

Does hypoxia and stress erythropoiesis compromise cardiac function in healthy adults? A randomized trial

Antonio L Arrebola

Cardiology Department, HLA Inmaculada Hospital, Granada

Rafael A Casuso

Department of Physiology, University of Granada

Jacob Bejder

Department of Nutrition, Exercise and Sports (NEXS), University of Copenhagen

Thomas Christian Bonne

Department of Nutrition, Exercise and Sports (NEXS), University of Copenhagen

Andreas B. Andersen

Department of Nutrition, Exercise and Sports (NEXS), University of Copenhagen

Jerónimo Aragón-Vela

Department of Physiology, University of Granada

Nikolai B. Nordsborg

Department of Nutrition, Exercise and Sports (NEXS), University of Copenhagen

Jesus R. Huertas (✉ jhuertas@ugr.es)

Universidad de Granada <https://orcid.org/0000-0002-7446-0857>

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Abstract

Objectives

To investigate whether recombinant human erythropoietin (rHuEPO) injections during an altitude training camp impact heart function.

Methods

Thirty-nine (16 women) moderately trained subjects stayed at 2,320 m altitude for 4 weeks while training. Subjects were randomized to placebo (isotonic saline) or rHuEPO (20 IU/kg body weight) i.v. injections. Transthoracic echocardiography imaging was acquired 3 days after arrival to altitude and prior to the first placebo or rHuEPO injection as well as one day after the last rHuEPO injection three weeks later.

Results

rHuEPO did not alter cardiovascular morphology parameters, systolic or diastolic function. In the placebo group, altitude exposure improved left ventricle (LV) systolic function due to an increased twist angle but rHuEPO had no additional effects. Pulmonary arterial systolic pressure was unaffected in either group. Notably, rHuEPO hampered LV untwist rate without affecting LV early filling.

Conclusion

rHuEPO alters LV twist rate during a high altitude training camp in a mechanism likely mediated by impaired restorative forces and/or by the haematological and metabolic effects induced rHuEPO outside the cardiovascular system. However, this alteration in heart mechanics is unlikely to have a meaningful clinical effect.

Key Points

Recombinant human erythropoietin (rHuEPO) administration during an altitude training camp does not clinically compromise cardiac function.

While rHuEPO alter left ventricle mechanics, this is likely due to a secondary response of its haematological effects.

1. Introduction

Human sea-level residents frequently travel to altitude for recreational and work purposes [1]. In addition, athletes have for decades engaged in altitude training aimed at legally enhancing sea level performance

via an increase in haemoglobin concentration and total haemoglobin mass [2–4]. However, reports exist to suggest that some athletes exploit altitude training camps to conceal a misuse of recombinant human erythropoietin (rHuEPO) [5]. The marked increase in endogenous erythropoietin (EPO) production during the first days even at moderate altitude [6, 7] in combination with prolonged (rHuEPO) treatment will increase activation of EPO receptors (EPOR).

EPOR is also expressed outside the hematopoietic system, including in cardiomyocytes [8, 9]. In rodents, a consequence of myocyte EPOR stimulation is improved hemodynamic characteristics [10, 11], reduced oxidative stress in a hypoxic situation [12] and remodelling [13]. Specifically, EPO administration improves LV function both during ischemia-reperfusion [14] and after myocardial infarction [15] in rats. In fact, it has been proposed that low dose rHuEPO administration would improve human cardiac disease through EPOR stimulation [16].

Human heart adapts its function and mechanics to preserve systolic function during altitude exposure. For instance, after several days to altitude exposure there is a reduced stroke volume [17–19] which is not due to impaired myocardial function but likely due to a decreased ventricular filling pressure [20, 21]. Moreover, to compensate for a decreased diastolic filling, there is an increase in LV twist and untwisting rate thereby maintaining LV ejection fraction [17]. Importantly, a decreased LV diastolic filling is inversely related to pulmonary arterial systolic pressure [22]. However, it is currently unknown if the mild hypoxic stress in combination with rHuEPO treatment causes clinical relevant myocardial adaptations in healthy humans. The question is of relevance from a basic physiological understanding perspective, a perspective of protecting athletes' health as well as a clinical perspective of inducing cardiac remodelling in patients. Therefore, here we aimed to study whether rHuEPO injections alter normal heart function and mechanics during mild altitude exposure.

