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ORIGINAL ARTICLE

Oxidative damage is present in plasma and circulating neutrophils 4 weeks after a high mountain expedition

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Abstract It is well known that exposure to extreme environments, such as in high-mountain expeditions, is associated with increased production of reactive oxygen species and related oxidative damage. However, there is little information concerning antioxidant recovery after this type of expedition. Thus, the aim of this study is to analyze the antioxidant recovery status at sea level of five expert alpinists 4 weeks after climbing Cho-Oyu (8,201 m). Body composition, cardiorespiratory capacity, and circulating parameters were almost similar to the values obtained at the beginning of the study. However, the alpinists presented high erythrocyte number, related hemogram values, and ferritin. Sodium, alkaline phosphatase, and γ-glutamyltransferase plasma levels were lower. Concerning oxidative stress, plasma uric acid levels were significantly increased, as well as malondialdehyde and protein carbonyls. Neutrophils displayed significantly higher levels of malondialdehyde and lower catalase activity. Therefore, these data indicate that the oxidative stress during a high mountain expedition is the most probable cause to explain an incomplete recovery in plasma and neutrophil antioxidant status.

Keywords Alpinism · Altitude · Antioxidants · Blood cells

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Introduction

During the last decades there is an increasing interest for high-altitude mountain climbing, despite the extremely hazardous conditions. In these situations, the human body is exposed to stress in which hypoxia plays a prominent role. Furthermore, the stress is exacerbated due to the physical activity performed by the alpinists, having to endure adverse environmental conditions such as low temperatures, high UV-radiation and deficient food supply (Radak et al. 1994; Wozniak et al. 2001; Dosek et al. 2007; Drust and Waterhouse 2010; Martarelli et al. 2011).

Exposure to high altitudes affects antioxidant enzymes, resulting in impaired free-radical scavenging (Heinicke et al. 2009; Dosek et al. 2007; Joanny et al. 2001). Radak et al. (1994) described for the first time that intermittent exposure to 4,000 m for 6 months resulted in a decrease in the activity of mitochondrial Mn-superoxide dismutase (Mn-SOD) in rat skeletal muscle as a consequence of altered gene transcription due to hypoxia. These results were confirmed by Nakanishi et al. (1995) who obtained similar results in liver and lungs of animals exposed to a simulated altitude of 5,500 m. In the same study, a decrease in glutathione (GSH)-peroxidase was detected in the liver (Nakanishi et al. 1995). This result corroborates with the observation that serum GSH-peroxidase activity is lower in people living at high altitudes (more than 4,000 m) compared

to those living at sea level (Imai et al. 1995). This observation seems to be related to a disruption of the glutathione system at high altitudes, affecting GSH-peroxidase and favoring the presence of oxidized glutathione (GSSG) (Ilavazhagan et al. 2001; Joanny et al. 2001). However, these antioxidant responses seem to be tissue specific, since blood serum SOD activity, as opposed to skeletal muscle and liver, increases at high altitudes (Nakanishi et al. 1995).

Hypoxia seems to be the main cause of increased free radical production at high altitudes. In this context, hypoxia leads to reductive stress, producing more free radicals through auto-oxidation of mitochondrial chain complexes (Mohanraj et al. 1998; Khan and O'Brien 1995). Furthermore, the free radicals cannot be efficiently eliminated due to the low effectiveness of the antioxidant enzymes, leading to oxidative stress (Dosek et al. 2007). Indeed, human and animal studies report increased free radical production and oxidative damage to lipids, proteins, and DNA (Dosek et al. 2007; Pialoux et al. 2009). Altogether, it seems that the degree of oxidative damage upon exposure to high altitudes reflects the balance between reactive oxygen and nitrogen species (RONS) production with the status of the antioxidant systems.

Little information is available regarding the plasticity of the antioxidant system as a result of exposure to extreme conditions such as those found at high mountain expeditions. In this sense, antioxidant defenses can, with time, adapt to these environments, contributing to the acclimatization at high altitudes (Vij et al. 2005). However, a less-explored question concerns the recovery of the redox balance when returning to moderate altitudes after a high-mountain expedition. In this context, we will consider the terms "low altitude" for ranges between 500 and 2,000 m above sea level, "moderate altitude" for 2,000–3,000 m, "high altitude" for 3,000–5,500 m, and "extreme altitude" above 5,500 m (Bärtsch and Saltin 2008). In this study we analyzed in more detail the persistence of oxidative damage after a mountain expedition in extreme-altitude conditions.

Materials and methods

Subjects and training/expedition program

A longitudinal study was performed in five expert climbers from a total of six subjects that participated in the experiment. One subject was withdrawn from the study since the alpinist suffered frostbite during the expedition. Volunteers were informed about the objective and demands of the study and gave their written consent to participate. The protocol was in accordance with local legal requirements and the Helsinki Declaration for research on human beings, and approved by the Ethics Committee of the corresponding autonomic governments. The subjects were 41.0 ± 3.5 years of age with a BMI of 23.1 ± 0.5 kg/m^2 at the beginning of the study.

Pre- and post-expedition anthropometric determinations (fat, muscle, bone and residual mass) were performed at the beginning and end of the study according to ISAK (International Society for Advancement of Kinanthropometry) recommendations (Marfell-Jones et al. 2006). In the calculation of the body composition of each individual, the following variables have been taken into account: height, weight, limb and trunk circumferences, skeletal widths, as well as the sum of the thickness of eight skinfolds (triceps, biceps, forearm, subscapular, suprailiac, midthigh, medial calf, and lateral calf). Metric tape, balance (Pesperson, Spain), skinfold calliper (Holtain, UK) and bicondylar calliper (Holtain, UK) were used for the different determinations and calibrated according to the manufacturers' instructions. Fat weight and percentage were calculated using Yuhaz's equation modified by Faulkner (1968). Bone mass and percentage were calculated according to Rocha (Prentice and Jebb 2001). Muscle mass and percentage were calculated using Martin's equation (Martin et al. 2003).

