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Highlights

- Perinatal high-dose erythropoietin (EPO) may aid neurodevelopment in preterm babies
- Long-term effects of perinatal EPO on retinal and visual function are unknown
- We used electroretinogram (ERG) to measure retinal function in schoolchildren
- No ERG differences between children who had received EPO and placebo
- Effects of premature birth on ERG partially mitigated by EPO

Original Articles

The effect of high-dose erythropoietin perinatally on retinal function in school-aged children born extremely or very preterm

Short title: Retinal effects of high-dose EPO after premature birth

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Abstract

Purpose To investigate the long-term effects of high-dose recombinant human erythropoietin (rhEPO) administered during the perinatal period on retinal and visual function in children born extremely or very preterm.

Design Randomized, double-blind clinical trial follow-up plus cohort study.

Methods

Setting: Department of Ophthalmology, University Hospital Zurich, Zurich, Switzerland.

Study Population: extremely or very preterm-born children aged 7-15 years, previously randomized to receive either high-dose rhEPO or placebo in the perinatal period. Inclusion criteria: participation in an ongoing neuropediatric study (EpoKids), written informed consent (IC). Exclusion criteria: previous ocular trauma or surgery; retinal or developmental disease unrelated to prematurity. Healthy control (HC) children of comparable age were recruited. Inclusion criteria: term birth, IC. Exclusion criteria: any ocular/visual abnormality, high refractive error. Intervention status (rhEPO/placebo) was unknown to examiners and subjects at examination, with examiners unblinded only after completion of all analyses.

Observation Procedures: Electroretinography (ERG) was performed with the RETeval device (LKC Technologies, Inc., Gaithersburg MD). Ophthalmological and orthoptic examinations excluded comorbidity in the prematurely born cohort and ocular diseases in the HC group.

Main Outcome Measures: Scotopic and photopic ERG response amplitudes and peak times (6 amplitudes; 6 peak times). Secondary outcomes were habitual visual acuity and color discrimination performance (for descriptive summary only).

Results No differences in ERG parameters between EPO (n=52; 104 eyes) and placebo (n=35; 70 eyes) subgroups were observed (all corrected p>0.05). Two cone system-mediated peak times were slightly slower in the placebo than HC (n=52; 104 eyes) subgroup (coefficient/95% confidence interval (CI) = 0.53/0.21 to 0.85 and 0.36/0.13 to 0.60; p = 0.012 and 0.022); a predominantly rod system-mediated peak time was slightly faster in the EPO than the HC subgroup (coefficient/95% CI = -4.33/-6.88 to -1.78; p = 0.011). Secondary outcomes were comparable across subgroups.

Conclusions Administration of high-dose rhEPO to infants born extremely or very preterm during the perinatal period has no measurable effects on retinal function in childhood compared to placebo. Premature birth may cause small, likely clinically insignificant effects on retinal function in childhood, which may be partially mitigated by administration of rhEPO during the perinatal period.

Introduction

Preterm birth, defined as a gestational age (GA) of less than 37 weeks, can show a wide variation in short- and long-term sequelae. Premature birth is categorized by the World Health Organization (WHO) as moderate (GA 32-37 weeks), very (GA 28 - <32 weeks), and extremely (GA < 28 weeks) preterm.[1] The earlier the child is born, the more vulnerable is the retina and visual system to abnormalities of development and maturation.[2] Recent studies have investigated the relationships between preterm birth and long-term ocular and visual development, and described an increased risk of associated abnormalities and dysfunction.[3-5] These include not only central visual acuity (VA), but also stereopsis, color vision, contrast sensitivity, visual perception, and ocular alignment.[4-9]

Most studies have documented reduced VA in children born moderately or very preterm when examined at an age when the visual system has matured.[3,5-10] Infants with extremely low weight at birth (< 1000g) are three times more likely to have a VA worse than 6/60 compared with infants born at term,[8] and Haugen et al. documented a subnormal VA in 46% of their cohort of extremely and very preterm-born children tested at the age of 6 to 7 years old.[3] Quantification of retinal morphology and function may help to explain reduced vision in formerly premature children. Retinal function in premature infants has been studied using the electroretinogram (ERG). For example, Molnar et al.[10] documented a reduction in rod and cone function in extremely preterm infants compared to term-born infants examined at the age of 6.5 years. ERG responses in moderate to extremely preterm infants recorded between the ages of 5 to 18 years showed smaller amplitudes and longer implicit times of rod- and cone system responses.[10-14] These studies are not directly comparable due to demographical and methodological differences such as GA and age at ERG

recording. Importantly, retinopathy of prematurity (ROP), as a confounding influence on retinal development, is not always reported in detail (e.g., zone and stage). A recent study demonstrated significantly longer cone-mediated flicker ERG implicit times in extremely preterm infants both with and without a history of ROP tested at the age of 6 to 12 years compared to term-born children of similar age, with ROP eyes also exhibiting longer peak times than those without previous ROP.[12] However, rod photoreceptor sensitivity is even more susceptible to the impact of ROP than cone photoreceptor function, as demonstrated by Fulton et al.[15,16]

Erythropoietin (EPO) is a glycoprotein hormone considered to be a regulator of erythropoiesis by stimulating survival, proliferation, and differentiation of erythroid cells.[17] It is secreted in the retina,[18] in addition to other organs and tissues such as the liver and kidney[19] and the brain.[20] Previous studies have demonstrated neuroprotective effects of recombinant human erythropoietin (rhEPO) both in vitro[21] and in animal models,[22,23] leading researchers to investigate potential benefits of early prophylactic treatment with high-dose rhEPO in prematurely born infants. At an age equivalent to full term, magnetic resonance imaging (MRI) revealed reduced loss of white matter in preterm infants who had received high-dose rhEPO within the first days of life, relative to those treated with placebo.[24] However, when the same cohort was examined again at the age of 2 years, there were no significant differences in neurodevelopmental outcomes such as cognitive or motor development.[25] At 5 years of age, there remained no measurable differences in neurodevelopmental outcomes between the two subgroups.[26] However, the potential effects of perinatal high-dose rhEPO administration on retinal and visual function remain unknown at the time of writing.

