Biological and Nutritional Consequences of Work at High Altitude in Search and Rescue Dogs: The Scientific Expedition Chiens des Cimes-Licancabur 1996[1,2]

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EXPANDED ABSTRACT

KEY WORDS: • *high altitude* • *peroxidation* • *rescue dogs* • *vitamin E* • *fish oil*

Reactive oxygen species (ROS)[4] such as the superoxide anion, $O_2^{\bullet -}$, the hydroxyl radical, $OH^{\bullet}$, and H_2O_2, are constantly produced by metabolic reactions (Halliwell 1994). When they are not removed through the action of biological antioxidants, they are harmful to cells, induce membrane lipid peroxidation and damage proteins and nucleic acids. ROS are counteracted by a wide range of antioxidants synthesized in the cell, including glutathione peroxidase (GSH-PX) and reductase, superoxide dismutase (SOD) and catalase, or supplied by the diet such as vitamins E and C or flavonoids. An excess of ROS, known as oxidative stress, can occur in many circumstances including inflammation, cigarette smoking, acute physical exercise and exposure to high altitude.

Effects of altitude on the generation of ROS have been extensively investigated. In rats exposed to hypobaric hypoxia "equivalent to an altitude of 5500 m", Nakanashi et al. (1995) reported increases in oxidative stress and concentrations of the antioxidant enzymes GSH-PX and SOD. In humans, Vasankari et al. (1997) showed that levels of serum conjugated dienes (CD), a lipid peroxidation marker, were increased during living, training and racing at moderate altitude. Working and racing dogs are often subjected to high levels of metabolic stress that generate ROS. However, the antioxidant/oxidant balance at rest and after exercise for these dogs remains unknown. The search and rescue dog teams such as the canine unit of the Paris Fire Brigade have to work without advance notice or any kind of adaptation period in situations of natural catastrophes (e.g., earthquakes or land slides), very often at high altitude (e.g., Central Asia or South America). Bearing this in mind and with the objective of offering the dogs better preparation for this type of situation, we organized a dedicated expedition to Chile in 1996.

Five rescue teams from Carabinieros de Chile and five from the Paris Fire Department participated in the study. They reached the volcano Licancabur, starting from sea level, in less than 24 h. They stayed 3 d at 4800 m, then they climbed the volcano, reaching an altitude of 5980 m on d 4 and returning to sea level at the end of d 5. This study was designed as an open comparative study between two groups of well-trained dogs fed different diets.

Approval to use laboratory animals was given by the French Ministry of Agriculture, and the protocol complied with NIH guidelines (NRC 1985).

Materials and methods. *Diet.* Group 1, the French dogs, received 400 g/d of a dry diet designed to cope with stress (Alpicroc, Royal Canin, France). They received also a daily supplement of 1 g of fish oil (Maxepa, Pierre Fabre, Castres, France) and 500 mg of vitamin E (α-tocopherol; Toco 500, Pharma 2000, Buc, France). Group 2, the Chilean dogs, received 600 g/d of a standard diet. Both group of dogs normally lived at sea level. **Table 1** contains the composition of the two diets.

Work tests. The dogs had to search for and discover two victims hidden by debris in a defined area. Lag times to find the first (T1) and second victim (T2) as well as the time interval ($\Delta T = T2 - T1$) were determined. Cardiac frequency and blood pressure were measured at rest and after exercise. On d 2 of the study, the blood draw and the search for victims took place at sea level; on d 4, they were at an altitude of 4800 m and on d 5 at an altitude of 5980 m. After the search on d 5, the dogs returned to sea level.

Biological parameters. Blood samples were collected at the different altitudes 30 min after work. Plasma was separated

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[4]Abbreviations used: *t*-BOOH, *tertio*-butyl hydroperoxide; CD, conjugated dienes; GLC, gas liquid chromatography; GSH-PX, glutathione peroxidase; MDA, malondialdehyde ; $O_2^{\bullet -}$, superoxide anion; $OH^{\bullet}$, hydroxyl radical; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species; SOD, superoxide dismutase.

