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Male reproductive system and antioxidants in oxidative stress induced by hypobaric hypoxia

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Summary

In Chile, due to the intensive activity developed in confining areas of the Andes Mountains ranging in altitude over 4000 asl, there has been an increasing intermittent movement of human resources to high altitude conditions. This unusual condition, defined as hypobaric hypoxia, affects notoriously in any living organism and there shows a series of physiological responses. Studies performed in rats under chronic hypobaric hypoxia and intermittent hypobaric hypoxia have registered changes in testicular morphology together with loss of spermatogenic cells in all stages of spermatogenic cycle. Furthermore, recent tests reinforced the existence of an oxidative metabolism in epididymis of rats subjected to hypobaric hypoxia due to the increase in the regulator enzyme expression of reactive oxygen species (ROS), This increase in the production of ROS induced a rise in apoptosis at germinal cell level, leading to a state of hypo-spermatogenesis that may jeopardise masculine fertility. Therefore, the eventual development of oxidative stress in spermatogenic cells and consequently the spermatozoids of workers subjected to high altitude, either chronic or intermittent, turns out to be critical when it poses as an imminent risk to the viability and quality of the reproductive cells of workers subjected to intermittent hypobaric hypoxia.

General concepts

Oxidative stress can be triggered by a series of endogenous and exogenous factors and exposure to high altitude is among them. Exposure to high altitudes has been associated with an increase in the production of reactive oxygen species (ROS) which are generated during the re-oxygenation phase of intermittent continuous hypobaric hypoxia and contribute to physiological responses (Farias *et al.*, 2005a; Nanduri *et al.*, 2008).

In Chile, due to intensive mining activity developed in confining areas of the Andes, mountains that range at altitude over 4000 asl, there has been an increasing intermittent movement of human resources to conditions of high altitude, a phenomenon that is being facilitated due to the narrow nature of the national territory (Richalet *et al.*, 2002). With the exception of the unique native population of Himalayas and Andes, human exposure to high altitude is not a common situation. This unusual condition, defined as hypobaric hypoxia, affects notoriously any living organism, whereas in high altitude the environmental oxygen available is reduced when the barometric or atmospheric pressure is lowered (exponentially descends in function of altitude), thereby the lower partial pressure consequently brings a decrease in the quantity of oxygen transported by the blood stream to all cells of the organism (Brito & Herruzo, 2007).

According to this, hypobaric hypoxia gives rise to a series of physiological responses which finally induce hypoxia of cellular type that is produced when the demand for molecular oxygen, necessary to generate sufficient levels of ATP for normal the cells physiological functions, exceeds the vascular supply (Cummins & Taylor, 2005). One of these responses is associated with the physiology and testicular functions studied in animal model. In fact, studies performed in rats under chronic and intermittent hypobaric hypoxia have registered changes in testicular morphology together with loss of spermatogenic cells in all stages of the cell cycle (Farias *et al.*, 2005a). Furthermore, recent tests reinforce the existence of an oxidative metabolism in rat epididymis subjected to hypobaric hypoxia due to the increase in the

regulator enzyme expression of ROS (Farias *et al.*, 2008). This increase in the production of ROS, induce an increase in apoptosis at germinal cell level, leading to a state of hypo-spermatogenesis (Turner & Lysiak, 2008), which may jeopardise the man fertility (Griveau & Le Lannou, 1997). In this way, the existence of an oxidative metabolism in hypobaric hypoxia conditions can lead to a situation of oxidative stress, which occurs when exist excessive production of ROS, overcoming the action of the antioxidant mechanisms of the cell, giving rise to generalised damage in proteins, lipids and DNA (Griveau & Le Lannou, 1997). Oxidative stress is produced by excessive formation of ROS which derive from the molecular oxygen that has been incompletely reduced by oxidases that participate in the electron-transporting chain giving rise to the production of species such as the superoxide $\left(O_2^{\cdot-}\right)$, hydrogen peroxide ($H_2O_2$) and the radical hydroxyl ($OH^{\cdot}$) among others (Mathews *et al.*, 2004). However, the ROS are not the only mediators of the oxidative phenomenon in the cell, as species known since free radical also participate, which correspond to molecules that possess at least one nonpaired electron that confers a high instability and promote the electron transfer to other molecules (Sorg, 2004). Therefore, in the presence of the variety of molecules and reactive species that are normally produced in aerobic biological systems, induce endogenous mechanisms of protection against oxidative aggression, within which enzymatic mechanisms can be found such as dismutase superoxide, catalase and glutathione system, as well as mechanisms nonenzymatic, which consider molecules with antioxidant properties such as ascorbic acid (vitamin C), carotenoids and retinoids (vitamin A) among others (Mathews *et al.*, 2004).

