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# Haematological acclimation and re-acclimation to hypoxia in the mouse

Meaghan J. MacNutt, James L. Rupert, A. William Sheel*

*School of Kinesiology, University of British Columbia, Vancouver, BC, Canada*

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ABSTRACT

Haematological responses throughout 4 w of initial acclimation (IA) and three paradigms of re-acclimation (RA) to hypoxia ($FI_{O_2} = 0.12$) were examined in female mice. We hypothesised that (i) haematological responses would be increased during re-exposure, resulting in greater $O_2$-carrying capacity in RA compared to IA; and (ii) further improvements would occur when abbreviating the de-acclimation period to 1 w ($RA_{\downarrow DA}$) or extending the IA period to 8 w ($RA_{\uparrow IA}$). The serum [EPO] response was blunted in all RA groups compared to IA but the resulting reticulocyte response was similar in all experimental groups. The [Hb] response was the same in RA and $RA_{\downarrow DA}$ as in IA but was blunted in $RA_{\uparrow IA}$ due to a reduction in mean corpuscular Hb. The sensitivity of EPO-producing cells appears blunted but the sensitivity of erythroid precursors to EPO is enhanced by recent hypoxic exposure. Erythropoietic regulation is altered during RA in a manner that is dependent on the paradigm of initial exposure.



## 1. Introduction

The haematological response to hypoxia represents one of the most important features of the acclimation process. Haematological acclimation to hypoxia leads to progressive increases in erythrocyte count (RBC), hematocrit (Hct) and, most importantly, haemoglobin concentration ([Hb]). Consequently, along with increases in the arterial partial pressure of oxygen ($Pa_{O_2}$) brought about by increased alveolar ventilation, perfusion and diffusion capacity, arterial content ($Ca_{O_2}$) is maintained as closely as possible to normoxic levels and $O_2$ delivery to the tissues is protected (Grover and Bärtsch, 1996).

As with many components of the acclimation response, the mechanisms underlying haematological adjustments during a single sustained exposure to hypoxia have been clearly elucidated. With hypoxia, a rapid increase in [Hb] is brought about by an immediate hemoconcentration: a reduction in plasma volume (PV) and blood volume caused by a shift of fluid from the extracellular to intracellular space (Hannon et al., 1969) and/or a frank diuresis (reviewed in Hoyt and Honig, 1996). The process of erythropoiesis is also initiated almost immediately, as demonstrated by a rise in serum erythropoietin concentration ([EPO]) within 6 h of hypoxia onset (Knaupp et al., 1992) and by an increase in circulating reticulocyte count (RC) in as little as 24 h (Schobersberger et al., 2005). Reticulocytes mature over the following weeks, leading to relatively linear increases in Hct and [Hb]. When the hypoxic stimulus is removed, haematological variables gradually return to normal. The process of haematological de-acclimation from hypoxia has been previously described and is mediated by neocytolysis, whereby the most recently formed erythrocytes are targeted for destruction and phagocytised by macrophages in the spleen (Rice et al., 2001).

There is some evidence that both the hemoconcentration (Lyons et al., 1995) and erythropoietic responses (Savourey et al., 1996, 2004) to acute hypoxia are altered by previous hypoxic acclimation or acclimatisation; however, the process of haematological acclimation to sustained re-exposure has received very little attention. In trekkers who were recently monitored throughout two identical treks to high altitude (up to 5460 m) separated by 10 d at low altitude (1300 m), a significantly greater [Hb] response was observed during the second exposure (MacNutt et al., 2009, 2012). Given the number of potentially confounding factors in such field studies (i.e. diet, exercise, temperature and psychological state), we sought to determine whether or not these results would be repeatable in a controlled laboratory setting using an animal model. Previous studies of intermittent hypoxic (IH) exposure in rabbits (6 h $d^{-1}$ at ~6100 m for 30 d) demonstrated a greater and more rapid haematological response during re-acclimation (RA) compared to an initial acclimation (IA) (Jain et al., 1978). However, as there are differences between physiological consequences of intermittent

* Corresponding author at: 6108, Thunderbird Blvd, Vancouver, BC, Canada V6T-1Z3. Tel.: +1 604 822 4459.
*E-mail address:* bill.sheel@ubc.ca (A. William Sheel).

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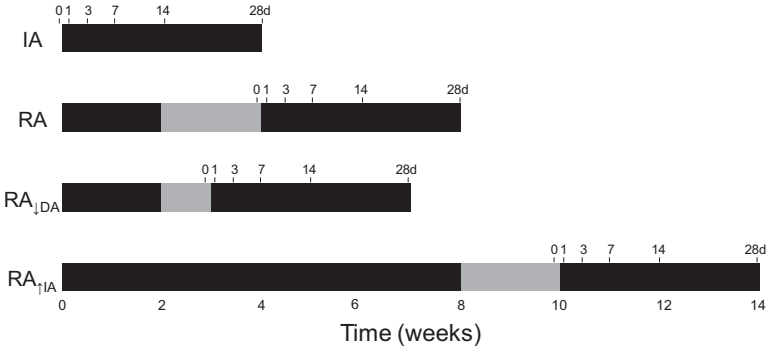