The volunteers were healthy and non-smokers. They had been previously exposed in 2009 to high altitudes several times before this experiment, climbing frequently in the Pyrenees (2,000–3,400 m), Alps (2,000–4,400 m), and Andes (3,000–6,000 m).

The participants live at sea level in Elche (Spain). In this study, they stayed for 6 weeks in the Himalayan Mountains, between 3,400 and 8,201 m, climbing Cho-Oyu in 2009. The planning of the acclimatization included 2 weeks at 3,400 m, trekking in the Ladakh region (India), passing the Kongmarula Pass (5,185 m) and climbing the Stok Kangri (6,135 m). The remaining 4 weeks were devoted to the Cho-Oyu ascension, including base camp (5,700 m) and intermediate camps I (6,380 m), II (7,100 m), III (7,500 m) and summit (8,201 m). Acclimatization was performed following a saw teeth strategy: climbing during the day and sleeping at night at a lower altitude. From sea level to camp III, the subjects were trained to perform daily monitorization of blood pressure, heart rate and indirect blood oxygen saturation using a portable sphygmomanometer and pulse oximeter. These measurements allowed us to control via Internet the adaptation of the alpinists during the ascension. Finally, the alpinists descended to 1,500 m in 1 week and remained below 1,000 m during 3 weeks, when they returned to Elche (sea level).

Circulating parameters were measured at 0 m, 15 days before the subjects traveled to the Himalayas (day 1) and when they returned (4 weeks after summit) (day 70). Determinations at intermediate days were impossible to be

performed. These determinations included a complete blood analysis, measurements of antioxidant enzyme activities in cell fractions and determination of oxidative stress in plasma and cell fractions (see below). Two ergometries were performed at days 1 and 70, in order to check the cardiorespiratory status of alpinists the same days of blood extraction.

Two months before the expedition, all subjects performed a supervised aerobic routine consisting of running for 40–60 min at 60–70% of maximal cardiac frequency, three alternative days a week. Weight-lifting (60–70% of maximal weight lifted in each exercise) and trekking (only during weekends) sessions were performed once at week. Caloric expenditure was theoretically estimated and calculated diets were adapted accordingly for each particular subject. Diet was designed using Diet source software (Novartis, Barcelona, Spain) and adapted to maintain muscle mass, since catabolism increases at high altitudes. Diets consisted in $2{,}466.6 \pm 135.0$ kcal distributed in $53.0 \pm 4.4\%$ carbohydrates, $31.0 \pm 3.6\%$ lipids and $15.8 \pm 1.0\%$ proteins. No specific supplements were taken by the subjects.

A control period of 70 days was monitored during summer 2010 at sea level. The same group of alpinists was submitted to a similar diet and training program. Hematology, circulating metabolites (glucose, uric acid, urea, creatinine, and lactate), circulating elements/electrolytes (Fe, Na^+, and K^+), circulating lipid profile (total, LDL-, HDL-cholesterol, and triglycerides), and plasma protein markers (ferritin, creatine phosphokinase (CK), myoglobin (Mb), alkaline phosphatase (AP), aspartate aminotransferase, alanine aminotransferase, and γ-glutamyltransferase (GGT)) were determined at days 1 and 70 (see below).

Blood sampling

Blood samples were obtained from the antecubital vein after overnight fasting in EDTA vacutainers at days 1 and 70, respectively. Blood cells were purified following an adaptation of the method described by Boyum (1964). Blood was carefully introduced in a Corning tube on Ficoll (GE Healthcare, Sweden) in a proportion of 1.5:1 vol, centrifuged at 900 *g* for 30 min at 4°C. The precipitate containing the erythrocytes and the neutrophils was incubated at 4°C with 0.15 M ammonium chloride to lyse the erythrocytes. The suspension was centrifuged at 750 *g*, at 4°C for 15 min and the supernatant was discarded. The bottom phase containing the neutrophils was washed first with ammonium chloride and then with phosphate-buffered saline solution at pH 7.4. The intermediate lymphocyte layer obtained from the Ficoll gradient was carefully removed, washed twice with PBS and centrifuged at 1,000 *g* for 10 min at 4°C. Cell precipitates (neutrophils and lymphocytes) were lysed with bi-distilled water to measure enzymatic activities, malondialdehyde (MDA) and protein carbonyls. Plasma and erythrocytes were obtained from another blood sample after 1,000 *g* centrifugation for 15 min at 4°C. The plasma, which corresponded to the supernatant after centrifugation, was stored at −80ºC. The erythrocytes present in the pellet were washed with PBS, resuspended, incubated in ice, centrifuged under the same conditions and resuspended in the same volume as the original plasma.

Hematological analysis and determination of serum parameters

Blood cells were determined by an automatic hematology analyzer (Roche Diagnostics, Barcelona, Spain).

Circulating glucose was determined by the glucose oxidase method coupled to the peroxidase reaction (Trinder 1969). HDL-cholesterol was determined by a direct enzymatic colorimetric method. HDL was dissolved with a detergent, while HDL-cholesterol was released to react with cholesterol esterase. Afterwards, free cholesterol was oxidized with cholesterol oxidase to cholest-4-ene-3-one and hydrogen peroxide, which was colorimetrically determined by the peroxidase reaction. The non-HDL lipoproteins were inhibited from reacting with the enzymes due to the absorption of the detergent (Naito and David 1984). Circulating triglycerides were determined from coupled reactions of lipoprotein-lipase, glycerol-kinase, glycerol phosphate oxidase and peroxidase giving a color end-adduct, as previously shown (Bucolo and David 1973).