2. Methods

2.1. Subjects

Thirty-nine (23 males, 16 females) moderately trained, sea level inhabitants and non-smoking subjects were recruited. Their anthropometric data has been described elsewhere [6]. None of the participants had been exposed to an altitude above 1,000 m for >1 month or donated blood for >3 months before the study. All the subjects gave their written informed consent to participate in a randomized, single-blinded, placebo-controlled study approved by the ethics committee of Copenhagen, Denmark (H-17036662), registered on www.clinicaltrials.gov (NCT04227665) and performed in accordance with the Declaration of Helsinki.

2.2. Design

The present study was performed during the altitude trial arm of a larger study which aimed to determine biological markers for detection of blood doping at altitude [6]. Pre data was acquired after three days upon arrival to moderate altitude (2,320 m), before the first rHuEPO injection. Participants were randomly

assigned either to blinded treatment with rHuEPO, consisting of intravenous injections of $20 \text{ IU} \times \text{kg bw}^{-1}$ epoetin alpha (Eprex, Janssen, Birkerød, Denmark), or to placebo consisting of intravenous injections of $\sim 0.3 \text{ mL}$ isotonic saline (B. Braun, Melsungen, Germany). Participants wore a blindfold during injections to maintain blinding. Treatment started on the third day following arrival to altitude and was given every second day for three weeks, resulting in a total of eleven injections. Post data was collected a day after the last injection. In this period, subjects trained daily including both resistance and endurance training. Details of the training program are reported elsewhere [23].

2.3. Echocardiography

Transthoracic echocardiography was performed using a commercially available system (Epiq-7 Philips, Andover, Netherlands) with a X5-1, 1 to 6-mHz phased-array, transducer. Images were obtained from parasternal short-axis and apical four chamber views with 2D frame rates of 60 to 100 frames/s and tissue Doppler frame rates >100 frames/s. Off-line data analysis (Qlab, Version 12.0, Philips) was performed by a cardiologist blinded to group allocation.

2.4. Two-Dimensional Echocardiography and Doppler Tissue Imaging

Two-dimensional (2D), pulsed-Doppler, and color tissue Doppler imaging were performed from parasternal short-axis. Images were obtained at the same frame rate to facilitate subsequent LV torsion and rotation analysis. The LV ejection fraction was calculated offline from 3D images (3DQ System, Philips). We defined LV length as the end-diastolic length from the mitral valve hinge point plane to the most distal endocardium at the LV apex and it was measured in the apical 4-chamber view. Pulsed-wave Doppler images were used to measure aortic valve opening/closure and mitral valve opening. Isovolumetric relaxation time (IVRT) was calculated as the difference between mitral valve opening and aortic valve closure. The left and right ventricular outflow tract diameter was measured from the parasternal long-axis view and was used to calculate stroke volume as previously [24, 25]. Cardiac output was calculated as the product of stroke volume and heart rate. Longitudinal tissue velocities were measured off-line from 2D color-coded tissue Doppler images and are reported as the average of 3 consecutive cardiac cycles.

2.5. Speckle-Tracking Echocardiographic Analysis

Myocardial deformation was measured as previously described [24]. Global LV peak systolic longitudinal strain (GLS) was calculated in an 18-segment model from the apical two-chamber, three-chamber, and four-chamber views. Global circumferential strain (GCS) measured from parasternal short-axis views at the base and apex. LV twist, systolic twist velocity and untwisting velocity were calculated by subtracting the apical frame-by-frame data from the basal data. The percentage of untwist during isovolumic relaxation time (IVRT; %UTIVRT) was calculated as follows [21]: $\% \text{UTIVRT} = [(twist \text{ at } \text{aortic} \text{ } \text{valve} \text{ } \text{closure} - \text{twist} \text{ at } \text{the} \text{ } \text{end} \text{ } \text{of} \text{ } \text{IVRT}) / \text{twist} \text{ at } \text{aortic} \text{ } \text{valve} \text{ } \text{closure}] \times 100$.

