Hormonal changes in acclimatized soldiers during a march at a high altitude with mountain skis

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Summary

Background: The aim of the present study is to identify the physiological impact of acute exposure to high altitudes on special acclimatized troops of the Chilean Army. Twenty-nine soldiers carried out a nocturnal winter march on mountain skis at an initial altitude of 2,800 m and up to 3,640 m. Two separate blood measurements were taken. The first one was taken the day before the march (Pre-sample) and the second one just after returning to the base camp (Post-sample). All subjects had been acclimatized prior to the study.

For hypothesis comparison purposes, the normality of the distribution was tested using the Shapiro-Wilk test. To determine if there were significant differences between the Pre and Post tests, a paired-samples Student t-test was applied for the variables with a normal distribution, and the Wilcoxon test was applied for the variables without a normal distribution. In all cases, a level of significance of 95% ($p<0.05$) was taken into consideration.

Results: Exposure of acclimatized troops to altitudes of 2,800 m to 3,640 m has an impact on the endocrine parameters and on the reduction of cortisol ($p<0.01$), total testosterone ($p<0.0001$), free testosterone ($p<0.0001$) and the free testosterone-cortisol ratio ($p<0.01$). Likewise, an increase in total leukocytes ($p<0.0001$), neutrophils ($p<0.0001$), monocytes ($p<0.0001$) and basophils ($p<0.001$), as well as a decrease of eosinophils ($p<0.0001$) and lymphocytes ($p<0.01$), was observed. No hematological changes were detected.

Conclusions: Endocrine changes were observed during high-altitude winter marches on mountain skis carried out by acclimatized Special Operation Troops, resulting in decreased cortisol and free and total testosterone levels. A stress condition due to the high altitudes also affected the anabolic/catabolic environment, which manifested as a significant decrease in the free testosterone/cortisol ratio. No hematological changes were identified. Marked changes were observed in some white cell series.

Key words:
Cortisol. Testosterone.
Special mountain troops.
High-altitude.

Cambios hormonales en soldados aclimatados durante una marcha en gran altitud con esquí de montaña

Resumen

Introducción: El objetivo del presente estudio es identificar el impacto fisiológico (con especial atención a los parámetros endocrinos y hematológicos) de la exposición aguda a gran altitud (GA) en tropas especiales aclimatadas del Ejército de Chile. Veintinueve soldados llevaron a cabo una marcha nocturna con esquí de montaña invernal a una GA de 2.800 m. hasta 3.640 m. Se tomaron dos muestras de sangre. La primera muestra fue tomada el día antes de la marcha (Pre test) y la segunda muestra justo después al regresar al campamento base Post test (a los 2.800 m). Todos los sujetos se encontraban aclimatados antes del estudio. Para cada análisis se testeo la normalidad de las distribuciones empleando el test de Shapiro-Wilk. Se calculó el promedio y la desviación estándar para cada medición. Para determinar si existían diferencias significativas entre el pre y post test se aplicó la prueba de t-Student pareada para las variables con distribución normal y el test de Wilcoxon para las variables que no tenían distribución normal. En todos los casos se consideró un nivel de confianza de 95% (valor $p < 0,05$).

Resultados: La exposición de las tropas aclimatadas a GA tiene un impacto en los parámetros endocrinos y en la reducción de cortisol ($p <0,01$), testosterona total ($p <0,0001$), testosterona libre ($p <0,0001$) y el ratio testosterona libre-cortisol ($p <0.01$). Asimismo, se observaron un aumento de leucocitos ($p <0,0001$), neutrófilos ($p <0,0001$), monocitos ($p <0,0001$) y basófilos ($p <0,001$), así como una decrease de eosinófilos ($p <0,0001$) y linfocitos ($p < 0.01$). No se observaron cambios en la serie roja.