**Fig. 1.** Schematic of exposure schedule for initial exposure (IA) and three paradigms of re-exposure (RA, $RA_{\downarrow DA}$ and $RA_{\uparrow IA}$) to hypoxia. Black shading denotes periods of hypoxic exposure and grey shading denotes periods of normoxic de-acclimation (DA). Animals in each treatment group were sampled as indicated after 0, 1, 3, 7, 14 and 28 d of hypoxic exposure or re-exposure.

versus continuous hypoxia (Sheel and MacNutt, 2008), the results from Jain and colleagues cannot necessarily be extrapolated to different paradigms of hypoxic re-exposure. For this reason, our first objective was to re-address the hypothesis that the haematological acclimation to hypoxia (HAH) would occur more rapidly and/or to a greater magnitude during RA compared to IA using a model of sustained exposure and re-exposure to continuous hypoxia in a controlled laboratory environment. The second objective was to examine the effects of manipulating the duration of IA and DA periods on the time course and magnitude of haematological responses during RA. Starting with a paradigm that closely resembled the repeated altitude exposures in trekkers (MacNutt et al., 2009, 2012), it was hypothesised that extending IA or abbreviating DA would lead to further facilitation of haematological acclimation during RA. Rudimentary assessments of haematological status were employed by Jain et al. (1978) in their work on rabbits as well as in previous field work in humans, offering little insight into physiological mechanisms underlying the results reported in both studies. Thus, the third objective of the current study was to explore the process of erythropoiesis and test the hypothesis that alterations in erythropoietic control would account for increased haematological responses. We hypothesised that: (1) RBC, Hct, and [Hb] would increase faster and to a greater magnitude during hypoxic re-exposure compared to an initial exposure, and (2) RA would be further facilitated by increasing the duration of the IA period and/or decreasing the duration of the DA period.

## 2. Methods

### 2.1. Overview

Experimental animals were initially acclimated (IA) to sustained normobaric hypoxia, allowed to de-acclimate in normoxia (DA), and then re-exposed to hypoxia (RA). This protocol was selected in order to permit comparisons with our human field study (MacNutt et al., 2012). Blood and tissue samples were collected by terminally sampling groups of animals at several time points throughout IA, DA and RA. All experimental protocols were approved by the University of British Columbia Animal Care Committee. Adult female mice (C57BL/6NCrl Charles River Laboratories, Pointe-Claire, QC) were housed five per cage, with each cage representing an experimental group. C57Bl/6 mice have a very similar haematological response to hypoxia as two of the other most commonly studied inbred strains (Balb/c and 129/Sv; Ward et al., 2007) and were chosen for their resistance to disease and general robustness (Hedrich, 2004). Animals experienced a 12:12 light:dark cycle and were fed commercial mouse chow ad libitum throughout the experiment. Animals were 10–24 w old and weighed 18–24 g at time of sacrifice.

### 2.2. Experimental treatments

#### 2.2.1. Control animals

Three groups of control animals (no hypoxic exposure) were sampled at ages 10, 12.5 and 20 w to test the effect of age and body mass on all outcome variables. Since no clear patterns were seen across this age range for any variables of interest, five animals were randomly selected from the three age groups to represent the control group. Data from the control group represent baseline (BL) values for each variable.

#### 2.2.2. Initial acclimation to hypoxia

Animals with no prior hypoxic exposure were sampled throughout a 4-w IA period after 0 (control group), 1, 3, 7, 14 and 28 d of hypoxic exposure.

#### 2.2.3. Re-acclimation to hypoxia

Animals that had been initially acclimated to hypoxia and de-acclimated in normoxia were sampled after 0, 1, 3, 7, 14 and 28 d of hypoxic re-exposure. The time domains of IA and DA were manipulated to compare three paradigms of haematological RA to IA.

"RA" = RA after 14 d IA and 14 d DA.
"$RA_{\downarrow DA}$" = RA after 14 d IA and an abbreviated (7-d) DA phase.
"$RA_{\uparrow IA}$" = RA after an extended (56-d) IA phase and 14 d DA.

Exposure paradigms for the four experimental groups are illustrated in Fig. 1. Data were collected from five animals at each of the time points indicated throughout IA, RA, $RA_{\downarrow DA}$ and $RA_{\uparrow IA}$.

#### 2.2.4. Hypoxic exposures

Up to eight cages were placed in a plexiglas chamber measuring 51 cm × 71 cm × 36 cm. Hypoxic air was produced using a commercially available oxygen extractor (Mountain Air Generator MAG-7, Higher Peak LLC, Winchester, MA, USA) and pumped through the chamber at ~30 L $min^{-1}$ to maintain a constant hypoxic environment ($FI_{O_2} = 0.12$) with negligible $CO_2$ accumulation. During DA, cages were placed on a rack beside the hypoxic chamber. Ambient conditions outside the chamber were monitored regularly and $FI_{O_2}$ never dropped below 0.20.

#### 2.2.5. Data collection

Animals were sacrificed with an overdose of inhaled isoflurane (AErrane®, Baxter Corporation, Mississauga, ON, Canada) after 20 ± 3 min (max 45 min) of removal from the hypoxic chamber.