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TABLE 1

Composition of the diet: specific dietary intakes[1]

	Group 1[2]	Group 2/Standard
Water, *% as fed*	6.8	10
Proteins, *% as fed*	31.8	30
Total fat, *% as fed*	28.6	22
Fiber, *% as fed*	12.2	6.4
Energy concentration, *MJ/kg of food*	20.2	17.3
(*n* − 6) fatty acids, *% of fat*	4.80	4.50
(*n* − 3) fatty acids, *% of fat*	0.65	0.20
Vitamin A, *IU/kg of food*	35840	20000
Vitamin E, *mg/kg of food*	166	110
	Quantities per dog per day	
Sea level		
Energy intake, *MJ*	8.1	10.3
(*n* − 3) fatty acids, *mg*	1044	264
Vitamin E, *mg*	566	66
Ratio (*n* − 6)/(*n* − 3)	5.26	22.75
Altitude		
Energy intake, *MJ*	9.1	14.0
(*n* − 3) fatty acids, *mg*	1137	352
Vitamin E, *mg*	575	88
Ratio (*n* − 6)/(*n* − 3)	5.43	22.75

[1] Group 1: French dogs; Group 2: Chilean dogs.
[2] The basic diet (alpicroc, Royal Canin; France) was supplemented with 300 mg of (*n* − 3) PUFA (1 g Maxepa) and 500 mg of vitamin E (1 tablet Toco500).

from RBC by centrifugation at 3000 rev/min and kept frozen at −20°C until analysis. Buffy coat was removed and RBC were washed twice with saline water.

Peroxidation resistance index. Washed RBC (20 μL) were submitted to oxidative stress by *tert*-butyl hydroperoxide (*t*-BOOH), a specific lipid peroxidative agent that breaks membrane lipids and causes the lysis of cells. We measured induced hemolysis time in seconds. This time is a function of (*t*-BOOH) concentration and RBC antioxidant capacity.

RBC fatty acids. Lipids were extracted from RBC by a mixture of isopropanol chloroform; BHT at 0.05% was added to prevent in vitro peroxidation. RBC phospholipids were separated using a Sep-Pak column. Transesterification of fatty acids was performed by BF_3/methanol at 70°C.

Fatty acid methyl esters were determined by gas liquid chromatography (GLC) on a polar capillary column.

Plasma vitamin E. Plasma vitamin E was measured by reverse-phase HPLC.

Statistical analysis. Data are expressed as means ± SD. Data were analyzed by two-factor ANOVA (group and altitude), and significant differences between groups were determined by Student-Neuman-Keuls test using SAS (SAS Institute, Cary, NC). Significance was set at $P < 0.05$.

Results. *Effect of altitude on work capacity*. At sea level (baseline), cardiac frequency (beats/min) was not different in the two groups at rest or after work (**Table 2**). It increased slightly with altitude and returned close to baseline values on d 5. Times required to find victims were much longer in Group 2 than Group 1, at baseline and at high altitude.

Peroxidation resistance index. RBC hemolysis time was not different in the two groups at baseline, and was significantly decreased at 4800 m, indicating that RBC became less resistant to induced peroxidation (**Table 3**). RBC from Group 2 were more sensitive than those from Group 1 at 5980 m.

Plasma vitamin E. Plasma vitamin E baseline values tended to be higher in Group 1 than in Group 2 due to daily supplementation of Group 1 dogs (**Table 4**). High altitude induced a marked (significant) increase in plasma tocopherol concentrations in Group 1, whereas there was only a moderate increase in Group 2.

TABLE 2

Effects of altitude on work capacity[1,2,3]

Parameter	Group	Altitude 0 m	Altitude 4800 m	Altitude 5980 m
Rest CF	1	92 ± 6	127 ± 10[a]	95 ± 8[b]
beats/min	2	91 ± 7	134 ± 15[a]	112 ± 9[b]
CF after	1	118 ± 11	163 ± 9[a]	115 ± 10[a]
exercise	2	121 ± 8	197 ± 14[a,c]	157 ± 12[b,c]
T1, *s*	1	62 ± 21	71 ± 18	100 ± 10[a]
	2	126 ± 17[c]	131 ± 10[c]	145 ± 12[b,c]
ΔT, *s*	1	112 ± 10	132 ± 17[a]	133 ± 16
	2	83 ± 11[c]	141 ± 14[a]	179 ± 18[b,c]
T2, *s*	1	173 ± 18	202 ± 24	233a ± 12[a]
(Total time)	2	209 ± 20[c]	272 ± 21[a,c]	324 ± 25[b,c]