The oxidation of the proteins leads to the loss of function or to the degradation in the peroxisomes, whereas the lipid peroxidation affects the biological function of the membrane. However, the most serious damage is detected at DNA level, as this may lead to mutation of genes, inducing translation of defective proteins, in addition to alteration in gene expression and eruption of apoptosis (Sorg, 2004). Therefore, the eventual development of oxidative stress in spermatogenic cells and consequently the spermatozoids of workers subjected to high altitude, either chronic or intermittent, it turns out critical when it has an imminent risk to the viability and quality of the reproductive cells of workers subjected to work at high altitude.

Hypobaric hypoxia

According to report issued in 2003 by the United Nation Environment Programmes (UNEP) approximately 25% of the Earth surface consists of mountains where about 12% of the world population live in those regions (Programme UNE, 2003). Nevertheless, more than 50% of the world populations depend directly or indirectly on resources from mountain zones, and Chile is not the exception. In fact, given the importance of the mining activity in confining zones of the Andes mountain ranging in altitude over 4000 asl, has caused an increasing intermittent movement of human resources to conditions of high altitude, a phenomenon that is being facilitated by the narrow nature of the chilean territory (Richalet *et al.*, 2002). Thus, this unusual condition, defined as hypobaric hypoxia, affects notoriously any living organism. As in high altitude, the environmental oxygen available is reduced when the barometric or atmospheric pressure is lowered (exponentially descends in function of altitude), the lower partial pressure consequently originates a decrease in the quantity of oxygen transported by the blood stream to all cells of the organism (Brito & Herruzo, 2007). Thus, the exposure to hypobaric hypoxia either intermittent or chronic gives rise to a series of physiological responses which finally falls back in hypoxia of cellular type. This is produced when the demand for molecular oxygen, necessary to generate sufficient levels of ATP for normal the cells physiological functions, exceeds the vascular supply (Cummins & Taylor, 2005).

Also, there are effects of exposition to hypobaric hypoxia over neurocrine system, where that condition induces higher levels of follicle-stimulating hormone during first days of exposition compared with normoxic controls, also it produces decreased levels of luteinising hormone (LH) and plasma testosterone (Farias *et al.*, 2008). The continuous decrease in LH has been related with the effect of hypoxia on the activity of the hypothalamus hypophysis and the diminished levels of testosterone could be related to spermatogenesis (Farias *et al.*, 2008). This condition also affects the cardiovascular system, where it induces a significant decrease in cardiac α-adrenoceptors expression, and it increases the expression of muscarinic receptors; however, this condition also acts increasing the haematological constants and producing an elevation of systolic blood pressure (Germack *et al.*, 2002).

Studies of hypobaric hypoxia on the masculine reproductive system

Due to the complexity in performing studies on humans, one of the strategies is to study hypobaric hypoxia with animal models. Thus, a variety of male Wistar rats used in these studies have demonstrated to respond to this phenomenon with cardiovascular changes which resemble