Conclusiones: La marcha invernal nocturna con esquí de montaña en GA para tropas de operaciones especiales aclimatadas presento cambios endocrinos con disminución del cortisol, testosterona libre y total. Una condición de estrés por la marcha en GA también afectó al ambiente anabólico/catabólico, lo que se ve reflejado en una disminución significativa en el cociente testosterona libre/cortisol. No se observaron cambios hematológicos. Se observaron cambios significativos en algunas células de la serie blanca.

Palabras clave:
Cortisol. Testosterona.
Tropas especiales de montaña.
Gran altitud.

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Introduction

The preparation of soldiers for combat at high altitudes and the likelihood of future deployments that may be unpredictable and thus provide little time for preparation largely depend on soldiers' physical acclimatization in the shortest possible time. High-altitude environments cause additional impact on the stress of military operations.

The British Army addressed the issue of altitude adaptation by implementing training strategies at an adequate intensity[1]. Likewise, a literature review from the U.S. Army found that an altitude exposure equal to or higher than 4,000 m and a daily exposure duration of at least 1.5 hours repeated over a week or more are required to have a high probability of developing altitude acclimatization[2]. For special operations units, acclimatization is even more important, given that rapid deployments to high-altitude (HA)[3] mountainous regions are usually for complex missions and with short preparation time.

Reduced oxygen levels at high altitudes imply a reduction in both alveolar and arterial oxygen partial pressure[4], producing a leftward shift of the hemoglobin disassociation curve[5] through time, as a compensation mechanism to the hypoxia, thus producing changes in the blood plasma volume and erythropoietic response[6-8].

The oxyhemoglobin disassociation curve during acute hypoxia is shifted to the left due to alkalosis and a decreased arterial CO_2 pressure, product of an acute ventilatory response, which in turn implies a decreased capacity for hemoglobin to release oxygen[9]. During chronic hypoxia exposure, the disassociation curve is shifted to the right; due to the increment in production by the erythrocytes of 2,3-Bisphosphoglyceric acid (2,3DPG) metabolites, a decrease in pH (renal metabolic compensation of respiratory alkalosis) and an increase in arterial CO_2 pressure[10]. This rightward shift implies a desaturation of oxygen by the hemoglobin, which facilitates the availability of this gas to the tissues. This phenomenon is known as the Bohr effect[11,12].

Results of studies on the impact of hypoxia on hormonal responses differ. In the case of cortisol, literature findings show contradicting results. While some authors have observed increased resting cortisol concentrations in HA environments[13-15], other studies have not found raised levels of resting cortisol[16,17]. Similar differences in cortisol values were found when the exposure times to HA differ. Some authors have observed that with progressive ascents, resting cortisol levels do not vary[18], whereas other studies report that subjects who are rapidly exposed to hypoxic conditions, whether through hypobaric chamber, vehicle or helicopter for a quick ascent, show a rise in cortisol levels[13,19,20].

A similar controversy arises with testosterone concentrations at HA. Some studies have reported a decrease in testosterone levels during mountain climbing programs[21], while other investigators postulate a rise in testosterone during acute exposure to HA[22]. It has been observed among the military population that intermittent exposure to altitudes of 5,300 m for 6 months initially reduces testosterone levels, which then progressively increase after the exposure to hypoxia[23].

Regarding the responses induced by training at high altitudes in some cells of the white cell series, Klokker (1993)[24] found an increase in leukocyte concentrations under hypobaric conditions over a period of 20 minutes due to higher concentration of lymphocytes. However, according to Niess, (2003)[25], the concentration of neutrophils increases after extensive intermittent training at an altitude of 1,800 m, compared to such training at sea level. Umeda (2011)[26] observed a variety of significant changes related to the different neutrophil subpopulations studied post-exercise and attributed these changes to a combination of internal and external factors.

With respect to the red blood series at high altitudes, some studies have reported that high-performance athletes versus non-trained subjects may show hematological anomalies and reduced hemoglobin levels either near or below the inferior limit of the normal range[2,27].

