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SHORT COMMUNICATION

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Chronic hypobaric hypoxia diminishes the expression of base excision repair OGG1 enzymes in spermatozoa

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Summary

Hypobaric hypoxia induces DNA damage in rat testicular cells, the production of defective spermatozoids and decreased sperm count, associated with an increase in oxidative stress. 8-Oxoguanine glycosylase (OGG1) enzymes are main members of the base excision repair (BER) system, a DNA repair mechanism. We determined the expression levels of mitochondrial and nuclear OGG1 isoforms in spermatozoa collected from cauda epididymis in rats exposed to chronic hypobaric hypoxia (CHH) for 5, 15 and 30 days. CHH attenuates OGG1 expression in a time-dependent fashion, with a greater reduction in the mitochondrial isoform OGG1-2a ($p < .05$). Attenuation of the BER system may contribute to DNA damage under hypoxia exposure.

KEYWORDS
8-oxoguanine, DNA damage, infertility, oxidative stress

1 | INTRODUCTION

Exposure to hypobaric hypoxia induces pathological changes in the male reproductive system including sperm DNA fragmentation, increases in reactive oxygen species (ROS) and apoptosis that may jeopardise male fertility (Zepeda et al., 2012). In fact in humans, sperm DNA damage has been associated with excessive ROS levels (Barroso, Morshedi, & Oehninger, 2000) and infertility (Saleh et al., 2003). One the most frequent products of purine oxidation is 7,8-dihydro-8-oxoguanine (oxo^{8}G), and DNA glycosylases continuously inspect the DNA for damaged bases and initiate the base excision repair (BER) response (Klungland and Bjelland, 2007). The enzyme 8-oxoguanine DNA glycosylase (OGG1) is a major BER enzyme present in nucleus and mitochondria in spermatozoa where it counteracts the effects of oxidative damage (Smith et al., 2013).

In this work, we studied the protein expression levels of nuclear and mitochondrial OGG1 isoforms in spermatozoa from chronically hypoxic male rats.

2 | MATERIALS AND METHODS

2.1 | Animals

Ten-week-old male Wistar rats (290 ± 10 g, $n = 20$) were separated into two groups: normobaric control (Nx) and chronic hypobaric hypoxia (CHH). CHH animals were exposed to a 4,600-m simulated altitude (428 torr; PO_2: 89.6 mmHg) for 5, 15 or 30 days. All animal procedures were approved by Universidad de La Frontera institutional review board.

2.2 | Preparation of protein extracts and Western blotting

At the times specified, animals were removed from hypoxia and killed by cervical dislocation and epididymides were collected. Spermatozoa from caudal regions were removed by vortexing gently in PBS and homogenised in extraction buffer (Glass-Col K4424, CA, USA) at

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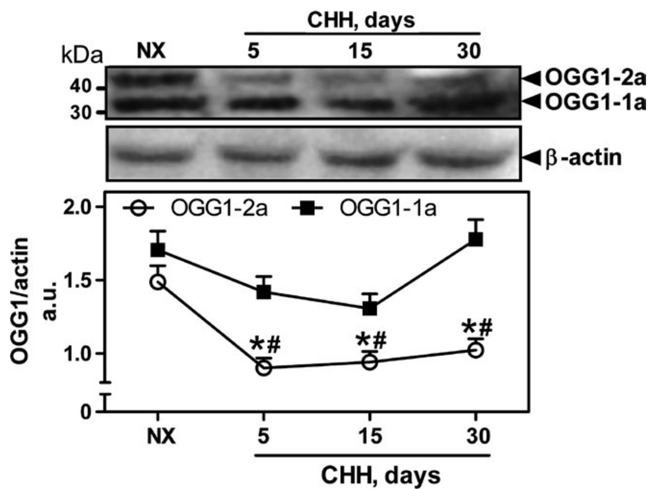

FIGURE 1 Expression levels of OGG1-1a and OGG1-2a isoforms in spermatozoa. Protein levels were determined by Western blotting in total extracts from spermatozoa obtained from rats exposed to 5, 15 and 30 days of chronic hypobaric hypoxia (CHH). Data are mean ± *SD*. $^{*}p < .05$ versus OGG1-1a; $\#p < .05$ versus normoxia (NX)

50 rpm. Extracts were centrifuged at 7,820 *g* for 30 min at 4°C. Protein concentration determinations and Western blotting procedures were performed as described (Zepeda et al., 2012). Primary antibodies for OGG1 (rabbit anti-rat Ogg1/2, 1:200 dilution, sc-3181, Santa Cruz Biotechnology, CA, USA) and actin (rabbit anti-rat beta-actin antibody, 1:1,000 dilution, Cat#8227, AbCam, USA) were used. Peroxidase-conjugated goat anti-rabbit IgG (1:1,000, Cat#31460, Thermofisher Scientific, MA, USA) was used as secondary antibody.

2.3 | Statistical analysis

Data were analysed by two-way ANOVA followed by Bonferroni test using GraphPad Prism Software v4.0 (San Diego, California) and presented as mean ± *SD*. $p < .05$ was used to assign statistical significance.

3 | RESULTS AND DISCUSSION

As shown in Figure 1a, the antibody used detected two bands in total protein extract from spermatozoa with molecular weight of 43 and 32 kDa corresponding to the mitochondrial OGG1-2a and nuclear OGG1-1a isoforms respectively (Hill, Hu, & Evans, 2008). Densitometric analyses (Figure 1b) showed that exposure to CHH induced a time-dependent decrease in expression levels of the mitochondrial OGG1-2a isoform ($p < .05$), whereas the decrease in the expression of the nuclear OGG1-1a did not reach statistical significance.

Spermatozoa are cells particularly susceptible to oxidative stress given their limited content of antioxidants (Aitken & Curry, 2011), and several models of hypoxia exposure have shown to produce DNA damage in sperm cells (Hartley, Castro-Sanchez, Ramos-Gonzalez, & Bustos-Obregon, 2009). Our results showing reduced expression of OGG1 enzymes in CHH suggest that attenuation of DNA repair mechanisms may contribute to the DNA damage observed. Similarly, lower levels of OGG1-2a suggest that mitochondrial DNA may be at higher risk of damage in hypoxia.

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