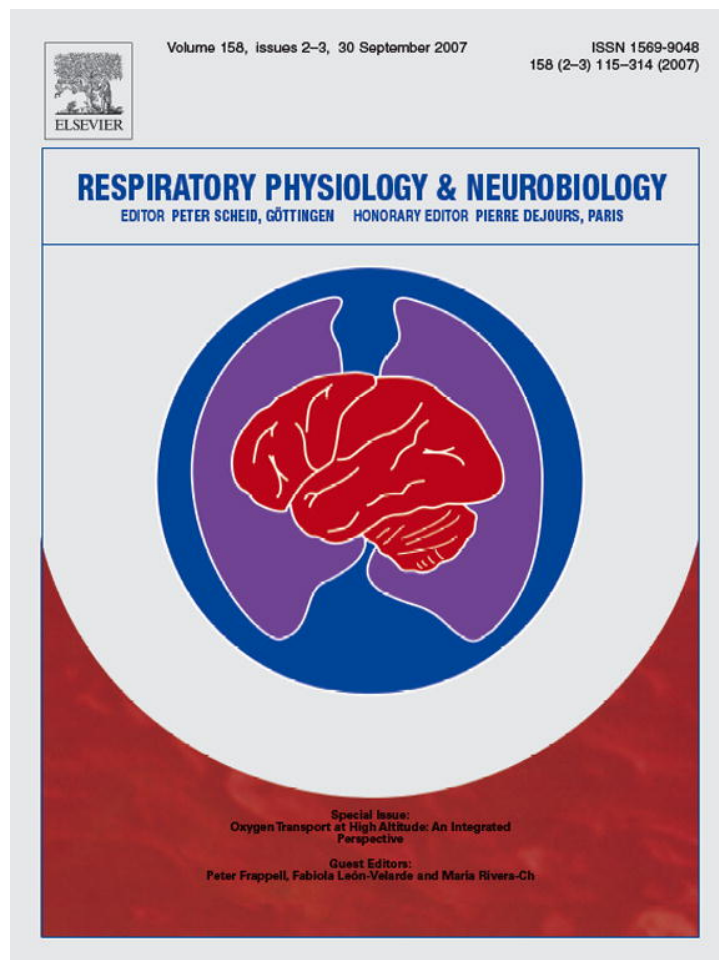


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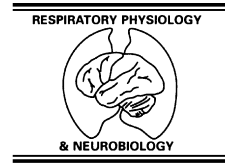


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## High altitude and oxidative stress

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### Abstract

Exposure to high altitude, which is associated with decreased oxygen pressure, could result in oxidative/reductive stress, enhanced generation of reactive oxygen and nitrogen species (RONS), and related oxidative damage to lipids, proteins, and DNA. The severity of oxidative challenge is related to the degree of altitude. A wide range of RONS generating systems are activated during exposure to high altitude, including the mitochondrial electron transport chain, xanthine oxidase, and nitric oxide synthase. High altitude appears to weaken the enzymatic and non-enzymatic antioxidant systems, and increased nutritional uptake of antioxidant vitamins are beneficial to reduce the altitude-induced oxidative damage. The pattern of high altitude exposure-associated oxidative damage resembles ischemia/reperfusion injury. The adaptive process to this oxidative challenge requires a relatively long period of time. Physical exercise or an enhanced level of physical activity at high altitude, exacerbates the extent of the oxidative challenge. Therefore, special attention is necessary to curb the degree of oxidative stress.

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**Keywords:** High altitude; Reactive oxygen and nitrogen species; Oxidative stress; Oxidative damage; Antioxidants; Acute mountain sickness

### 1. Introduction

Formation of reactive oxygen and nitrogen species (RONS) is a consequence of aerobic metabolism, since a number of RONS generating systems are created in the body. Indeed, RONS are natural and physiological modulators of the cellular redox milieu and thereby signal controlling factors of a wide range of known and unknown physiological and patho-physiological processes. Despite the multi line antioxidant system, the level of RONS generation can exceed the capability of the defense network, leading to oxidative stress (Askew, 2002). It is generally assumed that increases in aerobic metabolism or hyperoxia generate increased levels of RONS, causing alteration of redox homeostasis and oxidative damage to lipids, proteins, and DNA (Bailey et al., 2001a; Bailey and Davies, 2001b). Physical exercise, such as that associated with mountaineering itself, could lead to oxidative challenge and damage to different organs. Exercise and high altitude exposure very often result in oxidative damage (Radak

