in different cardiovascular and haematological diseases. In thrombotic thrombocytopenic purpura and haemolytic uremic syndrome large differences in P-selectin levels of 274% and 245% with healthy controls were observed.[26] In a case-control study deep vein thrombosis was associated with a much smaller difference of 19%.[24] This is only slightly higher than the increases we observed in our subjects. These findings combined indicate that exercise as well as rHuEPO use potentially are a risk factor for thrombosis.

Effects on E-selectin

The estimated mean levels of E-selectin after rHuEPO treatment were 5372 pg/mL compared to 4945 pg/mL in the placebo group, an increase of 9%. Exercise increased E-selectin by 7% in the placebo group, but post-exercise E-selectin levels were 15% higher still in the rHuEPO group at 5868 pg/mL, indicating there is an additive effect of rHuEPO and exercise. This leads to a combined increase in E-selectin of approximately 19% versus placebo in rest. Again, absolute baseline values of E-selectin are much smaller than reported previously for healthy volunteers (from 27800 to 54000 pg/mL in a review[29] to 314000 pg/mL in one other study[30]). This review describes significant differences between cases and controls, with increases in E-selectin of 30-50% for diabetes and hypertension. However, much larger effects are observed after a mild *ex vivo* LPS challenge (up to increases of 280%).[25] Predictive value of E-selectin for cardiovascular events is unclear. There was no predictive value of E-selectin for myocardial infarction or death in the follow-up of patients with acute ischemic-type chest pain.[31] However, patients with coronary artery disease showed higher risk of future death from cardiovascular causes if they had higher levels of iCAM, vCAM and E-selectin, the latter being 29% higher in the group of subjects that experienced events. Most of these reported differences and increases are only approximately twofold higher than the observed effect of the combination of rHuEPO and exercise, and so the combination of rHuEPO and exercise might increase the risk of cardiovascular events compared to exercise alone based on the endothelial marker E-selectin.

Mechanism

The origin of E- and P-selectin increments during exercise and particularly upon rHuEPO treatment remains somewhat uncertain. P-selectin is localised in alfa-granules of platelets, together with other platelet activation markers such as PF4. In addition, P-selectin is found in Weibel-Palade bodies of endothelial cells, together with von Willebrand Factor, so the effect on P-selectin could be mediated both through platelet activation and endothelial activation. However, neither PF4, nor vWF were increased after rHuEPO treatment in rest, contradicting either of these mechanisms (although there was an increase in PF4 after the combination of rHuEPO and exercise). E-selectin on the other hand, is restricted to endothelial cells, but so are iCAM and vCAM, which were not increased after rHuEPO treatment in rest. These increases in E- and P-selectin without changes in other markers reported here, are in agreement with previous findings.[32,33] One of these studies showed that E-selectin was already increased 24 hours after rHuEPO dosing,[32] indicating an acute mechanism and not an effect mediated through sheer stress by increases in haemoglobin; maturation of normoblasts in bone marrow lasts 5-7 days.[34] Although we did not measure levels in such short timeframe, it is interesting that E-selectin levels were already clearly increased at the first measurement 11 days after start of treatment (Figure 1), whereas P-selectin levels only started showing increased levels 25 days after start of treatment (Figure 2). This could indicate these

effects are mediated by two different mechanisms. In summary, the mechanism through which rHuEPO increases E- and P-selectin is not completely clarified and might be through enhancing both endothelial activation and platelet reactivity, as also suggested previously.[33]

A potentially interesting biomarker for this study is soluble CD40L, which is primarily produced and released by activated platelets. Numerous studies have reported increased sCD40L in various clinical conditions and diseases associated with platelet activation. When comparing two commercially available sCD40L ELISA kits (Thermo Fisher and RnD systems), with the aim to select one for evaluation of our clinical samples, all test samples collected in CTAD tubes showed sCD40L levels below or around the lower level of quantification. Further investigation indicated that when comparing CTAD and serum samples from the same donors, similar low sCD40L levels were detected in the CTAD samples, but significant sCD40L levels were present in the serum samples, demonstrating the efficacy of the used ELISA kits. These data indicate that careful sample handling procedures and collection in CTAD tubes resulted in minimal platelet activation and subsequent sCD40L release, and that minimal *in vivo* sCD40L release had taken place in the study participants.

Finally, there is clear evidence that elevated levels of vWF are a predictive factor for ischemic heart disease, cardiovascular mortality and stroke in healthy individuals,[35–37] and iCAM for myocardial infarction.[38] In our study, iCAM was unaffected by rHuEPO or the combination of rHuEPO and exercise, however, vWF showed a trend towards an increase after rHuEPO and exercise compared to exercise alone, albeit not significant.

