

## The influence of short-term high altitude training on inflammatory and prooxidative-antioxidative indices in alpine ski athletes

by

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*Exposure of alpine skiing athletes, while training, at altitude hypoxia and low ambient temperature can modify the response of the immune system and increase reactive oxygen and nitrogen species (RONS) generation. The aim of this study was to evaluate the impact of six day training model "live low – train high" on selected indicators of immune and antioxidant-prooxidant balance of alpine skiing competitors. The study was performed in 7 men, alpine skiers, who underwent 6-day training at Kaunertal glacier (3160 m). Before departure to glacier training, and after returning to sea level participants underwent series of tests. Somatic characteristics, anaerobic exercise capacity, blood morphological parameters and concentrations of interleukin 6 (IL-6), C-reactive protein (hsCRP), thyroid stimulating hormone (TSH), thiobarbituric acid reactive substances (TBARS), total antioxidant status (TAS), total iron (Fe) and total iron binding capacity (TIBC) were assessed. High altitude training has led to a significant increase in anaerobic capacity ( $p<0.05$ ) and serum concentrations of IL-6 and hsCRP ( $p<0.05$ ). A negative correlation among the difference in iron ( $\Delta$ Fe) concentration between two study terms and the change of hsCRP levels was also found ( $p<0.05$ ). Alpine training conditions led to a slight increase in immunological indices concentration in studied skiers. However, it did not cause any significant change in prooxidant-antioxidant balance, which could be related to earlier anaerobic training adaptation.*

**Key words:** interleukin 6, hsCRP, oxidative stress, high altitude, exercise, alpine skiing

### Introduction

Long-lasting exposure to altitude hypoxia and low ambient temperature is the specificity of

alpine skiing. Both of these environmental factors alter the immune response (Facco et al., 2005, Hartmann et al., 2000, Castellani et al., 2002, Walsh and Whitham, 2006). Upper respiratory

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tract infections are common in elite cross-country skiers (Facco et al., 2005). In exercising men, during cold exposure, changes in pro-inflammatory cytokine expression are often observed (Castellani et al., 2002).

High altitude hypoxia may stimulate expression of the inflammatory cytokines independently from ambient temperature. This kind of response to low oxygen concentration has been evolved as a physiological mechanism detecting tissue injury and improving tissue repair (Hartmann et al., 2000).

Performing physical exercise at high altitude enhances the immune response by increasing energy substrates demand and inducing significant myocytes damage under hypoxia conditions (Hagobian et al., 2006, Magalhães et al., 2005, Walsh, Whitham, 2006). Muscle damage in professional athletes during high-altitude training is not only due to the increased oxygen deficit, but also the result of a rapid increase in training intensity or volume (Cazzola et al., 2003).

It is generally admitted that a single bout of hypoxia can be responsible for an increase in reactive oxygen and nitrogen species (RONS) generation leading to higher oxidative stress values resulting in the increase of biological tissue oxidation (Pialoux et al., 2009). The available information suggests that RONS are involved, and may even play a causative role, in the high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE) (Bailey et al., 2001).

Various environmental factors such as cold temperature and high altitude can affect performance in winter sports athletes. The best acclimatization practices can help them in better preparation for training and competitions (Chapman et al., 2010). In Alpine skiing competitors, due to the specifics of this discipline, a high level of both aerobic and anaerobic capacity is essential (Bacharach and Duvillard, 1995). "Live low - train high" training model is used by the athletes to acclimatize for competing at high altitudes (Vogt, 2010). However, physical and physiological effects accompanying hypoxic conditions may have no beneficial effects on muscles or performance. There are no clear recommendations necessary to achieve sport success for athletes competing at high altitudes (Chapman et al., 2010).

Thus, better understanding of the impact caused by the environmental factors on certain metabolic processes and the stress response can lead to better preparation for training and competitive conditions. Therefore, the objective of this study was to assess the impact of the six day training model "live low – train high" on selected indicators of immune and antioxidant-prooxidant balance in alpine skiers.