The aim of this study was to evaluate the long-term effects of high-dose rhEPO administration within the perinatal period on retinal and visual function in extremely and very preterm infants by comparing functional outcome measures in children treated with EPO with those treated with placebo. In addition, we investigated the long-term effects of premature birth on retinal and visual function by comparing both prematurely born subgroups (EPO and placebo) with a control cohort comprising healthy term-born children of comparable age.

Methods

Study Design and Subjects

All prematurely-born children examined during the present study were subjects in both the Swiss EPO Neuroprotection randomized clinical trial (RCT) (NCT00413946) [24,25] and EpoKids Study[27]. The initial RCT investigated the effects of high-dose rhEPO administration in the perinatal period on neurodevelopmental outcomes in infants born between 26 weeks 0 days and 31 weeks 6 days GA. Subjects were randomly assigned to receive either high-dose rhEPO (3000IU/kg) or placebo (isotonic saline, 0.9%) within three hours of birth and at 12-18 hours and 36-42 hours after birth.[24] Long-term follow up of the cohort was initiated (via the EpoKids Study) to investigate neurodevelopment and executive function.[27] The present work represents a cross-sectional ophthalmological sub-study of the EpoKids Study in which the double-blind status of subjects and researchers was maintained throughout, with researchers being unblinded only after all analyses were complete. The sub-study was prospectively approved by the Cantonal Ethics Committee of Zurich (BASEC 2017-00521). Informed consent for participation in the sub-study was provided in writing by a parent/guardian of all subjects.

A total of 448 preterm infants, born between 26 weeks 0 days and 31 weeks 6 days gestational age (GA), were enrolled in the Swiss EPO Neuroprotection RCT[24] between 2005 and 2012, randomly assigned to one of the EPO and placebo groups, and received the corresponding intervention. Children who were enrolled in the RCT and participated in the 2 year follow up study[25] were eligible for the EpoKids Study (n=365).[27] Parents/guardians and children (at that point aged between 7 -15 years) who agreed to be contacted for information on, and possible enrollment in, our substudy, were approached. Inclusion criteria were: participation in the EpoKids Study and written informed consent. Exclusion criteria were: history of ocular trauma or surgery, and other retinal or developmental disease unrelated to premature birth. The recruitment of patients, and relationship of the sub-study to its parent studies, is summarized in Figure 1.

In addition to the infants born preterm, healthy control (HC) subjects aged between 7-14 years were recruited from among the children of friends and colleagues. HCs underwent the same examination as the prematurely born children. For HC subjects, inclusion criteria were term birth and written informed consent; exclusion criteria were any ocular or visual abnormality and refractive error >6 diopters spherical equivalent.

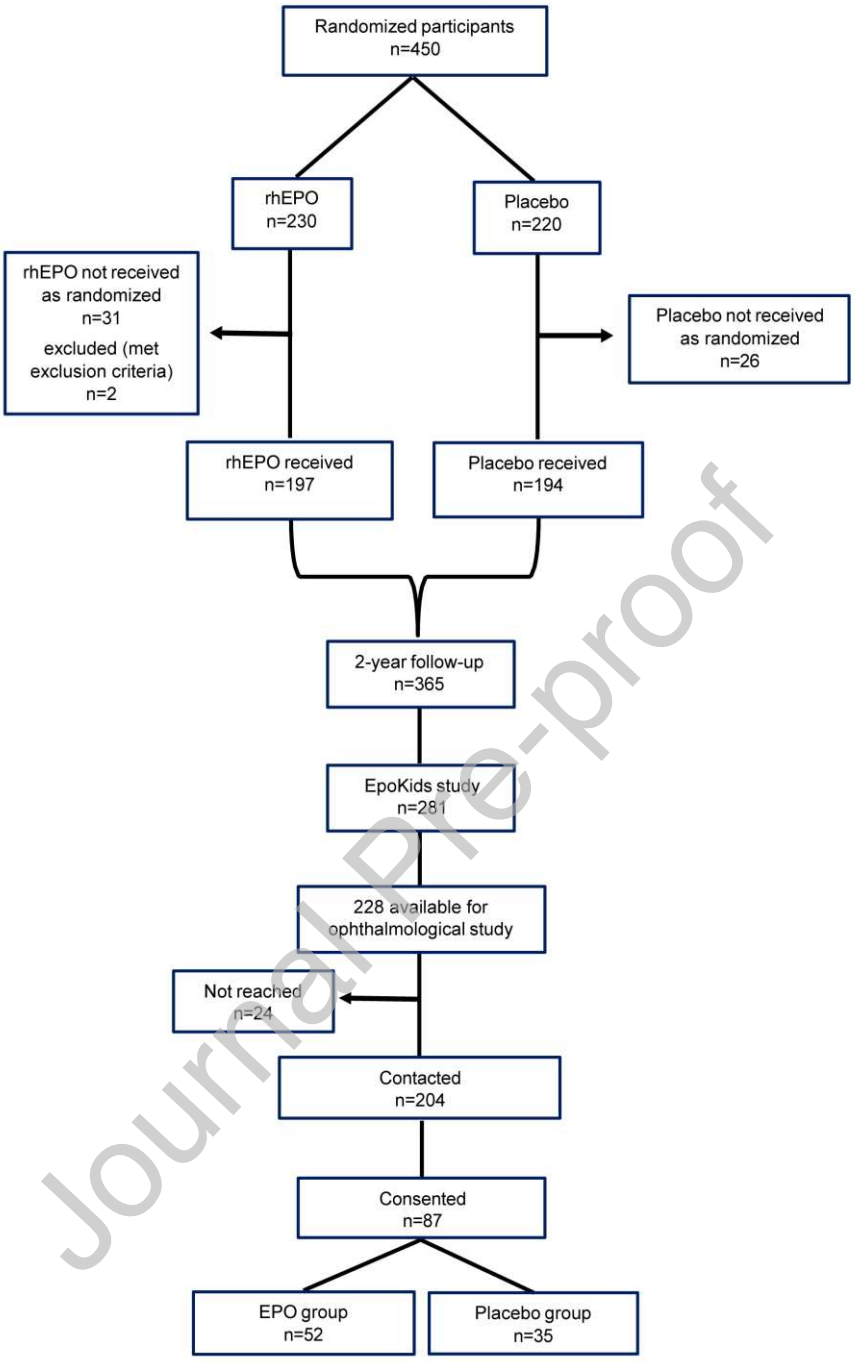

Figure 1. Participant flowchart of the study and relationship to parent studies. 448 infants born extremely or very preterm were recruited for the Swiss EPO Neuroprotection RCT between the years 2005-2012 and randomly assigned to receive either high-dose EPO or placebo. 393 of these subjects were included in the analysis published previously. 365 subjects were eligible to participate in the follow up EpoKids Study, in which 281 were able to be enrolled. Of those, 228 subjects were eligible for inclusion in our substudy. It was possible to contact parents/guardians of 204 of these subjects, of whom 87 consented to participate in our ophthalmological substudy. Blinding with respect to intervention subgroup (EPO/placebo) was maintained until after all analyses were complete.