Uric acid determination uses two coupled reactions catalyzed by uricase and peroxidase, giving rise to a colored compound (Fossati et al. 1980). Urea was determined by an enzymatic-colorimetric method using urease coupled to nitroprusside (Tabacco et al. 1979). Creatinine was determined by Jaffé direct reaction of creatinine with alkaline picrate forming a red complex (Bowers and Wong 1980). Serum iron was spectrophotometrically determined by ferrozine reaction (Itano 1978). Ferritin was determined using an enzyme-linked fluorescent assay (BioMerieux, Madrid) according to manufacturer's instructions. Lactate was determined by a lactate oxidase/peroxidase-coupled colorimetric reaction (Weisshaar et al. 1975). Serum Na^+ and K^+ were determined by potentiometry using selective Spotlyte electrodes (Menarini, Badalona, Spain).

Plasma proteins, such as CK, Mb, AP, aspartate aminotransferase/serum glutamic oxaloacetic transaminase (AST/GOT), alanine aminotransferase/serum glutamic pyruvic transaminase (ALT/GPT) and, GGT were measured using

automated standard laboratory procedures (Young and Friedman 2001).

Antioxidant enzymatic activities

All activities were determined on a microplate reader (SPECTROstar Omega, BMG LabTech GmbH, Offenburg, Germany) at 37°C. Catalase activity was determined according to Aebi's method (Aebi 1984). Glutathione (GSH)-reductase was determined according to Goldberg and Spooner (1983). Glutathione-peroxidase activity was determined according to Flohé and Gunzler (1984), with certain modifications. Superoxide dismutase (SOD) activity measurements were adapted from McCord and Fridovich (1969). Antioxidant activities were determined in all cell lysates.

Oxidative stress markers

Oxidative stress markers were determined in plasma and lymphocytes. Protein carbonyl derivatives were calculated by adapting the method developed by Levine et al. (1994). Malondialdehyde was determined by HPLC with fluorescence detection according to the method described by Laporta et al. (2007).

Exercise test

Maximal treadmill test was performed (Marquette Electronics, Milwaukee, WI) at sea level (inspired O_2 fraction = 21%), using the Bruce protocol. Cardiac frequency (CF) was monitored by continuous electrocardiography. Blood pressure was monitored during both exercise and recovery, using a sphygmomanometer attached to the arm. A calibrated, computerized metabolic cart (Medical Graphics, St. Paul, MN) was used for breath-by-breath analysis during exhalation. O_2 consumption (VO_2), CO_2 production, and minute ventilation were averaged during 30 s intervals. VO_2max was defined as the highest 30 s average during exercise. Before each session, the system was calibrated using known O_2 and CO_2 concentrations. During the treadmill test, each alpinist was encouraged to give a maximal effort. Tidal volume (TV, in mL), breathing frequency (BF, in breaths per min), ventilation per minute (VE, in L/min), oxygen consumption O_2 (VO_2, in L/min/kg), carbon dioxide production (VCO_2, in L/min) and respiratory quotient ($RQ = VCO_2/VO_2$) were measured breath by breath.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, v. 15.0 for Windows). The results were expressed as means and standard deviation (Mean ± SD). Since the sample was too small ($n = 5$), one-sample K–S test (Kolmogorov–Smirnov test) was performed in order to assess if each sample fits a normal distribution. Statistical significance was set at $p < 0.05$. All the data were tested for normality. Student's t test for paired data was used to identify differences in the participants at the beginning and end of the study. The Pearson and the Spearman's Rho coefficients were used to check correlation between circulating iron levels and persistence of oxidative damage in neutrophils and plasma.

Results

Anthropometric changes 4 weeks after returning from expedition

The objective of this study was to measure the biochemical parameters under recovery conditions, in order to determine to what extent stress persists. Four weeks after returning from the summit to sea level, the five climbers recovered their original body composition values: 12.4 ± 1.5% (day 1) versus 11.8 ± 0.7% (day 70) for fat mass, 45.5 ± 2.2% (day 1) versus 44.4 ± 2.0% (day 70) for muscle mass, 17.9 ± 0.9% (days 1 and 70) for bone mass and 24.2 ± 1.5% (days 1 and 70) for visceral mass. Differences were not significant. Body weight changed from 71.2 ± 5.7 to 69.9 ± 7.7 kg after returning to sea level, although the difference was not significant.

Changes in circulating parameters 4 weeks after returning from expedition

Cardiorespiratory parameters determined by ergometry at day 70 were similar to the values determined at day 1 (Table 1). Only blood pressure, heart rate, and indirect blood oxygen saturation were controlled by the alpinists and the resulting measurements were sent via Internet during ascension in order to monitor adaptation to altitude (Table 2). As a result, erythrocyte number (12.2%) was increased 4 weeks after returning to sea level (Table 3). This correlates with the changes observed in related parameters such as hemoglobin (8.8% increase), hematocrit (6.6% increase), mean corpuscular volume (MCV) (2.9% decrease) and mean corpuscular hemoglobin concentration (MCHC) (2.8% increase). No changes were observed in mean corpuscular hemoglobin (MCH), red cell distribution width (RDW-SD) or in platelets or leukocyte number (Table 3).

In addition, there was a 17.3% increase in the circulating levels of uric acid and a 2.9% decrease in sodium (Table 3). Concerning circulating proteins, ferritin increased significantly

Table 1 Ergometry at sea level-baseline (day 1) and 4 weeks post-altitude (day 70)

Parameter (units)	Day 1	Day 70
Maximal cardiac frequency (bpm)	178.2 ± 9.3	177.8 ± 7.7
Blood pressure (mm Hg)	121 ± 20	108 ± 8*
Maximal blood pressure (mm Hg)	181 ± 27	162 ± 19
	90.0 ± 10	74.0 ± 7
VO_2max (L/min)	4.1 ± 0.5	4.0 ± 0.4
Tidal volume (mL)	287.0 ± 41	278.0 ± 32
Breathing frequency (breaths per min)	44 ± 8	42 ± 5
Ventilation per min (L/min)	127 ± 21	121 ± 14
O_2 consumption (mL/min/kg)	56.3 ± 3.6	55.8 ± 2.1
CO_2 production (L/min)	4.1 ± 0.6	3.6 ± 0.3
Respiratory quotient (VCO_2/VO_2)	0.98 ± 0.03	0.89 ± 0.04

bpm beats per minute

* $p < 0.05$

Table 2 Means ± SEM of blood pressure (systolic/diastolic), heart rate (HR) and indirect blood oxygen saturation (SpO_2) registered by the alpinists during the ascension to Cho-Oyu from sea level to Camp III