In conclusion, rHuEPO treatment altered endothelial function in trained cyclists by selectively increasing levels of P- and E-selectin in rest. Exercise alone increased these markers as well, and the combination with rHuEPO use showed a cumulative effect for E-selectin, but not P-selectin. In addition, exercise induced hypercoagulability as measured by markers such as TAT, D-Dimer, aPTT, PF4 and beta-TG. PF4 showed a larger increase after the combination of exercise and rHuEPO than after exercise and placebo. Based on these markers, exercise potentially increases thrombotic risk, a risk that might be enhanced in combination with additional factors such as rHuEPO use, and this study adds knowledge on which pathways are involved in these processes.

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FIGURE 1 EXERCISE AND rHuEPO EFFECTS ON E-SELECTIN LEVELS Pre- and post- exercise levels of E-selectin for the maximal graded exercise test at baseline, 11, 25, 39 and 53 days and the Mont Ventoux race for rHuEPO and placebo groups.

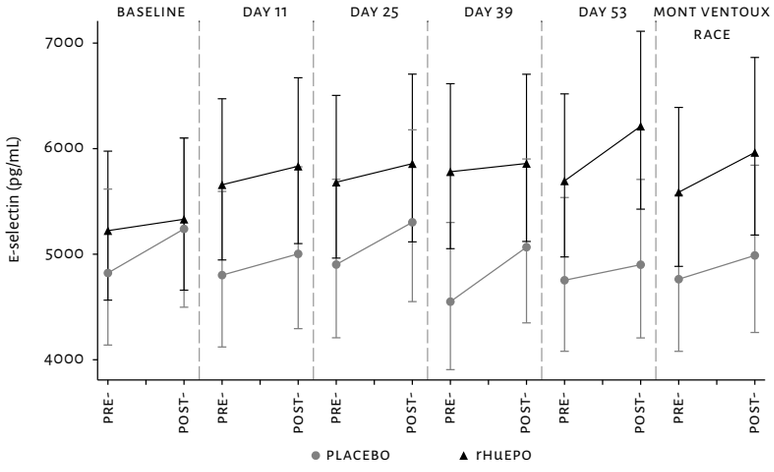

FIGURE 2 EXERCISE AND rHuEPO EFFECTS ON P-SELECTIN LEVELS Pre- and post- exercise levels of P-selectin for the maximal graded exercise test at baseline, 11, 25, 39 and 53 days and the Mont Ventoux race for rHuEPO and placebo groups.

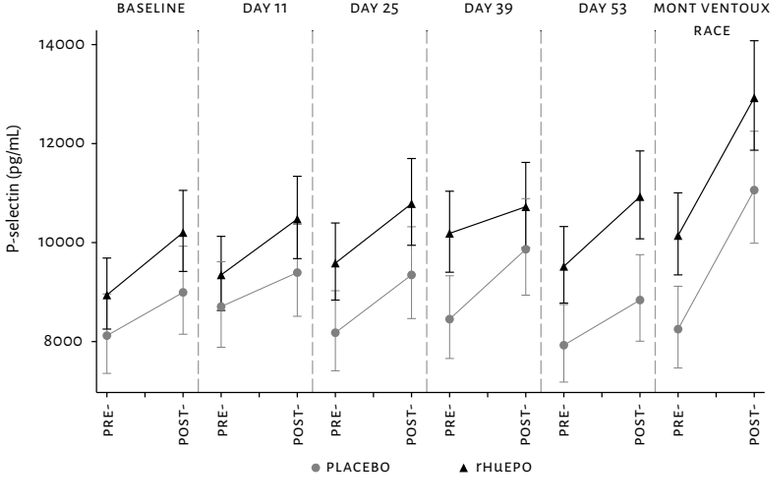

FIGURE 3 EXERCISE AND rHuEPO EFFECTS ON PLATELET FACTOR 4 Pre- and post- exercise levels of platelet factor 4 (PF4) for the maximal graded exercise test at baseline, 11, 25, 39 and 53 days and the Mont Ventoux race for rHuEPO and placebo groups.