Visual function

All examinations were carried out by a team composed of ophthalmologists, orthoptists, and a vision scientist. Habitual distance visual acuity (VA) was measured at a distance of 4m using Early Treatment Diabetic Retinopathy Study (ETDRS)-type charts (Precision Vision, Woodstock IL, U.S.A.) and recorded as the logarithm of the minimum angle of resolution (log MAR), with each correctly identified letter scored as 0.02 logMAR.[28] Color discrimination was assessed with the Mollon-Reffin “Minimal” Vision Test for color deficiencies (PA Vision Ltd, Ramsgate, U.K.) at a distance of 40cm.[29] The test includes caps with five different color saturation levels for protan, deutan, and tritan confusion axes.[30] Subjects had to identify the colored cap amongst distractors, with the examiner replacing the colored caps with ones of progressively less saturation. The least saturated chips that the child could distinguish from among the distractors were recorded separately for proton, deutan, and tritan axes (ranging from 5 [most saturated] to 1 [least saturated]). A single binocular training run to

illustrate the test principles to the child was followed by a monocular test run for each eye, starting with the right eye. Mollon and colleagues proposed that a score of 1 for each axis should be considered normal in adults;[31] as we were testing children, and based on previous evidence,[30] we chose instead to accept scores of 1 or 2 as normal. Scores of ≥ 3 were therefore considered abnormal.

Stereoacuity was measured using the TNO test, originally developed by the Netherlands Organisation for Applied Scientific Research, using red/green stereo viewing glasses and recorded in seconds of arc. A brief orthoptic examination consisting of cover test at distance and near with habitual correction and ocular motility testing was performed. This was undertaken primarily to ensure that no HC patients had undiagnosed binocular vision anomalies. Refractive error was measured at distance using retinoscopy under natural conditions (without use of mydriatic or cycloplegic drops) and recorded as mean spherical equivalent. The anterior segment was assessed using a slit lamp, which was also used for non-mydriatic fundus biomicroscopy.

Measurements of retinal and retinal vascular structure were also performed, using optical coherence tomography (OCT) and OCT-angiography (OCT-A) respectively, the results of which are reported in a separate publication.[32]

Finally, GA (in days) was recorded per patient, and ROP history per eye. Eyes with no history of ROP had a stage of 0 recorded, with the highest ROP stage reached recorded as the corresponding value. ROP staging was according to the nomenclature available at the time of the baseline study[33] and was extracted from the ophthalmological records. As all HC children were born at term, and had by definition no history of ROP, this information pertained to the prematurely born cohort only.

Electroretinogram (ERG)

Full-field ERG was recorded using the RETeval Complete device (LKC Technologies, Gaithersburg MD, U.S.A.) using a standard protocol supplied by the manufacturer with the device, “ISCEV 5 step Td light first”. The protocol permits an ERG recording comparable to published standards[34] without mydriasis, by continuously monitoring pupil diameter and presenting all stimuli at intensities calculated to ensure constant retinal illumination at each recording step.

Prior to recording, the skin below patients’ eyes was wiped clean using hand disinfectant and gently scrubbed with an abrasive paste, in order to minimize electrical impedance. Sensor Strip skin electrodes (LKC Technologies), containing recording, reference, and ground electrodes within a single adhesive strip, were then attached to the skin below the patients’ eyes as per the manufacturer’s instructions. As the patients had been in normal room illumination prior to the ERG recording, light-adapted responses were measured first. All stimuli and background illumination were composed of white light (Commission internationale de l’éclairage [CIE] chromaticity co-ordinates 0.33, 0.33). Subjects sat upright in a comfortable position whilst maintaining fixation on a small red light inside the RETeval. The first step of the protocol consisted of 30 single flashes of 85 trolands (Td) intensity presented twice per second (2 Hz) against a background of 850 Td (Cone Flash). This was followed by stimulation of 85 Td intensity delivered at 28.3 Hz against a background of 850 Td (Cone Flicker) until internal signal detection criteria of the device were met (approximately 5-10 seconds, up to a maximum of 15 seconds). Following these measurements, subjects sat in a completely darkened room for 20 minutes whilst wearing an eye mask. Following this period of dark adaptation, measurements were made without background illumination and consisted firstly of 12 single flashes of

0.28 Td presented at 0.5 Hz (Rod). Finally, five single flashes of 85 Td were presented at 0.1 Hz (Rod-Cone). A minimum of two measurements were made at each step in order to verify reproducibility, which were then averaged. All results were saved and then transferred to a computer for offline analysis.

For each patient, the RETeval produced a PDF report showing the individual and averaged ERG curves for each stimulus, including the timing and amplitudes of all the a- and b-waves. The positioning of these markers was carefully inspected. In the majority of cases, the PDF report displayed accurate data suitable for analysis. In cases where the markers did not line up precisely with the peaks or troughs of the averaged ERG curves for one or more steps, or were visibly affected by eye movements or blinks, manual processing was necessary. De-noised data were extracted from the results file using the proprietary RETeval RFF Extractor software (LKC Technologies) and saved as a .csv file. These data were imported into Excel 2016 (Microsoft, Redmond WA, U.S.A.), where the relevant curves could be re-plotted and the peaks and troughs manually determined. For the Cone and Rod-Cone conditions, the peak times (in milliseconds, ms) and amplitudes (in microvolts, μV) of both the a- and b-waves were recorded. For the Cone Flicker condition, the peak time and amplitude of the first positive peak was recorded. Finally, for the Rod condition, the peak time and amplitude of the b-wave were recorded. An example of an ERG recorded in a HC subject, illustrating the amplitudes and peak times included in the analyses, can be seen in Figure 2. All data extraction and post-processing was performed blind with respect to the subgroups of the prematurely born cohort. In other words, at this stage we were unaware which subjects had been randomized to the same group, and also which subjects had received EPO or placebo.

