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Endocrine responses to moderate altitude hypoxia in pregnant Ile de France sheep with low or high basal hematocrit levels

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Abstract

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The object of the present study was to investigate some hormonal responses to mild hypoxia. Thirty Ile De France ewes were selected according to their hematocrit level and were allocated into 3 groups: low hematocrit (LHct) (hematocrit range – 19.7–27.9%), high hematocrit (HHct) (hematocrit range – 32.0–36.9%) and mean hematocrit (MHct) (hematocrit range – 28.3–29.8%). Immediately after shearing, ewes were transported from the Institute farm (altitude 500 m) to a mountain pasture (altitude 1440 m). Blood samples were taken by jugular venepuncture at the following time points: before transportation (baseline level), on day 7, 20 and 42 after the transport. The traits investigated were blood cortisol, thyroid hormones (T3 and T4), growth hormone, reticulocyte count and lactate. Moderate altitude exposure resulted in significant increase on day 7 in plasma cortisol levels in LHct ewes ($P < 0.05$). Cortisol levels increased significantly on d 20 in HHct and MHct ewes ($P < 0.05$) compared to baseline levels. Thyroxine levels in LHct and MHct ewes were significantly higher on d 7 as compared to baseline levels ($P < 0.05$). Triiodothyronine in LHct and HHct ewes declined significantly at 20 d compared to baseline level. ($P < 0.05$). Growth hormone levels declined significantly in HHct ewes on d 42 as compared to baseline levels ($P < 0.05$), while in LHct and MHct ewes remained unchanged. Corrected reticulocyte count was significantly higher in HHct ewes compared to LHct ewes at 7 d ($P < 0.05$). There was a general trend of a slight decrease in T3 and T4 levels in all ewes at 20 d and 42 d. Blood lactate levels increased significantly in LHct ($P < 0.001$) and HHct ewes ($P < 0.05$) at d 7 compared to baseline levels. Lactate levels on d 20 d and d 42 in LHct ewes and HHcrt ewes declined but remained significantly higher compared to baseline leves ($P < 0.05$). There were significant correlations on day 7 between: GH and T3 ($r = 0.503$; $P < 0.05$); GH and lactate ($r = 0,574$; $P < 0.01$); T3 and lactate ($r = 0.517$); $P < 0.05$). In conclusion, Adaptation of shorn pregnant ewes to moderate altitude hypoxia was associated with a slight decrease in basal metabolism accompanied by a slight increase in lactate level.

Keywords: cortisol; GH; T4; T3; lactate; reticulocytes; stress; sheep

Introduction

Oxygen is the basis of life on Earth. A low-oxygen state (hypoxia) is associated with organ development, stem cell maintenance, inflammation, aging, lung disorder, cardiovascular disease, neuronal degeneration and cancer (Semenza, 2007). Maintaining oxygen homeostasis represents the essential cellular metabolic process for the structural integrity of tissues in different pathological conditions, including severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection (Hertzog et al., 2021).

Exposure to high altitude is a convenient model for studying hypoxia. Investigations on sheep adaptation to high altitude are scares. A relationship between blood-related phenotypes and EPAS1 genotypes (encoding hypoxia-inducible factor 2α) was only recently found. Also, scans for selection of candidate genes involved in the adaptation to high-altitude indicated that those genes were associated with hypoxia, energy metabolism, angiogenesis, Ca2 metabolism, cortisone synthesis, erythropoietin, and iron homeostasis (Wei et al., 2016).

At high altitude, in addition to hypoxia, there is also exposure to low environmental temperature. In this case, the respiratory system is influenced by conflicting factors, because of the respiratory heat loss associated with an increase in ventilation during hypoxia (Diesel et al., 1990; Mortola & Frappell, 2000). Thus, increased maintenance requirements to maintain homeostasis could have negative effect on both animal health and production.