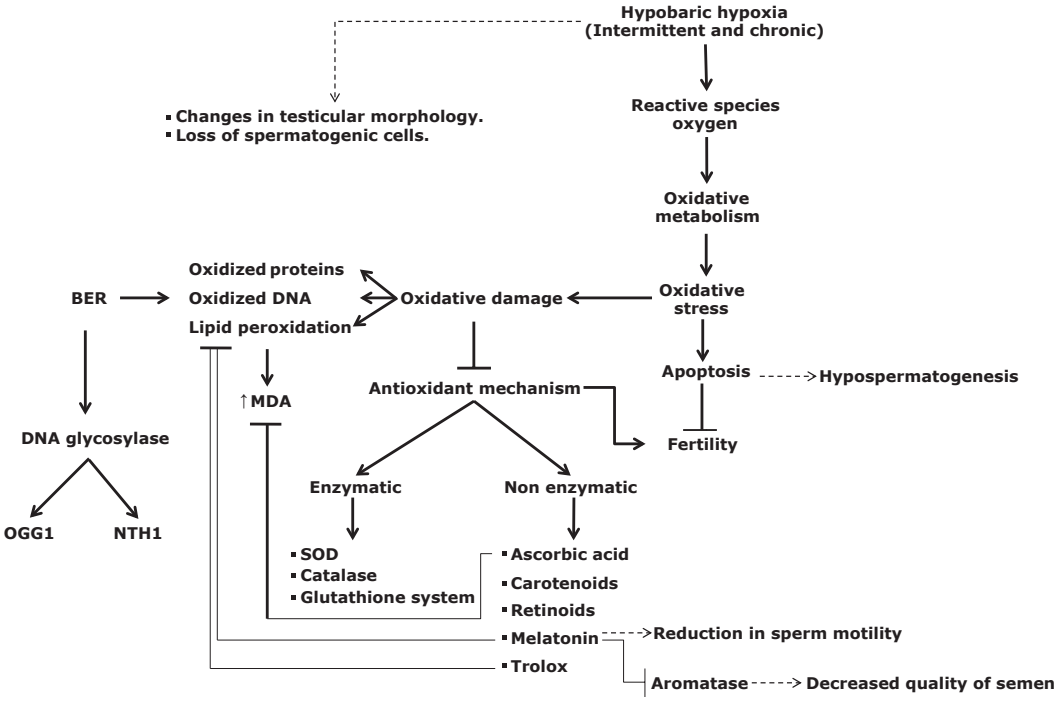

Fig. 1 Model to study the effect of hypobaric hypoxia exposure on epididymis and testicle. The dotted lines indicate the cellular changes; the solid lines indicate the induction of the reactions; and the bar-ending lines indicate the inhibition of the reactions. BER: base excision repair; OGG1: 8-oxo-guanine-DNA glycosylase 1; NTH1: endonuclease 1; MDA: malonaldehyde; SOD: superoxide dismutase.

to the human response exposed to conditions of high altitude. Therefore, it is feasible to study the physiological adaptation in rats exposed to hypobaric hypoxia (Farias *et al.*, 2005b). The study on male rats has revealed, for example, significant differences in the histology of the testis in comparison with normoxia state of control animals. When rats were compared with those exposed to treatment of hypobaric hypoxia either chronic or intermittent and chronic continuous hypobaric hypoxia, there was a decrease in the testicular mass, increment in the interstitial space, reduction of the seminal epithelium, depletion of cellular in epithelium and vacuolisation of the epithelial cells, changes that intensified further with the continuous treatment (Farias *et al.*, 2005a). Others, (Bustos-Obregon *et al.*, 2006), found similar results in rat testicles subjected similar conditions, in addition to characterise the changes in the amount and the form of spermatozoids present in epididymis, which are affected by the chronic exposure leading to teratozoospermia (situation in which 85% of the spermatozoids present abnormality in its morphology) and defective spermatozoids. However, it has been demonstrated on humans, that this series of changes induced by chronic hypoxia is reversible provided that the subject exposed stays under normoxia conditions for a minimum period of 6 months. Thus, masculine fertility normalises, with normal seminal count parameters, motility, form and maturity of spermatozoids (Verratti *et al.*, 2008) (Fig. 1).

Other researches carried out in rats testis have demonstrated that as time progress during exposure to continuous chronic hypobaric hypoxia (HHCC), a neovascularisation is gradually developing at interstitial space level due to the induction of the expression of the vascular endothelial growth factor (VEGF), leading to an increase in the intratesticular temperature of 1.5 °C in comparison with normoxia (Farias *et al.*, 2005b). The exposure to hypoxia produces an increased vascularisation, this phenomenon is associated with an increased expression of VEGF (Marti & Risau, 1998), so all that support when cells are exposed to HH would have an increased expression of VEGF.

Research related to oxidative stress

At present, there are a list publications carried out in rat model that confirm that hypobaric hypoxia induce oxidative stress induced in fluids and tissues of the organism (Askew, 2002). The testicular tissue, is not the exception, as it has been evidenced the presence of a severe oxidative stress in the round spermatids in rats subjected to HH,

due to the low basal oxygen compared with the normoxia condition (Farias *et al.*, 2005a). However, until very recent, it is known the mechanism that induces oxidative stress in the spermatogenic and spermatozoids cells.