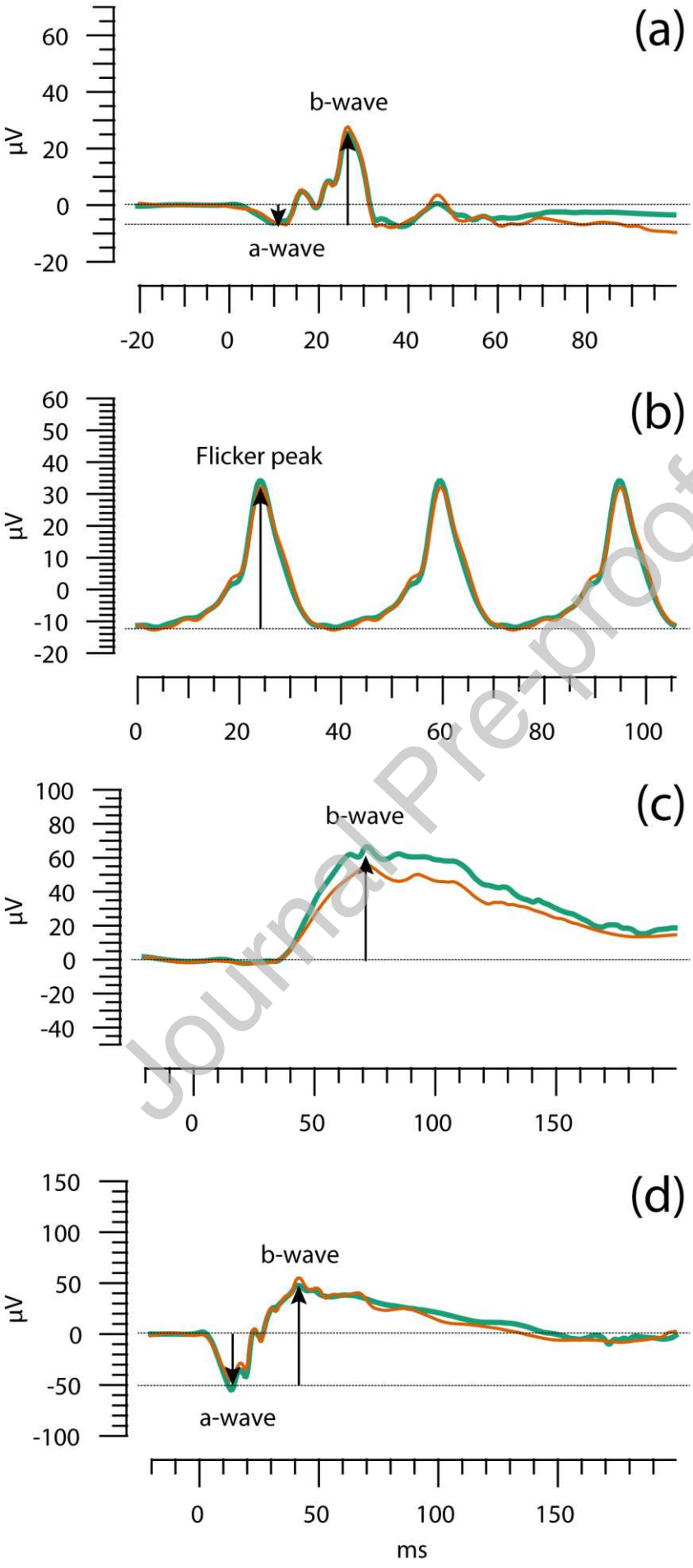

Figure 2 (a-d). Example of ERG waveforms recorded with the RETeval device in a single eye of a healthy control subject. Firstly the cone system was tested with light-adapted single flash (a) and 28.3 Hz flicker (b) stimulation. After a period of dark adaptation, dim (c) and bright (d) flashes were presented in darkness to measure responses of the rod and rod and cone systems, respectively. Red and green waveforms represent individual measurements, which were averaged for analysis. The amplitudes and peak times of the a- and b-waves and the peak of the flicker response (indicated by the relevant arrows) were included in the analysis. Further details of the stimulus conditions and data extraction are provided in the main text body.

Statistical analysis

During the whole study period subjects, their parents, and all investigators were blinded with regard to intervention status (EPO or placebo). For the statistical analysis, allocation to coded subgroups for each subject was known, without knowledge of which subgroup had received EPO or placebo. Complete unblinding was only performed after completion of all statistical analyses.

Subgroup (EPO, placebo, HC), age at examination, sex recorded at birth, and GA were recorded as subject-level variables and therefore identical for both eyes of each patient, with all other variables recorded per eye. GA was recorded only for the children born preterm. All outcome measures were descriptively summarized as mean and standard deviation (SD) for continuous variables and the number of cases per level for the categorical variables (e.g. color vision performance), for both the study population as a whole and the three subgroups. For the descriptive statistics, missing values were removed and the data were aggregated to the patient level by

averaging the numerical variables between the two eyes with the exception of ROP stage, which was recorded per eye. For color vision, we analyzed the data from the left eyes (which were examined after the right eyes) in order to minimize potential learning effects on color vision testing performance.[35] Generalized estimating equation (GEE) models adjusted for age at examination and sex[36] were used to compare ERG outcomes between the three groups (EPO, placebo, HC). For each parameter, a separate GEE model with normal error distribution was fitted. The coefficient of each GEE model was equal to the expected change for the relevant parameter between subgroups, i.e. the expected difference in mean values. Data from both eyes of subjects were included in the GEE models. P-values were corrected for multiple statistical comparisons according to the method of Benjamini and Hochberg.[37] Corrected values 0.05 - 0.01, 0.01 – 0.001, and <0.001 were considered as moderate, strong, and very strong evidence, respectively, of a difference between groups.[38] Due to the potentially confounding effects of GA and ROP on results in the EPO and placebo subgroups, we performed a sensitivity analysis using more complex GEE models that were adjusted for GA (in days) and ROP stage in addition to age at examination and sex, in order to assess whether the simpler models (adjusted only for age at examination and sex) were sufficient. All analyses were performed, and figures and tables generated, in the R programming language[39] using base and analysis-specific packages (listed in Supplementary Text). All ERG parameter distributions were summarized in separate boxplots.