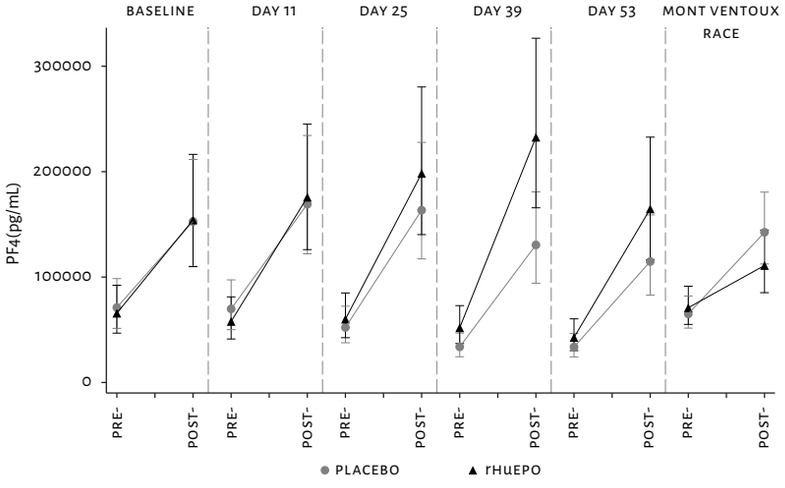

FIGURE 4 EXERCISE AND rHuEPO EFFECTS ON VON WILLEBRAND FACTOR Pre- and post- exercise levels of von Willebrand Factor for the maximal graded exercise test at baseline, 11, 25, 39 and 53 days and the Mont Ventoux race for rHuEPO and placebo groups.

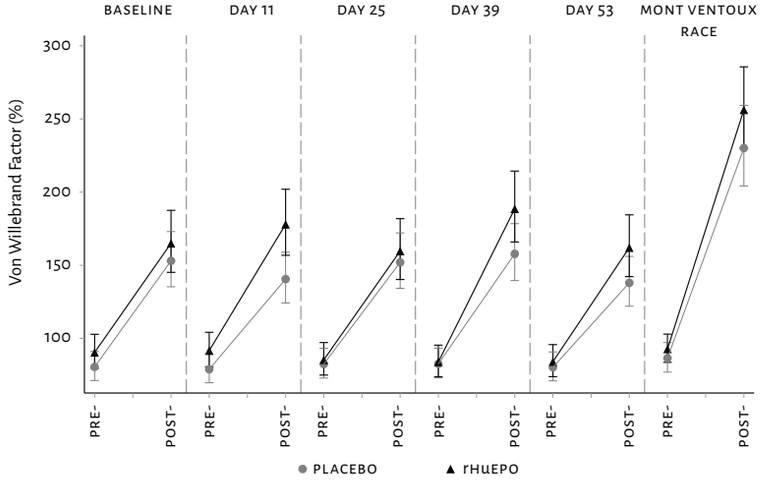

TABLE 1 EFFECTS OF rHuEPO ON MARKERS IN REST Raw baseline (and SD) and EM (Estimated Mean) values of coagulation and endothelial function markers in rest at the different time points for both treatment groups, including the estimated differences between the treatment groups (95% confidence interval) and p-value. 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
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	
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	
iCAM, pg/mL	Placebo	318333 (48913)	320475	313774	321983	326577	317702	297252	306471	320147	2.2% (-2.2%, 6.8%) p=0.33
	rHuEPO	335958 (73686)	322643	322951	328572	330397	330440	310291	311309	322155	
vCAM, pg/mL	Placebo	489750 (84683)	527396	482540	506971	502660	498766	493731	514184	493716	-0.0% (-4.2%, 4.4%) p=0.99
	rHuEPO	515000 (102348)	520718	480747	485750	496181	504203	508575	507573	515472	
Von Willebrand Factor, %	Placebo	88.4 (27.7)	81.953	86.168	85.932	91.320	86.046	88.634	83.627	90.113	-0.9 (-8.0%, 6.8%) p=0.8138
	rHuEPO	100.1 (25.4)	87.897	86.114	82.513	94.138	80.810	86.512	81.131	89.014	
PF4, pg/mL	Placebo	43019 (46073)	68339	23340	51235	28821	33209	35929	33026	63845	13.1% (-9.3%, 41.1%) p=0.2661
	rHuEPO	43988 (85138)	60195	23125	60555	31296	52786	36164	43549	72593	

TABLE 2 EFFECTS OF DIFFERENT TYPES OF EXERCISE ON HAEMOSTATIC PROFILE: CURRENT STUDY AND LITERATURE DATA Current study: Estimated percentage change due to exercise compared to rest in the placebo group [95% confidence interval of the effect size]. Literature data percentage change due to exercise compared to rest. Figures in bold depict a significant change. NA, not available.