A key adaptive response to chronic hypoxia is a switch from oxidative phosphorylation to anaerobic glycolysis (Gilany & Vafakhah, 2010). Exposure to moderate altitude increases blood lactate concentration compared with sea level values which results from greater reliance on anaerobic glycolysis (McArdle et al., 2010). Both, acute and chronic exposure to high altitude, activate sympathetic nervous system and hypothalamic-pituitary-adrenal axis (Bloom et al., 1976; Humpeler et al., 1980; Sawhney et al., 1991; Mazzeo et al., 1994, 1995, 2000). Stress-induced erythropoiesis involves glucocorticoid regulation of erythroid progenitor expansion and terminal differentiation arrest (Bauer et al., 1999; von Lindern et al., 1999). Increased erythroid proliferation and differentiation arrest leads to increased production of immature red blood cells (Luger et al., 2003).

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 & Whitlock, 1964). According to the optimal hematocrit hypothesis, blood viscosity increases with rising Hct levels, limiting the blood's O_2 transport capacity (Schuler et al., 2010).

The reports on the effects of acute hypoxic exposure on thyroid and growth hormone are few and often contradictory.

This study was thus designed to investigate some endocrine parameters in ewes before and after transportation from low to high altitude on day 7, 20 and 42 later.

Materials and Methods

Study site and environment data

The current study was conducted strictly in accordance with the guideline of the Institutional Animal Ethics Committee. Our investigation was carried out in 2021, at the Institute of Animal Science, Kostinbrod, Bulgaria.

The ewes were shorn in the end of May 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 air temperatures on the day of arrival at the mountain pasture were 12.9°C and 24.4°C for the region of Kostinbrod (low altitude), and 8.0°C–13.1°C for the region of Petrohan Pass (moderate altitude), respectively. The animals stayed at high altitude for 4 months where they were on pasture for 10 h during the day. At night, they stayed in a barn. In addition to pasture, they were offered concentrate mixture once per day. The ewes had free access to a NaCl licking stone and water. Mean air temperature range in the region of Petrohan Pass during the summer months of 2021 was 11.9 to 20.1°C.

Ewes

Institute′s research flock of 110 Ile De France ewes was used to select ewes with low, normal, and high level of hematocrit. Because of hematocrit variation, all animals were bled three times at 10-day intervals, one month before the start of the experiment in order to get correct hematocrit (Hct) values. Ewes were deprived of food the night before blood collection. In the beginning of May all ewes of the flock were artificially inseminated following estrus synchronization.

Thirty, clinically healthy Ile de France ewes were divided into 3 groups of 10 subjects each according to their hematocrit level, i.e. ewes with mean Hct level (hematocrit range of 28.3–29.8%), ewes with low Hct level (hematocrit range of 19.7–27.9%) and ewes with high hematocrit level (hematocrit range of 32.0–36.9%). The age-matched groups consisted of 3 to 5 years old ewes. During the day, the animals grazed on natural pasture and were kept in a barn at night. They received supplemental concentrate and meadow hay twice daily with free access to water.

Plasma collection

Blood samples were taken by direct jugular venepuncture at the following time points: before transportation (baseline level), on day 7, 20 and 42 after the transport to moderate altitude. All blood samples were centrifuged at 5000 x g for 5 min at 10°C, aliquoted and stored at – 20°C until assayed.

Estimation of blood variables

The hematological analyses were performed with whole blood samples with 5-part–differential using automated hematology analyzer (URIT-5160 Vet, URIT Medical Electronic Co., Ltd, China). Reticulocytes were stained with New methylene blue and counted microscopically. We followed the procedure of Briggs and Bain described by Bain et al. (2012).

Corrected reticulocyte count was calculated using the following formula:

Corrected Reticulocyte count = Reticulocyte percentage × (Hemoglobin/Normal Hemoglobin).

Blood lactate level was measured using Stat Strip Xpress lactate hospital meter (Nova Biomedical, USA) that corrects for interference due to changes in hematocrit.

Hormone measurements

Plasma cortisol, growth hormone (GH), thyroxine (T4) and triiodothyronine (T3) were estimated by commercial ELISA kits according to manufacturer's instructions (NovaTec Immunodiagnostica GmbH, Germany). The optical density was read at 450 nm against blank using the microplate reader (Biotek, USA).

Statistical analysis

Statistical significance was analyzed using one-way ANOVA. All data are presented as arithmetic means ± standard error of the mean (SEM). Results were considered significant when probability values (P) were less than 0.05.

