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# Effect of intermittent hypoxia and exercise on blood rheology and oxygen transport in trained rats

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ABSTRACT

Intermittent hypobaric hypoxia (IHH) exposure, accompanied or not with active recovery, can help to skeletal muscle repair. However, the erythropoietic response elicited can disturb blood rheology and thus alter the oxygen delivery to tissues. Male Sprague–Dawley rats were studied in two basal states: untrained and trained and compared with early (1–3 days) and late (7–14 days) stages of damage recovery in three groups of trained rats that had suffered skeletal muscle injury: Control, passive recovery rats; HYP, rats exposed to IHH after muscle damage; and EHYP, trained rats that performed light aerobic exercise sessions in addition to IHH. Hematocrit, RBC count and hemoglobin were only elevated in the late stage of recovery in HYP (13%; 14% and 8%) and EHYP (18%; 13% and 15%) groups. Blood viscosity increased about double for EHYP rats. It is concluded that intermittent exposure to hypobaric hypoxia in combination with light aerobic exercise in normoxia has an erythropoietic effect, but also provides advantageous hemorheological conditions for the perfusion of damaged muscle.



## 1. Introduction

Exposure to hypobaric hypoxia is recognized as an important factor that can elicit multiple changes at metabolic and physiological level, most of which are mediated by a signaling pathway dependent on hypoxia-inducible factors (HIFs) (Semenza et al., 2006). Many of these changes protect the body against hypoxia damage by favoring acclimation to altitude (Casas et al., 2000) and improving tissue oxygen availability (Leon-Velarde et al., 2000). It is well established that an advantageous way to obtain these favorable changes is intermittent exposure to hypobaric hypoxia (IHH). This kind of hypoxia stimulus has been widely used in sports and mountain medicine, but recently other potential benefits have been reported including an increase in circulating stem cells (Viscor et al., 2009; Zhu et al., 2005) and muscle tissue adaptations that favor physical training at altitude (Faiss et al., 2013; Hoppeler and Vogt, 2001). However, it is very important to take into account that many treatments involving repeated IHH exposure, and obviously chronic intermittent hypoxia, may have adverse effects on some rheological parameters, such as blood and plasma viscosity (Yelmen et al., 2011), which directly can affect the oxygen delivery to tissues. Some previous studies were performed in our laboratory to monitor possible hemorheological changes induced by environmental factors (Viscor et al., 2003; Esteva et al., 2009). However, to fully recognize the physiological meaning of hemorheological changes under IHH, measurements of whole blood viscoelasticity must describe the kinetics of blood flow more realistically. Measurements should include viscosity, elasticity and relaxation time under oscillatory flow (Thurston, 1989, 1990; Thurston et al., 2004). Viscoelasticity is an excellent indicator to determine the aggregation and deformability of red blood cells, as the rheological behavior of blood can be studied in the condition imposed for pulsatile circulation (Thurston, 1972, 1979).

Our hypothesis is based on the possible benefits of IHH exposure. We postulated that this type of exposure, combined or not with light aerobic exercise in normoxia, may be a complementary stimulus for the repair of damaged muscle tissues. However, given that blood flow depends on other factors such as hematocrit (Esteva et al., 2009) or changes in plasma components (Kwaan, 2010), it is very important to determine whether whole blood viscoelasticity during an IHH program could provoke excessive erythropoiesis and subsequent alterations in hemorheological behavior that could negatively affect microcirculation, tissue perfusion and oxygen delivery.

Although this paper is part of a larger study on the effects of hypoxia and exercise as a potential tool for skeletal muscle repair

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enhancement, in this report we focus on the possible rheological alterations in circulating blood and its effects on oxygen delivery to tissues.

## 2. Material and methods

### 2.1. Animals

This study was conducted in the Department of Physiology and Immunology at the University of Barcelona (UB). We used 64 adult male Sprague Dawley rats, with body mass at sampling time ranging from 350 to 410 g. All rats were maintained at 23 °C average temperature under a light–dark cycle of 12 h/12 h, with food and water *ad libitum*.

The animals were randomly pre-assigned to one of the experimental conditions and to a sampling time. All the analyses were carried out through a double blind system by means of barcoded sample identification. The experimental conditions were: Untrained Group (UNT), which was not subjected to any intervention or training and formed by rats rejecting to run on the treadmill; Trained Group (TRA), which was trained but had not suffered muscle damage before sampling. Three more groups were formed after muscle damage protocol: Control Group (CTRL), trained rats that suffered muscle damage but had no other intervention (passive recovery); Intermittent Hypoxia Group (HYP), trained rats that were exposed to intermittent hypobaric hypoxia sessions after the muscle damage protocol; and Exercise and Intermittent Hypoxia Group (EHYP), trained rats that were subjected to intermittent hypobaric hypoxia sessions plus a rehabilitation exercise program constituted by light aerobic exercise sessions after the muscle damage procedure.

