

EFFECT OF ALTITUDE HYPOXIA ON ADRENAL ACTIVITY AND NEUTROPHIL- TO - LYMPHOCYTE RATIO IN LOW AND HIGH HEMATOCRIT SHEEP

Penka Moneva*, Ivan Yanchev, Marina Dyavolova, Dimitar Gudev

Institute of Animal Science, BG-2232 Kostinbrod, Bulgaria,

*Corresponding author: pv_moneva@abv.bg

Abstract

The object of the present paper was to investigate the effect of altitude hypoxia on adrenal activity and blood leukocyte distribution in ewes having different baseline hematocrit levels. Ewes from Ile de France breed were allocated into two groups according to their baseline hematocrit level: group I – low baseline hematocrit and group II – high hematocrit level. Blood samples were taken before shearing (baseline level), immediately after shearing, 3 h after shearing, 14 d following exposure to moderate altitude (1440 m above sea level), immediately after transport to low altitude (500 m above sea level) following 4 months long exposure to altitude and 7 d later. All samples were analyzed for cortisol and neutrophil- to-lymphocyte ratio. Baseline hematocrit level was closely related with the rate of adrenal response to shearing and exposure to altitude. There was inverse relationship between plasma cortisol level and neutrophil-to-lymphocyte ratio following 14 d exposure to moderate altitude hypoxia and 7 d after transport to low altitude. Baseline neutrophil-to-lymphocyte ratio was not associated plasma level of cortisol. These results suggest that hematocrit related metabolic type of energy supply could have been implicated in the observed difference among the groups concerning sensitivity of leukocyte glucocorticoid receptors.

Keywords: cortisol, neutrophil-to-lymphocyte ratio, hematocrit, stress, sheep

Introduction

Neutrophil-to-lymphocyte ratio is widely used as a stress marker, particularly in poultry (heterophil-to-lymphocyte ratio). Stress has been shown to induce significant increase in N/L ratio (Maxwell, 1993; Davis et al., 2008; Damgaard et al., 2009). However, several studies did not find such correlation (Prieto et al., 2014; Cotter, 2015a; Cotter, 2015b).

There is evidence that the basal values of hematocrit are closely related to the adrenal response and the metabolic pathway for energy supply (aerobic, anaerobic) (Evans and Whitlock, 1964; Jones et al., 1967; Grace et al., 1992, Mason, 2000; Stark and Schuster, 2012; Shirasawa et al., 2013). In recent years immunologists have accentuated on the possibility of using bioenergetic profiles of leukocytes with the realization that leukocytes metabolism is closely tied to immunity (Kramer et al., 2014). Recent findings support the concept that circulating leukocytes can serve as early sensors of mitochondrial function under conditions of metabolic stress (Chacko et al., 2014). Stress hormones have been identified as the major endocrine mediators in leukocyte distribution during acute or chronic stress (Dhabhar, 2009a). Leukocyte subpopulations adopt specific metabolic profiles to cope with environmental and proliferative stressors (Macintyre and Rathmel, 2013). The increase of

hypoxia inducible factor 1 α during hypoxia stimulates glycolysis and thus takes part in the shift of the more dominant metabolic pathway for energy supply (Seagroves et al., 2001).

The object of the present paper was to investigate the effect of altitude hypoxia on adrenal activity and neutrophil-to-lymphocyte ratio distribution in ewes having different baseline hematocrit levels.