### ***Material and methods***

The study was performed on seven men aged from 20 to 29 years ( $23 \pm 3.6$  years,  $75.2 \pm 5.93$  kg,  $180 \pm 4.30$  cm), practicing alpine skiing. Training experience averaged  $7 \pm 2.1$  years (from 5 to 11

years). Skiers were subjected to 6-day training at Kaunertal glacier (3160 m). The study was conducted during the specific preparation phase. Before departure to the glacier, the athletes performed general conditioning three times a week. Subjects arrived to altitude 1300 m in the afternoon (about 6 pm) and the next day, in the morning (at 8 am), they started the 6-day training program. During the training period, the athletes rode every day from 1300 m to about 3000 m altitude above sea level, where they stayed for 7 hours. The total period of high intensity interval physical exercise was 4 hours per day and about 3 hours they spent on arrangement for the training, rest and final activity after the training (fig. 1). Daily training consisted of skiing through slalom and giant slalom gates. Athletes were skiing 30-40 gates long slaloms within 40-45 seconds. The exercise was repeated 16 times. During the glacier training, the air temperature ranged from -4 to -7 ° C.

The day before departure to glacier training (about 34 hours before arriving to the altitude 1300 m) and after returning to sea level (about 48 hours after leaving 1300 m altitude) participants were subjected to anthropometric measurements, anaerobic fitness evaluation and assessment of venous blood samples for biochemical analyses.

Anaerobic capacity assessment was conducted using the 30 s Wingate Anaerobic Test (Bar-Or, 1987). The test was performed on a Monark cycloergometer (Sweden). The workload

(mechanically set pedaling force) calculation was based on the following formula:

$$\text{Workload} = 0.075 \times \text{body weight (kg)}.$$

Blood samples for biochemical analyses were taken from basilic vein after an overnight fast (between 8 and 9 a.m.) The levels of morphological indices were determined in whole blood using the hematology analyzer (Coulter MD2, Sysmed Lab, USA). In the serum samples were assessed both total iron concentration with the colorimetric method (Emapol, Poland) and the value of total iron binding capacity (TIBC) using the method described by Persijn et al. (1971). The transferrin saturation (Tfs) was calculated using the formula:  $\text{Fe/TIBC} \times 100\%$ . The concentrations of thyroid stimulating hormone (TSH) and interleukin 6 (IL-6) were measured with a commercially available enzyme-linked immunoassays ELISA (Human, Germany and R&D Systems, USA kits, respectively).

Total antioxidant status (TAS) was measured in blood plasma samples with a commercially available assay (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK); C-reactive protein (hsCRP) concentrations were determined with a high sensitive nephelometer method (Dade Behring, Germany); thiobarbituric acid reactive substances (TBARS) concentrations were assessed using a spectrophotometric method with chromogen extraction with n-butanol, described by Buege and Aust (1991).

Capability of physical training participation of all subjects was confirmed by medical doctors

in written form. All participants gave written, informed consent to the training program. The protocol of the study was approved by the local Ethics Committee (Medical University in Poznan).

Statistical analyses were performed with Statistica 8.0 software package. All data are expressed as mean, standard deviation (SD), median, maximum and minimum values. The normality of the data distribution was verified with Shapiro-Wilk test. The differences between paired variables were measured with Wilcoxon test. Spearman's rank analysis was used to calculate correlation coefficients. P value <0.05 was considered significant.

## Results

Table 1 presents basic statistics of morphological and biochemical indices of blood, as well as the anaerobic peak power and relative peak power values measured before the glacier training, and after returning to sea level.

Comparative analysis of biochemical indices of blood obtained in both studied terms showed a significant increase in levels of hsCRP and IL-6 ( $p < 0.05$ ). There were no significant changes in TSH, TAS and TBARS concentrations. Although there was no significant change in the morphological indices after returning to sea level, in six athletes an increase in total iron concentration, the rate of its binding (TIBC), and the degree of transferrin saturation (Tfs) occurred. In one athlete, however, these indicators declined. The values of peak anaerobic power and relative

peak power increased significantly ( $p < 0.05$ ) over the research period. Difference between two studied terms in iron ( $\Delta Fe$ ) concentrations negatively correlated with changing concentrations of hsCRP ( $r = -0.84$ ,  $p = 0.0190$ ). There was a positive correlation ( $r = 0.96$ ,  $p = 0.0005$ ) between the change in iron concentration ( $\Delta Fe$ ) and total capacity of its binding ( $\Delta TIBC$ ).