Animals in the UNT and TRA groups were only sacrificed in the basal status, just one day before muscle damage of their peers (t00). In the rest of the experimental groups, sampling was programmed for days one (t01), three (t03), seven (t07) or fourteen (t14) after muscle damage. All procedures were performed in accordance with the internal protocols of our laboratory, which were authorized by the University of Barcelona's Ethical Committee for Animal Experimentation and ratified, in accordance with current Spanish legislation, by the *Departament de Medi Ambient i Habitatge* (file #1899) of the Catalan Government (Generalitat de Catalunya).

### 2.2. Exercise training protocol

Animals in the TRA, CTRL, HYP and EHYP experimental groups were trained under normal environmental conditions (sea level barometric pressure and 21 ± 2 °C room temperature) on a treadmill (LE 8710, Panlab, Barcelona, Spain). Actual training sessions were preceded by a ten-day preconditioning period, in which the total time, duration of the exercise and daily sessions (one or two) were gradually increased. The further training period consisted of two daily running sessions during the two subsequent weeks. In each 35-min training session, velocity was gradually increased up to 27 m $min^{-1}$. All the rats in the above mentioned experimental groups carried out this training protocol before the muscle damage session. In both phases, a recovery period of at least 6 h rest was scheduled between the end of first session and the beginning of the second session on the same day.

### 2.3. Muscle damage protocol

Skeletal muscle damage was induced by eccentric exercise (Armstrong et al., 1983) by means of downhill running at 30 m $min^{-1}$ and 15° of declination until exhaustion. This protocol began after three days at rest following completion of the training period, and was applied twice on the same day: one session in the morning and one in the afternoon, with a minimum rest period of 4 h between the end of the first session and the beginning of the second one.

### 2.4. Intermittent hypobaric hypoxia exposure

Intermittent hypobaric hypoxia sessions were performed in a hypobaric chamber with a volume of about 450 L, which provided ample space for three rat cages. The walls of the chamber were made of polymethylmethacrylate plastic, which is transparent and allows for the permanent observation of animals during the exposure protocol. A relative vacuum was created using a rotational vacuum pump (TRIVAC D5E, Leybold, Köln, Germany) and by regulating the airflow rate at the input with a micrometric valve. Inside pressure was controlled by two differential pressure sensors (ID 2000, Leybold, Köln, Germany) driving a diaphragm pressure regulator (MR16, Leybold, Köln, Germany). The target pressure was 462 Torr (equivalent to 4000 m), which was gradually decreased in about 15 min. Once this pressure had been reached, the chamber pressure was maintained and regulated for 4 h. At the end of the session, pressurization was progressively achieved in 15 min.

Only HYP and EHYP animals were submitted to this procedure on a daily schedule. The total days of hypobaric hypoxia exposure varied according to the sampling schedule. Animals assigned to "t01" were only submitted to one session, whereas "t14" were submitted to two weeks of daily exposure. Animals had *ad libitum* access to food and water kept in air-open reservoirs during the hypoxia sessions inside the hypobaric chamber.

### 2.5. Rehabilitation exercise program

Rats in the EHYP group were subjected to a rehabilitation exercise program consisting of a daily session of light aerobic exercise. Immediately after the hypobaric hypoxia session, these rats were placed on a treadmill to run in accordance with a program of low impact and concentric exercise. The exercise session lasted 20 min, during which rats ran progressively until 30 cm $seg^{-1}$, with a gradual increase in inclination from 0° to 5°.

### 2.6. Blood and plasma sampling

Before blood collection, rats were anesthetized with urethane solution (30 g $dL^{-1}$) at a dosage of 5 ml $kg^{-1}$. After laparotomy, a 5 mL blood sample was obtained by puncture of vena cava. The sample was immediately divided into two aliquot fractions. The first portion was separated in a sodium heparin tube for the hemorheological analysis. The second portion was stored in an EDTA tube, and was used for the blood count. Both aliquot samples were processed immediately after collection. Plasma was obtained by centrifugation of the blood and its viscosity was immediately measured.

### 2.7. Viscoelasticity and rheological parameters

Blood viscolelasticity was measured using a BioProfiler rheometer (Vilastic Scientific, Inc., Austin, TX, USA) with a 1 mm i.d. stainless steel measurement tube at a constant temperature of 37 °C. Measurements were obtained at a frequency of 2 Hz in a range from 0.2 to 100 $s^{-1}$ of shear rate ($\gamma$). The viscosity, elasticity and relaxation time were tabulated at shear rates of 2.6 $s^{-1}$, 12.3 $s^{-1}$ and 45.5 $s^{-1}$ corresponding to the strain of 0.2, 1 and 4 as representative values of physiological circulatory conditions. These three strain states correspond to aggregation effects, transition and deformability effects, respectively (Thurston, 1989, 1990). Due to the well-known Newtonian behavior of plasma, this was only measured at 450 $s^{-1}$ in a cone-plate microviscosimeter (Brookfield Digital Rheometer Model DV-III+, Middleborough, MA, USA)