Results

Plasma concentrations of cortisol in LHct ewes, unlike MHct and HHct ewes, were significantly higher ($P < 0.05$) at 7 d after moderate altitude exposure compared to baseline levels (Figure 1). Cortisol levels in HHct and MHct increased significantly ($P < 0.05$) and reached peak on day 20 after moderate altitude exposure compared to baseline levels ($P < 0.05$). Cortisol concentrations in all ewes declined significantly at 42 d ($P < 0.05$) compared to 20 d after the transport. Cortisol concentration in HHct ewes was significantly higher at that time compared to MHct ewes ($P < 0.05$).

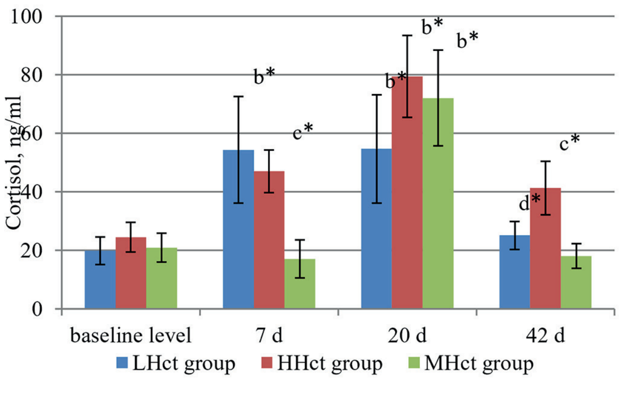

Fig. 1. Cortisol levels following moderate altitude exposure in pregnant sheep with low and high hematocrit values

* – $P < 0.05$; b – significantly different versus baseline level; c – significantly different versus MHct group; d – significantly different versus 7 d

There were no significant differences in baseline plasma T3 concentrations between groups (Figure 2). There was significantly higher T3 concentration in HHct than in LHct ewes on day 7 ($P < 0.05$). Plasma T3 concentrations increased insignificantly at 7 d in HHct ewes ($P>0.05$) and then declined significantly at 20 d after moderate altitude exposure compared to baseline concentrations ($P < 0.05$). There was significant decline of T3 concentration in LHct ewes at 20 d compared to baseline level ($P < 0.001$). Plasma T3 level in LHct was significantly lower as compared to MHct ewes at 20 d after moderate altitude exposure ($P < 0.05$).

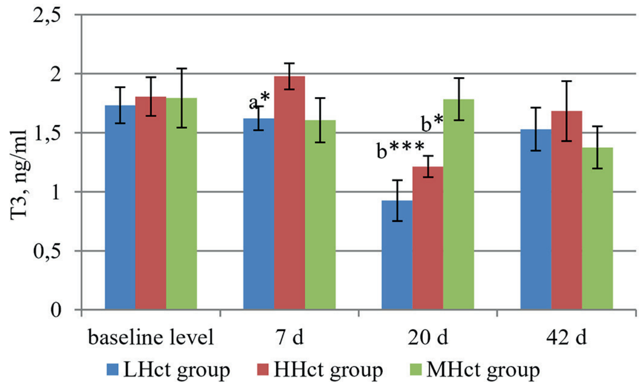

Fig. 2. T3 levels following moderate altitude exposure in pregnant sheep with low and high hematocrit values

* – $P < 0.05$; *** – $P < 0.001$; a – significantly different versus LHct group; b – significantly different versus baseline level

Plasma T4 levels increased significantly in LHct ($P < 0.05$) and MHct ($P < 0.01$) ewes as compared to respective baseline levels. Plasma T4 concentration in all ewes declined significantly as compared to 7 d. T4 levels at 20 d in HHct and LHct sheep were significantly lower ($P < 0.05$) than their respective baseline T4 levels. (Figure 3). There was a significant decrease of plasma T4 levels in LHct ($P < 0.05$) and HHct ($P < 0.01$) ewes at 42 d after moderate altitude exposure compared to baseline levels.

There was a significantly higher baseline GH level in HHct ewes as compared to baseline GH level in LHct ewes ($P < 0.05$). Plasma GH concentration in HHct ewes declined significantly at 42 d after moderate altitude exposure compared to baseline level ($P < 0.05$). GH concentration in MHct ewes was insignificantly higher at that time (42 d) compared to LHct and HHct ewes (Figure 4). There was significantly higher GH levels in LHct ewes compared to MHct ewes at 7 d after exposure to moderate altitude ($P < 0.05$).