The highly reactive and oxidant property that characterise ROS and the free radicals, brings as consequence a generalised damage that affect the principal cellular components, that is, carbohydrates, proteins, lipids and even to a DNA molecule (Blokhina *et al.*, 2003). The oxidation of protein leads to the loss of function or to its degradation in the peroxisomes, whereas the lipid peroxidation affects the biological function of the membrane. Likewise, the damage to DNA can lead to gene mutation, bringing consequently, the translation of defective proteins, in addition to the alteration of the genes and the eruption of apoptosis (Sorg, 2004). Given the wide spectrum of damage that derive from the oxidative attack, there have been realised multiple efforts in establishing specific methods which could determine the oxidative status of the cells that exist inside a particular tissue or fluid (Dotan *et al.*, 2004). Daily, it is estimated that a cell goes through 10^5 oxidative lesions in its DNA. According to this, it is not surprising that the greatest damage the DNA registered in its structure is caused by oxidative stress (Powell *et al.*, 2005). This type of damage leads to a series of chemical and structural modifications, such as modifications of the base and sugars, alteration in the protein-DNA bond, rupture of simple and double strings, also the favouring of base-free sites formation and lesions in tandem (Dizdaroglu, 2005). Within all these chemical modifications, the one most studied, frequent and harmful to the DNA integrity, is the residue formed by the hydroxyl denominated 8-oxoguanine (8-oxoG), also known as 7,8-dihydro-8-oxoguanine or 8-hydroxyguanine, when present in its tautomeric alternative (Perez, 2006). This lesion in the DNA molecule happens to be mutagen, due to the generation of transversion from G to T and from A to C at the moment of DNA replication (Klungland & Bjelland, 2007). However, it has been also considered of great interest, because the consecutive oxidation to the 8-oxoG lesion, as this appears to be more vulnerable to oxidation than the guanine itself, causing the production of other oxidative substances equally harmful to the DNA integrity (Hazra *et al.*, 2007; Klungland & Bjelland, 2007). For this reason, a continuous and persistent oxidative damage in the DNA can lead to altered signal cascade, gene expressions, or arrest of the transcription, causing errors in replication, giving genomic instability whose mutations can lead to carcinogenesis, neurodegenerative disorders and cardiovascular diseases (Powell *et al.*, 2005).

Given the fact that maintaining the genomic stability is fundamental in any cellular system, there are multiple routes for repairing and protecting DNA integrity (Powell *et al.*, 2005). However, of all the repairing routes, the principal responsibility in repairing the oxidative damages in the DNA is the base excision repair (BER), because it only removes the base that has been modified (Knudsen *et al.*, 2009). This mechanism has been widely maintained from bacteria to humans, having the common characteristic of participation of multiple proteins to achieve repairing one of the bases of the DNA (Dizdaroglu, 2005). The first participating enzymes in this route are the so-called DNA glycosylase, which catalyse the hydrolysis of bridge N-glycoside, located between the damaged base and the framework Sugar–Phosphate, giving as a result a apurinic/apyrimidinic (AP) site, which it can also be formed spontaneously (Powell *et al.*, 2005). Afterwards, the repairing route continues through two sub-routes, denominated short and long repairing route (which repair one or several bases respectively), those which process the AP site by means of the action of endonucleases, polymerases and ligases (Dizdaroglu, 2005).

DNA glycosylase correspond to small monomeric enzymes (*c.* 15–50 kDa), which can be structurally classified according to the presence or absence of folds and motifs maintained, the largest group being the one integrated by the endonuclease III (Nth) superfamily, where it includes the 8-oxoguanine-DNA glycosylase (OGG1) and the endonuclease 1 (NTH1) enzymes (Zharkov & Grollman, 2005).

The OGG1 enzyme is the principal enzyme responsible for repairing the oxidative lesion from 8-oxoguanine at nuclear level as well as mitochondrial level (Perez, 2006). It refers to a double-function enzyme, with a well defined and studied repairing route, capable of removing the 8-oxoG-specific lesions, when they are found in C:8-oxoG (Klungland & Bjelland, 2007). It is found widely distributed from yeasts to mammals (Boiteux *et al.*, 2002), being designated as hOGG1 for humans and Ogg1 for rodents (Radicella *et al.*, 1997; Rosenquist *et al.*, 1997). Irrespective of its origin, it is known that this enzyme presents eight isoforms (isomers), from which the most studied ones are those designated as α and β and are obtained due to the fact that they can form two different transcriptions from the nuclear gene, by means of alternative processing of the C-terminal region. In humans, the isoform hOGG1-1a is a protein of 345 amino acids (*c.* 36 kDa) and is located in the nucleus, while the isoform hOGG1-2a is composed by 424 amino acids (*c.* 40 kDa), and it is located exclusively in the mitochondria, whereas in rats, the molecular weight of both isoforms is *c.* 35 and 37 kDa (Olsen *et al.*, 2003; Perez, 2006).

With respect to NTH1, this enzyme is capable of removing a wide variety of oxidised pyrimidines, as thymine glycol, 5-hydroxyuracil and guanine and adenine

formamidopyrimidine (Rosenquist *et al.*, 2003). The protein codified in humans has a molecular weight of 34.3 kDa, and it has an extensive similarity with the sequence in prokaryotes (Dizdaroglu, 2005).

Oxidative stress and infertility

Under normal conditions, the small cytoplasm of spermatozoid contains relatively small quantities of ROS sequestrate, rendering these cells vulnerable and sensitive to oxidative stress (de Lamirande & O'Flaherty, 2008). It is for this reason that it is not surprising that from the total routine tests performed on humans consulting infertility problems, 25% register elevated levels of ROS (Griveau & Le Lannou, 1997), implying consequences of malfunctioning in the movement of abnormal flagella, in the recognition of the pellucid zone favours the inhibition of the spermatozoid fusion – oocytes (Sharma & Agarwal, 1996). Furthermore, it has been confirmed that there exist an increment of cellular apoptosis at a germinal cellular level, caused by the excess of ROS, which leads to hypospermatogenesis (Turner & Lysiak, 2008).