Altitude	Blood pressure (mmHg)	HR (bpm)	SpO_2(%)
Elche (0 m)	113.0 ± 3.7/77.6 ± 8.6	58.0 ± 4.4	98.9 ± 0.7
Base camp (5,700 m)	126.9 ± 9.7/86.5 ± 11.7	73.7 ± 12.1	85.0 ± 9.0
Camp I (6,380 m)	129.8 ± 15.6/88.4 ± 8.8	85.0 ± 13.2	84.7 ± 4.2
Camp II (7,100 m)	133.4 ± 9.5/90.1 ± 10.1	98.7 ± 12.1	82.5 ± 5.5
Camp III (7,500 m)	135.8 ± 11.0/92.6 ± 13.7	95.0 ± 15.8	81.6 ± 2.9

bpm beats per minute

a 106.3% after 4 weeks of exposure to high altitude, while AP and GGT both decreased a 15.3 and 22.7%, respectively (Table 3).

Finally, no significant changes were observed in glucose, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, creatinine, urea, potassium, lactate, serum iron, AST/GOT, ALT/GPT, CK, and Mb (Table 3). All determined parameters, except for Mb, were included into the corresponding reference values accepted for adult healthy populations.

Hematology, circulating metabolites and elements/electrolytes, lipid profile and plasma protein markers were determined in the alpinists during a similar period of time 1 year later under well-controlled conditions of diet and submitting them to the same exercise routine, but remaining at sea level (Table 4). As a result, there were significant decreases in MCHC (4.7%), HDL-cholesterol (12.6%) and serum iron levels (29.3%), as well as a significant increase in sodium levels (5.9%). All determined parameters, except for CK, were included into the corresponding reference values accepted for adult healthy populations.

Table 3 Blood analysis at sea level-baseline (Day 1) and 4 weeks post-altitude (Day 70)

Parameter (units)	Day 1	Day 70
Hemogram		
Erythrocytes (cells/μL)	4.9×10^6 ± 11,546	5.5×10^6 ± 190,601*
Hemoglobin (g/dL)	14.7 ± 0.2	16.0 ± 0.9*
Hematocrit (%)	45.4 ± 0.4	48.4 ± 2.4*
MCH (pg)	29.3 ± 0.7	29.1 ± 0.7
MCV (fl)	91.0 ± 2.4	88.4 ± 3.3*
MCHC (g/dL)	32.3 ± 0.7	33.2 ± 0.9*
RDW-SD (fl)	40.9 ± 8.6	40.6 ± 7.3
Platelets (cells/μL)	170,000 ± 29,329.1	166,400 ± 18,358.8
Neutrophils (cells/μL)	2,596.2 ± 615.6	2,545.6 ± 662.2
Lymphocytes (cells/μL)	2,294.8 ± 404.8	2,268.6 ± 323.0
Circulating metabolites/elements		
Glucose (mg/dL)	82.5 ± 8.6	80.4 ± 12.1
Triglycerides (mg/dL)	78.8 ± 22.9	69.8 ± 29.3
Total cholesterol (mg/dL)	187.2 ± 25.7	179.6 ± 41.8
HDL-cholesterol (mg/dL)	52.7 ± 4.4	51.8 ± 4.6
LDL-cholesterol (mg/dL)	118.8 ± 20.5	114.2 ± 34.3
Creatinine (mg/dL)	1.14 ± 0.1	1.04 ± 0.1
Urea (mg/dL)	37.2 ± 8.4	39.4 ± 9.5
Uric acid (mg/dL)	5.2 ± 1.8	6.1 ± 1.8*
Lactate (mg/dL)	7.1 ± 2.0	6.3 ± 2.0
Serum iron (μg/dL)	93.7 ± 11.4	127.6 ± 50.4
Na (mEq/L)	142.4 ± 0.9	138.3 ± 1.5*
K (mEq/L)	3.85 ± 0.2	4.02 ± 0.1
Circulating proteins		
Ferritin (ng/mL)	76.0 ± 36.1	156.8 ± 59.6*
Myoglobin (ng/mL)	69.0 ± 27.9	81.0 ± 22.9
Alkaline phosphatase (U/L)	105.8 ± 16.9	89.6 ± 14.7*
GGT (U/L)	22.0 ± 4.4	17.0 ± 4.6*
AST/GOT (U/L)	21.0 ± 3.3	24.6 ± 5.9
ALT/GPT (U/L)	18.3 ± 4.0	27.6 ± 8.1
CK (U/L)	152.5 ± 66.0	175.4 ± 103.2

AST/GOT aspartate aminotransferase/serum glutamic oxaloacetic transaminase, *ALT/GPT* alanine aminotransferase/serum glutamic pyruvic transaminase, *CK* creatine phosphokinase, *GGT* γ-glutamyltranferase, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *MCV* mean corpuscular volume, *RDW-SD* red cell distribution width