Marker	Current study		Literature data		
	Exercise test	*Race*	*Maximal exercise*	*Short (≤60 min) submaximal exercise*	*Long (>60 min) submaximal exercise*
Platelet count	NA	**10.6% [1.1%; 20.9%]**	**25 to 27%**[6,7]	**26%**[8]	**12%**[9]
Activated partial thromboplastin time	**-12.4% [-14.1%; -10.6%]**	**-15.5% [-28.2%; -0.5%]**	**-6%**[10,11]	**-5 to -8%**[10,11]	NA
Prothrombin time	**-2.1% [-2.9%; -1.3%]**	1.5% [-1.2%; 4.2%]	-1 to -3%[10,11]	2%[10,11]	NA
Fibrinogen	**7.0% [4.4%; 9.6%]**	-7.6% [-15.5%; 1.2%]	**12%**[12]	NA	2%[13]
D-dimer	**43.0% [25.5%; 62.9%]**	-2.5% [-24.3%; 25.6%]	NA	**~150%**[14]	NA
Beta Thrombo-globulin	**92.6% [63.8%; 126.3%]**	**21.2% [16.9%; 135.1%]**	**60 to 85%**[6,7]	21%[8]	NA
E-selectin	**7.1% [4.3%; 9.9%]**	4.7% [-16.1%; 30.7%]	4% to **10%**[15,16]	-4%[16]	**16%**[17]
ICAM	**8.6% [6.5%; 10.9%]**	4.4% [-4.7%; 14.4%]	**11% to 24%**[16,18]	-4%[16]	**5%**[17]
VCAM	**7.6% [5.7%; 9.4%]**	7.3% [-2.3%; 17.9%]	10%[16]	1%[16]	**22%**[17]
Prothrombin fragment 1+2	**88.0% [64.3%; 115.2%]**	9.8% [-12.6%; 38.1%]	**17% to 19%**[15,19]	NA	NA
Factor VIII	**128.7% [104.4%; 155.9%]**	**181.1% [147.8%; 218.9%]**	**63%**[10]	**34 to 38%**[10,20]	NA
P-selectin	**12.2% [9.1%; 15.4%]**	**34.1% [16.2%; 54.6%]**	**21%**[15]	**~70%**[14]	**44%**[17]
PF4	**192.4% [137.2%; 260.6%]**	**118.9% [57.2%; 205.0%]**	**85%**[7]	NA	**102%**[13]
Thrombin: Antithrombin	**504.6% [386.6%; 651.3%]**	**159.7% [65.2%; 308.5%]**	**32% to 108%**[10,19]	**16 to 43%**[8,10]	NA
Thrombomodulin	**13.5% [9.8%; 17.4%]**	6.0% [-20.6%; 41.5%]	NA	NA	NA
Von Willebrand Factor	**82.9% [64.1%; 103.8%]**	**166.0% [125.1%; 214.2%]**	NA	NA	NA

TABLE 3 EFFECTS OF EXERCISE ON MARKERS PER TREATMENT GROUP Raw baseline (and SD) and EM (Estimated Mean) values of coagulation and endothelial function markers after exercise at the different time points for both treatment groups, including the estimated differences between the treatment groups (95% confidence interval) and p-value. 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 25	EM Day 39	EM Day 53	Difference between groups
E-selectin, pg/mL	Placebo	5584 (2104)	5022	5333	5086	4919	15.3% (7.0%, 24.3%)
	rHuEPO	5613 (2001)	5731	5819	5800	6129	p=0.0004
P-selectin, pg/mL	Placebo	9214 (2105)	9789	9719	10280	9211	5.2% (-2.9%, 13.9%)
	rHuEPO	10300 (1884)	10027	10370	10203	10399	p=0.2077
ICAM, pg/mL	Placebo	339917 (46981)	338125	365918	350488	346019	-0.9% (-7.0%, 5.5%)
	rHuEPO	375217 (78950)	342709	357497	337634	349506	p=0.7677
VCAM, pg/mL	Placebo	520833 (76355)	551062	565671	546102	554070	-3.6% (-9.2%, 2.4%)
	rHuEPO	566739 (115781)	526734	535286	526161	550035	p=0.2302
Von Willebrand Factor, %	Placebo	164.0 (68.4)	143.4	155.1	161.0	140.8	11.0% (-1.6%, 25.3%)
	rHuEPO	174.0 (67.3)	174.6	156.8	181.0	154.7	p=0.0887
PF4, pg/mL	Placebo	194624 (116663)	169825	163287	131027	115203	32.1% (4.6%, 66.8%)
	rHuEPO	199970 (148748)	170564	198478	228441	164724	p=0.0207