Results

Patient characteristics

Of 228 eligible patients who agreed to be contacted for possible participation in our ophthalmological substudy we were able to contact 204, of whom 87 consented to participate and were examined during the study period. Study examinations took place at the Department of Ophthalmology of the University Hospital Zurich between November 2017 and September 2022. Unblinding confirmed that 52 patients (mean/standard deviation (SD) GA 202.77/13.35 days; 29 female) had received rhEPO and 35 (mean/SD GA 204.17/11.30 days; 16 female) placebo, as shown in Figure 1. GA was therefore equivalent to approximately 29 weeks in both intervention subgroups. A total of seven patients (5 EPO, 2 placebo) had a history of ROP, across a total of 10 eyes. Three eyes of two subjects (both from the EPO group) had ROP stage 3 and, of these, two eyes (of the same subject) required treatment for ROP, consisting of laser photocoagulation. We recruited and examined 52 children (21 female) as HCs. Mean/SD ages at examination were 11.86/1.42, 11.64/1.23, and 10.78/1.80 years for the EPO, placebo, and HC subgroups, respectively. Characteristics of the patient cohort and subgroups are summarized in Table 1.

Table 1: Demographic characteristics of the entire study population ('overall') and the healthy control, erythropoietin, and placebo subgroups. One eye of each patient was selected at random for the summary of categorical variables (ROP stage).

Variable	Level	Overall	HC	EPO	Placebo
n		139	52	52	35
GA in days; mean (SD)		203.33 (12.51)	NA	202.77 (13.35)	204.17 (11.30)
GA in weeks; mean, (SD)		29.05 (1.79)	NA	28.96 (1.79)	29.16 (1.61)
Age in years; mean (SD)		11.40 (1.60)	10.78 (1.80)	11.86 (1.42)	11.64 (1.23)
Sex (%)	F	66 (47.5)	21 (40.4)	29 (55.8)	16 (45.7)
	M	73 (52.5)	31 (59.6)	23 (44.2)	19 (54.3)
ROP stage (%)	0			46 (90.2)	31 (93.9)
	1			1 (2.0)	1 (3.0)
	2			2 (3.9)	1 (3.0)
	3			2 (3.9)	0 (0.0)
	4			0 (0.0)	0 (0.0)
	5			0 (0.0)	0 (0.0)
	NA*			1 (1.9)	2 (5.7)

F, female; GA, gestational age; HC, healthy control; M, male; NA, not applicable; ROP, retinopathy of prematurity; SD, standard deviation. * ROP data was not available in three patients.

Visual function

VA and color vision performance are summarized in Table 2. All subgroups had similar habitual VA: mean/SD -0.06/0.11, 0.00/0.11, and -0.04/0.11 logMAR in the HC, EPO, and placebo groups, respectively, corresponding to a difference of 1-3 letters between the various groups on the ETDRS-style visual acuity chart. Mean/SD refractive error was +0.43/1.16, +0.56/2.02, and +0.86/1.93 diopters (spherical equivalent) in the respective subgroups. Color discrimination was generally good, with a maximum of 7 eyes having a discrimination score of ≥ 3 along any of the confusion axes. For the proton and deutan axes, abnormal findings were present in all three subgroups, however only prematurely born children (4x EPO, 2x placebo) had abnormal tritan discrimination. Inspection of the raw data revealed that all eyes with abnormal color discrimination, whether prematurely born or HC, or proton, deutan, or tritan axes, belonged to male subjects (data not shown).

ERG

ERG results in the entire study population and in the three subgroups are descriptively summarized in Table 3 and can be visualized as boxplots in Figure 3. The GEE did not reveal any evidence of differences in any of the ERG parameters between the EPO and placebo subgroups (Table 4). However, we observed moderate evidence against equality between the other subgroups: the mixed rod-cone b-wave peak times were shorter in eyes treated with rhEPO than those of HC subjects, as evidenced by the negative coefficient when switching from HC to EPO (coefficient/95% confidence interval (CI) = -4.33/-6.88 to -1.78; p = 0.011).

Conversely, both cone flash b-wave and flicker peak times were slightly longer in the eyes of the placebo group than those of the HC subjects, as evidenced by the positive coefficient when switching from HC to placebo (coefficient/95% CI = 0.53/0.21 to 0.85 and 0.36/0.13 to 0.60; p = 0.012 and 0.022, respectively). Results of GEE models comparing the HC results with both the EPO and placebo results are shown in Table 5.

We also compared ERG findings in the subgroups using more complex GEE models, which were adjusted for GA and ROP stage in addition to age and sex. No differences between the simple and complex models were recorded, as shown in Supplementary Figure 1, a finding consistent with our results being unaffected by the potential confounders of GA and ROP.