2.6. Statistics

Data are reported as mean \pm standard deviation. Normality was evaluated by a Kolmogorov-Smirnov tests. Data were analysed using a 2 (Groups) \times 2 (Time) repeated measures ANOVA, within subject design. Significant interactions was further evaluated by Student's *t* test for independent samples (rHuEPO vs. placebo). Statistical significance was set at $P < .05$ and we considered $P < 0.08$ as a statistical tendency. Analyses were performed using SPSS version 20.

3. Results

3.1. General cardiovascular characteristics

Heart rate, diastolic blood pressure and mean blood pressure were reduced in both placebo and rHuEPO groups following 3 weeks of moderate altitude exposure, whereas systolic blood pressure remained unchanged (Table 1). No time \times treatment effect was observed for either of these parameters.

Table 1

Resting heart rate and blood pressure responses to rHuEPO administration during a high altitude training camp.

	Pre		Post		<i>Altitude training effect</i>	<i>Time X Treatment Effect</i>
	Placebo (n)	rHuEPO (n)	Placebo (n)	rHuEPO (n)		
Heart rate	61.9 \pm 9.47 (18)	63.1 \pm 8.68 (12)	59.3 \pm 11.77 (18)	53.9 \pm 10.0 (12)	0.033	0.224
Systolic BP	121.8 \pm 8.95 (18)	115.2 \pm 8.74 (11)	121.2 \pm 9.75 (18)	114.7 \pm 8.25 (11)	0.757	0.952
Diastolic BP	72.0 \pm 5.65 (18)	66.5 \pm 5.63 (11)	65.4 \pm 6.24 (18)	62.1 \pm 5.63 (11)	0.003	0.529
Mean BP	88.6 \pm 6.18 (18)	82.5 \pm 8.25 (11)	84.0 \pm 5.50 (18)	79.4 \pm 5.57 (11)	0.027	0.678

Results are means \pm SD. Abbreviations: BP, Blood pressure; rHuEPO, recombinant human erythropoietin

3.2. Morphological parameters

Table 2 summarize several representative cardiac morphological parameters. No effects were observed for any of the measured parameters including end-diastolic volume, ejection fraction, end-systolic volume, LV mass, RV volume and left atrial mass following moderate altitude exposure or rHuEPO injections.

Table 2
Cardiac morphology and functional parameters.

	Pre		Post		Altitude training Effect	Training X Treatment Effect
	Placebo (n)	rhEPO (n)	Placebo (n)	rhEPO (n)		
Morphological parameters						
EDV (ml)	123.5 ± 31.02 (19)	124.0 ± 24.69 (12)	125.7 ± 31.51 (19)	124.5 ± 27.81 (12)	0.858	0.914
EF (%)	63.9 ± 4.07 (19)	64.1 ± 7.04 (12)	62.2 ± 5.94 (19)	61.6 ± 6.22 (12)	0.163	0.796
ESV (ml)	44.5 ± 12.24 (19)	44.8 ± 13.36 (12)	47.0 ± 11.88 (19)	48.2 ± 14.45 (12)	0.383	0.894
LV mass Dias(g)	172.3 ± 47.70 (19)	170.2 ± 35.09 (12)	165.5 ± 48.29 (19)	157.6 ± 28.62 (12)	0.387	0.797
LV mass syst (g)	107.2 ± 36.76 (19)	106.4 ± 28.72 (12)	109.1 ± 37.07 (19)	102.9 ± 23.07 (12)	0.930	0.753
LA vol (ml)	53.6 ± 17.74 (14)	53.1 ± 15.7 (8)	51.5 ± 13.81 (14)	52.5 ± 10.99 (8)	0.771	0.871
RV diam basal	38.1 ± 7.53 (18)	39.6 ± 5.68 (11)	38.2 ± 6.18 (18)	39.9 ± 4.90 (11)	0.889	0.944
Systolic Function						
<i>Left ventricle</i>						
LV SV	98.2 ± 24.87 (19)	105.3 ± 22.43 (12)	101.5 ± 26.08 (19)	109.6 ± 25.54 (12)	0.561	0.935
LV CO	6.03 ± 1.57 (18)	6.59 ± 1.38 (12)	5.92 ± 1.25 (18)	5.84 ± 1.53 (12)	0.260	0.420