## Discussion

In the presented study, after the glacier training, there was a slight, statistically significant, increase in concentrations of proinflammatory indicators: C-reactive protein and IL-6 ( $p < 0.05$ ). Hartmann et al. (2000) showed that exposure to high altitude, even at rest, induces an inflammatory response and suggested that proinflammatory cytokines (mainly IL-6) released in hypoxia may contribute to the pulmonary edema development. Hartmann et al. (2000) noted a significant increase in IL-6 level (up to 2 pg/ml) already on the second day of stay at 3500 meters altitude and a similar increase in mentioned cytokine on the fifth day at 4500 m above the sea level. The increased C-reactive protein concentration was observed after the 3<sup>rd</sup> and 4<sup>th</sup> day of stay at these heights (3.5 – 5.8  $\mu\text{g/ml}$ ). After a week spent at sea level the concentration of these indicators decreased to the initial level. In the presented research, in studied skiers a smaller increase of IL-6 and hsCRP was noticed. It could be related to a lower altitude (3000 m) at which athletes trained comparing to Hartman et al. (2000) studies and that the

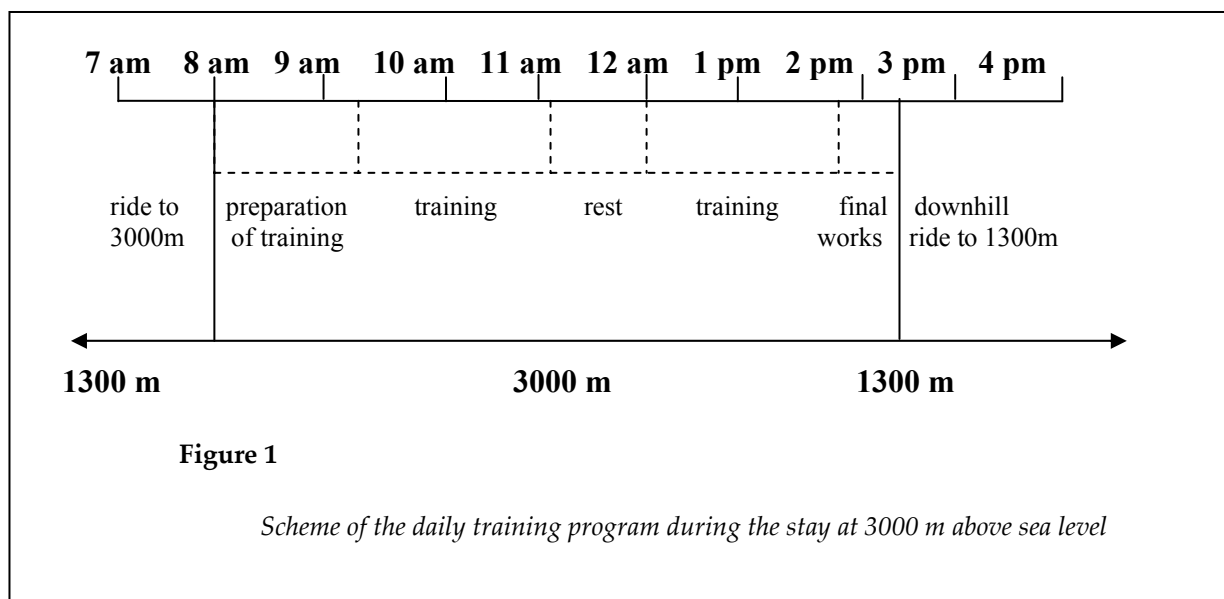
concentration of these cytokines was measured 48 hrs after returning to sea level altitude. Hagobian et al. (2006) have also observed elevated plasma IL-6 and CRP concentrations following exercise at high altitude (4300 m), but those changes were most significant at the beginning of the training camp. In contrary, Klausen et al. (1997) observed

an increase in IL-6 concentration on the fourth day of stay at 4350 m altitude, yet there was no change in C-reactive protein concentration. According to these authors, increased level of IL-6 had no connection with the inflammatory process, but probably served to stimulate erythropoiesis.