A mature and fertile spermatozoid is characterised by its chromatin highly compacted and stable, which protects against damage in DNA (Sakkas *et al.*, 1999). Nevertheless, due to effects of oxidative stress, the sperm DNA remains susceptible to ROS and oxidant radicals attack jeopardising the integrity of the gamete genetic material. This preservation of the integrity in the chromatin implies the absence of ruptures in the single and double strings, as well as avoiding chemical modifications in the DNA structure, are all indicators that are used to correct functioning of the spermatozoid (Shamsi *et al.*, 2008). Additionally, it has been shown that a high level of damage in DNA of male gamete show an externalisation of phosphatidylserine molecules, a reduction of the mitochondrial membrane potential and the activation of the caspases route, which leads to developing apoptosis (Marchetti *et al.*, 2002). For this reason, spermatozoid protection in the tail of epididymis is fundamental to preserve the DNA. Therefore, the presence of antioxidant mechanisms (especially the expression of the antioxidant enzymes SOD, GPX y catalase) becomes important during the spermatozoid voyage to achieve fertilisation (Oa *et al.*, 2006). Nevertheless, it has been registered that the deficiency of ascorbic acid and excessive smoking can cause an increase in oxidation in DNA in humans and low levels of antioxidants in sperm and in testicular cells (Fraga *et al.*, 1996).

Studies have been carried out evidencing the damages in the DNA of testicular cells resulting from oxidative stress, and then this situation affects the production of defective spermatozoids. Using organic hydroperoxides, pro-oxidants which induce oxidative conditions (Kumar & Muralidhara, 2007) and quantifying the damage in the strands of the DNA by measuring the single strand, in addition to measuring lipid peroxidation, we can conclude that there is a reduction in the sperm count of the epididymis and an increase in the percentage of defective cells by the oxidative stress.

Intano *et al.* (2001) carried out Western blot tests to detect genes implicated in the repairing in thymocytes, Sertoli cells and in a mixture of rat testicular germinal cells and concluded that the germinal cells mixture exhibit a greater BER enzymatic defence in comparison with other cellular types. In an *in vitro* study, where the oxidative damage was induced in the spermatozoid DNA by H_2O_2 and Fe^{2+}, a quantitative study was carried out using PCR in real time to determine the expression of specific genes of nuclear and mitochondrial DNA implicated in the repairing of this molecule. The results showed that H_2O_2 has a cytotoxic power greater than the Fe^{2+} and that the mitochondrial DNA is more sensitive to oxidative damage than the nuclear DNA. Also, by the Comet test, it was determined that the damage in DNA was proportional to the concentration of H_2O_2 (Sawyer *et al.*, 2003). Furthermore, it has been seen that there are differences between cellular populations present in testicle. Such is the case of the research carried out by Wellejus *et al.* (2004), where haploid, diploid cells were separated from the rats S and tetraploides phase that were exposed to oestrogen as oxidant agent and revealed that haploids cells resulted to be more susceptible to DNA oxidative damage, being an approximation to understand what occurs at a spermatozoid level. However, it was not observed significant variations in messenger levels of Ogg1 in testicles.

Spermatozoids, exposure to high altitude and oxidative stress

As it was mentioned previously, little is known about how hypobaric hypoxia and oxidative stress affects the spermatozoids after establishing that precisely there is an oxidative metabolism in testicles (Farias *et al.*, 2005a). Nevertheless, in 2006, it was found in testicular tissues and rat spermatozoid, under chronic treatment of simulated hypoxia at 4000 m asl, which as exposure time progressed, the phenomenon of lipid peroxidation resulted to be lower and more variable with respect to normoxia control (Bustos-Obregon *et al.*, 2006). Conversely, in the treatment of intermittent hypobaric hypoxia in rats, it was registered that in testicles and epididymis where existed an increase in the MDA content with respect to control, but it reverted by the administration of ascorbic acid as nonenzymatic antioxidant. In addition, it was observed that the expression of glutathione reductase enzyme (GR) was lower in both organs with respect to

control, but in epididymis, the expression of GR activity was greater (Farias *et al.*, 2010). The glutathione system has an essential function because it prevents the oxidative damage, and GSH acts as an electron donor in reduction reaction, and GR maintains the reduced form of GSH. The importance of GSH is the ability to remove ROS and to protect the cell (Townsend *et al.*, 2003). Besides, the epididymis has the function to transport spermatozoa and to support a microenvironment for sperm maturation (Cornwall, 2009). Probably for those functions, the epididymis requires increased levels of GR, and it allows to maintain and to protect spermatozoa of oxidative stress caused for exposure to hypoxia.