Results are means ± SD. Abbreviations: EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; LV mass Dias, left ventricle mass in diastole; LV mass syst, left ventricle mass in systole; LA vol, left atrial volume; RV diam basal, right ventricle basal diameter; LV SV, left ventricle systolic volume; LV CO, left ventricle cardiac output; S' mean, mean S' wave in the medial and lateral mitral annulus; RV SV, right ventricle systolic volume; RV CO, right ventricle cardiac output; S' RV, S' wave in the lateral tricuspid annulus; PAAT, pulmonary arterial acceleration time; Peak E, peak E wave in the mitral flow; Peak A, peak A wave in the mitral flow; E/A ratio, peak E and peak A ratio; E' mean, mean lateral and medial E' wave in the mitral annulus; A' mean, mean lateral and medial A' wave in the mitral annulus; E/E'lat, ratio between E wave in the mitral flow and E' wave in the lateral mitral annulus; E'RV, peak E' wave in the lateral tricuspid annulus; A'RV, peak A' wave in the lateral tricuspid annulus.

	Pre		Post		Altitude training Effect	Training X Treatment Effect
	Placebo (n)	rhEPO (n)	Placebo (n)	rhEPO (n)		
S' mean (cm s ⁻¹)	8.33 ± 1.15 (17)	9.17 ± 1.17 (8)	8.71 ± 1.39 (17)	8.42 ± 0.97 (8)	0.621	0.131
<i>Right ventricle</i>						
RV SV	107.2 ± 41.79 (8)	72.6 ± 26.09 (8)	88.4 ± 23.19 (8)	82.2 ± 16.62 (8)	0.649	0.168
RV CO	6.09 ± 2.53 (8)	4.44 ± 1.45 (8)	4.98 ± 1.80 (8)	4.38 ± 1.38 (8)	0.380	0.427
S' RV (cm s ⁻¹)	13.0 ± 2.34 (17)	13.5 ± 1.72 (11)	12.6 ± 1.65 (17)	12.1 ± 0.96 (11)	0.077	0.302
PAAT (ms)	138.6 ± 28.08 (10)	133.3 ± 24.66 (9)	130.0 ± 12.79 (10)	125.7 ± 13.83 (9)	0.241	0.946
<i>Diastolic Function</i>						
<i>Left ventricle</i>						
Peak E (cm s ⁻¹)	79.8 ± 16.29 (14)	78.2 ± 14.4 (10)	79.8 ± 16.04 (14)	76.3 ± 12.27 (10)	0.832	0.836
Peak A (cm s ⁻¹)	45.1 ± 11.48 (14)	50.4 ± 10.91 (10)	40.4 ± 7.85 (14)	41.4 ± 5.03 (10)	0.016	0.440
E/A ratio	1.85 ± 0.56 (14)	1.59 ± 0.31 (10)	2.02 ± 0.48 (14)	1.85 ± 0.27 (10)	0.097	0.724
E' mean (cm s ⁻¹)	14.0 ± 2.06 (17)	12.5 ± 2.18 (8)	14.4 ± 2.35 (17)	13.9 ± 1.55 (8)	0.146	0.419
A' mean (cm s ⁻¹)	6.59 ± 1.50 (17)	6.91 ± 1.60 (8)	5.96 ± 1.28 (17)	5.85 ± 0.90 (8)	0.047	0.597