**Table 1**  
*Descriptive statistics of morphological parameters and blood concentrations of biochemical indices in skiers before and after the return from glacier training*

Parameter	Pre-training	Post-training	p value
	$\bar{X} \pm SD$ (Me; min-max)	$\bar{X} \pm SD$ (Me; min-max)	
WBC [ $10 \times 3 / \square l$ ]	5.2 $\pm$ 1.00 (5.4; 3.7-7.0)	5.8 $\pm$ 1.88 (4.9; 3.7-8.4)	0.3454
Lymphocyte [%]	31.3 $\pm$ 7.17 (33.5; 19.8-39.6)	25.6 $\pm$ 8.16 (21.6; 17.6-39.0)	0.0630
RBC [ $10 \times 6 / \square l$ ]	5.4 $\pm$ 0.27 (5.4; 5.0-5.8)	5.3 $\pm$ 0.28 (5.3; 5.1-5.9)	0.3980
Hb [mmol/l]	10.2 $\pm$ 0.56 (10.1; 9.5-11.1)	10.2 $\pm$ 0.68 (10.2; 9.6-11.6)	0.6750
Hct [%]	46.5 $\pm$ 2.68 (46.0; 43.4-51.2)	46.5 $\pm$ 3.08 (46.0; 44.3-53.0)	0.9326
Fe [ $\square g / dl$ ]	91.7 $\pm$ 37.49 (85.0; 59.0-169.0)	119.6 $\pm$ 23.61 (115.0; 90.0-155.0)	0.1763
TIBC [ $\square g / dl$ ]	366.9 $\pm$ 103.51 (361.0; 262.0-570.0)	426.3 $\pm$ 50.95 (451.0; 341.0-480.0)	0.1763
Tfs [%]	24.4 $\pm$ 2.79 (23.3; 21.9-29.6)	28.0 $\pm$ 3.82 (28.9; 22.5-32.6)	0.1563
TSH [mIU/l]	2.7 $\pm$ 2.39 (1.8; 0.1-7.1)	1.9 $\pm$ 1.02 (2.2; 0.1-3.1)	0.2945
TAS [mmol/l]	1.7 $\pm$ 0.27 (1.7; 1.4-2.1)	1.5 $\pm$ 0.32 (1.5; 0.94-1.8)	0.3105
TBARS [ $\mu mol / l$ ]	3.3 $\pm$ 1.02 (3.6; 1.1-4.2)	4.0 $\pm$ 0.96 (3.9; 2.5-5.4)	0.1763
IL-6 [pg/ml]	0.57 $\pm$ 0.386 (0.52; 0.27-1.4)	0.76 $\pm$ 0.384 (0.66; 0.46-1.6)	0.0280*
hsCRP [mg/l]	0.56 $\pm$ 0.462 (0.45; 0.16-1.46)	1.40 $\pm$ 1.290 (0.91; 0.31-3.9)	0.0180*
Anaer. Peak Pow. [W]	734.9 $\pm$ 94.79 (763.0; 560.0- 838.0)	778.1 $\pm$ 108.18 (797.0; 567.0-901.0)	0.0425*
Relative Peak Pow. [W/kg]	9.7 $\pm$ 0.71 (9.8; 8.8 – 10.5)	10.4 $\pm$ 0.92 (10.6; 9.0 – 11.4)	0.0313*
Overall work [kJ]	16.7 $\pm$ 2.83 (17.6; 12.6 – 19.3)	17.9 $\pm$ 2.40 (17.9; 13.8 – 20.7)	0.1094

\*  $p < 0.05$  - statistically significant difference between terms of the study, WBC-white blood cells, RBC - red blood cells, Hb - hemoglobin, Hct – hematocrit, TIBC - total iron binding capacity, Tfs - transferrin saturation, TSH - thyroid stimulating hormone, TAS - total antioxidant system, TBARS - thiobarbituric acid reactive substances, hsCRP - high sensitivity C-reactive protein



In a long-term study at high altitude in Antarctica (from 2077 m to 3032 m), Otani and Kusagaya (2003) found an increase in IL-6 and CRP levels, while the changes in CRP levels were very slight. They concluded that the inflammation was not associated with changes in IL-6. These authors indicated psychological stress and other factors as related to IL-6 levels. However there was no correlation between changes in IL-6 and erythropoietin level. In this study, similarly to Otani and Kusagaya (2003), the correlation between IL-6 and hsCRP concentrations was not observed and this may underline the existence of different mechanism of both immunological indices changes.