Materials and Methods

One hundred one Ile de France ewes (1-7 years old) were used in the present experiment. All ewes of the flock were artificially inseminated in May following estrus synchronization. The animals were allocated into two groups following hematocrit measurement in all ewes. Group I comprised individuals with low level of hematocrit (low hematocrit group; n=10) and group II comprised individuals which had high level of hematocrit (high hematocrit group; n=10). Two additional measurement of baseline hematocrit at intervals of 10 days were performed in the sheep of both groups to verify hematocrit values of both groups, since hematocrit is known to be influenced by many factors and fluctuates from day to day. The average age of the ewes in groups I and II was 3.9 ± 0.795 and 3.1 ± 0.745 years respectively. The ewes were shorn on June 2nd and were immediately transported from the experimental base of the Institute of Animal Science, Kostinbrod (500 m above sea level) to the Petrohan Pass region (Balkan mountains), located at 1440 m above sea level. Minimum and maximum temperatures on that day were 13.9 - 25°C for the region of Kostinbrod (low altitude) and 8.2-13.6°C for the region of Petrohan Pass (high altitude) respectively. The animals remained at high altitude for 4 months where they were on pasture for 10 h during the day. At night they stayed in a barn. The ewes had free access to a NaCl licking stone and water. In addition to pasture, they were offered concentrate once per day. Mean air temperature range in the region of Petrohan pass during the summer months of 2015 was 12 to 20°C. At the end of the grazing season the ewes were transported back to low altitude. At that time the ewes in groups I and II were at 131 ± 6.652 and 140 ± 4.015 d of gestation respectively as estimated by the day of parturition.

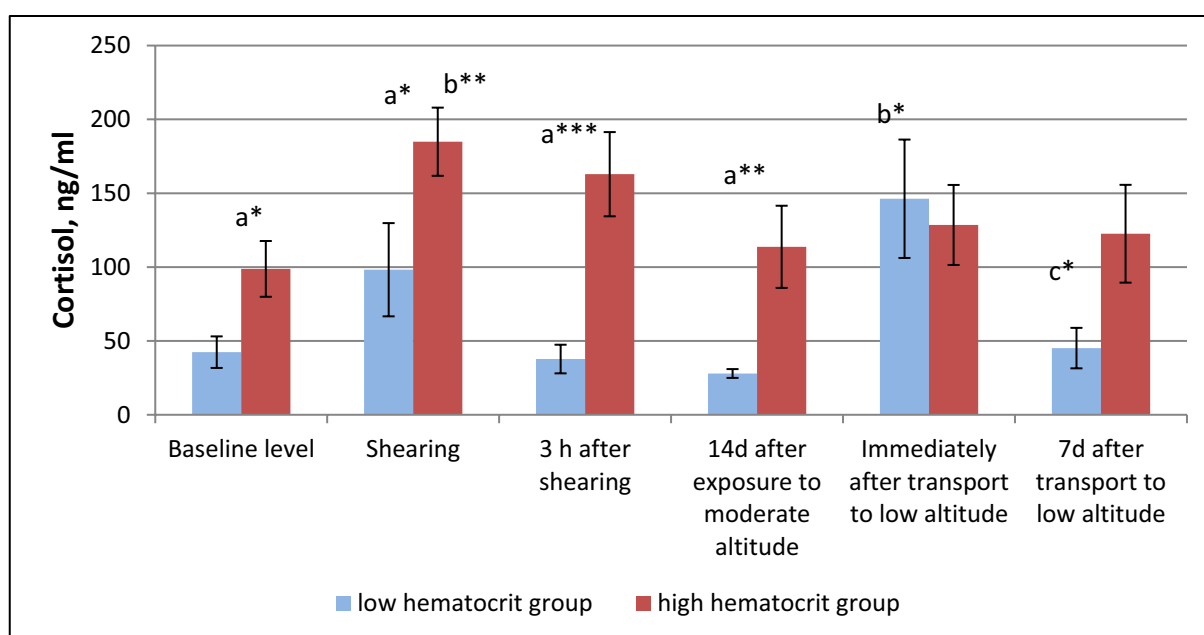
Blood samples were collected before shearing, immediately after shearing, at 14 d following exposure to moderate altitude, immediately after transport to low altitude and following 7d of stay at low altitude.

All samples were taken via jugular venipuncture within 3 min in the morning before feeding in order to minimize handling stress and avoid possible interference caused by cortisol diurnal variation. Blood samples were collected in EDTA tubes, centrifuged and stored at -20°C until analyzed. Plasma cortisol was measured using a commercial cortisol ELISA kit. Leukocyte subpopulations were counted microscopically in smears made after staining with Giemsa-Romanovsky. The results of one factor analysis are expressed as means $\pm$ S.E.M. and were analyzed by ANOVA.