* $p < 0.05$

Table 4 Blood analysis at day 1 and after 70 days staying at sea level

Parameter (units)	Day 1	Day 70
Hemogram		
Erythrocytes (cells/μL)	$4.7 \times 10^6 \pm 27{,}386$	$4.7 \times 10^6 \pm 130{,}372$
Hemoglobin (g/dL)	14.5 ± 0.9	14.5 ± 0.9
Hematocrit (%)	43.0 ± 2.4	44.8 ± 2.2
MCH (pg)	31.4 ± 1.5	30.4 ± 1.5
MCV (fl)	91.7 ± 3.5	94.0 ± 2.9
MCHC (g/dL)	34.0 ± 0.9	32.4 ± 1.3*
RDW-SD (fl)	46.4 ± 2.2	46.6 ± 2.2
Platelets (cells/μL)	199,600 ± 45,932.0	170,800 ± 37,171.2
Neutrophils (cells/μL)	3028.0 ± 806.1	3,354.6 ± 290.3
Lymphocytes (cells/μL)	2,482.4 ± 347.6	2,172.8 ± 539.2
Circulating metabolites/elements		
Glucose (mg/dL)	88.8 ± 4.6	88.2 ± 11.9
Triglycerides (mg/dL)	66.6 ± 21.6	56.2 ± 11.4
Total cholesterol (mg/dL)	184.2 ± 30.1	188.6 ± 24.2
HDL-cholesterol (mg/dL)	62.5 ± 8.1	54.6 ± 2.0*
LDL-cholesterol (mg/dL)	111.7 ± 21.3	121.2 ± 25.1
Creatinine (mg/dL)	1.07 ± 0.1	1.02 ± 0.04
Urea (mg/dL)	31.2 ± 3.7	30.8 ± 4.6
Uric acid (mg/dL)	4.8 ± 0.9	5.0 ± 1.1
Lactate (mg/dL)	7.1 ± 1.3	7.6 ± 1.8
Serum iron (μg/dL)	107.0 ± 16.1	75.7 ± 22.4*
Na (mEq/L)	132.8 ± 1.3	140.7 ± 0.9*
K (mEq/L)	3.82 ± 1.3	4.1 ± 0.4
Circulating proteins		
Ferritin (ng/mL)	78.1 ± 39.8	85.8 ± 35.6
Myoglobin (ng/mL)	43.2 ± 27.9	50.8 ± 22.0
Alkaline phosphatase (U/L)	95.5 ± 20.4	99.8 ± 19.1
GGT (U/L)	19.5 ± 4.6	22.0 ± 2.4
AST/GOT (U/L)	26.4 ± 11.0	30.8 ± 12.5
ALT/GPT (U/L)	17.6 ± 4.6	22.8 ± 4.8
CK (U/L)	158.2 ± 71.1	229.4 ± 150.7

AST/GOT aspartate aminotransferase/serum glutamic oxaloacetic transaminase, *ALT/GPT* alanine aminotransferase/serum glutamic pyruvic transaminase, *CK* creatine phosphokinase, *GGT* γ-glutamyltranferase, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *MCV* mean corpuscular volume, *RDW-SD* red cell distribution width

* $p < 0.05$

Changes in oxidative stress markers 4 weeks after returning from expedition

Although neutropenia was not evident 4 weeks after returning to sea level, the volunteers still presented significantly higher amounts of MDA (approximately 3 times more with respect to sea level-baseline condition) in this cell type (Table 5). No oxidative alterations (MDA and protein carbonyls) were observed in lymphocytes 4 weeks after returning from expedition. On the other hand, oxidative damage persisted in plasma, expressing significantly higher values of MDA (approximately 2.75 times with respect to sea level-baseline condition) and protein carbonyls (approximately 1.5 times with respect to sea level-baseline condition) (Table 5).

Table 5 Oxidative stress markers in neutrophils, lymphocytes and plasma at sea level-baseline (day 1) and 4 weeks post-altitude (day 70)

Marker (units)	Day 1	Day 70
Neutrophils		
MDA (μmols/10^6 cells)	24.1 ± 15.2	72.4 ± 17.8*
Protein carbonyls (μmols/L)	4.0 ± 0.9	4.6 ± 1.1
Lymphocytes		
MDA (μmols/10^6 cells)	55.4 ± 23.8	51.5 ± 16.5
Protein carbonils (μmols/L)	5.0 ± 0.9	4.3 ± 0.4
Plasma		
MDA (μmols/L)	171.9 ± 127.8	474.6 ± 81.2*
Protein carbonyls (μmols/L)	78.5 ± 13.9	120.5 ± 22.4*

MDA malondialdehyde

* $p < 0.05$

Table 6 Mean percentage values of antioxidant enzyme activities in different circulating cell types 4 weeks post-altitude. The values at sea level-baseline (day 1) were considered 100%

Enzyme	Lymphocytes (%)	Neutrophils (%)	Erythrocytes (%)
Catalase	64.5	44.9*	122.7
Glutathione peroxidase	74.9	47.7	57.4
Glutathione reductase	103.4	21.1	137.4
Superoxide dismutase	107.9	97.9	140.9

* $p < 0.05$

Changes in antioxidant enzymes 4 weeks after returning from expedition

Catalase, SOD, GSH-peroxidase, and GSH-reductase activities were determined in erythrocytes, lymphocytes, and neutrophils. Catalase in neutrophils was the only antioxidant enzymatic activity that presented significantly different values, decreasing 55.1% 4 weeks after exposure to extreme altitude: 48.4 ± 15.8 k5/10^9 cells (day 1) versus 26.7 ± 4.8 k5/10^9 cells (day 70). In the other cell types (erythrocytes and lymphocytes), there was a huge variability in catalase levels as well as in the other enzymatic activities analyzed (Table 6).

Discussion

The main objective of this work was to study the recovery rate from stressors in alpinists after a 6-week expedition to extreme altitudes. Since persistent hypoxic conditions are related to oxidative stress, this is one of the most important stressors in alpinism (Dosek et al. 2007). The major finding in our work is that oxidative stress markers still persist in neutrophils and plasma 4 weeks after returning to sea level.

Previously, it has been shown that exposure to extreme altitudes enhances weight loss due to malnutrition and protein catabolism, since lipids cannot be used efficiently in energy metabolism due to the hypoxic conditions (Bärtsch and Saltin 2008). In this study, all subjects lost 4–5 kg when returning from the summit (base camp).