et al., 2001; Wozniak et al., 2001). It appears that the increased incidence of RONS production is due to the involvement of a number of different RONS generating systems. Although low oxygen pressure seems to be favorable to low RONS production, it appears that high altitude exposure is associated with an increase in oxidative damage as a consequence of the altered activity of the RONS generating and antioxidant systems. Moreover, not just the enzymatic but the non-enzymatic system is effected by exposure to high altitude (Imai et al., 1995; Chao et al., 1999). The present review draws upon the available literature on high altitude and exercise, and high altitude and oxidative stress.

### 2. RONS generating systems at high altitude

It has been well demonstrated that an increased oxygen supply results in increased production of mitochondrial ROS. Furthermore, it has been suggested that 1–2% of the oxygen which enters the mitochondrion, is released as a ROS. On the other hand, it appears that hypoxia can lead to reductive stress, which also results in increased ROS production by the mitochondrial electron transport system (Mohanraj et al., 1998). It is believed that ROS are generated at complex I and complex III of the electron transport chain. During hypoxia, less O<sub>2</sub> is

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available to be reduced to H<sub>2</sub>O at cytochrome oxidase, thus causing accumulation of reducing equivalents within the mitochondrial respiratory sequence. This accumulation is known as reductive stress and this reaction leads to ROS formation by the auto-oxidation of one or more mitochondrial complexes, such as the ubiquinone–ubiquinol redox couple. Khan and O'Brien (1995) previously demonstrated increases in the cellular NADH/NAD<sup>+</sup> ratio during hypoxia associated reductive stress.

When an extremely low availability of oxygen occurs, such as during ischemia or exposure to very low oxygen pressure, such as altitude over 6000 m, cells tend to generate ATP. This reaction occurs via the interaction of two ADP, which are catalysed by adenylate kinase. This process also generates AMP, which cannot be recycled, and it is catabolyzed and hypoxanthine is formed. In the presence of calcium-related proteases xanthine dehydrogenase can be converted to xanthine oxidase, which uses molecular oxygen instead of NAD<sup>+</sup> as the electron acceptor, with the consequent production of xanthine plus superoxide anion or H<sub>2</sub>O<sub>2</sub>. The xanthine dehydrogenase/oxidase system is a potent ROS generator during hypoxia/reperfusion conditions. Intermittent exposure to high altitude has similar characteristics as ischemia/reperfusion (Radak et al., 1994). On the other hand, the changing pattern of ROS and nitric oxide (NO) is different during ischemia/reperfusion and exposure to high altitude (Schneider et al., 2001). In contrast to ischemia/reperfusion, ROS levels increase during hypoxia and assume pre-hypoxic values upon a return to normoxia. Acclimatization involves up-regulation of inducible nitric oxide synthase (iNOS), suggesting that hypoxia leads to an alteration of the ROS/NO balance, which is eventually restored during the acclimatization process (Gonzalez and Wood, 2001). This phenomenon may have relevance to the microcirculatory alterations associated with hypoxic exposure, including acute mountain sickness, and high altitude pulmonary and cerebral edema. The findings of Serrano et al. (2003) indicate that the involvement of a different type of NOS is dissimilar in NO production during high altitude, which can lead to an increased formation of nitrotyrosine, at least in the rat cerebellum after reoxygenation at sea level. RONS are involved in the regulation of transcription factors, but an overview of this issue is not the topic of the present review.

Besides the above mentioned RONS generating systems, it is well known that UV radiation is significantly increased at high altitude, resulting in enhanced formation of RONS. According to our current understanding, it seems that high altitude is associated with an increase in ROS generation and this is due to different factors, including the mitochondrial respiratory chain, xanthine oxidase, and iNOS.