Results and Discussion

Baseline plasma cortisol level in the ewes of group II (high hematocrit group) was significantly higher ($P < 0.05$) than that in ewes of group I (low hematocrit group). Shearing caused increase in the levels of cortisol in both groups. However, plasma level of cortisol in the ewes of group I unlike that in group II did not reach level of significance (Figure 1). The higher cortisol level in the ewes of group II as compared to that in the ewes of group I persisted immediately after shearing ($P < 0.05$), three hours after shearing ($P < 0.001$) and at 14

d following exposure to moderate altitude ($P<0.01$). Transportation of sheep to low altitude increased cortisol level significantly ($P<0.05$) in the ewes of group I only as compared to the baseline level while in the ewe of group II the increase was insignificant. Cortisol level in the ewes of Group II remained unchanged at 7 d following transport relative to the level immediately after transport. On the contrary, plasma cortisol level in the ewes of group I declined at 7 d following transport and was similar to the baseline level. Our data suggest that basal and stress-induced cortisol levels were closely related to baseline values of hematocrit. Besides, it seems that hematocrit related response to stress depends on the nature of the stress stimuli. In general, cortisol levels in the sheep of Group II were higher than those in the sheep of group I.



* $P<0.05$, ** $P<0.01$, *** $P<0.001$

a - significantly different among the groups

b - significantly different versus baseline level

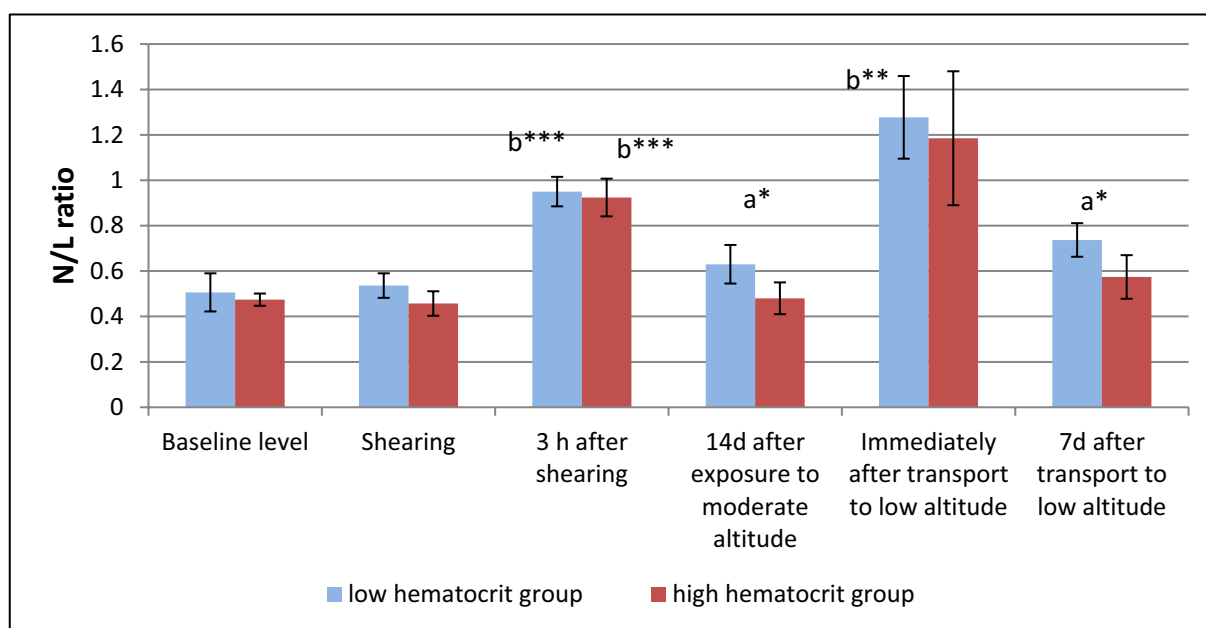
c - significantly different versus immediately after transport to low altitude

Figure 1. Cortisol in sheep with low and high hematocrit values following shearing, exposure to moderate altitude and transport to low altitude.

In our previous study we suggested that hematocrit was closely related to the metabolic energy supply. High hematocrit sheep was proposed to have predominantly anaerobic energy supply as compared to low hematocrit sheep (aerobic energy supply). Consequently, hematocrit associated response to stress found in this study may be attributed to the specific metabolic pathway for energy supply inherent in each hematocrit type.

