Respiratory Physiology & Neurobiology 225 (2016) 38–47

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Effects of erythropoietin on systemic hematocrit and oxygen transport in the splenectomized horse

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ARTICLE INFO

Article history:
Received 5 September 2015
Received in revised form 30 January 2016
Accepted 2 February 2016
Available online 4 February 2016

Keywords:
Maximal aerobic capacity
Perfusive and diffusive O_2 transport
Cardiac output
Arteriovenous O_2 difference

ABSTRACT

To test the hypotheses that erythropoietin (rhuEPO) treatment increases systemic hematocrit, maximal O_2 uptake ($\dot{V}O_2$max, by elevated perfusive and diffusive O_2 conductances) and performance five female horses (4–13 years) received 15 IU/kg rhuEPO (erythropoietin) three times per week for three weeks. These horses had been splenectomized over 1 year previously to avoid confounding effects from the mobilization of splenic red blood cell reserves. Each horse performed three maximal exercise tests (one per month) on an inclined (4°) treadmill to the limit of tolerance; two control trials and one following EPO treatment. Measurements of hemoglobin concentration ([Hb] and hematocrit), plasma and blood volume, $\dot{V}O_2$, cardiac output as well as arterial and mixed venous blood gases were made at rest and during maximal exercise. EPO increased resting [Hb] by 18% from 13.3 ± 0.6 to 15.7 ± 0.8 g/dL (mean ± SD) corresponding to an increased hematocrit from 36 ± 2 to 46 ± 2% concurrent with 23 and 10% reductions in plasma and blood volume, respectively (all $P < 0.05$). EPO elevated $\dot{V}O_2$max by 20% from 25.7 ± 1.7 to 30.9 ± 3.4 L/min ($P < 0.05$) *via* a 17% increase in arterial O_2 content and 18% greater arteriovenous O_2 difference in the face of an unchanged cardiac output. To achieve the greater $\dot{V}O_2$max after EPO, diffusive O_2 conductance increased ~30% (from 580 ± 76 to 752 ± 166 mL O_2/mmHg/min, $P < 0.05$) which was substantially greater than the elevation of perfusive O_2 conductance. These effects of EPO were associated with an increased exercise performance (total running time: control, 216 ± 72; EPO, 264 ± 48 s, $P < 0.05$). We conclude that EPO substantially increases $\dot{V}O_2$max and performance in the splenectomized horse *via* improved perfusive and diffusive O_2 transport.

1. Introduction

The maximal O_2 uptake ($\dot{V}O_2$max) defines the upper limit for O_2 transport from the atmosphere to its site of reduction in the mitochondrial electron transport chain. Across a broad range of species $\dot{V}O_2$max is determined by O_2 supply as distinct from the mitochondrial oxidative capacity (rev. Wagner et al., 1997; Poole and Erickson, 2011; but see also Weibel and Hoppeler, 2005). Supporting the highly integrated functioning of the O_2 transport system, for the whole body (Roca et al., 1989, 1992) or exercising muscle(s) (Hogan et al., 1988, 1989, 1990, 1991), perfusive (blood flow x arterial [O_2]) and diffusive (transmembrane O_2 transport) conductances conflate to yield a given $\dot{V}O_2$max (rev. Wagner et al., 1997).

It is generally, but not always (Gonzalez et al., 1994; Prommer et al., 2007), recognized that total hemoglobin (and therefore red blood cell) mass, an index of O_2 delivery potential, correlates closely with $\dot{V}O_2$max in humans (Buick et al., 1980; Convertino, 1991; Ekblom et al., 1976; Gledhill, 1985; Saltin and Strange, 1992; Schaffartzik et al., 1993; Woodson et al., 1978; rev. Schmidt and Prommer, 2010), horses (Wagner et al., 1995) rats (Gonzalez et al., 1994) and dogs (Hsia et al., 2007). In marked contrast, relatively scant attention has been afforded the contribution of the whole body or muscle(s) O_2 diffusing capacity and how it might be impacted by altered [hemoglobin] and what research there is on

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http://dx.doi.org/10.1016/j.resp.2016.02.001
1569-9048/

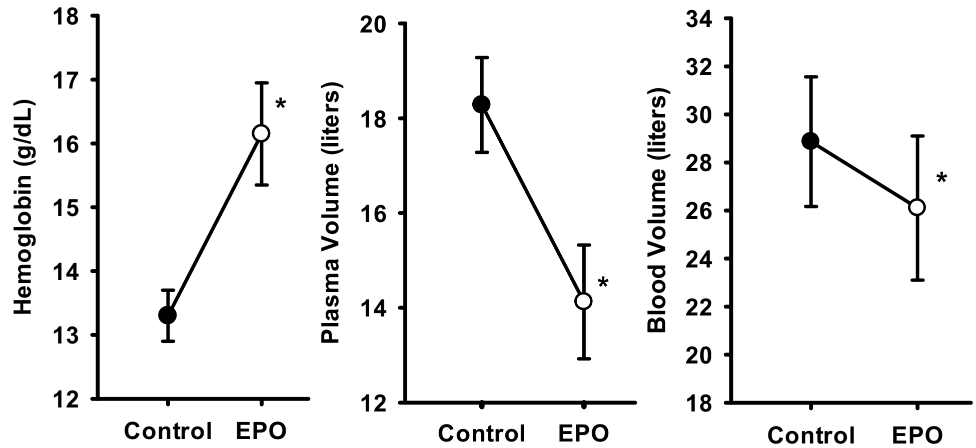

Fig. 1. Effects of erythropoietin rhuEPO administration on mean (±SD) resting hemoglobin concentration, plasma volume and total blood volume. *$p < 0.05$ erythropoietin (EPO) *versus* Control.