### 3. The effect of high altitude on antioxidant systems

There are an increased number of investigations on the effects of high altitude on the antioxidant system. Unfortunately, the findings seem to vary considerably. It is meaningful that some native highlander tribes in India often eat the seeds of *Trichopus zeylanicus*, which have been shown to scavenge free radicals and reduce the levels of lipid peroxidation and DNA damage by their

antioxidant capacity, depending upon the polyphenol and sulfhydryl content (Tharakan et al., 2005). One of the first investigations to study the effects of altitude on the enzymatic antioxidant system came from our laboratory, where we reported that 6 months of intermittent exposure to high altitude (4000 m) resulted in a decreased activity and protein content of mitochondrial SOD in skeletal muscle of rats (Radak et al., 1994). The decreased level of Mn-SOD protein meant that intermittent exposure to high altitude affected the transcription of the enzyme as well. Our data were confirmed by Nakanishi et al. (1995), who found, at a simulated altitude of 5500 m, increased levels of immunoreactive Mn-SOD in serum and decreased levels in the liver and lungs of the animals. The activity of glutathione peroxidase (GPX) also decreased in liver, suggesting that liver might be especially sensitive to high altitude-induced oxidative stress (Nakanishi et al., 1995). The most apparent decrease was observed in mitochondrial SOD activity and content, which might indicate that low oxygen pressure, associated with high altitude exposure, results in down-regulation of Mn-SOD (Zamudio et al., in press), and the generation of an excess of H<sub>2</sub>O<sub>2</sub>. This could explain the associated oxidative damage. Moreover, organ specific responses occur with exposure to high altitude, since in the serum Mn-SOD activity increased (Nakanishi et al., 1995). According to our hypothesis, serum could be affected more significantly by the impaired NO synthesis by eNOS (Droma et al., 2002) and the related oxidative stress, than are liver and skeletal muscle. This possibly is one of the factors which results in different regulation of Mn-SOD in serum and other tissues.

In a subsequent study, we could not detect a significant effect of a 4-week exposure to 4000 m on the activities of antioxidant enzymes (Radak et al., 1997). However, the exposure to altitude was longer and less severe than in the former study, which could account for the discrepancy (Nakanishi et al., 1995). Imai et al. (1995) compared the activities of GPX in serum of native highlanders (4000 m) and subjects from sea level. They found that people residing at high altitude had lower levels of GPX activity. The activity and effectiveness of GPX is strongly dependent upon the state of the thiol system. Glutamyl-cysteinyl-glycine is one of the main thiol/antioxidant sources for the cell, and it is continuously synthesized by glutamyl cycle. High altitude exposure decreases the level of reduced glutathione (GSH) and increases oxidized glutathione concentration (Ilavazhagan et al., 2001; Joanny et al., 2001). Thus, it appears that the capacity of enzymatic and non-enzymatic antioxidant systems is somewhat decreased at high altitude.

Schmidt et al. (2002) have applied an antioxidant mixture, containing vitamin E, beta-carotene, ascorbic acid, selenium, alpha-lipoic acid, *N*-acetyl l-cysteine, catechin, lutein, and lycopene, to reduce oxidative stress caused by altitude. This mixture was found to be effective in reducing the level of oxidative damage. Supplementation of vitamin E (40 mg per rat d<sup>-1</sup>) taken orally, 5 days prior to and during the period of hypoxic exposure to 7576 m, significantly reduced the high altitude-induced increase in lipid peroxidation (Ilavazhagan et al., 2001). On the other hand, an antioxidant supplement mixture containing 20,000 IU beta-carotene, 400 IU vitamin E, 500 mg vitamin C, 100 µg selenium, and 30 mg zinc, (in a daily base)

did not prevent oxidative damage to macromolecules (Pfeiffer et al., 1999). Furthermore the findings of a recent study revealed that antioxidant supplementation attenuated the high altitude-induced decrease in ventilatory threshold in exercising humans (Subudhi et al., 2006).

It appears that exposure to high altitude decreases the activity and content of some antioxidant enzymes. Moreover, the effectiveness of the thiol system is also reduced at high altitude. As well, there are some indications that antioxidant supplementation reduces or prevents the high altitude-induced oxidative damage to macromolecules.

