Altitude training induced alterations in erythrocyte rheological properties: A controlled comparison study in rats

Article *in* Clinical hemorheology and microcirculation · March 2013

DOI: 10.3233/CH-131711 · Source: PubMed

5 authors, including:

Melek Bor-Kucukatay
Pamukkale University
68 PUBLICATIONS **1,014** CITATIONS

SEE PROFILE

Ridvan Colak
Ardahan Üniversitesi
9 PUBLICATIONS **33** CITATIONS

SEE PROFILE

Gulten Erken or Emmungil
Balikesir University
27 PUBLICATIONS **207** CITATIONS

SEE PROFILE

Emine Kilic-Toprak
Pamukkale University
34 PUBLICATIONS **147** CITATIONS

SEE PROFILE

Clinical Hemorheology and Microcirculation xx (20xx) x–xx
DOI:10.3233/CH-131711
IOS Press

Altitude training induced alterations in erythrocyte rheological properties: A controlled comparison study in rats

Melek Bor-Kucukatay[a,*], Ridvan Colak[b], Gülten Erken[c], Emine Kilic-Toprak[a] and Vural Kucukatay[a]

[a]*Faculty of Medicine, Department of Physiology, Pamukkale University, Kinikli, Denizli, Turkey*
[b]*Highshool of Physical Education and Sports, Department of Physical Education and Sports Teaching, Ardahan University, Ardahan, Turkey*
[c]*School of Medicine, Department of Physiology, Balikesir University, Balikesir, Turkey*

Abstract. Altitude training is frequently used by athletes to improve sea-level performance. However, the objective benefits of altitude training are controversial. This study aimed to investigate the possible alterations in hemorheological parameters in response to altitude training. Sprague Dawley rats, were divided into 6 groups: live low-train low (LLTL), live high–train high (LHTH), live high-train low (LHTL) and their controls live high and low (LHALC), live high (LHC), live low (LLC). LHC and LHTH groups were exposed to hypoxia (15% O_2, altitudes of 3000 m), 4 weeks. LHALC and LHTL were exposed to 12 hours hypoxia/normoxia per day, 4 weeks. Hypoxia was maintained by a hypoxic tent. The training protocol corresponded to 60–70% of maximal exercise capacity. Rats of training groups ran on treadmill for 20–30 min/day, 4 days/week, 4 weeks. Erythrocyte deformability of LHC group was increased compared to LHALC and LLC. Deformability of LHTH group was higher than LHALC and LLTL groups. No statistically significant alteration in erythrocyte aggregation parameters was observed. There were no significant relationships between RBC deformability and exercise performance. The results of this study show that, living (LHC) and training at altitude (LHTH) seems more advantageous in hemorheological point of view.

Keywords: Altitude training, exercise, RBC deformability, erythrocyte aggregation

1. Introduction

Living at "high" altitude (above 2500 m) and training at "low" altitude (below 1500 m) ("live high-train low," LHTL) has become a popular strategy for elite endurance athletes in recent years with the expectation that sea-level performance may be improved [28, 29, 31, 49]. Chronic exposure to hypobaric hypoxia is known to stimulate various physiological adaptations such as, loss of body weight [4], increment of capillary density [17], enhancement in hemoglobin (Hb), hematocrit (Hct) and red cell volume (RCV) [29, 36]. Increment in Hb and Hct may be considered as the most important adaptations, raising the

*Corresponding author: Melek Bor-Kucukatay, Faculty of Medicine, Department of Physiology, Pamukkale University, Kinikli, 20070 Denizli, Turkey. Tel.: +90 258 296 17 00; Fax: +90 258 296 24 33; E-mails: drzmbk@yahoo.com, mbor@pau.edu.tr; colak.ridvan@gmail.com (Ridvan Colak); gulemmun@gmail.com (Gülten Erken); pt_emine@yahoo.com (Emine Kilic-Toprak); vkucukatay@pau.edu.tr (Vural Kucukatay).

oxygen-carrying capacity of the blood and thus leading an improvement in low-altitude performance [27]. Therefore, the LHTL concept has been suggested to be superior to normal sea-level training or classical live high-train high (LHTH) altitude training since living at high altitude brings various physiological advantages and training at low altitude avoids hypoxic disorders and allows working with high intensity [36, 49]. On the other hand, the objective beneficial effects of LHTL are still controversial, since some studies previously made did not find any improvement either in performance or red blood cell (RBC) mass [1, 13, 28, 30].