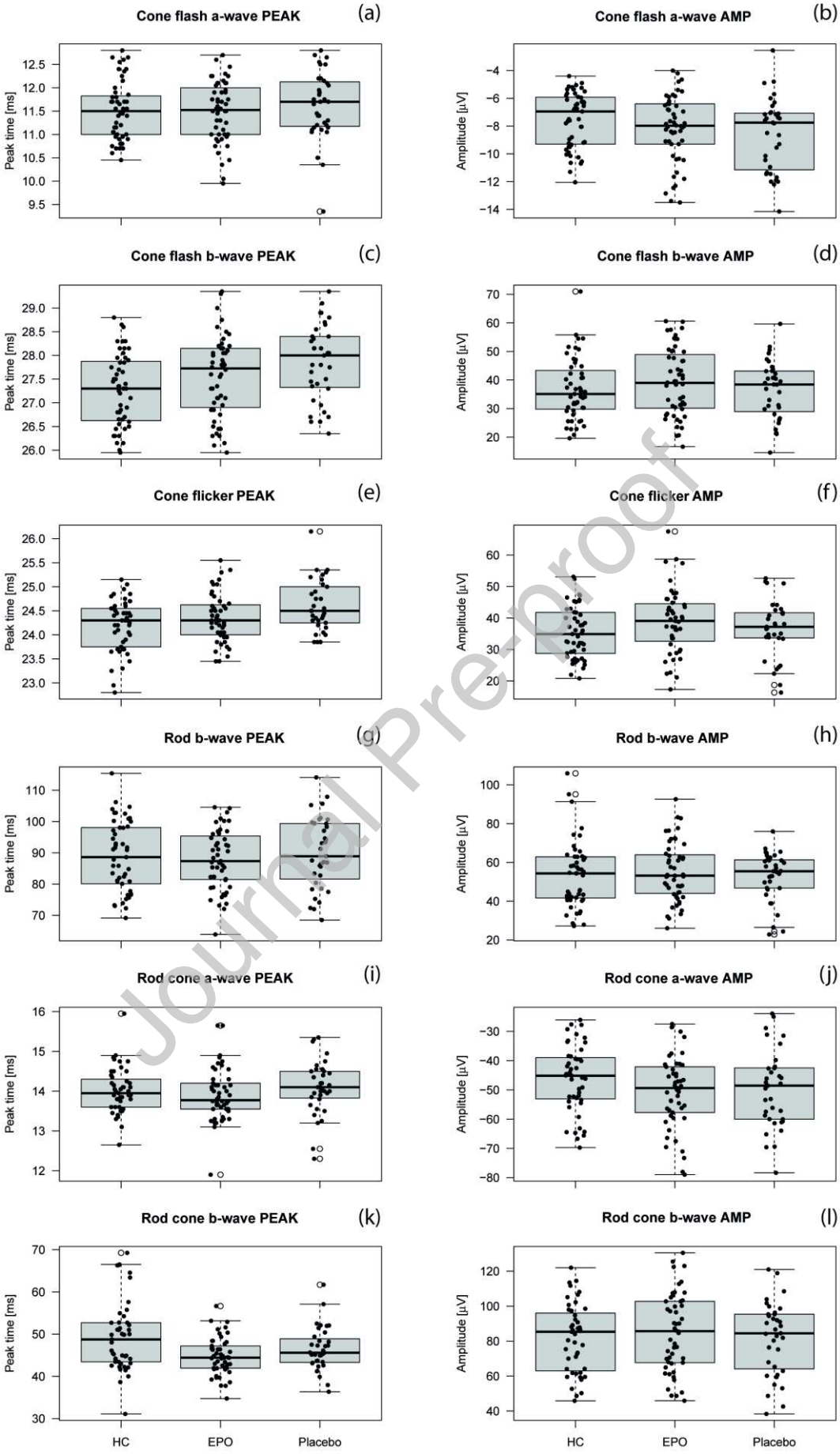

Figure 3 (a-l). Boxplots showing ERG results in healthy control (HC; leftmost boxes), EPO (middle boxes) and placebo (rightmost boxes) subgroups. Each plot is named according to the ERG parameter displayed, with PEAK indicating peak times and AMP indicating amplitudes. Median values and IQRs are indicated by horizontal lines and boxes, respectively; whiskers show the range after excluding outliers (defined as data points over 1.5 IQR from the box, i.e. Q3+1.5IQR and Q1-1.5IQR). Individual data points, including outliers when present, are represented by gray dots, horizontally jittered for clarity. All peak times are in milliseconds (ms) and all amplitudes in microvolts (μV).

Table 2: Descriptive statistics summarizing visual acuity, refractive error, and color discrimination performance (from 1 [best] to 5 [worst]) for the entire study population (overall) and the healthy control, erythropoietin, and placebo subgroups. One eye was selected at random for the descriptive summary with the exception of the color vision results, for which the left eye was used (see text for details).

Variable	Level	Overall	HC	EPO	Placebo
n		139*	52	52	35*
VA (mean (SD))		-0.03 (0.11)	-0.06 (0.11)	0.00 (0.11)	-0.04 (0.11)
Refraction (mean (SD))		0.59 (1.72)	0.43 (1.16)	0.56 (2.02)	0.86 (1.93)
Color P (%)	1	125	48 (92.3)	49 (94.2)	28 (91.2)
	2	10 (13.8)	4 (7.7)	1 (1.9)	5 (14.7)
	3 4 5	2 (1.4) 1 (0.7) 0 (0.0)	0 (0.0) 0 (0.0) 0 (0.0)	2 (3.8) 0 (0.0) 0 (0.0)	0 (0.0) 1 (2.9 0 (0.0)
Color D (%)	1	129 (93.5)	49 (94.2)	49 (94.2)	31 (91.2)
	2	5 (3.6)	1 (1.9)	2 (3.8)	2 (8.8)
	3 4 5	1 (0.7) 1 (0.7) 2 (1.4)	0 (0.0) 1 (1.9) 1 (1.9)	1 (1.9) 0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0) 1 (2.9)
Color T (%)	1	128 (92.8)	50 (96.2)	49 (94.2)	29 (85.3)
	2	7 (5.1)	2 (3.8)	1 (1.9)	4 (11.8)
	3 4 5	3 (2.2) 0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0) 0 (0.0)	2 (3.8) 0 (0.0) 0 (0.0)	1 (2.9) 0 (0.0) 0 (0.0)

D, deutan; EPO, erythropoietin; HC, healthy control; P, proton; T, tritan; VA, visual acuity (expressed as log of the minimum angle of resolution, logMAR). *Color vision test results were missing in a single participant, who had received placebo. Therefore, color discrimination results in the placebo group are from 34, not 35, eyes; similarly, the total number of study eyes in which color discrimination results were available was 138, not 139.

Table 3: ERG results for the entire study population (overall) and the healthy control, erythropoietin, and placebo subgroups. All values are expressed as mean (standard deviation).