Other studies have shown that some cells of the red series increase after three weeks of biathlon training[7]. Alternatively, Hematy (2014)[28] noted significant changes in red blood cells when observing subjects at the start of an ascent to 1,830 m and then 24 and 48 hours after the stay at 1,830 m (after returning from 4,000 m).

One of the stressors experienced by soldiers in the Chilean Army is the winter marches on mountain skis at different geographical altitudes. However, their physiological impact has not been addressed experimentally.

The aim of the present study is to identify the physiological impact of a nocturnal winter march on mountain skis at high altitude on Special Operation troops of the Chilean Army with special emphasis on endocrine and hematological parameters.

Material and method

Twenty nine soldiers (25.7 ± 4.50 years of age, 76.9 ± 7.12 kg weight and 178 ± 0.05 cm tall) participated in a nocturnal winter march on mountain skis in the region of Portillo, Chile, ascending from 2,800 m to 3,640 m and back to 2,800 m. Each soldier was loaded with 28 kg of equipment. The duration of the march was 5 hours and 35 minutes from base camp (2,800 m) up to (3,640 m) and back. The distance travelled was 20,6 km with an average slope of 9.3% and a maximum of 27,4%. The average environmental temperature during the march was -3 ºC to -12 ºC. The initial atmospheric pressure was 554 mmHg at 2,800 m. All subjects had been residing at base camp, located at 2,800 m altitude; for twelve weeks prior to carrying out the march, so they were acclimatized prior to the study. All subjects were informed of every aspect of the study and gave their written consent. The study was approved by the ethics committee of the Hospital Militar de Santiago, Chile and was carried out in compliance with the requirements of the Helsinki Declaration[29].

Two separate blood samples were taken at 06:00 hours. The first measurement was taken prior to the march (Pre) and the second after the march was completed (ascent to 3,640 m and return to the camp at 2,800 m) (Post).

All blood samples were taken by military nurses and they were obtained by venipuncture following the procedure stipulated in the Sampling Manual of the Clinical Laboratory of Hospital Militar de Santiago Chile.

The analytical processing of the blood measurements was performed in the Lab Core under the fully automated platform "LAB CELL" (Siemens) interfaced with Advia 2120, Advia 1800 and Advia Centaur XP equipment. Samples for blood count were collected in BD Vacutainer with EDTA and processed using optical laser-based and impedance flow cytometry. Samples for cortisol and testosterone (total and free) were collected in BD Vacutainer with clot activator and gel for serum separation. Samples were processed using chemiluminescence methodology for cortisol and total testosterone, and radioimmunoassay (gamma counter) for free testosterone.

Systolic and diastolic arterial pressures were measured manually via a sphygmomanometer (blood pressure kit, CEISO, USA, 2014). Mean arterial pressure (MAP) was calculated using the following formula: [(systolic – diastolic)/3] + diastolic[30]. Arterial oxygen saturation (SaO_2) was measured with a portable device (Nonin CMS50D, USA, 2014).

Prior to testing, weight and size were measured with a Tanita weighting machine (Tanita Ironman BC1500, Japan, 2015). Tympanic temperature was taken before and after the march via an infrared thermometer. (Boeringher, Germany, 2015).

Dehydration was calculated through the weight loss percentage.

Statistical analysis

For hypothesis contrast purposes, the normality of the distribution was tested using the Shapiro-Wilk test. Averages and standard deviations were calculated for each measurement. To determine if there were significant differences between the Pre and Post tests, a paired-samples Student *t*-test was used for the variables with a normal distribution, and the Wilcoxon test was used for the variables without a normal distribution. In all cases, a level of significance of 95% ($p < 0.05$) was taken into consideration.

Results

Table 1 shows Pre and Post data for SaO_2, MAP, temperature and weight with the value of p. All the changes were significant.

From the changes in weight, the level of dehydration induced by the march was calculated at 2.9%.