It is well known that blood flow in skeletal muscles is closely related to oxygen demand [21, 40]. Any alterations in RBC structural and mechanical properties may affect oxygen transfer to the actively used tissues, influencing athletic performance [3, 5, 7–9, 19, 39, 42]. Deformability of RBCs is one of the key factors in the perfusion of capillaries, whereas RBC aggregation affects the fluidity of blood in larger blood vessels where the shear rate is low enough to allow RBC to aggregate, such as in veins [6, 20, 22, 35, 41, 47, 51]. Studies investigating RBC deformability and erythrocyte aggregation in hypoxia found conflicting results depending on the duration of hypoxic exposure, the methods used to obtain hypoxia and determine RBC deformability and erythrocyte aggregation [18, 23, 38, 46, 52]. Additionally, although a limited number of studies in the literature have shown that RBC deformability is modified by altitude training, these studies were performed in 2 groups: hypoxic and normoxic exercise training groups [12, 34]. As far as we know, no study has been conducted to observe alterations in hemorheological parameters at different altitude training approaches such as LHTL, LHTH and live low-train low (LLTL).

In the light of above knowledge, the goal of this study was to investigate and compare the possible changes in RBC deformability and aggregation as well as hematological parameters at different altitude training approaches (LHTL, LHTH and LLTL), further providing a feasible strategy for developing an appropriate exercise regimen that minimizes the risk of hemorheological disorders.

2. Materials and methods

2.1. Animal model

This study was conducted in Pamukkale University Experimental Animal Unit. 37 adult male Sprague Dawley rats, weighing 200–250 g, were used. Eight-week-old rats were pre-selected by their ability to run on a motorized treadmill (MAY-TME 9805, Commat, Ankara, Turkey); at 0.3 km/h up to 0.5 km/h, 0% grade, 10 min/day, for 4–5 days [26]. The pre-selected animals were then randomly assigned to exercise trained or sedentary groups. Each group was further divided into three subgroups ($n \cong 6$ in each): Live high and low control (LHALC), Live high control (LHC), Live low control (LLC) for control groups and Live high train low (LHTL), Live high train high (LHTH), live low train low (LLTL) for training groups.

Normobaric hypoxia was obtained by using a hypoxic tent (Altitude Tech. Co., Canada; altitudes of 3000 m, 15% O_2). In each chamber, O_2 and CO_2 levels, humidity and temperature conditions were continuously estimated by using electronic sensors. Normoxic environment was supplied with room air (20.9% O_2) at the ~350 m altitude in which the laboratory exist. LHTH groups were exposed to hypoxia for 24 hours, LHTL were exposed to 12 hours hypoxia/normoxia per day while LLTL groups were exposed to normoxia for 24 hours, for 4 weeks. The control groups were exposed to hypoxia and normoxia at the same period of time with their own training groups.

All rats were maintained at 23°C under a light/dark cycle of 12 h/12h. Rat chow and tap water were provided ad libitum. Two days after the end of the 4 week training programme, the rats were anaesthetized

in normoxia with intraperitoneal ketamine (50 to 75 mg/kg) and xylazine (10 to 15 mg/kg) and blood samples anticoagulated with heparin (15 IU/ml) were quickly taken from the abdominal aorta of rats. The animals where then sacrificed under anesthesia. All procedures were performed in agreement with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publications No. 85-23, revised 1996) and with the approval of the Pamukkale University Ethics Committee of Animal Care and Usage.

2.1.1. Exercise training protocol

All rats in the training groups (LHTL, LHTH and LLTL) were given familiarization training for 4 weeks, 15–30 minutes per day at the environment in which the laboratory exists (Denizli/Turkey, ~350 m) to ensure them to be trained at the same level. At the end of this first training period all rats had been trained for 30 minutes to be able to run 1.5 km/h. In order to supervise training intensities that will be applied for the following 4 weeks with precision, maximal aerobic velocity (MAV) was evaluated for the training groups two days after the resting period. Both MAV obtained in normoxia and hypoxia were estimated using a treadmill during a continuous and progressive maximal exercise test. Under normobaric hypoxia (~3000 m, 15% O_2, LHTH group), the treadmill was set at a speed of 0.3 km/h at grade of 0% after which the speed was increased by 0.3 km/h every 3 min until the maximal intensity was attained for each rat until the rat could not maintain its running position. MAV in normoxia (~350 m, %20.9 O_2, LLTL and LHTL) was evaluated using the same protocol, but with a starting speed of 0.6 km/h [11, 26]. The training sessions were conducted for 4–5 days per week, at the running speeds equal to 60% of MAV for 20 min in the first week, 65% of MAV for 25 min in the second week and 70% of MAV for 30 min in third and 35 min in the fourth weeks. At the exercise training protocol, (MAV) was evaluated for the training groups two days after the resting period. An outline of the study design is shown in Fig. 1.

Blood anticoagulated with heparin (15 IU/ml) was collected from all experimental groups for the determination of hemorheological (RBC deformability and aggregation) and hematological parameters was used within 3 hours.