Results are means ± SD. Abbreviations: EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; LV mass Dias, left ventricle mass in diastole; LV mass syst; left ventricle mass in systole; LA vol; left atrial volume; RV diam basal, right ventricle basal diameter; LV SV, left ventricle systolic volume; LV CO; left ventricle cardiac output; S' mean, mean S' wave in the medial and lateral mitral annulus; RV SV, right ventricle systolic volume; RV CO, right ventricle cardiac output; S' RV, S' wave in the lateral tricuspid annulus; PAAT, pulmonary arterial acceleration time; Peak E, peak E wave in the mitral flow; Peak A, peak A wave in the mitral flow; E/A ratio, peak E and peak A ratio; E' mean; mean lateral and medial E' wave in the mitral annulus; A' mean, mean lateral and medial A' wave in the mitral annulus; E/E'lat, ratio between E wave in the mitral flow and E' wave in the lateral mitral annulus; E'RV, peak E' wave in the lateral tricuspid annulus; A'RV, peak A' wave in the lateral tricuspid annulus.

	Pre		Post		Altitude training Effect	Training X Treatment Effect
	Placebo (n)	rhEPO (n)	Placebo (n)	rhEPO (n)		
E' mean/A' mean ratio	2.24 ± 0.63 (17)	1.91 ± 0.61 (8)	2.52 ± 0.65 (17)	2.44 ± 0.49 (8)	0.033	0.500
E/E'lat	5.14 ± 1.49 (13)	5.7 ± 1.39 (6)	5.1 ± 1.61 (13)	4.66 ± 0.60 (6)	0.343	0.317
<i>Right ventricle</i>						
E'RV (cm s⁻¹)	12.8 ± 2.29 (16)	13.6 ± 2.64 (12)	11.7 ± 2.65 (16)	12.2 ± 2.31 (12)	0.066	0.873
A'RV (cm s⁻¹)	8.4 ± 2.54 (16)	9.1 ± 2.41 (12)	7.7 ± 1.9 (16)	8.5 ± 2.5 (16)	0.319	0.950

Results are means ± SD. Abbreviations: EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; LV mass Dias, left ventricle mass in diastole; LV mass syst; left ventricle mass in systole; LA vol; left atrial volume; RV diam basal, right ventricle basal diameter; LV SV, left ventricle systolic volume; LV CO, left ventricle cardiac output; S' mean, mean S' wave in the medial and lateral mitral annulus; RV SV, right ventricle systolic volume; RV CO, right ventricle cardiac output; S'RV, S' wave in the lateral tricuspid annulus; PAAT, pulmonary arterial acceleration time; Peak E, peak E wave in the mitral flow; Peak A, peak A wave in the mitral flow; E/A ratio, peak E and peak A ratio; E' mean; mean lateral and medial E' wave in the mitral annulus; A' mean, mean lateral and medial A' wave in the mitral annulus; E/E'lat, ratio between E wave in the mitral flow and E' wave in the lateral mitral annulus; E'RV, peak E' wave in the lateral tricuspid annulus; A'RV, peak A' wave in the lateral tricuspid annulus.

3.3. Left and right ventricle systolic function

Both LV stroke volume, CO and S' mean wave in mitral annulus (S'_{mean}) remained unchanged throughout the analysed time points and were not influenced by rHuEPO (Table 2). Similarly, RV stroke volume and CO were unaffected by moderate altitude exposure and by rHuEPO. In addition, pulmonary artery acceleration time (PAAT) was unaffected by either condition. However, we observed a trend ($p = 0.077$) towards an increase of the S' wave in the lateral tricuspid annulus (S'_{RV}) in the placebo group without being affected by rHuEPO (Table 2).

3.4. Left and right ventricle diastolic function

We found that late filling (peak A wave) was reduced by moderate altitude exposure while left ventricle early filling was unchanged (peak E wave) (Table 2). In this regard, we have not observed a reduced early-to-late transmitral filling velocity (E/A ratio). Nevertheless, there was a reduced transmitral tissue velocity as we noted a reduced ratio between mean lateral and medial E' wave in the mitral annulus (E'_{mean}) and A' mean (mean lateral and medial A' wave in the mitral annulus), mainly due to a decrease in A' mean (Table 2). Finally, there was no effect on a filling pressure index (E/E'_{lat}) in the analysed subjects. Importantly, none of these parameters were altered by rHuEPO injections (Table 2). Regarding the RV, no rHuEPO

effect was observed in transtricuspid tissue velocity, but moderate altitude exposure tended ($p = 0.066$) to decrease E'_{RV} (Table 2).