There was no difference in baseline neutrophil-to-lymphocyte ratio (N/L ratio) among the groups (Figure 2). Neutrophil-to-lymphocyte ratio remained unchanged immediately after shearing. This result could be due to the chronological order of catecholamines and cortisol secretion. Both catecholamines and cortisol have been reported to play a role in the distribution of leukocyte subpopulations (Dhabhar et al., 1995). Also, a certain role could be attributed to the rapid change in blood volume during shearing due to a quick turnover of

water (Piccione et al., 2006; Piccione et al. 2010; Hashem, 2014). The increased N/L ratio 3 hours after shearing in both experimental groups ($P<0.001$) was most probably due to the well documented effect of glucocorticoids on distribution of leukocyte subpopulation. It is well known that peak plasma catecholamine response occurs rapidly after onset of the stress and precedes that of glucocorticoid response to stress. Furthermore, the dynamic change in the release of catecholamines and cortisol has been shown to have opposite effects on leukocyte distribution during acute and chronic stress (Schedlowski et al., 1993; Dhabhar, 2009a; Dhabhar, 2009b).



* $P<0.05$, ** $P<0.01$, *** $P<0.001$

a- significantly different among the groups

b- significantly different versus baseline level

Figure 2. Neutrophil to lymphocyte ratio in sheep with low and high hematocrit values following shearing, exposure to moderate altitude and transport to low altitude.

Recently, leukocyte subpopulations are considered in the context of their bioenergetics. Neutrophils are known to rely on anaerobic glycolysis for energy supply, while lymphocytes in quiescent state use primarily oxidative phosphorylation to meet their energetic needs. However, lymphocyte activation is associated with an increase in both glycolytic function and mitochondrial oxygen consumption (Kramer et al., 2014).

It is worth noting that N/L ratio was higher at 14 d following exposure to altitude and 7d following transport to low altitude in the sheep having low baseline hematocrit as compared to those having high baseline hematocrit level. Therefore, it could be assumed that hematocrit related metabolic type of energy supply was implicated in the observed difference among the groups concerning these leukocyte subtypes. This view is further supported by the similar N/L ratio among the groups before and following shearing against the background of higher cortisol level in the ewes of group II, that is a reliable marker of increased metabolic rate in these ewes. Besides, the lack of association between N/L ratio and plasma level of cortisol at that time may be due to decreased leukocyte glucocorticoid receptors.

Conclusion

1. Baseline hematocrit level was closely related with the rate of adrenal response to shearing and exposure to altitude.
2. There was inverse relationship between plasma cortisol level and neutrophil to lymphocyte ratio following 14 d exposure to moderate altitude hypoxia and 7 d after transport to low altitude.
3. Baseline Neutrophil-to-lymphocyte ratio was not associated plasma level of cortisol.

References

- Chacko, B.K., P.A. Kramer, S. Ravi, G.A. Benavides, T. Mitchell, B.P. Dranka, D. Ferrick, A.K. Singal, S.W. Ballinger, S.M. Bailey, R.W. Hardy, J. Zhang, D. Zhi, V.M. Darley-USmar (2014): The Bioenergetic Health Index: a new concept in mitochondrial translational research. *Clin Sci (Lond)*, 127, 6: 367-373.
- Cotter, P.F. (2015a): An examination of the utility of heterophil-lymphocyte ratios in assessing stress of caged hens. *Poultry Science*, 94: 512–517.
- Cotter, P.F. (2015b): Atypical lymphocytes and leukocytes in the peripheral circulation of caged hens. *Poultry Science*, 94: 1439–1445.
- Damgaard, B.M., M. Studnitz, K.H. Jensen (2009): The effect of continuous grouping of pigs in large groups on stress response and haematological parameters. *Livestock Science*, 121: 137-140.
- Davis, A.K., D.L. Maney, J.C. Maerz (2008): The use of leukocyte profile to measure stress in vertebrates: a review for ecologists. *Functional Ecology*, 22: 760-772.
- Dhabhar, F.S. (2009a): Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. *Neuroimmunomodulation*, 16, 5: 300-317.
- Dhabhar, F.S. (2009b): A hassle a day may keep the pathogens away: The fight-or-flight stress response and the augmentation of immune function *Integrative and Comparative Biology*, 49, 3: 215–236.
- Dhabhar, F.S., A.H. Miller, B.S. McEwen, R.L. Spencer (1995): Effects of stress on immune cell distribution—dynamics and hormonal mechanisms. *Journal of Immunology*. 154: 5511–5527.
- Evans, J.V., J.H. Whitlock (1964): Genetic Relationship between Maximum Hematocrit Values and Hemoglobin Type in Sheep. *Science*, 145, 3638: 1318.
- Grace, R.J., P.A. Gover, D.F. Treacher, S.E. Heard, T.C. Pearson (1992): Venesection in haemoglobin Yakima, a high oxygen affinity haemoglobin. *Clinical and Laboratory Haematology*, 14, 3: 195-199.
- Hashem, A.L.S. (2014): Effect of Summer Shearing on Thermoregulatory, Hematological and Cortisol Responses in Balady and Damascus Goats in Desert of Sinai. *Egypt World Applied Sciences Journal*, 30, 5: 521-533.