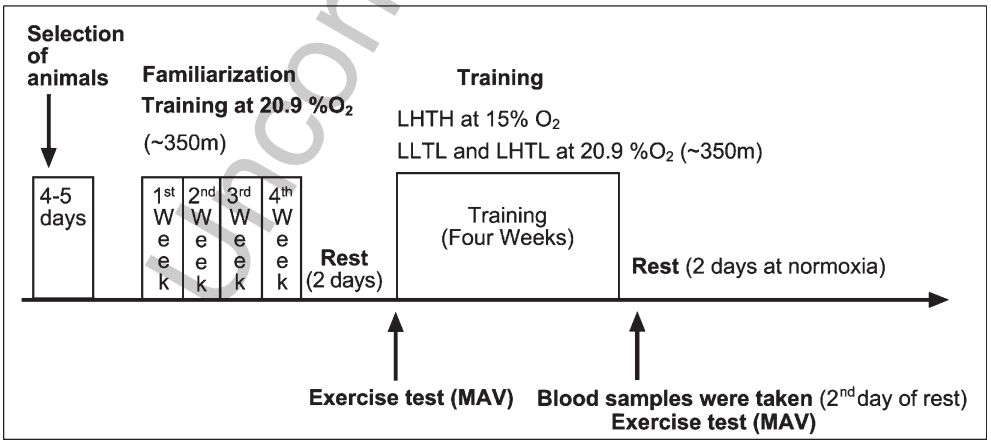

Fig. 1. Experimental design of the study.

2.2. Determination of hematological parameters

RBC count, Hb and Hct were determined using an electronic hematology analyzer (Cell-Dyn 3700, Illinois, USA).

2.3. RBC deformability measurements

RBC deformability (i.e., the ability of the entire cell to adopt a new configuration when subjected to applied mechanical forces) was determined by laser diffraction analysis using an ektacytometer (LORCA, RR Mechatronics; Hoorn, The Netherlands). The system has been described elsewhere in detail [2, 16]. Briefly, a low Hct suspension of RBC in 4% polyvinylpyrrolidone 360 solution (MW 360 kD, Sigma P 5288, ST. LOUIS, MI) was sheared in a Couette system composed of a glass cup and a precisely fitting bob. A laser beam was directed through the sheared sample, and the diffraction pattern produced by the deformed cells was analyzed by a microcomputer. On the basis of the geometry of the elliptical diffraction pattern, an elongation index (EI) was calculated for 9 shear stresses between 0.3 and 30 Pascal (Pa) as: $EI = (L-W)/(L+W)$, where L and W are the length and width of the diffraction pattern, respectively. An increased EI at a given shear stress indicates greater cell deformation and hence greater RBC deformability. All measurements were carried out at 37°C.

2.4. Assessment of RBC aggregation

RBC aggregation was also determined by LORCA as described elsewhere [15]. The measurement is based on the detection of laser back-scattering from the sheared (disaggregated), then unsheared (aggregating) blood, performed in a computer-assisted system at 37°C. Back-scattering data were evaluated by the computer and the aggregation index (AI), aggregation half time (t 1/2) which shows the kinetics of aggregation and the amplitude (AMP) which is a measure for the total extent of aggregation were calculated on the basis that there is less light back-scattered from aggregating red cells. The hematocrit (Hct) of the samples used for aggregation measurements was adjusted to 40% and blood was fully oxygenated.

2.5. Statistical analysis

Results were expressed as means ± standard error (SE). Statistical comparisons among groups were done by "one way ANOVA" and *Post hoc* comparisons of the means were carried out using the LSD post test, with p values <0.05 accepted as statistically significant. Pearson correlation coefficient was performed between EI values measured at 0.53 Pa and physical performance of training groups. All analyses were carried out with the computerized SPSS 10.0 program (Statistical Package for Social Sciences, SPSS Inc).

3. Results

Exercise indexes of training groups are demonstrated in Table 1. Although no differences existed at the beginning of the study between groups for running speed, the latter increased significantly in only LHTL group ($p < 0.01$). The maximal speed reached was 20.57% higher for LHTL group, 5.40% for LHTH and 3.47% for LLTL group when compared to the speed observed in the first test. The posttest running speed

Table 1
Indexes of exercise of training groups. Values are expressed as means ± SE

Groups	Pretest running speed (km/h)	Posttest running speed (km/h)
LHTL	2.40 ± 0.093	2.87 ± 0.03$^{\neq}$
LHTH	2.45 ± 0.092	2.58 ± 0.12*
LLTL	2.45 ± 0.050	2.53 ± 0.12*

LHTL: Live high train low; LHTH: Live high train high; LLTL: live low train low. *:$p < 0.05$ difference from posttest of LHTL group. $^{\neq}$:$p < 0.001$ from pretest of LHTL group.