Figure 1 shows the changes in cortisol, free testosterone, total testosterone and the free testosterone-cortisol ratio. All the changes were significant.

Figure 2 shows the results of red blood cells, erythrocytes, hemoglobin, hematocrit, and medium corpuscular volume (MCV) Pre and Post march. Significant changes are only observed in the MCV between the Pre and Post measurements.

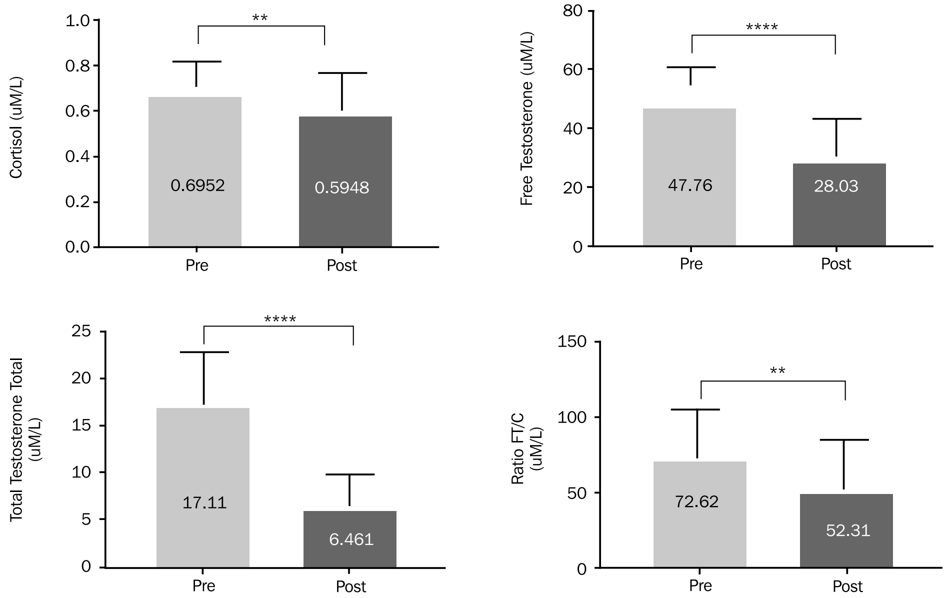
Figure 3 shows changes in total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils, all of which were significant between the Pre and Post situation.

Table 1. Shows pre and post saturation data, mean arterial pressure, temperature and weight with the value of p.

	Pre		Post		Pre *vs* Post
	Avg.	**SD**	**Avg.**	**SD**	***p***
Sa02 (%)	96.79	1.85	94.93	2.21	0.001
MAP (mmHg)	85,7	8,4	89,5	11,4	No significativo
Temperature (°C)	35.9	0.36	36.3	0.38	0.023
Weight (kg)	76.90	7.12	74.64	7.03	<0.0001