Variable	Overall	HC	EPO	Placebo
n	139	52	52	35
Cone flash a-wave PEAK	11.53 (0.65)	11.51 (0.62)	11.48 (0.64)	11.62 (0.73)
Cone flash a-wave AMP	-8.06 (2.34)	-7.57 (2.00)	-8.17 (2.40)	-8.59 (2.62)
Cone flash b-wave PEAK	27.52 (0.83)	27.27 (0.76)	27.55 (0.84)	27.85 (0.80)
Cone flash b-wave AMP	37.64 (10.95)	36.80 (10.73)	39.18 (11.70)	36.55 (10.12)
Cone flicker PEAK	24.35 (0.55)	24.18 (0.54)	24.35 (0.51)	24.60 (0.54)
Cone flicker AMP	36.90 (9.37)	35.31 (8.16)	38.82 (10.42)	36.52 (9.25)
Rod.b-wave PEAK	88.68 (10.60)	88.75 (11.20)	87.76 (9.49)	89.92 (11.42)
Rod b-wave AMP	53.83 (15.24)	53.25 (17.19)	55.01 (15.14)	52.91 (12.56)
Rod Cone a-wave PEAK	13.99 (0.63)	14.00 (0.57)	13.90 (0.66)	14.09 (0.68)
Rod Cone.a-wave AMP	-48.83 (12.38)	-46.21 (11.47)	-50.58 (12.43)	-49.84 (13.22)
Rod Cone b-wave PEAK	46.82 (6.46)	49.34 (8.22)	44.71 (4.39)	46.46 (5.13)
Rod Cone.b-wave AMP	82.87 (20.92)	81.46 (19.65)	85.11 (22.34)	81.57 (20.80)

AMP, amplitude (µV); EPO, erythropoietin; HC, healthy control; n, number; PEAK peak times (ms).

Table 4: Results of generalized estimating equation models adjusted for age and sex comparing the ERG findings in the EPO and placebo groups. For each comparison the estimated coefficient (switching from EPO to placebo), 95% confidence intervals, and corrected p-values are given.

Variable	Coefficient	95% CI	P
Cone flash a-wave PEAK	0.12	from -0.18 to 0.42	0.70
Cone flash a-wave AMP	-0.18	from -1.11 to 0.75	0.70
Cone flash b-wave PEAK	0.28	from -0.05 to 0.61	0.70
Cone flash b-wave AMP	-3.72	from -7.65 to 0.21	0.63
Cone flicker PEAK	0.23	from -0.00 to 0.46	0.60
Cone flicker AMP	-2.98	from -6.65 to 0.69	0.70
Rod b-wave PEAK	1.64	from -2.81 to 6.10	0.70
Rod b-wave AMP	-3.7	from -8.72 to 1.31	0.70
Rod Cone a-wave PEAK	0.098	from -0.17 to 0.37	0.70
Rod Cone a-wave AMP	2.99	from -1.57 to 7.55	0.70
Rod Cone b-wave PEAK	2.15	from 0.20 to 4.10	0.37
Rod Cone b-wave AMP	-6.46	from -14.50 to 1.58	0.70

AMP, amplitude (µV); CI, confidence interval; EPO, erythropoietin; PEAK peak times (ms).

Table 5: Results of generalized estimating equation models adjusted for age and sex comparing the ERG findings in the HC and placebo, and HC and EPO, groups. For each comparison the estimated coefficient (switching from HC to the indicated intervention subgroups), 95% confidence intervals, and corrected p-values are given. Corrected p-values <0.05 are highlighted in bold.

		HC vs. EPO			HC vs. placebo	
Variable	**Coefficient**	**95% CI**	**P**	**Coefficient**	**95% CI**	**P**
Cone flash a-wave PEAK	-0.086	from -0.33 to 0.16	0.96	0.068	from -0.23 to 0.36	0.72

Cone flash a-wave AMP	-0.99	from -1.87 to -0.11	0.22	-1.29	from -2.24 to -0.34	0.08
Cone flash b-wave PEAK	0.26	from -0.03 to 0.56	0.5	0.53	from 0.21 to 0.85	**0.012**
Cone flash b-wave AMP	4.85	from 0.65 to 9.05	0.21	0.99	from -3.17 to 5.15	0.72
Cone flicker PEAK	0.16	from -0.06 to 0.37	0.68	0.36	from 0.13 to 0.60	**0.022**
Cone flicker AMP	4.98	from 1.39 to 8.57	0.07	1.84	from -1.64 to 5.33	0.72
Rod b-wave PEAK	-0.11	from -4.20 to 3.98	0.96	2.04	from -2.80 to 6.89	0.72
Rod b-wave AMP	4.12	from -1.82 to 10.06	0.68	1.09	from -4.43 to 6.61	0.72
Rod Cone a-wave PEAK	-0.05	from -0.30 to 0.20	0.96	0.091	from -0.17 to 0.35	0.72
Rod Cone a-wave AMP	-6.54	from -11.24 to -1.84	0.066	-4.62	from -9.68 to 0.44	0.58
Rod Cone b-wave PEAK	-4.33	from -6.88 to -1.78	**0.011**	-2.7	from -5.47 to 0.07	0.5
Rod Cone b-wave AMP	7.2	from -0.87 to 15.26	0.5	1.46	from -6.66 to 9.59	0.72

AMP, amplitude (µV); CI, confidence intervals; EPO, erythropoietin; HC, healthy control; PEAK, peak time (ms).

Discussion

Our study did not show a measurable effect of prophylactic treatment with high-dose rhEPO in the first days after birth on retinal function in extremely and very prematurely born children, either positive or negative, as demonstrated by the lack of evidence for any significant differences in ERG findings between the EPO and placebo subgroups. Hence, potential adverse effects on future retinal function can likely be discounted when assessing the utility of EPO treatment in prematurely born infants.

However, we did observe differences between the groups which may be instructive. Subjects treated with high-dose rhEPO were found to have moderate evidence of faster mixed rod-cone ERG b-wave peak times compared to HCs. This finding is

consistent with a beneficial effect of rhEPO administration on rod system function, which is the primary driver of mixed rod-cone ERG responses due to the far greater number of rods than cones.[40] As the isolated rod b-wave tends to have a much broader peak than the combined rod-cone b-wave (for example, see Figure 2c-d), peak time measurements for this parameter tend to be less precise. We therefore do not interpret the lack of evidence for differences in isolated rod b-wave peak time as inconsistent with a potential beneficial effect of rhEPO on rod function. Although this difference in function at the retinal level may or may not be clinically relevant, it is nevertheless potentially encouraging when considering retinal effects of rhEPO.