Table 2
Hematological parameters of control and training groups. Values are expressed as means ± SE

	LHALC	LHC	LLC	LHTL	LHTH	LLTL
RBC count (10^6/μL)	9.53 ± 0.78	9.97 ± 0.26	9.19 ± 0.50	9.60 ± 0.31	9.40 ± 0.61	9.20 ± 0.33
Hb (g/dL)	15.88 ± 0.67	16.48 ± 0.35	14.37 ± 0.49$^{\emptyset}$	15.73 ± 0.45	16.43 ± 0.90	14.91 ± 0.45
Hct (%)	80.14 ± 6.36	84.50 ± 1.73	48.53 ± 2.79$^{*,£,\beta}$	78.87 ± 2.53	80.33 ± 3.84	80.15 ± 2.20

RBC, Red blood cell; Hb, hemoglobin; Hct, hematoctit; LHALC: Live high and low control; LHC: Live high control; LLC: Live low control; LHTL: Live high train low; LHTH: Live high train high; LLTL: live low train low. *:$p < 0.001$ difference from group LHALC; $^{£}$:$p < 0.001$ difference from group LHC; $^{\emptyset}$:$p < 0.05$ difference from group LHALC and LHC; $^{\beta}$:$p < 0.001$ difference from group LLTL.

reached by LHTH and LLTL groups was significantly higher than LHTL group ($p < 0.05$). Table 2 shows hematological parameters of the groups. Hb value of the LLC group was significantly lower compared to LHALC and LHC ($p < 0.05$) and Hct of this group was decreased compared to groups LHALC, LHC, LLTL ($p < 0.001$).

RBC deformability (i.e., the elongation index EI) for the RBCs of all experimental groups was measured at 9 shear stresses between 0.3 and 30 Pa and presented in Table 3. RBC deformability of the control of live high (LHC) group measured at 0.53, 0.95 and 1.69 Pa were higher than control of live high and low (LHALC; $p < 0.05$) and control of live low (LLC; $p < 0.05$) groups. On the other hand, although the difference at RBC deformability between live high train high (LHTH) and live high train low (LHTL) groups was not statistically significant, erythrocyte deformability of the LHTH group was higher compared to live low trian low (LLTL) group ($p < 0.05$). Lastly mentioned alteration was statistically significant only at 0.53 Pa shear stress. The exercise protocols applied at different altitudes measured at 9 different shear stresses did not cause any statistically significant alteration in RBC deformability compared to their own controls (ie; LHTL group versus LHALC and LHC groups, LHTH group versus LHC and LHALC groups; LLTL group compared to LLC and LHALC groups) except LHTH group measured at 0.53 Pa. RBC deformability of LHTH group measured at 0.53 Pa shear stress was significantly higher compared to LHALC group ($p < 0.05$, data not shown). No statistically significant alterations among groups at RBC deformabilities measured below 0.53 Pa and above 1.69 Pa were observed. Pearson correlation coefficient was performed between EI values measured at 0.53 Pa and posttest running speed of traning groups. No statistically significant relationship was observed ($p > 0.05$). The alterations observed in aggregation parameters were not statistically significant, as well (Table 4).

Table 3
Erythrocyte Elongation Index (EI) values of the groups. Values are expressed as means ± SE

	LHALC	LHC	LLC	LHTL	LHTH	LLTL
EI (0.30)	0.098 ± 0.006	0.108 ± 0.003	0.088 ± 0.003	0.097 ± 0.003	0.110 ± 0.003	0.081 ± 0.014
EI (0.53)	0.139 ± 0.006	0.158 ± 0.005*,**	0.128 ± 0.006	0.143 ± 0.005	0.158 ± 0.005*,***	0.137 ± 0.006
EI (0.95)	0.216 ± 0.008	0.236 ± 0.006*,**	0.205 ± 0.006	0.223 ± 0.006	0.234 ± 0.005	0.213 ± 0.007
EI (1.69)	0.302 ± 0.007	0.323 ± 0.007*,**	0.297 ± 0.007	0.314 ± 0.007	0.322 ± 0.006	0.302 ± 0.007
EI (3.00)	0.386 ± 0.006	0.403 ± 0.007	0.401 ± 0.007	0.401 ± 0.007	0.387 ± 0.006	0.386 ± 0.006
EI (5.33)	0.457 ± 0.005	0.440 ± 0.006	0.461 ± 0.005	0.472 ± 0.006	0.482 ± 0.140	0.436 ± 0.019
EI (9.49)	0.513 ± 0.005	0.518 ± 0.006	0.517 ± 0.005	0.523 ± 0.005	0.515 ± 0.006	0.510 ± 0.003
EI (16.87)	0.568 ± 0.019	0.558 ± 0.005	0.558 ± 0.005	0.562 ± 0.005	0.553 ± 0.005	0.530 ± 0.018
EI (30.00)	0.580 ± 0.005	0.591 ± 0.005	0.586 ± 0.007	0.596 ± 0.007	0.584 ± 0.005	0.577 ± 0.001

LHALC: Live high and low control; LHC: Live high control; LLC: Live low control. LHTL: Live high train low; LHTH: Live high train high; LLTL: live low train low. *:$p < 0.05$ difference from group LHALC, **:$p < 0.05$ difference from group LLC, ***:$p < 0.05$ difference from group LLTL.