As previously mentioned, exposure to high and extreme altitudes is correlated with an impairment in antioxidant activity (Dosek et al. 2007); however, little is known concerning the recovery of the body redox balance once returning to low altitudes. One recent report (Pialoux et al. 2010) studied the recovery of antioxidant capacity in 11 cross-country skiers, training at a moderate altitude and living in simulated increasing hypoxic conditions (2,500, 3,000, and 3500 m) versus a control group training and living at a low altitude (1,200 m). Two weeks after the exposure, the Trolox equivalent antioxidant capacity of the experimental group of athletes remained low when compared to the control group. These changes were attributed in part to low circulating levels of lycopene and β-carotene possibly due to insufficient intakes of vitamins A and E. However, the conditions underwent by the volunteers of this study were not exactly the severe conditions experienced in high mountain expeditions. Exposure to high and extreme altitudes includes a number of stressors such as hypoxia, low atmospheric pressure, low temperature, extreme weather conditions, high UV radiation, tissue ischemia–reperfusion, as well as high metabolic demands due to the intense physical activity, and impaired nutrition and liquid intakes (Vij et al. 2005; Dosek et al. 2007; Bärtsch and Saltin 2008; Pialoux et al. 2009; Drust and Waterhouse 2010; Martarelli et al. 2011). Altogether, these conditions may contribute to the disruption of the redox body balance, although the degree of participation of each particular stressor is still an open question in altitude biomedicine.

In the present study, we analyzed in detail the recovery of cell-circulating antioxidant enzymes and subsequently the persistence of oxidative damage after extreme alpinism practice. To this end, we performed all determinations in a group of five expert alpinists, 4 weeks after climbing the Cho-Oyu. As a result, the subjects presented anthropometric and cardiorespiratory parameters that were very similar to those determined at the beginning of the study (day 1). However, other parameters analyzed were affected. For example, erythrocyte number was higher at day 70 than at day 1. This coincided with an increase in hemoglobin, hematocrit, MCV and MCHC (Table 3). The most probable reason for this increase is erythropoietin secretion in response to the high altitude exposure, stimulating the synthesis of new red blood cells in order to increase oxygen capture (Drust and Waterhouse 2010). In our study, the values of these parameters remained modified for at least 4 weeks after exposure to high altitudes. Ferritin, an indicator of iron body storage, was also increased, possibly to favor the extra demand of hemoglobin synthesis.

Changes were not observed in plasma protein markers for tissue damage such as CK and Mb for muscle tissue, or AST/GOT and ALT/GPT for liver and muscle tissues. Interestingly, GGT, a liver marker and AP, a marker for liver, bone, and intestine, decreased 4 weeks after exposure to high altitudes. Altogether, these results indicate that tissue integrity was nearly recovered after returning from high mountain expedition.

Interestingly, circulating sodium levels decreased modestly. Although we do not have a direct explanation for this observation, this could be due to changes in kidney function after extreme altitude exposure. The natriuretic response to acute hypoxemia is characterized by a decreased re-absorption of sodium and bicarbonate in the proximal renal tubules as a result of hyperventilation and hypocapnia (Olsen 1995). However, this situation is transient and normalizes very quickly (in several hours) after exposure to high or extreme altitudes. Although this point deserves to be studied in more detail, we hypothesize that the additional adverse conditions underwent in this type of expeditions (including extreme weather conditions and impaired nutrition and liquid intakes) may contribute to a change in Na homeostasis that remained altered 4 weeks after returning to sea level.

Uric acid levels were elevated 4 weeks after returning from the high mountain climbing expedition. A hereditary reason could explain this observation; however, uric acid would have been altered before and after the expedition in volunteers. In addition, several pathologies lead to increased circulating levels of uric acid, such as diabetes/metabolic syndrome and gout (Hovind et al. 2011; Edwards 2009; Smith et al. 2010). However, the volunteers of this study were healthy individuals free from these pathologic conditions. Thus, the most plausible explanation is the contribution of a poor diet balance typical of this type of expeditions or increased tissue turnover. Nevertheless, we cannot obviate that uric acid is the product of xanthine oxidase, an enzyme that is activated in oxidative stress situations such as in the exposure to hypoxic conditions (Dosek et al. 2007) or after intense exercise performance (Tauler et al. 2003). In this sense, the increased circulating uric acid values may reflect the existence of a plasma oxidation