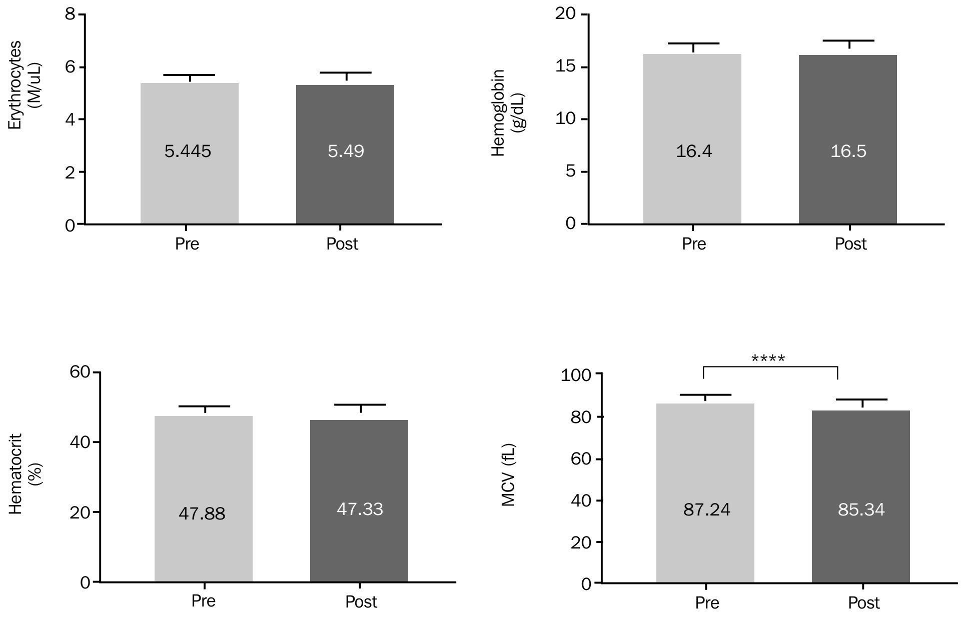
Changes in oxygen saturation, mean arterial pressure, temperature and weight caused by the HA march. (*=p<0.05 **p<0.01; ***p<0.001; ****p<0.0001).

Figure 1. Changes in cortisol, free testosterone, total testosterone and the free testosterone-cortisol ratio. All the changes were significant.



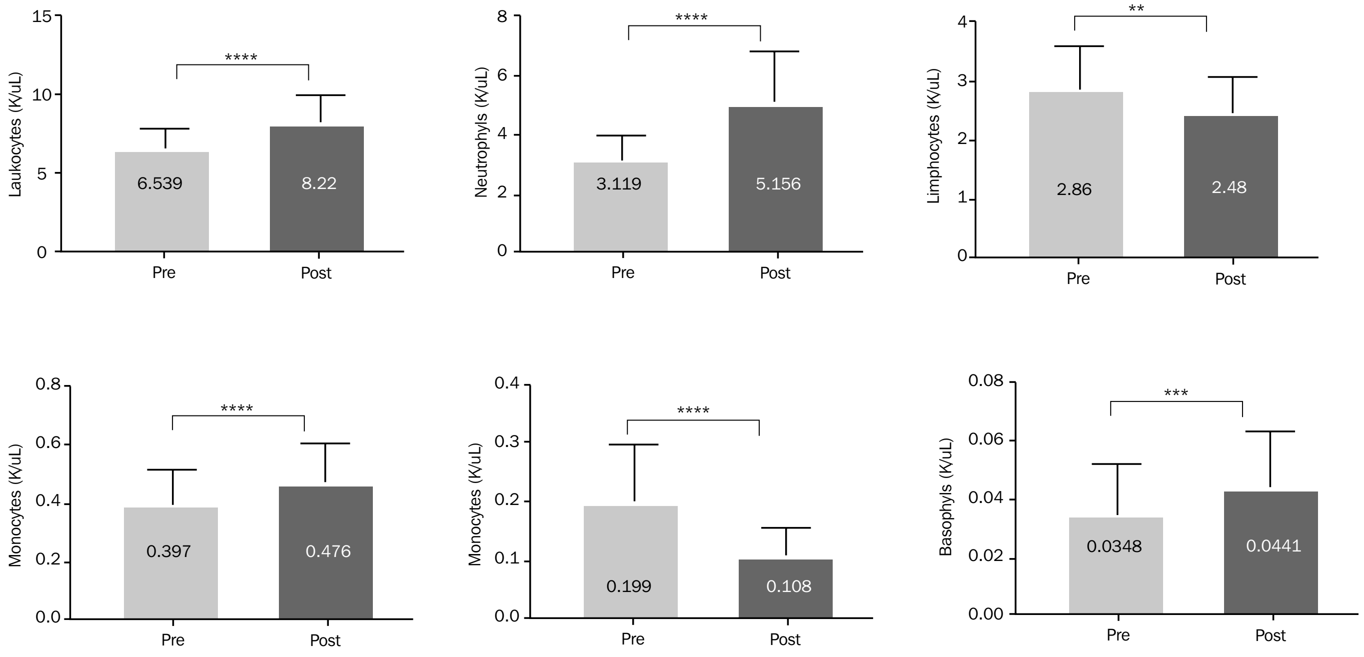
*= p <0,5; **p <0.01 ***p <0.001; ****p <0.0001