Table 4
Erytrocyte aggregation parameters of control and training groups. Values are expressed as means ± SE

	LHALC	LHC	LLC	LHTL	LHTH	LLTL
AI (%)	63.57 ± 1.60	59.56 ± 1.87	61.52 ± 1.57	62.58 ± 2.21	59.38 ± 1.92	61.15 ± 4.05
t½ (s)	2.04 ± 0.12	2.45 ± 0.25	2.21 ± 0.14	2.19 ± 0.27	2.55 ± 0.28	2.49 ± 0.57
Amp (au)	17.19 ± 0.97	19.41 ± 2.42	20.04 ± 0.93	21.15 ± 1.04	17.60 ± 0.99	19.82 ± 1.68

AI, aggregation index; *t½*, aggregation half time; Amp, amplitude of aggregation. LHALC: Live high and low control; LHC: Live high control; LLC: Live low control; LHTL: Live high train low; LHTH: Live high train high; LLTL: live low train low.

4. Discussion

Effects of living and training at different altitudes on RBC deformability, aggregation and hematological parameters were investigated in the current study. Hb and Hct of groups living at altitude (LHALC and LHC) were higher, than the group living at ∼350 m altitude (LLC). Enhanced oxygen transport to tissues via increased number of RBC and Hb appears to be the dominant mechanism for adaptation to living at altitude. Distinct results in the literature were reported concerning hypoxia and altitude training induced alterations in hematological parameters depending on the type and duration of the exercise and hypoxia [10, 34], some of which are consistent with our results [13, 48, 50].

The ability of the entire RBC to deform is of crucial importance for performing its function of oxygen delivery and it is also a determinant of the cell survival time in the circulation [45]. The results of the current study indicate that, RBC deformability of LHC group measured at 0.53, 0.95 and 1.69 Pa are increased compared to LHALC and LLC groups. RBC deformability of LHTH group measured at just 0.53 Pa shear stress was found to be improved compared to LHALC and LLTL groups (Table 3). No other statistically significant alteration between the exercise groups and their controls were observed. Guezennec et al. investigated the effect of hypoxic exercise training on hemorheological regulation. They submitted human male subjects to two physical exercises of 1 hour cycling, at 70% of their VO_2 max. One test was performed at sea level, the other at a simulated altitude of 3000 m in a hypobaric chamber. They

measured RBC deformability by filtration on polycarbonate membrane and found that RBC deformability decreased after exercise under hypoxic conditions but remained unchanged after the same exercise at sea level [12]. Similarly, in Mao TY et al.'s study sedantary males were trained on 60% of maximum work rate under 15% (hypoxic) or 21% (normoxic) O_2 condition for 30 min/day, 5 days/week, 5 weeks. They have found that although hypoxic training for 5 weeks lowered RBC deformability, about of exercise test at hypoxic conditions and 4 weeks of exercise at normoxic conditions did not cause any significant changes in basal and Gardos channel-modulated RBC deformability measured by an ektacytometer (RheoScan-D system) [34]. To our knowledge, current study is the first one investigating the effects of living and training at different altitudes on RBC deformability. Our results demonstrating that, the training protocol corresponding to 60–70% of rat's maximal exercise capacity for 20–30 min a day, 4 days a week, for 4 weeks did not cause a significant alteration in RBC deformability measured by an ektacytometer etiher in hypoxic, or in normoxic, or hypoxic-normoxic conditions are consistent with at least a portion of previous observations summarized above.

Effects of different types of hypoxia on RBC deformability has been studied. Exposure to acute hypoxia was generally shown to cause a decrement in RBC deformability [32, 33]. On the other hand, Yelmen et al. placed rats in a hypobaric chamber (430 mmHg; 5 hours/day, 5 days/week, 5 weeks) to obtain chronic long-term intermittent hypobaric hypoxia and demonstrated that erythrocyte rigidity index was unaltered after this exposure [52]. Similarly, Kaniewski et al. by using ektacytometry to measure RBC deformability have shown that deformability of human, cat, rat, rabbit and dog RBCs at lower shear stresses is unaltered by hypoxia [23]. Nie HJ et al. have exposed rats to hypoxia for 0,1,28 days by bleeding from their hearts and demonstrated that acute hypoxia induces a decrement in RBC deformability, while acclimatization to hypoxia causes increment of this parameter [38]. Rats were exposed to chronic normobaric hypoxia (4 weeks) using a hypoxic tent in the current study. Similar to the results of the above mentioned studies, RBC deformability of LHALC group in which rats were exposed to 12 hours hypoxia/normoxia per day was not different from LLC group which was obtained by exposing rats to normoxia for 24 hours. On the other hand, erythrocyte deformability of LHC group in which rats were exposed to chronic hypoxia for 24 hours during 4 weeks was increased compared to LHALC and LLC groups at 0.53–1.69 Pa.