#### 4. High altitude and oxidative damage

The reactivity of RONS makes them difficult to measure. It is usual that, from the accumulation of the end-product of RONS and lipids, proteins, and DNA interaction, the extent of oxidative stress is judged. It should be mentioned that the grade of oxidative damage reflects the balance between the generation of RONS and the antioxidant/repair systems. We have shown that intermittent exposure (12 h/day) to simulated altitude of 4000 m results in increased lipid peroxidation in skeletal muscle (Radak et al., 1994). We further observed that the level of lipid peroxidation, although it increased in both fiber types, was related to the metabolic capacity of the muscle (Radak et al., 1994). Interestingly, when we applied 4 weeks of continuous exposure to the same altitude, we did not observe increased lipid peroxidation, which indicates that the intermittent exposure either increased the RONS more significantly, probably by xanthine oxidase, or that the capability of the antioxidant system declined more significantly than during continuous exposure to altitude. Simultaneously we have observed that exposure to 4000 m increased the level of oxidative protein damage, as measured by carbonyl derivatives in skeletal muscle of rats (Radak et al., 1997). We used immunoblot to detect the molecular weight of these proteins which had accumulated carbonyl bonds, and found that actin was seriously affected. On the other hand, the level of carbonylation decreased in rat brain with exposure to 4000 m (Radak et al., 1998). Kumar et al. (1999) reported that short exposure (5 days) to an altitude of 7576 m caused increased lipid peroxidation in plasma of rats. This result was confirmed using the same experimental protocol, but adding vitamin E supplemented groups (Ilavazhagan et al., 2001). These investigators reported that 3 and 7 days of exposure to 6100 m significantly increased the level of RONS, and lipid peroxidation in different brain regions (Maiti et al., 2006). Exposure to an altitude of 8235 m for 7 h and reoxygenation resulted in increased activity of nNOS, and eNOS with an associated increase in nitrotyrosine content in rat cerebellum (Serrano et al., 2003). Although there is an organ specific response to exposure to high altitude, the effects seem to be systemic, which has been well demonstrated by Nakanishi and co-workers, who reported that exposure to 5500 m resulted in increased levels of malondialdehyde in serum, lung, liver, heart and kidney (1995).

Human studies have revealed similar results to those observed in other mammals. Re-oxygenation, after returning from an altitude of 3500 m, resulted in oxidative damage to the membrane of

erythrocytes (Gonzalez et al., 2005). Moller et al. (2001) exposed 12 healthy subjects to an altitude of 4559 m, which caused a significant increase in DNA strand breaks, measured in urine. The damage was more prominent at the endonuclease-III sites. When subjects were exposed simultaneously to high altitude (2700 m) and cold, the level of urinary lipid peroxidation, and DNA damage increased significantly (Schmidt et al., 2002). Arendea and co-workers (2005) reported increased H<sub>2</sub>O<sub>2</sub> levels, and lipid peroxidation products in exhaled breath condensate of mountain bikers performing a maximal cycloergometric exercise at 670 m and at 2160 m, as well as soldiers climbing to 6125 m in the Andes mountains of Northern Chile.

The Operation Everest III study revealed that the level of lipid peroxidation increased by 23% at 6000 m, and by 79% at 8848 m, indicating that the level of oxidative stress is parallel to the increase in altitude (Joanny et al., 2001). In a recent study, the acclimatization to altitude at 4500 m was studied and blood samples were collected after 3 and 13 month of exposure (Vij et al., 2005), and it appears that 3 months is an adequate time frame to cause increased lipid peroxidation and decreased enzymatic and non-enzymatic defense, while a 13 month sojourn normalizes the redox balance. Indeed, when well trained cyclists, who were residents of a moderate altitude, have been subjected to intensive interval exercise, at an altitude of 1860 m, oxidative stress markers did not change, emphasizing the importance of genetic adaptation (Wilber et al., 2004).

Thus, both human and animal studies are relatively consistent in reporting that high altitude-associated hypoxia causes oxidative damage to lipids, proteins, and DNA. This damage can be due to the increased levels of ROS production and/or the decreased levels of the antioxidant capacity. The oxidative stress seems to be linearly related to the altitude: higher altitude leads to greater oxidative challenge to the body. It also appears that long term acclimatization and/or genetic adaptation attenuate or eliminate the high altitude-induced oxidative stress. On the other hand, physical exercise at high altitude could further increase the altitude-induced oxidative stress and the associated oxidative damage.

#### 5. Summary

Exposure to high altitude disrupts the efficiency of the antioxidant system and, due to the increased level of RONS production, can lead to oxidative damage to macromolecules. Physical exercise can exacerbate the effects of high altitude and, further, can increase the related oxidative stress. Antioxidant supplementation has been shown to have beneficial effects and can attenuate and/or prevent the oxidative damage associated with high altitude and exercise.