Jones, R.T., E.E. Osgood, B. Brimhall, R.D. Koler (1967): Hemoglobin Yakina. I. Clinical and biochemical studies. *Journal of Clinical Investigation*, 46, 11: 1840-1847.

Kramer, P.A., S. Ravi, B. Chacko, M.S. Johnson, V.M. Darley-USmar (2014): A review of the mitochondrial and glycolytic metabolism in human platelets and leukocytes: implications for their use as bioenergetic biomarkers. *Redox Biology*, 10, 2: 206-210.

Macintyre, A.N., J. C. Rathmel (2013): Activated lymphocytes as a metabolic model for carcinogenesis. *Cancer & Metabolism*, 1, 5: 1-12.

Mason, N.P. (2000): The physiology of high altitude: an introduction to the cardio-respiratory changes occurring on ascent to altitude. *Current Anaesthesia & Critical Care*, 11, 1: 34–41.

Maxwell, M.H. (1993): Avian blood leucocyte responses to stress. *World's Poultry Science Journal*, 49: 34–43.

Piccione, G., F. Grasso, F. Fazio, E. Giudice, P. Pennini (2006): Evaluation of some physiological and haematological parameters in the ewes: influence of shearing and sheltering. *Folia Veterinaria*, 50: 13-16.

Piccione, G., S. Casella, D. Alberghina, A. Zumbo, P. Pennisi (2010): Impact of shearing on body weight and serum total proteins in ewes. *Spanish Journal of Agricultural Research*, 8, 2: 342-346.

Prieto, M.T., J.L. Campo, S.G.Davila, M. G. Gil, O. Torres (2014): Effect of regrouping on heterophil to lymphocyte ratio, tonic immobility duration, and fluctuating asymmetry in layer stocks. *Europ.Poult.Sci.*, 78., ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2013.1

Schedlowski, M., A. Falk, A. Rohne, T. O. F. Wagner, R. Jacobs, U. Tewes, R. E. Schmidt (1993): Catecholamines induce alterations of distribution and activity of human natural killer (NK) cells. *Journal of Clinical Immunology*, 13:344–351.

Seagroves, T.N., H.E. Ryan, H. Lu, B.G. Wouters, M. Knapp, P. Thibault, K. Laderoute, R.S. Johnson (2001): Transcription factor HIF-1 is a necessary mediator of the pasteur effect in mammalian cells. *Molecular and Cellular Biology*, 21, 10: 3436-44.

Shirasawa, T., M. Izumizaki, Y. Suzuki, A. Ishihara, T. Shimizu, M. Tamaki, F. Huang, K. Koizumi, M. Iwase, H. Sakai, E. Tsuchida, K. Ueshima, H. Inoue, H. Koseki, T. Senda, T. Kuriyama, I. Homma (2003): Oxygen Affinity of Hemoglobin Regulates O₂ Consumption, Metabolism, and Physical Activity. *The Journal of Biological Chemistry*, 278, 7: 5035–5043.

Stark, H., S. Schuster (2012): Comparison of various approaches to calculating the optimal hematocrit in vertebrates. *Journal of Applied Physiology*, 113, 3: 355-367.