The results of the current study also show that, RBC deformability of individuals living and training at altitude (LHTH) is higher than individuals living and training close to sea level (∼350 m-LLTL) and living at altitude and training close to sea level (LHTL). It was demonstrated that, training under hypoxic conditions causes erythrocyte senescence and erythropoises accompanied by elevated erythropoietin (Epo) concentration has been found after both long-term high altitude exposure and training under hypoxic conditions [14, 34]. The influence of EPO on RBC deformability was analyzed recently [25, 43, 53]. Although neither age distrubition of RBCs nor determination of EPO level were performed in the current study, when our data are evaluated together the increment in RBC deformability observed in both group LHC and LHTH may be explained as increased RBC turnover since young RBCs are known to deform more [40, 44]. The increments observed in RBC deformability in response to hypoxia may be considered as a favorable adaptation under hypoxic conditions at low shear stresses. However, the RBC deformability improvement observed during LHTH protocol was not accompanied by greater exercise performance which was determined as running speed (Table 1).

Another hemorheological parameter determined in this study is the RBC aggregation which is a reversible process meaning a temporary linear or branched aggregate formation of the erythrocytes under critically low shear stress conditions [24]. As far as we know, our study is the first one in the literature exploring the effects of hypoxic exercise training on RBC aggregation. The results of the current study

demonstrate that, living and training at neither hypoxic nor normoxic conditions induced statistically significant alterations in RBC aggregation parameters.

In conclusion, the results of this study indicate that increased RBC deformability observed in living (LHC) and training (LHTH) at altitude groups may serve as a favorable adaptive mechanism to contribute blood flow in response to hypoxia at low shear stresses. At higher shear stresses (above 3.00 Pa) which are usually observed at the muscle tissue capillary level, this adaptive mechanism can not be observed. This difference may be due to the type, duration, intensity of the exercise applied. To our knowledge, the present study is the first one in the literature investigating the effects of living and training at different altitudes on hemorheological parameters. Further investigations will be necessary to clarify which exercise regimen is more effective and may be recommended to athletes for cardiovascular health and improving their athletic performance.

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

Acknowledgments

This study was supported by the Pamukkale University Research Fund (Project No. 2009BSP021).

References

[1] D.M. Bailey and B. Davies, Physiological implications of altitude training for endurance performance at sea level: A review, *Br J Sports Med*, **31** (1997), 183–190.
[2] O.K. Baskurt, M. Boynard, G.C. Cokelet, P. Connes, B.M. Cooke, S. Forconi, F. Liao, M.R. Hardeman, F. Jung, H.J. Meiselman, G. Nash, N. Nemeth, B. Neu, B. Sandhagen, S. Shin, G. Thurston and J.L. Wautier, International expert panel for standardization of hemorheological methods, New guidelines for hemorheological laboratory techniques, *Clin Hemorheol Microcirc* **42** (2009), 75–97.
[3] O.K. Baskurt, P. Ulker and H.J. Meiselman, Nitric oxide, erythrocytes and exercise, *Clin Hemorheol Microcirc* **49** (2011), 175–181.
[4] A.X. Bigard, A. Brunet, B. Serrurier, C.Y. Guezennec and H. Monodo, Effects of endurance training at high altitude on diaphragm muscle properties, *Pflugers Arch* **422** (1992), 239–244.
[5] S. Chien, Red cell deformability and its relevance to blood flow, *Annu Rev Physiol* **49** (1987), 177–192.
[6] S. Chien, The microcirculatory society eugene M. Landis award lecture. Role of blood cells in microcirculatory regulation, *Microvasc Res* **29** (1985), 129–151.
[7] P. Connes, A. Pichon, M.D. Hardy-Dessources, X. Waltz, Y. Lamarre, M.J. Simmonds and J. Tripette, Blood viscosity and hemodynamics during exercise, *Clin Hemorheol Microcirc* **51** (2012), 101–109.
[8] P. Connes, M.J. Simmonds, J.F. Brun and O.K. Baskurt, Exercise hemorheology: Classical data, recent findings and unresolved issues, *Clin Hemorheol Microcirc* **53** (2013), 187–199.
[9] M.S. El-Sayed, N. Ali and A.A. Omar, Effects of posture and ergometer-specific exercise modality on plasma viscosity and plasma fibrinogen: The role of plasma volume changes, *Clin Hemorheol Microcirc* **47** (2011), 219–228.
[10] L.A. Garvican, T. Pottgiesser, D.T. Martin, Y.O. Schumacher, M. Barras and C.J. Gore, The contribution of haemoglobin mass to increases in cycling performance induced by simulated LHTL, *Eur J Appl Physiol* **111** (2011), 1089–1101.
[11] L. Goret, C. Reboul, S. Tanguy, M. Dauzat and P. Obert, Training does not affect the alteration in pulmonary artery vasoreactivity in pulmonary hypertensive rats, *Eur J Pharmacol* **527** (2005), 121–128.
[12] C.Y. Guezennec, J.F. Nadaud, P. Satabin, F. Leger and P. Lafargue, Influence of polyunsaturated fatty acid diet on the hemorrheological response to physical exercise in hypoxia, *Int J Sports Med* **10** (1989), 286–291.