Figure 2. Changes in red blood cells, Erythrocytes, hematocrit, MCV and hemoglobin caused by the HA winter march.



*= p <0,5; **p <0.01 ***p <0.001; ****p <0.0001

Figure 3. Changes in Leukocytes, Neutrophils, Monocytes and Eosinophils caused by the HA winter march.



*= p <0,5; **p <0.01 ***p <0.001; ****p <0.0001

Discussion

The main finding of this study is that a nocturnal winter march with exposure from 2,800 m to 3,640 m among acclimatized Special Operation Troops has an impact on the endocrine and white cell series.

At the end of the march, significant weight loss was observed between the Pre- and Post march situations (2.9%), as well as significant oxygen desaturation and a significant increase in body temperature (Table 1). In our data, the resulting effect of a nocturnal march on mountain skis supposes an increase of energy exertion on the aerobic and anaerobic systems due to the slopes previously described, as well as muscle wear upon descent on skis, carrying military equipment. These conditions may explain the increase in the temperature of the subjects Post arrival at base camp (2800 m). The decreases in SaO_2 could be explained by the reduction of the partial pressure of O_2 at these altitudes, whereas the capacity of the hemoglobin to bond with oxygen may be seen to be affected[11,12]. This has also been observed in studies conducted on football players acclimatized to high altitudes[4] and players coming from low altitudes, not acclimatized to high altitudes[10].

With regard to cortisol, a significant decrease was observed after the march (Figure 1). Similar results have been observed in other studies. Woods (2012)[18] showed a salivary cortisol decrease during a six-hour march from 1,900 m, to 3,400 m and 4,270 m. Mc Lean (1989)[16] demonstrated a reduction in basal cortisol with submaximal exercises over 15 days at 4,450 m. Even after eight weeks of mountain training and an ascent to over 7,546 m, basal cortisol levels decreased in comparison to the values before training[21]. In contrast, Benso (2007)[17] did not observe any changes in cortisol levels after gradual exposures from sea level to 5,400 m and above 8,000 m.

It appears that the determining factor of the changes of cortisol at HA is the gradual altitude exposure. Acute exposure to altitude seems to increase cortisol levels[13-20]. Nevertheless, various studies have shown that if subjects who are previously acclimatized to the altitude practice physical activity, cortisol levels decrease[17,18,21,31]. In this study, the subjects remained at the base camp located at 2,800 m for a period of 12 weeks, and their cortisol levels decreased after the march at 3,640 m. It would appear that the gradual exposure and acclimatization at the base altitude and the response to physical exercise have a predominant role in reducing cortisol levels.

Testosterone, both free and total (protein-bound), decreased with the march (Figure 1), an expected finding that supports the stress conditions induced by the exercise and hypoxia exposure. This finding was further supported by the free testosterone/cortisol ratio, in which a marked decrease was observed (Figure 1). Nevertheless, the results obtained do not correspond to the high testosterone values documented in high-performance athletes who were overtrained[22,32,33]. Results similar to those of this study were observed in post training and post-high-altitude ascents when compared to the conditions prior to training[21]. Other authors[17,23,34] have also observed decreases in testosterone one and three months after expeditions above 7,800 m. The testosterone data of this study tend to be consistent with the bibliography, which refers to decreased testosterone levels in high-altitude conditions.