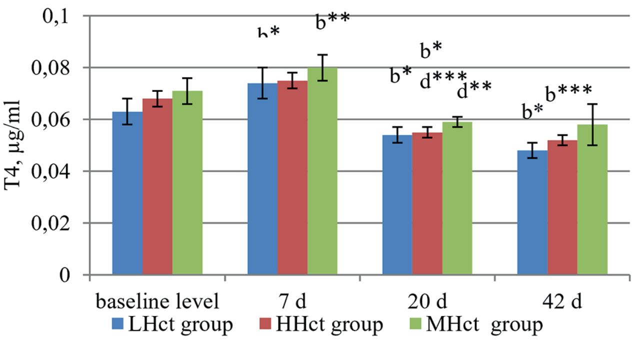

Fig. 3. T4 levels following moderate altitude exposure in pregnant sheep with low and high hematocrit values.
* – P < 0.05; ** – P < 0.01; *** – P < 0.001; b – significantly different versus baseline level; d – significantly different versus 7 d

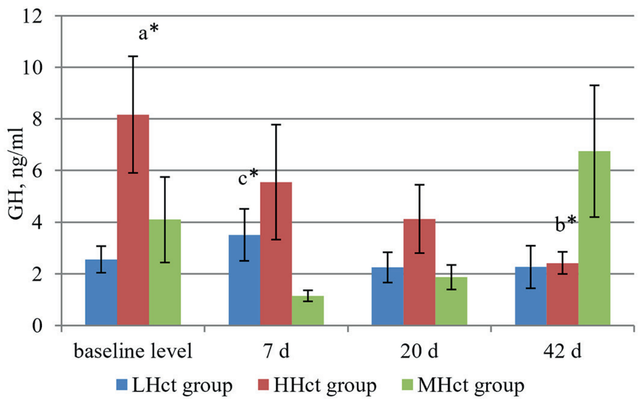

Fig. 4. GH levels following moderate altitude exposure in pregnant sheep with low and high hematocrit values
* – P < 0.05; a – significantly different versus LHct group; c – significantly different versus MHct group

There was a significant difference in baseline lactate levels between LHct and HHct ewes (P < 0.05). Lactate levels increased significantly in LHct (P < 0.001) and HHct (P < 0.05) groups on day 7 after transport to moderate altitide compared to baseline levels and recovered at 20 d and 42 d after moderate altitude exposure (Figure 5). The ewes with low hematocrit levels (LHct) showed the highest rate of lactate increase (1.75 mmol/L ± 0.29), followed by HHct (1.368 mmol/L ± 0.36) and MHct (0.443 mmol/L ± 0.53) ewes.). Plasma lactate levels in LHct ewes were significantly higher at 7 d after moderate altitude exposure compared to MHct ewes (P < 0.05). Lactate levels decreased on d 20 and d 42 (at moderate altitude), but remained statistically higher (P < 0.05) in LHT and HHct groups compared to basal (before transport to moderate altitude) levels.

The reticulocyte percentage and corrected reticulocyte count are presented in Figure 6 and Figure 7 The results

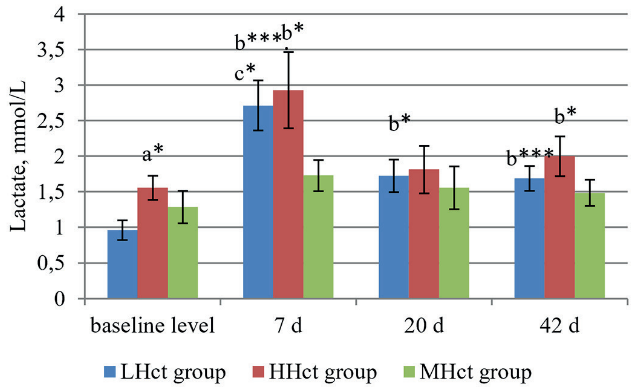

Fig. 5. Lactate levels following moderate altitude exposure in pregnant sheep with low and high hematocrit values
* – P < 0.05; *** – P < 0.001; a – significantly different versus LHct group; b – significantly different versus baseline level; c – significantly different versus MHct ewes