Antioxidants for spermatogenesis under intermittent hypobaric hypoxia

The development of oxidative stress in spermatogenic cells and consequently the spermatozoids of workers subjected to high altitude condition, either chronic or intermittent, it turns out to be critical when it has an imminent risk to the viability and quality of the reproductive cells of workers subjected to perform work at high altitude. For this reason, it is fundamental to find new antioxidant that can be incorporated into the diet of people exposed to intermittent hypobaric hypoxia. Rats subjected to intermittent hypobaric hypoxia showed that testicles and epididymis suffered of an increase in MDA content with respect to control, but it was reverted with administration of ascorbic acid (10 mg kg^{-1} body weight) as nonenzymatic antioxidant. In addition, it was found that the expression of glutathione reductase enzyme was lower in both organs with respect to control, but in epididymis, the expression and GR activity was greater (Farias *et al.*, 2010). It have been also described that melatonin (10 mg kg^{-1} body weight) counteracted the testis and spermatogenesis damage in rats subjected to intermittent hypobaric hypoxia (Hartley *et al.*, 2009; Bustos-Obregon *et al.*, 2010). However, our results showed that melatonin had no protective effect in testis and epididymis damaged (Farias *et al.*, 2012). Melatonin has been shown to exert a protective effect against lipid peroxidation under oxidative stress (Serel *et al.*, 2004). However, it has been reported that melatonin treatment induced a marked reduction in sperm motility (Oosthuizen *et al.*, 1986), and it did not prevent the reduction in sperm concentration under an ischaemia/reperfusion condition (Kurcer *et al.*, 2010). Also, this hormone is 40 fold less efficient that Trolox, an analogue of vitamin E in achieving lipid peroxidation reduction in infertile men (Gavella & Lipovac, 2000). Authors (Luboshitzky *et al.*, 2002) reported that melatonin administration (3 mg) was associated with decreased quality of semen in healthy men, probably through the inhibition of aromatase at testicular level. The aromatase is the terminal enzyme responsible for oestrogen formation from androgens and play a physiological role in maintenance of male gonadal functions (Carreau *et al.*, 2001). These findings support the assumption that melatonin may adversely affect sperm quality. The protective oxidative damage of melatonin under an intermittent hypobaric hypoxia is organ-dependent and had no protective effect in testis and epididymis damage (Farias *et al.*, 2012).

Conclusions

It is not completely clear so far whether high altitude is an inductor of oxidative stress at spermatozoid level; therefore, it is necessary to determine the degree that affects its structure and function. It is for that reason that by the use of animal model, it is possible to know the damage that can generate the oxidative metabolism in the masculine gamete, affecting the function of the spermatozoid. On the other hand, antioxidants constitute an interesting alternative in the diet of people subjected to intermittent hypobaric hypoxia as protector of oxidative damage on testis and spermatogenic cells.

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References

Askew EW (2002) Work at high altitude and oxidative stress: antioxidant nutrients. *Toxicology* 180:107–119.

Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann Bot* 91:179–194.

Boiteux S, Gellon L, Guibourt N (2002) Repair of 8-oxoguanine in *Saccharomyces cerevisiae*: interplay of DNA repair and replication mechanisms. *Free Radic Biol Med* 32:1244–1253.

Brito J, Herruzo R (2007) Hipoxia hipobárica intermittent crónica en gran altura: construcción de la historia natural de una nueva situación epidemiológica y biológica [Tesis Doctorado en Medicina Preventiva y Salud Pública]. Universidad Autónoma de Madrid, Madrid, p 169.

Bustos-Obregon EC, Contreras J, Maurer I, Sarabia L (2006) Effects of chronic simulated hypobaric hypoxia on mouse spermatogenesis. *Int J Morphol* 24:481–488.

Bustos-Obregon E, Castro-Sanchez R, Ramos-Gonzalez B, Torres-Diaz L (2010) Rat spermatogenesis damage in intermittent hypobaric hypoxia and the protective role of melatonin II: testicular parameters. *Int J Morphol* 28:537–547.

Carreau S, Bourguiba S, Lambard S, Galeraud-Denis I, Genissel C, Bilinska B, Benahmed M, Levallet J (2001) Aromatase expression in male germ cells. *J Steroid Biochem Mol Biol* 79:203–208.

Cornwall GA (2009) New insights into epididymal biology and function. *Hum Reprod Update* 15:213–227.