The immune system boost in high altitude conditions may be caused by the increased secretion of thyroid hormones. A significant contribution of thyroid hormones in the erythropoietin expression in hypoxic conditions (Ma et al. 2004) was revealed. Basu et al. (1995)

recorded a decrease in the concentration of thyroid stimulating hormone (TSH) and increased levels of thyroid hormones (total T3, free T4, free T3) in people staying at altitudes above 5000 m. According to this author, it indicates a subtle degree of tissue hyperthyroidism which may play an important role in overcoming the extreme cold and hypoxic environment of high altitude. Though at lower altitudes (3750 m), the changes in TSH concentration were not noted. Alike with the results of this study. Thyroid hormones affect the immune system by increasing the proliferation and lymphocytes secretion (Kruger, 1996). In this study, the tendency to decrease the amount of lymphocytes was found, yet statistical significance did not occur.

Under reduced oxygen availability, the body is exposed to greater risk of oxidative stress (Bakonyi, Radak 2004). Human studies indicate that high altitude associated hypoxia causes oxidative damage to lipids, proteins and DNA

(Moller et al., 2001, Magalhães et al., 2005, Joanny et al., 2001). On the other hand, the induction of RONS concentration changes during exercise is an important process ensuring the proper functioning of the cell, its growth, proliferation and remodeling (Ji et al., 2008). Exercise induced regulation of DNA binding of transcription factors, sensitive to cell oxidation-reduction environment changes, may determine main adaptive response conditioning the survival under adverse external conditions (Vasilaki et al., 2006).

It should also be noted that when training intensity or volume exceeds the organisms' adaptive capacity, the recovery system becomes inefficient. At the same time, increased synthesis of RONS and inflammatory mediators may lead to transition from the controlled body defense to an uncontrolled tissue damage process (Margonis et al., 2007). Although, up to date, there is no standardized method of monitoring the training loads on the basis of prooxidant-antioxidant balance markers changes, some authors suggest that differences in TBARS and TAS levels may be useful in detecting overload in athletes, especially in skeletal muscles (Margonis et al., 2007). TBARS are formed during free radical damage in lipid membranes of skeletal muscle and red blood cells, while the initiating defense in oxidative stress conditions may lead to the TAS level differences.

Results obtained in this study did not show significant changes in plasma antioxidant status (TAS) and TBARS levels after the training period

in alpine skiers. The development of higher concentrations of RONS immediately after athletes' returning from training carried out in hypoxic conditions at about 3000 m above sea level to a resting place with greater oxygen availability (1300 m) can not be excluded. It is probable that completed subsequent reperfusion could activate RONS synthesis, which were a stimulus causing a physiological response (insignificant post-training increase in plasma TBARS concentration). Striving for cell metabolic balance restoration resulted in the post-training adaptation, which was evidenced by the increase in skiers' anaerobic capacity, evaluated by the Wingate test, after training conducted in high mountains. However, improved performance during the test in athletes could be the effect of changes in oxidative energy production. According to Beneke et al. (2002), the aerobic component in metabolic profile of the Wingate test is about 20 %.

Inflammatory response to hypoxia is one of the mechanisms, in which iron metabolism is involved. IL-6 is the primary mediator for the up-regulation of liver hepcidin activity – the key regulator of iron metabolism (Kemna et al., 2008). In hypoxia, hepcidin regulates the availability of iron for erythropoiesis (Vyoral and Petrák 2005). Adequate altitude (minimum 2100-2500 m) and duration of the stay are important factors for achieving significant increase in red blood cell mass (Pottgieser et al., 2009).

In this study, probably due to a short period of stay at high altitude, there was no significant change in the quantity of erythrocytes and hemoglobin. However, there was a tendency towards changes in iron concentration and its binding ability. It should be emphasized that this trend was noticed in six athletes, thus a small number of subjects probably affected the lack of statistical significance. Although, it is difficult to determine whether changes in serum iron level were associated with the mechanism leading to an increase in erythropoiesis (in the later stage), or with other existing mechanisms, such as a change in prooxidant-antioxidant balance occurring as a metabolic response to exercise (Nagata et al., 2007).