[1] Group 1: French dogs; Group 2: Chilean dogs.
[2] CF, cardiac frequency in beats per minutes; T1, time to discover the 1st victim (s); T2, time to discover the 2nd victim (s) − total time for finding two victims; ΔT, time interval between discovering the 2 victims (s).
[3] Values are means ± SD, $n = 5$; there were no significant group × altitude interactions for any variable.
[a] significantly different from 0 m in same group; [b] significantly different from 4800 m in same group; [c] significantly different from group 1 at same altitude; there were no significant group × altitude interactions for any variable.

TABLE 3

Peroxidation resistance index[1,2]

Group	0 m	4800 m	5980 m
1	40.6 ± 3.9	28.0 ± 4.8[a]	16.6 ± 4.5[b]
2	43.8 ± 2.7	27.2 ± 3.7[a]	12.8 ± 7.1[b,c]

[1] Group 1: French dogs; Group 2: Chilean dogs.
Reported in RBC hemolysis time (in seconds)
[2] Results are expressed as means ± SD, $n = 5$. There was no significant group by altitude interaction; [a] significantly different from 0 m in same group; [b] significantly different from 4800 m in same group; [c] significantly different from Group 1 at same altitude.

TABLE 4

Plasma vitamin E[1,2]

Group	0 m	4800 m	5980 m
		μmol/L	
1	43.3 ± 8.9	58.2 ± 8.1[a]	57.4 ± 7.5[b]
2	33.6 ± 6.7[c]	39.0 ± 7.3[a,c]	32.2 ± 6.2[c]

[1] Group 1: French dogs; Group 2: Chilean dogs.
[2] Results are expressed as means ± SD, $n = 5$. There was no significant group by altitude interaction; [a] significantly different from 0m in same group; [b] significantly different from 4800m in same group; [c] significantly different from group 1 at same altitude.

TABLE 5

Red cell membrane fatty acids[1,2]

Fatty acid	Group	0 m	4500 m	5980 m	Sign. main effect
		% total membrane fatty acids			
16:0	1	20.8 ± 1.2	19.5 ± 0.9	21.7 ± 0.8	
	2	19.4 ± 1.3	18.8 ± 1.4	22.4 ± 0.7	
16:1($n-7$)	1	0.81 ± 0.15	0.84 ± 0.20	0.80 ± 0.03	
	2	0.83 ± 0.17	0.95 ± 0.23	0.93 ± 0.10	
18:0	1	23.5 ± 1.5	22.6 ± 1.3	22.6 ± 1.4	
	2	23.6 ± 1.4	22.1 ± 1.3	22.3 ± 1.4	
18:1($n-9$)	1	10.3 ± 0.6	10.1 ± 0.5	10.6 ± 0.3	
	2	10.0 ± 0.6	10.5 ± 0.7	10.3 ± 0.4	
18:2($n-6$)	1	15.6 ± 1.8	17.7 ± 1.3	15.8 ± 1.6	
	2	14.0 ± 1.2	16.8 ± 1.3	14.1 ± 1.2	
20:3($n-6$)	1	1.30 ± 0.1	1.27 ± 0.08	1.23 ± 0.1	
	2	1.49 ± 0.1	1.38 ± 0.09	1.32 ± 0.1	
20:4($n-6$)	1	21.7 ± 1.4	21.8 ± 1.9	21.4 ± 1.5	
					Group
	2	24.5 ± 1.8	23.2 ± 1.9	22.6 ± 1.5	
22:4($n-6$)	1	0.95 ± 0.5	0.92 ± 0.4	0.93 ± 0.5	
					Group
	2	1.68 ± 0.6	1.63 ± 0.6	1.47 ± 0.7	
20:5($n-3$)	1	0.44 ± 0.05	0.61 ± 0.01	0.54 ± 0.03	
					Group
	2	0.11 ± 0.04	0.13 ± 0.03	0.18 ± 0.02	
22:5($n-3$)	1	0.42 ± 0.01	0.40 ± 0.04	0.39 ± 0.02	
					Group
	2	0.36 ± 0.02	0.41 ± 0.01	0.34 ± 0.03	
22:6($n-3$)	1	0.79 ± 0.11	0.82 ± 0.12	0.78 ± 0.1	
					Group
	2	0.13 ± 0.10	0.13 ± 0.11	0.14 ± 0.1	
Total ($n-3$)	1	1.65 ± 0.4	1.83 ± 0.2	1.71 ± 0.1	
					Group
	2	0.60 ± 0.3	0.66 ± 0.2	0.66 ± 0.1	