#### References

- Arendea, O.F., Garcia, C., Lagos, N., Quiroga, G., Cajigal, J., Salazar, M.P., Behn, C., 2005. Lung oxidative stress as related to exercise and altitude. Lipid peroxidation evidence in exhaled breath condensate: a possible predictor of acute mountain sickness. *Eur. J. Appl. Physiol.* 95, 383–390.
- Askew, E.W., 2002. Work at high altitude and oxidative stress: antioxidant nutrients. *Toxicology* 180, 107–119.

- Bailey, D.M., Davies, B., 2001b. Acute mountain sickness; prophylactic benefits of antioxidant vitamin supplementation at high altitude. *High Alt. Med. Biol.* 2, 21–29.
- Bailey, D.M., Davies, B., Young, I.S., Hullin, D.A., Seddon, P.S., 2001a. A potential role for free radical-mediated skeletal muscle soreness in the pathophysiology of acute mountain sickness. *Aviat. Space Environ. Med.* 72, 513–521.
- Chao, W.H., Askew, E.W., Roberts, D.E., Wood, S.M., Perkins, J.B., 1999. Oxidative stress in humans during work at moderate altitude. *J. Nutr.* 129, 2009–2012.
- Droma, Y., Hanaoka, M., Ota, M., Katsuyama, Y., Koizumi, T., Fujimoto, K., Kobayashi, T., Kubo, K., 2002. Positive association of the endothelial nitric oxide synthase gene polymorphisms with high-altitude pulmonary edema. *Circulation* 106, 826–830.
- Gonzalez, N.C., Wood, J.G., 2001. Leukocyte-endothelial interactions in environmental hypoxia. *Adv. Exp. Med. Biol.* 502, 39–60.
- Gonzalez, G., Celedon, G., Escobar, M., Sotomayor, C., Ferrer, V., Benitez, D., Behn, C., 2005. Red cell membrane lipid changes at 3,500 m and on return to sea level. *High Alt. Med. Biol.* 6, 320–326.
- Ilavazhagan, G., Bansal, A., Prasad, D., Thomas, P., Sharma, S.K., Kain, A.K., Kumar, D., Selvamurthy, W., 2001. Effect of vitamin E supplementation on hypoxia-induced oxidative damage in male albino rats. *Aviat. Space Environ. Med.* 72, 899–903.
- Imai, H., Kashiwazaki, H., Suzuki, T., Kabuto, M., Himeno, S., Watanabe, C., Moji, K., Kim, S.W., Rivera, J.O., Takemoto, T., 1995. Selenium levels and glutathione peroxidase activities in blood in an andean high-altitude population. *J. Nutr. Sci. Vitaminol. (Tokyo)* 41, 349–361.
- Joanny, P., Steinberg, J., Robach, P., Richalet, J.P., Gortan, C., Gardette, B., Jammes, Y., 2001. 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* 49, 307–314.
- Khan, S., O'Brien, P.J., 1995. Modulating hypoxia-induced hepatocyte injury by affecting intracellular redox state. *Biochim. Biophys. Acta* 1269, 153–161.
- Kumar, D., Bansal, A., Thomas, P., Sairam, M., Sharma, S.K., Mongia, S.S., Singh, R., Selvamurthy, W., 1999. Biochemical and immunological changes on oral glutamate feeding in male albino rats. *Int. J. Biometeorol.* 42, 201–204.
- Maiti, P., Singh, S.B., Sharma, A.K., Muthuraju, S., Banerjee, P.K., Ilavazhagan, G., 2006. Hypobaric hypoxia induces oxidative stress in rat brain. *Neurochem. Int.* 49, 709–716.
- Mohanraj, P., Merola, A.J., Wright, V.P., Clanton, T.L., 1998. Antioxidants protect rat diaphragmatic muscle function under hypoxic conditions. *J. Appl. Physiol.* 84, 1960–1966.
- Moller, P., Loft, S., Lundby, C., Olsen, N.V., 2001. Acute hypoxia and hypoxic exercise induce DNA strand breaks and oxidative DNA damage in humans. *FASEB J.* 15, 1181–1186.