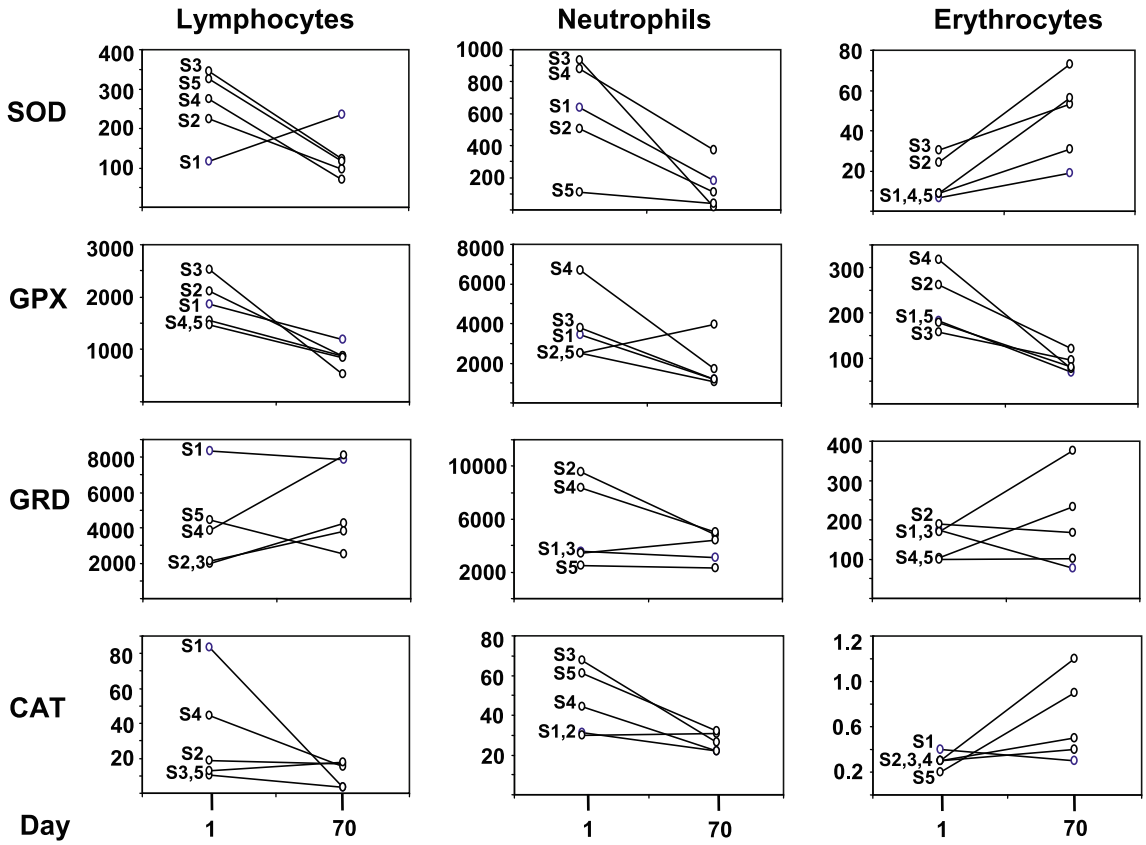

Fig. 1 Variations in antioxidant enzymatic activities determined in lymphocytes, neutrophils and erythrocytes between the five subjects (S1–5) that participate in the study. The activities determined were superoxide dismutase (SOD) in pkat/10^9 cells in lymphocytes and neutrophils and pkat/g hemoglobin in erythrocytes, glutathione-peroxidase (GPX) and glutathione-reductase (GRD) in nkat/10^9 cells in lymphocytes and neutrophils and nkat/g hemoglobin in erythrocytes, and catalase (CAT) in k5/10^9 cells in lymphocytes and neutrophils and k5/g hemoglobin in erythrocytes

status due to the participation of different stressors during the expedition. Nevertheless, it must be also mentioned that uric acid acts as a free-radical scavenger under these circumstances, acting as a defence against antioxidant demise (Baillie et al. 2007). Therefore, the exact role of increased uric acid levels after exposure to extreme altitudes remains to be elucidated.

In accordance with this observation, MDA and protein carbonyl values in plasma were high 4 weeks after exposure to extreme altitudes. The damage persisted in neutrophils where MDA values were high and catalase activity low compared to day 1. Therefore, while several parameters and systems recovered favorably, others remained altered 4 weeks after returning to sea level. In particular, plasma and neutrophils exhibited significant oxidative damage. In this context, neutrophils constitute the most abundant nucleated circulating cell type (50–70% of leukocytes). These cells play a crucial role by phagocytic cell debris, where there is tissue damage and inflammation (Nikolaidis and Jamurtas 2009).

Regarding other antioxidant enzymes (SOD, GSH-peroxidase, GSH-reductase) in other circulating cell types (lymphocytes, erythrocytes) we observed a large variability between individuals, thus it was impossible to reach a consistent conclusion. Several authors have shown that the activity of these enzymes tend to be lower in simulated hypoxic conditions (Dosek et al. 2007). Therefore, it is very likely that in our volunteers these antioxidant enzymes were partly inhibited immediately after descending from summit, unfortunately this determination was not performed. On the other hand, in the analyses performed 4 weeks after exposure to extreme altitudes, all antioxidant enzymes tended to decrease in the case of neutrophils, while in erythrocytes and lymphocytes some activities were either restored, increased or diminished (Fig. 1). In any case, we must emphasize that these changes are not significant and that the results must be interpreted with caution.

The increase observed in several oxidative markers could be ascribed to the increases observed for serum iron, which is a potent pro-oxidant agent. However, we did not observe any correlation, determined by either Pearson's or Spearman's Rho coefficients, between serum iron or ferritin and MDA and carbonyls measured in neutrophils and plasma.

It is important to mention that our experimental design cannot be ascribed exactly to a recovery protocol, since blood samples were not extracted immediately when alpinists reached 1,500 m. Technical and economic factors were the cause of this limitation. In any case, we have monitored the same volunteers during similar periods of time (70 days) at sea level performing aerobic exercise and following a controlled diet, observing very similar values for hematology, circulating metabolites, and elements/electrolytes, lipid profile, plasma protein markers, antioxidant activities and oxidative markers at the beginning and end of the study (Table 4). Therefore, we could conclude that high-mountain expeditions represent a convergence of stressors that deeply alter redox balance of several body systems, such as plasma and neutrophils reported in this study. These alterations persist over time causing an incomplete recovery.

Conclusion

The data presented suggest that the recovery after returning from a high-mountain expedition occurs at different rates, depending on the parameter studied. In this sense, anthropometric, cardiorespiratory status, hematology, circulating metabolites, and elements/electrolytes, lipid profile and plasma protein markers were restored after 4 weeks at sea level. However, erythrocyte number, ferritin and sodium seemed to require longer recovery times. The same observation was noticed for neutrophils, which presented oxidative changes in the form of increased MDA levels and low levels of catalase. Also, in plasma levels of uric acid, MDA and protein carbonyls were increased. Therefore, recovery strategies must consider these differences.

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Conflict of interest The authors declare that no conflicting financial interest exists.