From a hematological perspective, no changes were observed in the red blood cell indices tests performed after the march (erythrocytes (Er), hemoglobin (Hb) and hematocrit (Hct) (Figure 2), as was expected for subjects with an acclimatization period of 12 weeks. This finding is consistent with those of other studies that have observed that after 21 days of training at altitudes of 1,500 m and 1,816 m, no significant differences in hemoglobin or hematocrit are registered[35]. Likewise, these results are also consistent with the findings of studies by Rietjens, *et al* (2002)[36], who observed that training over two weeks at altitudes of 1,500 m and 1,850 m initially caused significant increases in Hb, Hct and MCV; however, training at 2,600 m did not produce a considerable increase in the value of such variables, being in the lower extreme of the normal range. Regardless, the data in this study are not consistent with those of Heinicke (2003)[7] or Hematy (2014)[28], who observed increases in the red blood count over periods of up to three weeks at altitudes of 1,800 m and 4,000 m. Only mean corpuscular volume (MCV) (Figure 2) had a significant decrease in the Pre- and Post test values. Although the authors have not found a satisfactory answer for this change, these results are consistent with those of the studies by Sewchand (1980)[37], who reported a noticeable decrease (12-14%) in the MCV of subjects after five hours of exposure to low pressure. However, their MCV returned close to baseline values after 40 hours.

The red blood count response in this study would seem to be essentially determined by the altitude acclimatization process. In our opinion, the absence of changes in the red series may be due to a hemodilution effect seen in marathon runners[38] and to the acclimatization of the soldiers during the period prior to this study.

In relation to the white cell series (Figure 3), a significant increase was observed in leukocytes, neutrophils, monocytes and basophils, as well as a marked decrease in lymphocytes and eosinophils. Changes in leukocyte concentrations are interesting because stress is commonly associated with immunosuppression and, therefore, a high risk of acquiring infectious diseases[39]. Thake (2004)[40] observed similar results to those found in our study, in particular in relation to some cells of the white cell series through laboratory testing of long- and short-duration exercises equivalent to 4,000 m, which resulted in relative lymphopenia and a decrease in eosinophils. Likewise, Niess (2003)[25] observed increases in neutrophils post-exercise, which were markedly more pronounced at 1,800 m than at sea level. The investigation by Umeda (2011)[26] is also quite interesting, as he found significant results in various neutrophil immune functions after carrying out different types of exercises, attributing the increases and decreases to the balance between external factors (e.g., intensity and exercise modality) and internal factors (e.g., physical pain and fatigue).

Although these findings relate to a global count of white cell series, and the authors have not delved into subpopulation studies, the data in this study coincide with the literature in terms of the expected changes post-exercise under acclimatization conditions.

The main limitation of this study lies in the inability to differentiate the effect of the physical work load carried out from the additional stressors (such as the cold, sleep deprivation or accumulated fatigue). An additional constraint was raised by the non-monitoring of liquid intake, the degree of dehydration was only measured through weigh-ins pre and post march.

It would be interesting to include a psychological evaluation with the purpose of identifying the appropriate stress management mechanisms of those subjects with fewer changes in their testosterone and cortisol hormones, as well as heart rate variability (HRV) measurements as an indicator of sympathetic stress (Naranjo *et al*., 2015)[41].

Conclusions

A nocturnal winter march on mountain skis, with combat equipment at high altitudes and a 840 m slope, produced endocrine changes in acclimatized Special Operations Troops, resulting in decreased cortisol and free and total testosterone levels. Stress due to the HA march also affected the anabolic/catabolic environment, which manifested as a significant decrease in the free testosterone/cortisone ratio. No significant decrease was found in red blood cells, hemoglobin or hematocrits, which may be due to a hemodilution effect and the acclimatization of the subjects. Marked changes were observed in some white cell series. The Leukocyte population increased considerably during the winter march, specifically, there was a marked increase in neutrophil and monocyte, while also accompanied by a consistent decrease in lymphocyte.

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