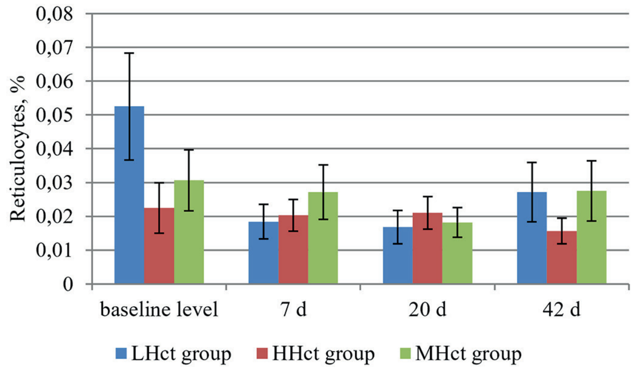

Fig. 6. Reticulocytes numbers following moderate altitude exposure in pregnant sheep with low and high hematocrit values

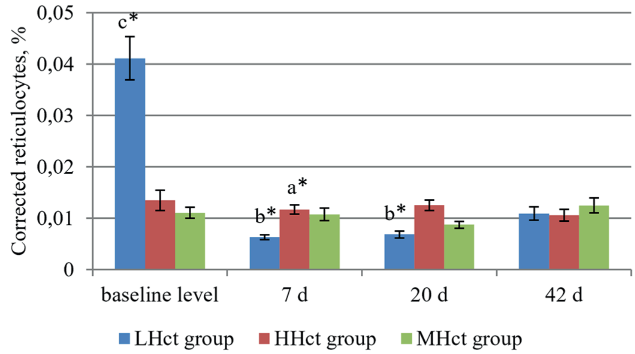

Fig. 7. Corrected Reticulocytes numbers following moderate altitude exposure in pregnant sheep with low and high hematocrit values
* – P < 0.05; a – significantly different versus LHct group; b – significantly different versus baseline level

indicate significantly higher corrected reticulocyte count in HHct ewes relative to LHct ewes ($P < 0.05$) on day 7.

Discussion

Exposure to moderate altitude for 7 days caused significant increase in cortisol levels ($P < 0.05$) in LHct ewes only (Figure 1). This difference in the amplitude of the cortisol response between the groups is most likely related to the existence of a different threshold of activation of the sympathetic-adrenomedullary system, which is caused by stress factors (transport, low temperature), whose action is sufficient to claim that it is a complex of stress stimuli, the total effect of which is the activation of the stress system. Lowlanders exhibited sympathetic nervous system stimulation on acute exposure to hypoxia, whereas Tibetans expressed a significant vagal dominance (Zhuang et al., 1993; West et al., 2013). Consequently, the observed difference in cortisol level increment between the groups after the transport to altitude could be attributed to a possible difference in sympathetic nervous system stimulation since altitude increases sympathetic activation (Hainsworth et al., 2007). Acute hypoxia enhances muscle sympathetic activity (Duplain et al., 1999; Hansen & Sander, 2003).

Cortisol values increased up to day 20 of moderate altitude exposure relative to basal levels in HHct and MHct ewes ($P < 0.05$), while in LHct ewes the cortisol level was similar to that on day 7. The further dynamics of cortisol in the three groups is characterized by a decrease, which in LHct ewes reaches statistical significance on d 42 compared to d 7 ($P < 0.05$). Higher cortisol levels in HHct ewes compared to LHct and MHct ewes on day 20 were maintained on day 42, with the difference between HHct and MHct ewes being demonstrated on day 42 ($P < 0.05$). It was hypothesized that the high levels of NO in response to hypoxia served as a potent inhibitor of steroidogenesis on ascent to high altitude (Gilbert-Kawai et al., 2014). Therefore, it could be speculated that NO was also involved in the lower adrenal response to altitude in animals with lower hematocrit values.