3.5. Left ventricle mechanics

GCS and peak twist were increased during altitude exposure without an effect of rHuEPO (Table 3). No effect of altitude was observed for LV relaxation parameters such as % Untwist under IVRT or peak untwisting velocity. However, an interaction effect was observed for peak untwisting velocity (Table 3). In fact, rHuEPO subjects showed a ~30 percentage points reduction over time, which resulted in a significant lower peak untwisting velocity if compared with placebo subjects at post.

Table 3
Left ventricle ventricular strain and twisting mechanics.

	Pre		Post		<i>Altitude training effect</i>	<i>Time X Treatment effect</i>
	Placebo (n)	rHuEPO (n)	Placebo (n)	rHEPO (n)		
GCS (%)	24.3 ± 2.91 (19)	25.1 ± 3.43 (12)	27.9 ± 4.16 (19)	26.8 ± 2.80 (12)	0.005	0.280
GLS (%)	18.9 ± 2.15 (19)	18.3 ± 2.13 (12)	18.8 ± 1.94 (19)	19.0 ± 1.99 (12)	0.545	0.441
Twist peak (°)	5.03 ± 2.14 (18)	4.78 ± 3.14 (11)	6.32 ± 2.31 (18)	6.67 ± 1.91 (11)	0.020	0.934
Peak twisting velocity (°/seg)	50.4 ± 23.84 (18)	51.2 ± 24.75 (11)	51.4 ± 21.67 (18)	46.0 ± 11.38 (11)	0.725	0.599
% Untwist under IVRT	55.0 ± 17.54 (17)	62.4 ± 22.89 (12)	65.4 ± 19.66 (17)	54.9 ± 22.89 (12)	0.792	0.107
Peak untwisting velocity (°/seg)	81.5 ± 32.35 (18)	97.7 ± 51.00 (12)	91.8 ± 33.82 (18)	67.3 ± 26.57 (12)*	0.296	0.038
Untwist velocity/peak twist	22.5 ± 20.57 (17)	24.7 ± 17.03 (12)	16.7 ± 13.83 (17)	12.6 ± 8.54 (12)	0.040	0.464

Results are means ± SD. Abbreviations: GCS, global circumferential strain; GLS, global longitudinal strain; IVRT, interventricular relaxation time. * $p < 0.05$ vs Placebo Post.

4. Discussion

The present study analysed heart function and mechanics during moderate altitude exposure in subjects administered with rHuEPO or isotonic saline. Here, we show that rHuEPO injections during an altitude training camp do not importantly alter heart function. We observed a blunted untwisting velocity induced

by rHuEPO which may suggest an impaired LV relaxation during diastole. However, this effect could also reflect that subjects receiving rHuEPO do not need to activate a compensatory mechanism such us untwisting in order to maintain a given CO.

4.1. Methodological considerations

Several important methodological considerations should be taken into account when interpreting our data. Indeed, preliminary data was collected during acclimatisation (i.e. 3 days after arrival to 2230m), immediately before rHuEPO and placebo injections. This is of importance as most of the acute effects of hypoxia on the heart are evident within the first 4 days following exposure [17]. For instance, early (E wave) and late (A wave) left ventricular filling are not altered after 2 days of HA exposure [26] while a reduced early ventricular filling is evident after 2-6 days in hypoxia [17]. In the present study, we observed an increment of the E/A ratio by means of reduced late filling. While this seems to be inconsistent with the literature [20, 21, 25, 27], this is probably a result of a long-term normalization following altitude acclimatization. In agreement, a rapid increase in late filling has been reported upon arrival to 4320m above sea level [17]. In this regard, it must be highlighted that the present study design does not allow to reach conclusions on the effects of mild hypoxic exposure on heart function but may provide indications of longer term adaptation to mild hypoxia.