Cummins EP, Taylor CT (2005) Hypoxia-responsive transcription factors. *Pflugers Arch* 450:363–371.

Dizdaroglu M (2005) Base-excision repair of oxidative DNA damage by DNA glycosylases. *Mutat Res* 591:45–59.

Dotan Y, Lichtenberg D, Pinchuk I (2004) Lipid peroxidation cannot be used as a universal criterion of oxidative stress. *Prog Lipid Res* 43:200–227.

Farias JG, Bustos-Obregon E, Orellana R, Bucarey JL, Quiroz E, Reyes J (2005a) Effects of chronic hypobaric hypoxia on testis histology and round spermatid oxidative metabolism. *Andrologia* 37:47–52.

Farias JG, Bustos-Obregon E, Reyes JG (2005b) Increase in testicular temperature and vascularization induced by hypobaric hypoxia in rats. *J Androl* 26:693–697.

Farias JG, Bustos-Obregon E, Tapia PJ, Gutierrez E, Zepeda A, Juantok C, Cruz G, Soto G, Benites J, Reyes J (2008) Time course of endocrine changes in the hypophysis–gonad axis induced by hypobaric hypoxia in male rats. *J Reprod Dev* 54:18–21.

Farias JG, Puebla M, Acevedo A, Tapia PJ, Gutierrez E, Zepeda AB, Juantok C, Calaf G, Reyes J (2010) Oxidative stress in rat testis and epididymis under intermittent hypobaric hypoxia: protective role of ascorbate supplementation. *J Androl* 31:314–321.

Farias JG, Zepeda A, Calaf G (2012) Melatonin protects heart, lung and kidneys from oxidative stress under intermittent hypobaric hypoxia in rats. *Biol Res* 45:81–85.

Fraga CG, Motchnik PA, Wyrobek AJ, Rempel DM, Ames BN (1996) Smoking and low antioxidant levels increase oxidative damage to sperm DNA. *Mutat Res* 351:199–203.

Gavella M, Lipovac V (2000) Antioxidative effect of melatonin on human spermatozoa. *Arch Androl* 44:23–27.

Germack R, Leon-Velarde F, ValdesDeLa Barra R, Farias J, Soto G, Richalet JP (2002) Effect of intermittent hypoxia on cardiovascular function, adrenoceptors and muscarinic receptors in Wistar rats. *Exp Physiol* 87:453–460.

Griveau JF, Le Lannou D (1997) Reactive oxygen species and human spermatozoa: physiology and pathology. *Int J Androl* 20:61–69.

Hartley R, Castro-Sanchez R, Ramos-Gonzalez B, Bustos-Obregon E (2009) Rat spermatogenesis damage in intermittent hypobaric hypoxia and the protective role of melatonin. I Cauda epididymal spermatozoa. *Int J Morphol* 27:1275–1284.

Hazra TK, Das A, Das S, Choudhury S, Kow YW, Roy R (2007) Oxidative DNA damage repair in mammalian cells: a new perspective. *DNA Repair (Amst)* 6:470–480.

Intano GW, McMahan CA, Walter RB, McCarrey JR, Walter CA (2001) Mixed spermatogenic germ cell nuclear extracts exhibit high base excision repair activity. *Nucleic Acids Res* 29:1366–1372.

Klungland A, Bjelland S (2007) Oxidative damage to purines in DNA: role of mammalian Ogg1. *DNA Repair (Amst)* 6:481–488.

Knudsen NO, Andersen SD, Lutzen A, Nielsen FC, Rasmussen LJ (2009) Nuclear translocation contributes to regulation of DNA excision repair activities. *DNA Repair (Amst)* 8:682–689.

Kumar TR, Muralidhara (2007) Induction of oxidative stress by organic hydroperoxides in testis and epididymal sperm of rats *in vivo*. *J Androl* 28:77–85.

Kurcer Z, Hekimoglu A, Aral F, Baba F, Sahna E (2010) Effect of melatonin on epididymal sperm quality after testicular ischemia/reperfusion in rats. *Fertil Steril* 93:1545–1549.

deLamirande E, O'Flaherty C (2008) Sperm activation: role of reactive oxygen species and kinases. *Biochim Biophys Acta* 1784:106–115.

Luboshitzky R, Shen-Orr Z, Nave R, Lavi S, Lavie P (2002) Melatonin administration alters semen quality in healthy men. *J Androl* 23:572–578.

Marchetti C, Obert G, Deffosez A, Formstecher P, Marchetti P (2002) Study of mitochondrial membrane potential, reactive oxygen species, DNA fragmentation and cell viability by flow cytometry in human sperm. *Hum Reprod* 17:1257–1265.