Baseline lactate level was significantly higher in HHct ewes as compared to LHct ewes ($P < 0.05$) The increase of lactate levels in LHct ($P < 0.05$) and HHct ewes ($P < 0.05$) at 7 d after transport to high altitude compared to the respective baseline levels may reflect an attempt of the body to cope with the extra energy requirement to regulate body temperature, when the animals experience some degree of cold and hypoxia (Figure 5). Both physical and psychological stressors are known to increase blood lactate due to stress-induced oxygen debt, determined by the high energy requirement (Hermann et al., 2019).

Lactate levels declined on d 20, but remained higher in HHct ewes as compared to LHct and MHct ewes, and such a trend was maintained throughout the experimental period at moderate altitude (Figure 5). The higher lactate levels in HHct as compared to LHct ewes might be due to a lower aerobic metabolism and reduced clearance of lactate (Stainsby & Brooks, 1990). Under conditions of hypoxia both hepatic lactate uptake and glucose production are increased (Palmer & Clegg, 2014). Therefore, an increase in Cori cycle activity under hypoxia can supply the necessary substrate to fuel the greater dependency of blood glucose brought about by increased hypoxia inducible factor (Palmer & Clegg, 2014). Increased gluconeogenesis under conditions of hypoxia serves to minimize accumulation of lactate in muscles and provide adequate amounts of glucose to facilitate the switch in fuel utilization (Palmer & Clegg, 2014).

It is worth noting that the degree of increase in the lactate levels in LHct group (64.53 %) was significantly higher than in HHct group (46.77 %) on d 7 compared to the basal level. The shift to anaerobic metabolism under stress conditions was expected to cause more pronounced stimulation of glycolysis and gluconeogenesis in LHct ewes, because of their higher dependence on oxidative phosphorylation. Increased energy deficit in these ewes caused by stress (transport, cold, hypoxia) would require fast production of energy via glucocorticoid stimulated glyconeogenesis. This assumption corresponds to the significantly higher cortisol response ($P < 0.05$) found on day 7 as compared to baseline level in LHct ewes (Figure 1). On the contrary, adrenal response in HHct ewes did not change significantly. This was probably due to the preferential reliance on glycolysis and therefore to higher capacity for quick supply of energy in HHct ewes. According to the lactate shuttle concept, skeletal muscle both produces and uses lactate as a fuel with much of the lactate formed in glycolytic fibers being taken up and oxidized in adjacent oxidative fibers. This use of lactate as a fuel requires mitochondrial respiration, thus it follows that an ability to rapidly utilize lactate requires ample mitochondrial abundance and respiratory capacity (Brooks, 2002; Brooks, 2018). Therefore, a possible difference in muscle mitochondrial density among the groups may influence lactate removal. Furthermore, it has been suggested that selection for increased body weight entails higher conversion from type IIa (fast oxido-glycolytic) to type IIb (fast glycolytic muscle fibers). The most pronounced shift from an oxidative to a more glycolytic metabolism has been observed in double-muscled compared to non-double muscled Belgian blue bulls. Moreover, due to the reduction of capillary density, the oxygen supply has also been reduced, which may impair elimination rate of lactate (Fiems, 2012). These findings suggest that HHct ewes may have higher percentage of type IIb (glycolytic) fibers and therefore higher need of oxygen to remove increased lactate production.

The dynamics of reticulocytes in HHct ewes differs from that of LHct ewes with a pronounced tendency to a higher value on the d 7 and d 20 and a lower value on d 42 (Figure 6). These differences were mathematically proven only in the corrected reticulocytes on day 7 due to the large individual differences (Figure 7). Recent studies have shown that hypoxic stress causes an increase in erythropoidal precursor populations (Wang et al., 2021; Bapata et al., 2021). The assumption that the observed changes in reticulocyte dynamics are directly related to cortisol dynamics is in line with the trend of a higher percentage of reticulocytes on day 7 in HHct ewes compared to LHct ewes, but does not correspond to day 42 of the stay on the mountain pasture, during which the percentage of reticulocytes shows a tendency to lower values than those of LHct ewes despite higher values of cortisol during this period.

Thyroid hormones appear to play a crucial role in stimulating reticulocyte production in normalizing cortisol levels. This is evident from the marked tendency of a higher percentage of reticulocytes on d 42 in LHct ewes (Figure 6), accompanied by a higher degree of increase in the value of triiodothyronine (Figure 2) to d 42 versus d 20 of exposure at moderate altitudes.