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Baillie JK, Bates MGD, Thompson AAR, Waring WS, Partridge RW, Simpson A, Maxwell SRJ (2007) Endogenous urate production augments plasma antioxidant capacity in healthy lowland subjects exposed to high altitude. Chest 131:1473–1478

Bärtsch P, Saltin B (2008) General introduction to altitude adaptation and mountain sickness. Scand J Med Sci Sports 18(Suppl 1):1–10

Bowers LD, Wong ET (1980) Kinetic serum creatinine assays. II. A critical evaluation and review. Clin Chem 26:555–561

Boyum A (1964) Separation of white blood cells. Nature 204:793–794

Bucolo G, David H (1973) Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 19:476–482

Dosek A, Ohno H, Acs Z, Taylor AW, Radak Z (2007) High altitude and oxidative stress. Resp Physiol Neurobiol 158:128–131

Drust B, Waterhouse J (2010) Exercise and altitude. Scott Med J 55:31–34

Edwards NL (2009) The role of hyperuricemia in vascular disorders. Curr Opin Rheumatol 21:132–137

Faulkner JA (1968) Physiology of swimming and diving. In: Falls H (ed) Exercise physiology. Academic Press, Baltimore, pp 415–445

Flohé L, Gunzler WA (1984) Assays of glutathione peroxidase. Methods Enzymol 105:114–121

Fossati P, Prencipe L, Berti G (1980) Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem 26:227–231

Goldberg DM, Spooner RJ (1983) Glutathione reductase. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Verlag Chemie, Basel, pp 258–265

Heinicke I, Boehler A, Rechsteiner T, Bogdanova A, Jelkmann W, Hofer M, Rawlings P, Araneda OF, Behn C, Gassmann M, Heinicke K (2009) Moderate altitude but not additional endurance training increases markers of oxidative stress in exhaled breath condensate. Eur J Appl Physiol 106:599–604

Hovind P, Rossing P, Johnson RJ, Parving HH (2011) Serum uric acid as a new player in the development of diabetic nephropathy. J Ren Nutr 21:124–127

Ilavazhagan G, Bansal A, Prasad D, Thomas P, Sharma SK, Kain AK, 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 SW, Rivera JO, 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

Itano M (1978) CAP comprehensive chemistry. Serum iron survey. Am J Clin Pathol 70:516–522

Joanny P, Steinberg J, Robach P, Richalet JP, Gortan C, Gardette B, Jammes Y (2001) Operation Everest III (Comex'97): the effect of simulated sever 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 PJ (1995) Modulating hypoxia-induced hepatocyte injury by affecting intracellular redox state. Biochim Biophys Acta 1269:153–161

Laporta O, Funes L, Garzon MT, Villalain J, Micol V (2007) Role of membranes on the antibacterial and anti-Inflammatory activities of the bioactive compounds from Hypoxis rooperi corm extract. Arch Biochem Biophys 467:119–131

Levine RL, Williams JA, Stadtman ER, Shacter E (1994) Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 233:346–357

Marfell-Jones M, Olds T, Steward A, Carter L (2006) International standards for anthropometric assessment. ISAK, Potchefstroom

Martarelli D, Cocchioni M, Scuri S, Spataro A, Pompei P (2011) Cold exposure increases exercise-induced oxidative stress. J Sports Med Phys Fitness 51:299–304

Martin AD, Daniel M, Clarys JP, Marfell-Jones MJ (2003) Cadaver-assessed validity of anthropometric indicators of adipose tissue distribution. Int J Obes Relat Metab Disord 27:1052–1058

McCord JM, Fridovich I (1969) Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 244:6049–6055

Mohanraj P, Merola AJ, Wright VP, Clanton TL (1998) Antioxidants protect rat diaphragmatic muscle function under hypoxic conditions. J Appl Physiol 84:1960–1966

Naito HK, David JA (1984) Laboratory considerations: determination of cholesterol, triglyceride, phospholipid, and other lipids in blood and tissues. Lab Res Methods Biol Med 10:1–76

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

Nikolaidis MG, Jamurtas AZ (2009) Blood as a reactive species generator and redox status regulator during exercise. Arch Biochem Biophys 490:77–84

Olsen NV (1995) Effect of hypoxaemia on water and sodium homeostatic hormones and renal function. Acta Anaesthesiol Scand Suppl 107:165–170

Pialoux V, Mounier R, Brown AD, Steinback CD, Rawling JM, Poulin MJ (2009) Relationship between oxidative stress and HIF-1 alpha mRNA during sustained hypoxia in humans. Free Radic Biol Med 46:321–326

Pialoux V, Brugniaux JV, Rock E, Mazur A, Schmitt L, Richalet JP, Robach P, Clottes E, Coudert J, Fellmann N, Mounier R (2010) Antioxidant status of elite athletes remains impaired 2 weeks after a simulated altitude training camp. Eur J Nutr 49:285–292

Prentice AM, Jebb SA (2001) Beyond body mass index. Ob Rev 2:141–147

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

Smith EU, Diaz-Tomé C, Pérez-Ruiz F, March LM (2010) Epidemiology of gout: an update. Best Pract Res Clin Rheumatol 24: 811–827

Tabacco A, Meiattini F, Moda E, Tarli P (1979) Simplified enzymic/colorimetric serum urea nitrogen determination. Clin Chem 25:336–337

Tauler P, Aguilo A, Gimeno I, Fuentespina E, Tur JA, Pons A (2003) Influence of vitamin C diet supplementation on endogenous antioxidant defences during exhaustive exercise. Pflugers Arch 446:658–664

Trinder P (1969) Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J Clin Pathol 22:158–161

Vij AG, Dutta R, Satija NK (2005) Acclimatization to oxidative stress at high altitude. High Alt Med Biol 6:301–310

Weisshaar D, Gossrau E, Faderl B (1975) Normal ranges of alpha-HB-DH, LDH, AP, and LAP as measured with substrate-optimated test charges. Med Welt 26:387–392

Wozniak A, Drewa G, Chesy G, Rakowski A, Rozwodowska M, Olszewska D (2001) Effect of altitude training on the peroxidation and antioxidant enzymes in sportmen. Med Sci Sports Exerc 33:1109–1113

Young DS, Friedman RB (2001) Effects of disease on clinical laboratory tests. AACC Press, Washington DC