In addition, PAAT, an accurate marker of pulmonary arterial systolic pressure [28], was unaltered under our experimental conditions. Thus, although inferred from an indirect measurement, pulmonary arterial systolic pressure appears unaltered by moderate altitude as well as by rHuEPO.

4.2. Left ventricle filling and mechanics in response to rHuEPO

The store and release of energy through elastic components is a key process linking systole and diastole. The diastole phase consists of two phases: initially, due to the stored energy of the elastic component, the pressure gradient between the atria and the ventricles is high and ventricular filling is rapid (the phase of rapid early filling). Under normal circumstances about 70% of ventricular filling occurs during this phase. As diastole progresses, ventricular pressure rises and the rate of filling slows (i.e. diastasis phase). The final ~25% of filling during ventricular diastole results from atrial contraction (i.e. atrial systole phase) [29].

Untwisting occurs during the diastole phase of rapid early filling and is directly influenced by the relaxation of cardio myocytes and the release of potential energy stored during systole [30]. Interestingly, we found that untwisting velocity decreased in the rHuEPO group following 3 weeks of altitude exposure. Moreover, while placebo group increased %UTIVRT by ~20%, the rHuEPO group showed a ~13% decrement. There are two potential reasons for these observations. In one hand, rHuEPO may alter early diastolic filling during HA exposure; however, E'_{mean} was not altered by rHuEPO thus excluding a potential alteration in myocardial relaxation. Moreover, we found no effect of rHuEPO in early diastolic filling (E wave) or filling pressures. Therefore, it seems unlikely that rHuEPO alterations of myocardial mechanics

results in altered early LV filling. However, further studies perhaps examining subendocardial and subpericardial are needed in order to elucidate whether rHuEPO could differently affect either layer during altitude exposure.

On the other hand, rHuEPO is better known for its effect outside the cardiovarcular system in humans. For instance, increased red cell production, angiogenesis, myogenesis, shift in muscle fibre types, and oxidative enzyme activities in skeletal muscle has been reported [31] that may also lead to improve maximal oxygen uptake [32]. In this scenario, rHuEPO may help to maintain a given CO through haematological and metabolic mechanisms without an increased untwisting rate. In agreement with this hypothesis, subjects in the rHuEPO group showed higher erythropoietic response than subjects in placebo group [6]. Therefore, while the rHuEPO dose used in the present study is of physiological relevance [32], it seems unlikely to alter cardiomyocyte function.

Finally, it has been reported that untwist velocity increases immediately in response to altitude exposure, an effect that is maintained for 4 days, but which is normalized to sea levels after 6 days [17]. Thus, the lack of effect of altitude on this heart mechanics parameter could be easily explained by the fact that our preliminar measurements were obtained 3 days after arrival to altitude. In contrast, we were able to detect the common observation of increased twist angle in response to altitude exposure [17, 21, 25, 29]. In this regard, it is important to highlight that endurance training decreases twist angle without altering untwist velocity [34]. Thus, as our sample was already trained subjects then, an increased twist angle would be easier to detect even with the design limitations discussed above.

5. Conclusions

Here we show that low dose rHuEPO injections provided during mild hypoxic exposure do not cause any major effects on heart function. Thus, no immediate health hazards are obvious for athletes training at altitude and no obvious beneficial effects of mild hypoxic exposure and / or rHuEPO treatment can be expected as evaluated on a young symptom free healthy population.

Declarations

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Conflict of interest: The authors declare no conflict of interest.

Ethical approval: All procedures performed were in accordance with the ethical standards outlined by the institutional research committees of the ethics committee of University of Copenhagen, Denmark (H-

17036662) and University of Granada and in conformity with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Data availability: All data related to this study are presented within the manuscript.

Author contributions: NBN, JRH, JB, TCB and ABA conceived the study. ALR and JAV collected the data. RAC and ALR analysed the data. All authors interpreted the data. RAC wrote the first draft of the manuscript. All authors critically revised and contributed to the manuscript. All authors approved the final version of the manuscript.

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