There is no doubt that, along with the many mediators involved in the control of erythropoiesis, the hormones of the adrenal glands and thyroid gland play a significant role in controlling this process. The mechanisms of mutual control of the two glands are still unclear. There is evidence that corticotropin-releasing factor stimulates thyrotropin secretion. On the other hand, elevated levels of corticosteroids caused by stimulation with corticotropin-releasing hormone suppress thyroid function through their negative feedback on corticotropin-releasing hormone increment. A number of *in vitro* studies have shown that both glucocorticoid hormones and thyroid hormones stimulate erythropoietin secretion, erythropoiesis, and reticulocyte proliferation (Udupa et al., 1985; Wessely et al., 1997; Stellaccia et al., 2009).

The intense stress, such as transport followed by cold stress and hypoxia during the first days at moderate altitude in our case, leading to a generalized response, expressed in a rise in cortisol levels (Figure 1) did not cause an increase in the biologically active thyroid hormone, which is triiodothyronine (Figure 2) in LHct ewes. This suggests that the stimulatory effect of corticotropin-releasing hormone on thyrotropin is partially offset by elevated cortisol levels, which predetermines the marked trend of a slight increase in triiodothyronine levels in HHct ewes on day 7 after transport of sheep to the the mountain pasture. (Figure 2). However, during the acclimatization process (d 42), cortisol levels normalized, suggesting that the initial generalized response to transport was discontinued. The resultant effect of the reduced level of cortisol and the expected reduced level of the corticotropin-releasing hormone is expressed in a marked tendency of lowering of triiodothyronine (T3) in the process of acclimatization (20–42 d). It can definitely be argued that higher values of cortisol in HHct ewes are accompanied by a marked tendency to lower values of T3 (20 d, 42 d), which suggests that in the process of acclimatization the nonspecific response to the central nervous system is transformed into the activation of functional systems involved in minimizing the effect of specific stress stimuli, such as hypoxia and low temperature. In this case, the inhibitory effect of cortisol on T3 secretion is thought to dominate over the thyroid-stimulating effect of corticotropin-releasing hormone. Exceptions to this trend are cortisol and T3 values in HHct ewes on day 7 of acclimatization. Deviation from the observed trend of dependence between the two hormones may be associated with a stronger adrenal response after transport of sheep with high hematocrit, in which the level of cortisol is higher than that of sheep with low hematocrit at 20 d. Therefore, it can be assumed that the inhibitory effect of higher cortisol levels on thyroid function in HHct ewes on day 20 compared to LHct ewes was dominated by the stimulatory effect of corticotropin-releasing hormone on thyroid function.

It is noteworthy that the lower cortisol level (Figure 1) in MHct ewes on day 7 coincided with the statistically proven higher values of thyroxine (T4) over the same time interval (Figure 3). Therefore, the increase in the percentage of reticulocytes on d 7 and d 42 of acclimatization in these sheep may be associated with a lower ratio of cortisol to thyroxine, while in HHct ewes the ratio between these two hormones is higher. The biological significance of the literature data for higher values of free T4 and reduced levels of free T3 at high altitudes remains unclear. Stress-induced increase in type 3 deiodinase has been shown to shift conversion of T4 from biologically active T3 to biologically inactive reverse T3 (Simonides et al., 2008).

It is well known that the hormones cortisol and T3 stimulate metabolism. Higher cortisol levels on d 20 in HHct ewes suggest a higher intensity of metabolic processes in them, which is accompanied by higher heat production, which in turn allows to compensate for hypoxia by increasing the degree of lung ventilation. Lower cortisol levels in LHct ewes on day 20 suggest a lower intensity of metabolic processes and heat production, which requires, among other physiological mechanisms to maintain thermoregulation at low temperatures, to reduce pulmonary ventilation, because inhaled cold air makes it difficult to maintain thermal homeostasis. Lower levels of cortisol and T3 on day 7 in MHct ewes suggest that in them, unlike animals with high hematocrit, the specific thermoregulatory mechanisms during this period are oriented to reduce metabolic processes in order to reduce the need for oxygen and respiratory cooling.