- Nakanishi, K., Tajima, F., Nakamura, A., Yagura, S., Ookawara, T., Yamashita, H., Suzuki, K., Taniguchi, N., Ohno, H., 1995. Antioxidant system in hypobaric-hypoxia. *J. Physiol.* 489, 869–876.
- Pfeiffer, J.M., Askew, E.W., Roberts, D.E., Wood, S.M., Benson, J.E., Johnson, S.C., Freedman, M.S., 1999. Effect of antioxidant supplementation on urine and blood markers of oxidative stress during extended moderate-altitude training. *Wilderness Environ. Med.* 10, 66–74.
- Radak, Z., Lee, K., Choi, W., Sunoo, S., Kizaki, T., Oh-Ishi, S., Suzuki, K., Taniguchi, N., Ohno, H., Asano, K., 1994. Oxidative stress induced by intermittent exposure at a simulated altitude of 4000 m decreases mitochondrial superoxide dismutase content in soleus muscle of rats. *Eur. J. Appl. Physiol. Occup. Physiol.* 69, 392–395.
- Radak, Z., Asano, K., Lee, K.C., Ohno, H., Nakamura, A., Nakamoto, H., Goto, S., 1997. High altitude training increases reactive carbonyl derivatives but not lipid peroxidation in skeletal muscle of rats. *Free Radic. Biol. Med.* 22, 1109–1114.
- Radak, Z., Asano, K., Fu, Y., Nakamura, A., Nakamoto, H., Ohno, H., Goto, S., 1998. The effect of high altitude and caloric restriction on reactive carbonyl derivatives and activity of glutamine synthetase in rat brain. *Life Sci.* 62, 1317–1322.
- Radak, Z., Taylor, A.W., Ohno, H., Goto, S., 2001. Adaptation to exercise-induced oxidative stress: from muscle to brain. *Exerc. Immunol. Rev.* 7, 90–107.
- Schmidt, M.C., Askew, E.W., Roberts, D.E., Prior, R.L., Ensign Jr., W.Y., Hesslink Jr., R.E., 2002. Oxidative stress in humans training in a cold, moderate altitude environment and their response to a phytochemical antioxidant supplement. *Wilderness Environ. Med.* 13, 94–105.
- Schneider, J.C., Blazy, I., Dechaux, M., Rabier, D., Mason, N.P., Richalet, J.P., 2001. Response of nitric oxide pathway to L-arginine infusion at the altitude of 4,350 m. *Eur. Respir. J.* 18, 286–292.
- Serrano, J., Encinas, J.M., Salas, E., Fernandez, A.P., Castro-Blanco, S., Fernandez-Vizarrá, P., Bentura, M.L., Rodrigo, J., 2003. Hypobaric hypoxia modifies constitutive nitric oxide synthase activity and protein nitration in the rat cerebellum. *Brain Res.* 976, 109–119.
- Subudhi, A.W., Jacobs, K.A., Hagobia, T.A., Fattor, J.A., Muza, S.R., Fulco, C.S., Cymermon, A., Friedlander, A.L., 2006. Changes in ventilatory threshold at high altitude: effect of antioxidants. *Med. Sci. Sports Exerc.* 38, 1425–1431.
- Tharakn, B., Dhanasekan, M., Manyam, B.V., 2005. Antioxidant and DNA protecting properties of anti-fatigue herb *Trichopus zeylanicus*. *Phytother. Res.* 19, 669–673.
- Vij, A.G., Dutta, R., Satija, N.K., 2005. Acclimatization to oxidative stress at high altitude. *High Alt. Med. Biol.* 6, 301–310.
- Wilber, R.L., Holm, P., Morris, D.M., Dallam, G.M., Subudhi, A.W., Morray, D.M., Callan, S.D., 2004. Effect of FIO<sub>2</sub> on oxidative stress during interval training at moderate altitude. *Med. Sci. Sports Exerc.* 36, 1888–1894.
- Wozniak, A., Drewa, G., Chesy, G., Rakowski, A., Rozwodowska, M., Olszewska, D., 2001. Effect of altitude training on the peroxidation and antioxidant enzymes in sportsmen. *Med. Sci. Sports Exerc.* 33, 1109–1113.
- Zamudio, S., Kovalenko, O., Vanderlelie, J., Illsley, N.P., Heller, D., Belliappa, S., Perkins, A.V. in press. Chronic hypoxia in vivo reduces placental oxidative stress. *Placenta*.