Conversely, we recorded moderate evidence of post-receptoral cone system dysfunction in prematurely born children receiving placebo, as indicated by slower cone b-wave and flicker peak times relative to HC, but not in those treated with rhEPO. This finding is consistent with both a negative effect of premature birth on cone system function,[15] and mitigation of this negative effect by treatment with rhEPO. Inspection of Table 5 shows that this significant effect of prematurity on the cone system appears rather small, and thus may not be clinically relevant. Nevertheless, this result is broadly consistent with previous findings[10,12] recorded in childhood. We did not observe any evidence of significant differences in ERG amplitudes between the subgroups, which may be at least in part due to the skin electrodes employed here producing relatively low ERG amplitudes compared to corneal electrodes[41] used in other studies.[10,14] This could potentially make detection of small inter-group amplitude differences more challenging. Other authors, using the same ERG device and skin electrodes employed in the present study, also did not observe any effects of premature birth on flicker ERG amplitude.[12]

Findings of reduced ERG amplitudes and/or prolonged peak times (relative to normative/reference ranges) would generally be considered undesirable, particularly when evaluated in the context of clinical signs and/or symptoms suggestive of retinal disease. The consensus regarding supernormal (shorter) peak times such as those described here in the EPO group relative to HC, or indeed amplitudes above normative limits, is less clear. However, we are only aware of one example where a shorter peak time would be viewed as pathological: in complete (Type 1) congenital stationary night blindness, the rod-cone b-wave peak time is frequently supernormal and comparable to that of the corresponding cone flash b-wave peak time (as illustrated in a recent review[42]). In such a case, the peak time is usually much shorter than normal and accompanied by an obvious qualitative change to the ERG waveform and symptoms of night blindness, rather than the quantitatively smaller degree of peak time reduction and normal ERG waveforms recorded here in asymptomatic individuals. Therefore, whilst caution precludes us from interpreting the slightly faster peak times recorded here in the EPO group relative to HC subjects as clinically relevant, we consider it highly unlikely that this result is indicative of negative retinal effects of perinatal high-dose rhEPO.

We did not record an influence of ROP and GA on results in children born preterm, as evidenced by the strong similarity between results in our standard models adjusted for age and sex, and complex models adjusted for age, sex, GA, and ROP (Supplementary Figure 1). We note, however, that very few eyes included in the analysis had a history of ROP, doubtless reflecting the low incidence of ROP in Switzerland[43,44] at the time of the initial study. Thus, our analysis likely would not have had sufficient power to detect potential subtle effects of ROP on the ERG. Previous work using more complex, non-standard scotopic ERG-derived measures of retinal function recorded differences in younger children that, at least in milder ROP,

partially resolved by the age at which our subjects were tested.[45] However, due to the different methods employed and parameters analysed, we cannot directly compare these previous results to ours. Conversely, other authors recorded prolonged flicker ERG peak times in children with previous ROP compared with those born preterm with no history of ROP when tested at approximately seven years of age, without any differences in flicker ERG amplitudes.[12] However, only six eyes with previous ROP were analysed in this previous study. Recruitment of a sufficiently large cohort of children with previous ROP would likely be extremely challenging in the majority of higher-income countries due to the low incidence of ROP, as discussed above.

Similarly, GA appeared not to exert an observable influence on our findings (Supplementary Figure 1). Our intervention cohort had a GA ranging from 162 to 223 days, equivalent to roughly 23 to 32 weeks, a broad range encompassing both very- and extremely preterm babies. We interpret this finding as indicating that potential effects of increasing prematurity on retinal function[14] have resolved by the age at which our cohort was tested, i.e. after retinal maturation is complete.

As all of the patients in whom abnormal color discrimination was recorded were male, we interpret these results (at least in the proton and deutan axes) as most likely reflecting congenital color vision anomalies, which are x- linked, typically prot- or deuteranomalous, and present in approximately 8 to 10 % % of males.[46] Although all patients with likely tritan defects (n=3) were born prematurely, we cannot definitively ascribe these results to premature birth due to the very small number of patients. For the same reason, it is not possible to infer whether treatment with EPO is more likely to cause tritan deficiency than placebo (n=2 and 1, respectively). Congenital tritan defects are also known to exist, but are much rarer than proton or deutan anomalies.[46]

Advantages of our study included the randomized, double blind nature of recruitment, assessment, and analysis, and the high number of patients and HC examined. Recruitment was likely facilitated by our ability to perform all examinations without the instillation of mydriatic agents, due to our use of the handheld device for recording the ERG. Limitations included the fact that the EPO and placebo subgroups were not of equal size (n= 52 and 35, respectively). However, this was a factor beyond our control due to the double-blind nature of the study. As the ERG in our study was recorded using skin electrodes, the response amplitudes were likely smaller than those obtained using corneal or conjunctival electrodes, and it is possible that this may have reduced our ability to detect smaller differences in ERG amplitudes between subgroups. As ROP is rare in Switzerland,[43,44] the number of our subjects with ROP was correspondingly small, and hence our conclusions with regard to possible confounding effects of ROP were limited (see also discussion above). However, the influence of ROP was not the study objective. Were ROP to have a confounding effect on our results, this effect would be correspondingly limited by the small number of study eyes with previous ROP. Our pre-planned analyses were focused upon standard and long-established ERG outcome measures of amplitude and peak time; we cannot exclude the possibility that more novel parameters (such as the time required for the ERG to reach an arbitrary criterion voltage[47]) may have provided supplementary information regarding the underlying retinal neural responses in the subgroups.

In summary, administration of high-dose rhEPO during the first days of life appears to have no measurable effects on retinal function in children born extremely or very preterm relative to placebo. Instead, it may partially mitigate the retinal effects of premature birth. Future consideration and evaluation of the utility of high-dose rhEPO administration in extremely and very preterm infants may focus on other (e.g.

neurological/neurodevelopmental) outcomes due to the exclusion of potential adverse ocular effects.

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Data Statement

Anonymized raw data will be made available to qualified investigators upon written request to the corresponding author

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Table of Contents Statement

In this double-blind randomized cross-sectional study, the authors investigated long-term effects of perinatal high-dose erythropoietin on retinal function in children aged 7-15 years born very or extremely preterm. No functional differences between erythropoietin and placebo groups were recorded. However, comparison with healthy control subjects revealed subtle effects of premature birth on retinal function, partially mitigated by erythropoietin. Potentially neuroprotective effects of erythropoietin in premature birth may be sought without anticipation of long-term retinal dysfunction.