Our data show that the acclimatization strategy of animals with lower hematocrit is associated with lower energy consumption during the first days of the acclimatization period. This view is supported by the well-defined trend of a higher percentage of reticulocytes on day 7 of acclimatization in HHct ewes, which shows that the increased oxygen deficiency caused by increased metabolism cannot be compensated by increased pulmonary ventilation under conditions of the reduced atmospheric pressure at this altitude. The fact that in HHct ewes the level of T3 shows a tendency to increase, and in LHct and MHct ewes a tendency to decrease on d 7 compared to basal levels suggests that in sheep with high hematocrit, the supply of tissues with oxygen allows these animals to increase heat production in response to cold stress by stimulating metabolism (oxygen consumption) and not by reducing oxygen consumption and metabolism, as is likely to be the case in sheep with low hematocrit. This interpretation is also confirmed by the level of T4, which is significantly higher in sheep with low hematocrit (LHct and MHct) on day 7 compared to the basal level ($P < 0.05$), which suggests that the conversion of T4 to T3 in sheep with low hematocrit in the first days of acclimatization is suppressed in response to the need to lower metabolism and compensate for oxygen deficiency under conditions of cold stress. A significant difference between the groups in the T3 level on d 20 of acclimatization, when some relief of thermoregulation is expected due to increased hair cover and improved body insulation, shows the relationship of hemoglobin type with endocrine dynamics in the process of adaptation, namely statistically proven decrease in T4 on d 20 compared to day 7 in MHct ewes (control group) is accompanied by unchanged levels of T3 during this period (maintenance of metabolic activity), and in HHct ewes the level of T3 decreased significantly ($P < 0.05$). The close values of T3 and T4 between the groups on d 42, when a significant increase in the wool and improvement of the thermal insulation are expected, are an indication that the acclimatization process has already been performed. Therefore, differences in the dynamics of thyroid hormones between animals with different hematocrit are observed in both acute and chronic hypoxia.

Growth hormone is known to stimulate sweat secretion and heat evaporation during exercise. The increase in GH release during exercise is associated with the concomitant increase in body temperature. However, exercise performed at 4°C results in a suppression of GH secretion (Jørgensen et al., 2003).We did not find significant changes in GH levels throughout the entire experimental period in LHct and MHct ewes.

The major findings of a recent review of the research on multiple effects of GH suggest a role of GH in the regulation of angiogenesis, immune function, hematopoietic system, normal differentiation and function of blood cells (Devesa et al., 2016). Therefore, the tendency of increased GH level in MHct ewes on d 42 can be associated with one, or more of its many functions. The functional role of central GH signaling for energy homeostasis has not been fully defined. The trend of a slight increase in GH levels and decreased T3 levels on day 7 in LHct sheep indicates that GH influences metabolic responses that conserve energy at altitude.

Baseline GH level of HHct ewes was significantly higher ($P < 0.05$) compared to LHct ewes (Figure 4). GH levels decreased significantly at 42 d compared to baseline levels in HHct ewes only ($P < 0.05$). The reason for the changes in GH at high altitude is unclear In hyperthyroidism, the GHRH-induced release of GH is reduced, probably by an increase in hypothalamic somatostatin tone (Giustina & Veldhuis,1998).

There were significant Pearson correlation coefficients on day 7 between GH and lactate ($r = 0.573698$; $P < 0.01$) and also between T3 and lactate ($r = 0.51661$; $P < 0.05$). There was slight, but significant correlations between GH and T3 on day 7 ($r = 0.503005$ ($P < 0.05$). The data show the existence of relationship between these variables during the first days of acclimatization to moderate altitude. The data suggest that GH could play a role in modifying the metabolism to satisfy increased needs at altitude during short-term and long-term exposure.

Overall, the results of hormonal dynamics give further support of the view that exposure of ewes to moderate altitude results in a mild hypoxia accompanied by mild cold stress.

Conclusions

Exposure of sheep to moderate altitude elicited significant rise in plasma cortisol and lactate, which is widely believed to be marker of energy deficiency and inadequate oxygen delivery.

Adaptation of sheep to moderate altitude resulted in a slight decline in basal metabolism accompanied by a slight increase in lactate level.

Basal hematocrit level is related with the pattern of adrenal, thyroid and growth hormone adjustments of newly shorn ewes to moderate altitude.

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