Marti HH, Risau W (1998) Systemic hypoxia changes the organ-specific distribution of vascular endothelial growth factor and its receptors. *Proc Natl Acad Sci USA* 95:15809–15814.

Mathews CK, VanHolde KE, Ahern KG (2004) Biochemistry. Pearson Addison Wesley, Spain, p 1335.

Nanduri J, Yuan G, Kumar GK, Semenza GL, Prabhakar NR (2008) Transcriptional responses to intermittent hypoxia. *Respir Physiol Neurobiol* 164:277–281.

Oa WS, Chen H, Chow PH (2006) Male genital tract antioxidant enzymes – their ability to preserve sperm DNA integrity. *Mol Cell Endocrinol* 250:80–83.

Olsen AK, Duale N, Bjoras M, Larsen CT, Wiger R, Holme J, Seeberg E, Brunborg G (2003) Limited repair of 8-hydroxy-7,8-dihydroguanine residues in human testicular cells. *Nucleic Acids Res* 31:1351–1363.

Oosthuizen JM, Bornman MS, Schulenburg GW (1986) Melatonin impairs sperm motility – a novel finding. *S Afr Med J* 70:566.

Perez G (2006) Genetic polymorphism and thyroid cancer. Universitat Autònoma de Barcelona, Bellaterra, Spain, p 268.

Powell CL, Swenberg JA, Rusyn I (2005) Expression of base excision DNA repair genes as a biomarker of oxidative DNA damage. *Cancer Lett* 229:1–11.

Programme UNE (2003) Mountain biodiversity: status and trends of mountain biodiversity and threats to it. (Diversidad biológica de montañas: situación y tendencias de la diversidad biológica de montañas y amenazas a la misma). Meeting 8th edn. UNEP/CBD/SBSTTA, Montreal, Canada.

Radicella JP, Dherin C, Desmaze C, Fox MS, Boiteux S (1997) Cloning and characterization of hOGG1, a human homolog of the OGG1 gene of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 94:8010–8015.

Richalet JP, Donoso MV, Jimenez D, Antezana AM, Hudson C, Cortés G, Osorio J, León A (2002) Chilean miners commuting from sea level to 4500 m: a prospective study. *High Alt Med Biol* 3:159–166.

Rosenquist TA, Zharkov DO, Grollman AP (1997) Cloning and characterization of a mammalian 8–oxoguanine DNA glycosylase. *Proc Natl Acad Sci USA* 94:7429–7434.

Rosenquist TA, Zaika E, Fernandes AS, Zharkov DO, Miller H, Grollman AP (2003) The novel DNA glycosylase, NEIL1, protects mammalian cells from radiation-mediated cell death. *DNA Repair (Amst)* 2:581–591.

Sakkas D, Mariethoz E, Manicardi G, Bizzaro D, Bianchi PG, Bianchi U (1999) Origin of DNA damage in ejaculated human spermatozoa. *Rev Reprod* 4:31–37.

Sawyer DE, Mercer BG, Wiklendt AM, Aitken RJ (2003) Quantitative analysis of gene-specific DNA damage in human spermatozoa. *Mutat Res* 529:21–34.

Serel TA, Ozguner F, Soyupek S (2004) Prevention of shock wave-induced renal oxidative stress by melatonin: an experimental study. *Urol Res* 32:69–71.

Shamsi MB, Kumar R, Dada R (2008) Evaluation of nuclear DNA damage in human spermatozoa in men opting for assisted reproduction. *Indian J Med Res* 127:115–123.

Sharma RK, Agarwal A (1996) Role of reactive oxygen species in male infertility. *Urology* 48:835–850.

Sorg O (2004) Oxidative stress: a theoretical model or a biological reality? *C R Biol* 327:649–662.

Townsend DM, Tew KD, Tapiero H (2003) The importance of glutathione in human disease. *Biomed Pharmacother* 57:145–155.

Turner TT, Lysiak JJ (2008) Oxidative stress: a common factor in testicular dysfunction. *J Androl* 29:488–498.

Verratti V, Berardinelli F, Di Giulio C, Bosco G, Cacchio M, Pellicciotta M, Nicolai M, Martinott S, Tenaglia R (2008) Evidence that chronic hypoxia causes reversible impairment on male fertility. *Asian J Androl* 10:602–606.

Wellejus A, Bornholdt J, Vogel UB, Risom L, Wiger R, Loft S (2004) Cell-specific oxidative DNA damage induced by estrogen in rat testicular cells *in vitro*. *Toxicol Lett* 150:317–323.

Zharkov DO, Grollman AP (2005) The DNA trackwalkers: principles of lesion search and recognition by DNA glycosylases. *Mutat Res* 577:24–54.