[13] A.G. Hahn, C.J. Gore, D.T. Martin, M.J. Ashenden, A.D. Roberts and P.A. Logan, An evaluation of the concept of living at moderate altitude and training at sea level, *Comp Biochem Physiol A Mol Integr Physiol* **128** (2001), 777–789.
[14] R. Hainsworth and M.J. Drinkhill, Cardiovascular adjustments for life at high altitude, *Respir Physiol Neurobiol* **30** (2007), 204–211.
[15] M.R. Hardeman, J.G.G. Dobbe and C. Ince, The laser-assisted optical rotational cell analyzer (LORCA) as red blood cell aggregometer, *Clin Hemorheol Microcirc* **25** (2001), 1–11.
[16] M.R. Hardeman, P.T. Goedhart, J.G.G. Dobbe and K.P. Lettinga, Laser assisted optical rotational cell analyzer (LORCA): A new instrument for measurement of various structural hemorhelogical parameters, *Clin Hemorheol* **14** (1994), 605–618.
[17] O. Hudlicka, M.D. Brown, H. Walter, J.B. Weiss and A. Bate, Factors involved in capillary growth in the heart, *Mol Cell Biochem* **147** (1995), 57–68.
[18] G. Ilavazhagan, A. Bansal, D. Prasad, P. Thomas, S.K. Sharma, A.K. Kain, D. Kumar and W. Selvamurthy, Effect of vitamin E supplementation on hypoxia-induced oxidative damage in male albino rats, *Aviat Space Environ Med* **72** (2001), 899–903.
[19] B. Jia, X. Wang, A. Kang, X. Wang, Z. Wen, W. Yao and L. Xie, The effects of long term aerobic exercise on the hemorheology in rats fed with high-fat diet, *Clin Hemorheol Microcirc* **51** (2012), 117–127.
[20] F. Jung, From hemorheology to microcirculation and regenerative medicine: Fahraeus Lecture 2009, *Clin Hemorheol Microcirc* **45** (2010), 79–99.
[21] F. Jung, H. Kessler, G. Pindur, R. Sternitzky and R.P. Franke, Intramuscular oxygen partial pressure in the healthy during exercise, *Clin Hemorheol Microcirc* **21** (1999), 25–33.
[22] F. Jung, C. Mrowietz, B. Hiebl, R.P. Franke, G. Pindur and R. Sternitzky, Influence of rheological parameters on the velocity of erythrocytes passing nailfold capillaries in humans, *Clin Hemorheol Microcirc* **48** (2011), 129–139.
[23] W.S. Kaniewski, T.S. Hakim and J.C. Freedman, Cellular deformability of normoxic and hypoxic mammalian red blood cells, *Biorheology* **31** (1994), 91–101.
[24] F. Kiss, N. Nemeth, E. Sajtos, E. Brath, K. Peto, O.K. Baskurt, I. Furka and I. Miko, Examination of aggregation of various red blood cell populations can be informative in comparison of splenectomy and spleen autotransplantation in animal experiments, *Clin Hemorheol Microcirc* **45** (2010), 273–280.
[25] M. Klipp, A.U. Holzwarth, J.M. Poeschl, M. Nelle and O. Linderkamp, Effects of erythropoietin on erythrocyte deformability in non-transfused preterm infants, *Acta Paediatr* **96** (2007), 253–256.
[26] R.H. Lambertucci, A.C. Levada-Pires, L.V. Rossoni, R. Curi and T.C. Pithon-Curi, Effects of aerobic exercise training on antioxidant enzyme activities and mRNA levels in soleus muscle from young and aged rats, *Mech of Ageing Dev* **128** (2007), 267–275.
[27] B.D. Levine, R.C. Roach and C.S. Houston, Work and training at altitude, in: *Proceedings of the 7th International Hypoxia Symposium held at Lake Louise, Canada February 1991*, J.R. Sutton, G. Goates and C.S. Houston, ed., Section V, Queen City, Burlington, Vt, 1992, pp. 192–201
[28] B.D. Levine and J. Stray-Gundersen, "Living high-training low": Effect of moderate-altitude acclimatization with low-altitude training on performance, *J Appl Physiol* **83** (1997), 102–112.
[29] B.D. Levine and J. Stray-Gundersen, A practical approach to altitude training: Where to live and train for optimal performance enhancement, *Int J Sports Med* **13**(Suppl 1) (1992), S209–S212.
[30] B.D. Levine and J. Stray-Gundersen, Exercise at high altitudes, in: *Current Therapy in Sports Medicine*, (3rd ed.), J.S. Torg and R.J. Shepard, ed., Mosby-Year Book: St. Louis, MO, 1995, pp. 588–593.
[31] B.D. Levine and J. Stray-Gundersen, High-altitude training and competition, in: *The Team Physician's Handbook*, (2nd ed.), M.B. Mellion, W.M. Walsh and G.L. Shelton, ed., Hanley & Belfus: Philadelphia, PA, 1997, pp. 186–193.
[32] X.B. Li, X.Q. Guo and Z.J. Liang, Effect of acute hypoxia on blood viscosity, red blood cell deformability and the left ventricular function in rats, *Sheng Li Xue Bao* **47** (1995), 165–172.
[33] W. Liang, D. Luo, Y. Gao and G. Zhang, Studies of mechanism of erythrocyte deformability injury during hypobaric hypoxia in rats, *Zhongguo Ying Yong Sheng Li Xue Za Zhi* **13** (1997), 306–308.
[34] T.Y. Mao, L.L. Fu and J.S. Wang, Hypoxic exercise training causes erythrocyte senescence and rheological dysfunction by depressed Gardos channel activity, *J Appl Physiol* **111** (2011), 382–391.
[35] G. McHedlishvili, Basic factors determining the hemorheological disorders in the microcirculation, *Clin Hemorheol Microcirc* **30** (2004), 179–180.
[36] S. Miyazaki and A. Sakai, The effect of "living high-training low" on physical performance in rats, *Int J Biometeorol* **44** (2000), 24–30.