Table 1
Body mass, blood measurements, and vascular volumes.

	[a]Control GXT #1	Control GXT #2	Average control GXTs 1 & 2	Control C.V.	Post-EPO GXT
Body mass (kg)	429 ± 23	423 ± 23	426 ± 20	1.9%	411 ± 21
Hemoglobin (g/dL)	13.8 ± 0.6	12.9 ± 0.4	13.3 ± 0.6	5.3%	15.7 ± 0.8*
Hematocrit (%)	36 ± 2	37 ± 2	36 ± 2	1.3%	46 ± 2*
Plasma total solids(g/dL)	9.6 ± 0.6	10.0 ± 0.4	9.8 ± 0.5	2.3%	10.0 ± 0.2
Plasma volume (L)	18.4 ± 1.6	18.3 ± 1.4	18.3 ± 1.5	2.6%	14.1 ± 1.2*
Red cell volume (L)	10.4 ± 1.4	10.6 ± 1.2	10.5 ± 1.1	2.7%	12.0 ± 1.5*
Blood volume (L)	28.8 ± 3.0	28.9 ± 2.5	28.8 ± 2.7	2.3%	26.1 ± 2.0*

[a] Values represent means ± SD for each variable measured during graded exercise tests to fatigue (GXT). Control GXT 1 and Control GXT 2 were performed 4 weeks apart and prior to administration of EPO. The "control" coefficient of variation (CV) is presented to demonstrate the variability between measurements made in Control GXT #1 *versus* Control GXT #2. Means with and asterisk (*) are different ($P < 0.05$).

Table 2
Oxygen uptake and cardiovascular data.

		[a]Control GXT #1	Control GXT #2	Average control GXTs 1 & 2	Control C.V.	Post-EPO GXT
Oxygen uptake (mL/Kg/min)	Rest	3.8 ± 0.4	3.8 ± 0.7	3.8 ± 0.2	23.5%	4.4 ± 0.6
	$\dot{V}O_2$max	60.6 ± 14.7	60.4 ± 12.8	60.5 ± 13.8	5.7%	72.0 ± 15.3*
Cardiac output (L/min)	Rest	34 ± 7	32 ± 10	33 ± 9	32.0%	37 ± 9
	$\dot{V}O_2$max	150 ± 32	149 ± 28	148 ± 33	8.5%	151 ± 33
Heart rate (beats/min)	Rest	43 ± 14	46 ± 10	44 ± 12	20.2%	44 ± 11
	$\dot{V}O_2$max	201 ± 3	200 ± 3	201 ± 3	1.7%	198 ± 4
Mean arterial pressure (mmHg)	Rest	124 ± 10	118 ± 13	121 ± 12	8.1%	120 ± 12
	$\dot{V}O_2$max	132 ± 21	135 ± 15	133 ± 18	6.8%	136 ± 19
Total peripheral resistance (mmHg/L/min)	Rest	4.0 ± 0.9	4.0 ± 1.3	4.0 ± 1.1	25.5%	3.0 ± 1.0
	$\dot{V}O_2$max	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	12.8%	0.9 ± 0.1
Right atrial pressure (mmHg)	Rest	5.0 ± 0.9	0.9 ± 1.6	2.3 ± 2.4	99.7%	2.0 ± 2.0
	$\dot{V}O_2$max	2.4 ± 3.2	0.9 ± 3.4	1.4 ± 3.2	263.4%	−5.0 ± 4.0
Right ventricular pressure (mmHg)	Rest	54 ± 8	53 ± 5	53 ± 5	4.8%	55 ± 7
	$\dot{V}O_2$max	125 ± 12	118 ± 16	121 ± 11	4.7%	122 ± 15

[a] Values represent means ± SD for each variable measured during incremental exercise tests to fatigue (GXT). Control GXT 1 and Control GXT 2 were performed 4 weeks apart and prior to administration of EPO. The "control" coefficient of variation (CV) is presented to demonstrate the variability between measurements made in Control GXT #1 *versus* Control GXT #2. Means with and asterisk (*) are different ($P < 0.05$).

the topic paints a controversial picture. Currently accepted models (Federspiel and Popel, 1986; Groebe and Thews, 1990) and experimental evidence in frog skin (Malvin and Wood, 1992) support that O_2 diffusing capacity is determined by the number of red blood cells in the capillaries immediately adjacent to the muscle fibers or tissue. Thus, if microvascular hematocrit were to change in concert with systemic hematocrit, alterations in systemic [hemoglobin] induced by blood transfusion or the action of erythropoietin, for example, should translate directly to proportional changes in O_2 diffusing capacity during maximal exercise. However, direct measurements in the microcirculation suggest that capillary hematocrit (and changes thereof) may be dissociated from systemic (Sarelius, 1989). It is not surprising, therefore, that manipulations of systemic hematocrit may (dog, Hsia et al., 2007; control rat, Gonzalez et al., 1994; horse (splenectomy), Wagner et al., 1995) or may not (humans, Lundby et al., 2008a,b; altitude acclimatized, Gonzalez et al., 1994; horse (splenectomy + transfusion), Wagner et al., 1995) impact O_2 diffusing capacity during maximal exercise.

The horse (Wagner et al., 1995; Poole and Erickson, 2011) and other highly aerobic vertebrates such as the rainbow trout