[1] Group 1: French dogs; Group 2: Chilean dogs.
[2] Results are expressed as means ± SD, $n = 5$.

RBC fatty acid composition. RBC fatty acid composition was not modified by high altitude and remained constant throughout the study (**Table 5**). Group 1 dogs had higher levels of (n-3) fatty acids and lower levels of (n-6) fatty acids than Group 2 dogs. These differences reflected dietary intake.

Discussion. The acute exposure to high altitude, without adaptation, induces a physiologic stress that reduces oxygen supply to cells. In our study, hypobaric-hypoxic conditions led to enhanced cardiac frequency, at rest and after exercise, and decreased work efficiency. However, these parameters were less affected in dogs supplemented with fish oil and vitamin E than in control dogs. Fish oils have been reported to have a beneficial effect on cardiovascular diseases, presumably via increased membrane fluidity (Driss et al. 1988) and an increased prostacyclin/thromboxane A_2 ratio (Schmidt and Dyerberg 1994).

Therefore, the better performance of supplemented dogs could be related to (n-3) fatty acid–induced endothelial vasorelaxation and enhanced red cell deformability, both conditions that improve oxygen uptake by muscle cells. Moreover, vitamin E could also have played a protective role against membrane peroxidation in response to high altitude–induced stress. Lipid peroxidation is usually assessed by techniques based on the determination of malondialdehyde (MDA) or CD (Esterbauer and Cheeseman, 1990), which are known to give controversial results because of their poor sensitivity and lower specificity. In our study, red cell membrane polyunsaturated fatty acid (PUFA) levels were used as a biomarker of lipid peroxidation. PUFA levels were not lowered under our experimental conditions, indicating that lipid peroxidation was not initiated or that ROS production had not yet overwhelmed the antioxidant capacities of red cell membranes. Our results are consistent with those of Radak et al. (1997) who did not detect lipid peroxidation in rats exposed to high altitude during a period of 20 d. They reported that tissue amino acids were more sensitive to oxidative modification than PUFA. The peroxidation resistance index test used in our study gives a better understanding of the oxidant/antioxidant balance of red cells. The hemolysis time was not different in the two groups at sea level and was considerably decreased at 4800 m. The altitude lowering effect was more pronounced after heavy exercise such as climbing the volcano. However, in this particular case, resistance to induced peroxidation was greater in dogs fed vitamin E than in those not fed vitamin E. Enzymatic and nonenzymatic antioxidants can be produced as an adaptive response to cellular oxidative stress. Ji (1993) reported increased levels of SOD and GSH, and Pincemail et al. (1998) reported increased plasma vitamin E in response to oxidative stress. The exact mechanism of such an increase has not been elucidated in detail, but redistribution from cellular compartments is highly probable. This notion is supported by the higher peroxidation susceptibility of red cells observed in our study.

The clinical manifestations induced by altitude hypoxia were similar to those reported in racing sled dogs (Grandjean and Sept 1991). Only few cases of diarrhea, myoglobinuria and

rhabdomyolysis occurred. These disappeared immediately after returning to sea level. Whether they were related to oxidative stress or not requires more investigation.

ROS, generated by work during a short-term exposure to high altitude, induced biological and physiologic modifications, which were partly prevented in dogs fed dry diet and supplemented with fish oil and vitamin E. The French dogs performed equally well at sea level and at 4800 m, whereas the Chilean dogs did not perform as well at the higher altitude as at sea level. The performance of both groups of dogs decreased at the higher altitude. Despite minor or severe pathologic changes, these supplemented dogs were able to perform search and rescue tasks without further adaptation.

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