[37] A.V. Muravyov, S.V. Draygin, N.N. Eremin and A.A. Muravyov, The microrheological behavior of young and old red blood cells in athletes, *Clin Hemorheol Microcirc* **26** (2002), 183–188.
[38] H.J. Nie, Y.M. Tian, D.X. Zhang and H. Wang, Changes of erythrocyte deformability in rats acclimatized to hypoxia and its molemechanism, *Zhongguo Ying Yong Sheng Li Xue Za Zhi* **27** (2011), 23–28.
[39] A.J. Romain, J.F. Brun, E. Varlet-Marie and E. Raynaud de Mauverger, Effects of exercise training on blood rheology: A meta-analysis, *Clin Hemorheol Microcirc* **49** (2011), 199–205.
[40] B. Saltin, G. Radegran, M.D. Koskolou and R.C. Roach, Skeletal muscle blood flow in humans and its regulation during exercise, *Acta Physiol Scand* **162** (1998), 421–436.
[41] H. Schmid-Schönbein, Blood rheology and physiology of microcirculation, *Ric Clin Lab* **11** (1981), 13–33.
[42] H. Schmid-Schönbein, Fluid dynamics and hemorheology *in vivo*: The interactions of hemodynamic parameters and hemorheological "properties" in determining the flow behavior of blood in microvascular Networks, in: *Clinical Blood Rheology*, G.D.O Lowe, ed., CRC: Boca Raton, FL, 1988, pp. 129–219.
[43] M. Simó, M. Santaolaria, J. Murado, M.L. Pérez, D. Corella and A. Vayá, Erythrocyte deformability in anaemic patients with reticulocytosis determined by means of ektacytometry techniques, *Clin Hemorheol Microcirc* **37** (2007), 263–267.
[44] J.A. Smith, Exercise, training and red blood cell turnover, *Sports Med* **19** (1995), 9–31.
[45] J. Stuart and G.B. Nash, Red cell deformability and haematological disorders, *Blood Rev* **4** (1990), 141–147.
[46] L.A. Subbotina, V.K. Stepanov and M.V. Dvornikov, Aggregate state of human blood in the period of normobaric interval hypoxic training, *Aviakosm Ekolog Med* **39** (2005), 36–39.
[47] I.A. Tikhomirova, A.O. Oslyakova and S.G. Mikhailova, Microcirculation and blood rheology in patients with cerebrovascular disorders, *Clin Hemorheol Microcirc* **49** (2011), 295–305.
[48] J.S. Wang, M.H. Wu, T.Y. Mao, T.C. Fu and C.C. Hsu, Effects of normoxic and hypoxic exercise regimens on cardiac, muscular, and cerebral hemodynamics suppressed by severe hypoxia in humans, *J Appl Physiol* **109** (2010), 219–229.
[49] R.L. Wilber, *Altitude Training and Athletic Performance*, Human Kinetics: Champaign, IL, 2004.
[50] J.S. Windsor and G.W. Rodway, Heights and haematology: The story of haemoglobin at altitude, *Postgrad Med J* **83** (2007), 148–151.
[51] O. Yalcin, M. Bor-Kucukatay, U.K. Senturk and O.K. Baskurt, Effects of swimming exercise on red blood cell rheology in trained and untrained rats, *J Appl Physiol* **88** (2000), 2074–2080.
[52] N. Yelmen, S. Ozdemir, I. Guner, S. Toplan, G. Sahin, O.M. Yaman and S. Sipahi, The effects of chronic long-term intermittent hypobaric hypoxia on blood rheology parameters, *Gen Physiol Biophys* **30** (2011), 389–395.
[53] J. Zhao, Y. Tian, J. Cao, L. Jin and L. Ji, Mechanism of endurance training-induced erythrocyte deformability in rats involves erythropoiesis, *Clin Hemorheol Microcirc* (2012), DOI: 10.3233/CH-2012-1549