The increase in TIBC and transferrin saturation levels could play a protective role against prooxidative (toxic) iron-catalyzed reactions. In the presented research however, the relationship between serum iron, TIBC and Tf

with prooxidant-antioxidant indicators was not noted, but a negative correlation between the increase in iron concentration and the value of change in hsCRP concentration was found after the stay in the mountains. The resulting correlation can therefore indicate the participation of proinflammatory factors in the distribution of iron in the body, which was confirmed in other studies (Feelders, 1998).

### ***Conclusion***

In conclusion, after a short training period at high altitude, there was a subtle increase in immunological indices and at the same time, no changes in prooxidative-antioxidant parameters in alpine skiers. Six-days high altitude training did not cause dramatic effects in homeostasis of athletes practicing disciplines with a high anaerobic component but was efficient in improving performance. However, the small number of athletes was the limiting factor of this study.

### ***References***

- Bacharach DW, Duvillard SP. Intermediate and long-term anaerobic performance of elite Alpine skiers. *Med Sci Sports Exerc*, 1995; 27(3): 305-309
- Bailey DM, Davies B, Young IS, Hullin DA, Seddon PS. A potential role for free radical-mediated skeletal muscle soreness in the pathophysiology of acute mountain sickness. *Aviation Space Environ Medicine*, 2001; 72: 513-521
- Bakonyi T, Radak Z. High altitude and free radicals. *J Sci Med Sport*, 2004, 3, 64-69
- Bar-Or O. The Wingate anaerobic test: An update on methodology, reliability and validity. *Sports Medicine*, 1987; (4): 381-394
- Basu M, Pal K, Malhotra AS., Prasad R, Sawhney RC. Free and total thyroid hormones in humans at extreme altitude. *Int J Biometeorol*, 1995; 39: 17-21



- Beneke R, Pollmann C, Bleif I, Leithäuser RM, Hütler M. How anaerobic is the Wingate Anaerobic Test for humans? *Eur J Appl Physiol* 2002; 87: 388-392
- Buege J, Aust SD. The thiobarbituric acid assay. In: Rice-Evans CA, Diplock AT, Symons MCR. (Eds) *Techniques in Free Radical Research*. Elsevier, Amsterdam, London, New York, Tokyo, 1991; 147-148
- Castellani JW, Brenner IKM, Rhind SG. Cold exposure: human immune responses and intracellular cytokine expression. *Med Sci Sports Exerc*, 2002; 34(12): 2013-2020
- Cazzola R, Russo-Volpe S, Cervato G, Cestaro B. Biochemical assessment of oxidative stress, erythrocyte membrane fluidity and antioxidant status in professional soccer players and sedentary controls. *Eur J Clin Invest*, 2003; 33: 924-930
- Chapman RF, Stickford JL, Levine BD. Altitude training considerations for the winter sport athlete. *Exp Physiol*, 2010; 95: 411-421
- Facco M, Zilli C, Siviero M, Ermolao A, Travain G, Baesso I, Bonamico S, Cabrelle A, Zaccaria M, Agostini C. Modulation of immune response by the acute and chronic exposure to high altitude. *Med Sci Sports Exerc*, 2005; 37 (5): 768-774
- Feelders RA, Vreugdenhil G, Eggermont AMM, Kuiper-Kramer PA, van Eijk HG, Swaak AJG. Regulation of iron metabolism in the acute-phase response: interferon  $\gamma$  and tumour necrosis factor  $\alpha$  induce hypoferraemia, ferritin production and a decrease in circulating transferrin receptors in cancer patients. *Eur J Clin Invest*, 1998; 28: 520-527
- Hagobian TA, Jacobs KA, Subudhi AW, Fattor JA, Rock PB, Muza SR, Fulco CS, Braun B, Grediagin A, Mazzeo RS, Cymerman A, Friedlander AL. Cytokine responses at high altitude: effects of exercise and antioxidants at 4300 m. *Med Sci Sports Exerc*, 2006; 38(2): 276-285
- Hartmann G, Tschöp M, Fischer R, Bidlingmaier C, Riepl R, Tschöp K, Hautmann H, Endres S, Toepfer M. High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and c-reactive protein. *Cytokine*, 2000; 12: 246-252
- Ji LL, Radak Z, Goto S. (2008) Hormesis and Exercise: How the Cell Copes with Oxidative stress. *Am J Pharm Toxicol*, 2008; 3(1): 41-55
- Joanny P, Steinberg J, Robach P, Richalet JP, Gortan C, Gardette B, Jammes Y. Operation Everest III (Comex'97): the effect of simulated severe hypobaric hypoxia on lipid peroxidation and antioxidant defence systems in human blood at rest and after maximal exercise. *Resuscitation*, 2001; 49: 307-314
- Kemna EHJM, Tjalsma H, Willems HL, Swinkels DW. Hepcidin: from discovery to differential diagnosis. *Haematologica*, 2008; 93(1): 90-97. DOI:10.3324/haematol.11705
- Klausen T, Olsen NV, Poulsen TD, Richalet JP, Pedersen BK. Hypoxemia increases serum interleukin-6 in humans. *Eur J Appl Physiol*, 1997; 76: 480-482
- Kruger TE. Immunomodulation of peripheral lymphocytes by hormones of the hypothalamus-pituitary-thyroid axis. *Adv Neuroimmunol*, 1996; 6: 387-395
- Ma Y, Freitag P, Zhou J, Brüne B, Frede S, Fandrey J. Thyroid hormone induces erythropoietin gene expression through augmented accumulation of hypoxia-inducible factor-1. *Am J Physiol Regul Integr Comp Physiol*, 2004; 287: R600-R607

- Magalhães J, Ascensão A, Soares JMC, Ferreira R, Neuparth MJ, Marques F, Duarte JA. Acute and severe hypobaric hypoxia increases oxidative stress and impairs mitochondrial function in mouse skeletal muscle. *J Appl Physiol*, 2005; 99: 1247-1253
- Margonis K, Fatouros IG, Jamurtas AZ, Nikolaidis MG, Douroudos I, Chatzinikolaou A, Mitrakou A, Mastorakos G, Papassotriou I, Taxildaris K, Kouretas D. Oxidative stress biomarkers responses to physical overtraining: implication for diagnosis. *Free Radic Biol Med*, 2007; 43: 901-910
- Moller P, Loft S, Lundby C, and Olsen NV. Acute hypoxia and hypoxic exercise induce DNA strand breaks and oxidative DNA damage in humans. *FASEB J* 2001; 15: 1181-1186
- Nagata M, Arimitsu N, Ito T, Sekimizu K. Antioxidant N-acetylcysteine inhibits erythropoietin-induced differentiation of erythroid progenitors derived from mouse fetal liver. *Cell Biol Int*, 2007; 31: 252-256
- Otani S, Kusagaya H. Changes in cytokines at extreme surroundings in Antarctica. *Yonago Acta Medica*, 2003; 46: 29-34
- Persijn JP, van der Slik W, Riethorst A. Determination of serum iron and latent iron-binding capacity (LIBC). *Clin Chim Acta*, 1971; 35(1): 91-98
- Pialoux V, Mounier R, Rock E, Mazur A, Schmitt L, Richalet JP, Robach P, Brugniaux J, Coudert J, Fellmann N. Effects of acute hypoxic exposure on prooxidant/antioxidant balance in elite endurance athletes. *Int J Sports Med*, 2009; 30: 87-93
- Pottgieser T, Ahlgrim C, Ruthardt S, Dickhuth H-H, Schumacher YO. Hemoglobin mass after 21 days of conventional altitude training at 1816 m. *J Sci Med Sport*, 2009; 12: 673-675
- Vasilaki A, McArdle F, Iwanejko JM, McArdle A. Adaptative responses of mouse skeletal muscle to contractile activity: the effect of age. *Mech Ageing Dev*, 2006; 127: 830-839
- Vogt M, Hoppeler H. Is hypoxia training good for muscles and exercise performance? *Progress in Cardiovascular Diseases*, 2010; 52: 525-533
- Vyoral D, Petrák J. Hepcidin: a direct link between iron metabolism and immunity. *Int J Biochem Cell Biol*, 2005; 37(9): 1768-1773
- Walsh NP, Whitham M. Exercising in environmental extremes. *Sports Med*, 2006; 36(11): 941-976

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