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ORIGINAL CONTRIBUTION

Antioxidant status of elite athletes remains impaired 2 weeks after a simulated altitude training camp

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Abstract
Background It has been shown that the antioxidant status was altered by the "live high-train low" (LHTL) method, however, no information is available regarding the antioxidant restoration during the recovery period.
Aim of the study We tested the hypothesis that the antioxidant status is impaired by 18 days LHTL in elite athletes and remained altered after 14 days of recovery.
Methods Eleven elite cross-country skiers from the French Skiing Federation were submitted to 18-day endurance training. Six (hypoxic group; HG) trained at 1,200 m and lived in hypoxia (simulated altitude of 2,500 m–3,000 m–3,500 m) and 5 (control group; CG) trained and lived at 1,200 m. Plasma levels of advanced oxidation protein products (AOPP), malondialdehydes (MDA), ferric reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC) lipid-soluble antioxidants (α-tocopherol, β-carotene and lycopene) were measured at rest, before (PRE), the first day after (POST1) and again 2 weeks (POST14) after the training. Intakes of vitamins A and E were evaluated from the dietary recording.
Results In POST1, FRAP and TEAC decreased in both groups, however, the TEAC decrease persisted in POST14 for HG only. Lycopene and β-carotene decreased in POST1 for HG and remained lower in POST14. Finally, AOPP increased only for HG in POST1. The general decline of antioxidant status for both groups might result from insufficient intakes in vitamins A and E.
Conclusion This is the first study to show that the antioxidant status did not return to baseline 2 weeks after 18 days of LHTL training.

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Introduction

Altitude exposure is used in endurance training to increase the oxygen transport capacity. Since no clear significant advantage of "continuous altitude camps" (i.e., live and train at moderate altitude) were demonstrated [10, 16], altitude training models have been proposed to improve the performance at the sea-level using the "living high-training low" (LHTL) method. LHTL combines exposure to hypoxia during resting periods and exercise training sessions in normoxia [17]. It is now generally admitted that a single bout of hypoxia can be responsible for an increase in reactive oxygen species (ROS) generation leading to higher values of oxidative stress resulting to the increase of biological tissue oxidation. This phenomenon has been shown in sedentary [2, 11, 21, 31] but also in elite endurance athletes [25]. Similarly, it is known that acute exercise by itself increases oxidative stress [30].

We recently demonstrated that 18 days of LHTL decreases the plasma antioxidant values (measured by lipid soluble antioxidant and by the ferric reducing antioxidant power) of elite runners when it was measured the day after the training [24]. Thus, the association of hypoxic exposure and exercise in normoxia during such altitude training, likely overwhelms the antioxidant defenses (i.e., enzymatic and non-enzymatic antioxidants) and might subsequently depress the antioxidant status, especially if the athletes are submitted to this regimen for a sufficient period of time. Indeed, a 13-day LHTL was not sufficient to affect the antioxidant/prooxidant balance in elite swimmers [22]. It is noteworthy that the drop in antioxidant status observed for the runners immediately after the 18 days of LHTL did not impair the improvement of performance [5]. The low antioxidant intake observed in athletes involved in endurance training [27] as well in those undertaking altitude training [24] may be insufficient to compensate for the antioxidant deficit during the LHTL camp and therefore, could be detrimental in the long-term performance. Indeed, an antioxidant nutrient imbalance could result in an increase susceptibility of exercise-induced damage by ROS and thus lead to decrease in performance as it was shown in human with impaired vitamin C status [8]. In our preceding studies [22, 24] the antioxidant status was not followed-up after a period recovery. To our knowledge, little is known regarding the restoration of the antioxidant/prooxidant balance after intense training camp and after LHTL solicitation.

Thus, we investigated the impact of 18 days of LHTL and 14 days of recovery, as previously described [26], on the antioxidant status in elite cross-country skiers.

We tested here, the hypothesis that the antioxidant status is impaired by 18 days LHTL in elite athletes and remained altered after 14 days of recovery. To study the underlying mechanisms of these two stimuli, we measured (1) the oxidative stress by analyzing lipids and protein oxidation (malondialdehydes [MDA] and advanced oxidation protein products [AOPP]), and (2) the antioxidant status using two global antioxidant markers (ferric reducing antioxidant power [FRAP] and trolox Equivalent antioxidant capacity [TEAC]), as well as α-tocopherol, β-carotene and lycopene, before and after the 18 days of LHTL and after 14 days of recovery.

Materials and methods

Subjects

The study was approved by the Necker Hospital Ethics Committee (Paris, France). All subjects who gave written informed consent were low-altitude residents and were not acclimatized to altitude prior to the study. Eleven elite cross-country skiers were recruited from the national teams of the French Skiing Federation. The subjects were divided into two groups matched according to their maximal oxygen uptake ($\dot{V}O_{2max}$): a group submitted to LHTL (HG, $n = 6$ with 3 males and 3 females) and a control group (CG, $n = 5$ with 2 males and 3 females) submitted to a "living low-training low" model, i.e., same training as HG in normoxia but without exposure to hypoxia during resting periods. All subjects were involved in international cross-country ski competitions during the year of the study.

Experimental design

The subjects were submitted to an 18-day period of supervised aerobic training with (HG) or without (CG) hypoxia exposure in the "Centre National de ski Nordique" (altitude = 1,200 m; Prémanon, Jura, France) during the aerobic preparation period of skiers, prior to the competitive season.

The HG group trained 2 h per day (supervised by the national coaches) at the altitude of Prémanon while, they spent, during resting and sleeping periods, 11 h per day in hypoxia at a simulated altitude of 2,500 m for 6 nights, 3,000 m for the 6 subsequent nights and 3,500 m for the 6 final nights. CG lived and followed the same training program as HG skiers in normoxia at the altitude of Prémanon. More details can be found elsewhere [26].

Normobaric poikilocapnic hypoxia in the rooms was obtained using an oxygen extraction system (OBS, Husøysund, Norway). For safety reasons, nocturnal arterial oxygen saturation for each athlete was continuously monitored by finger pulse oximetry (NPB-290, Nellcor Puritan Bennett, Pleasanton, CA, USA).

Resting blood tests were performed before [pre-training period (PRE)] and then repeated the first day [post-training period (POST1)] and again 2 weeks (POST 14) after the training period cessation.

Measurements

Intake

The intake of vitamins C, E and A (retinol + β-carotene), daily energy intakes and percentage of energy intake of carbohydrates, lipids and proteins were determined from the dietary recording using GENI software (MICRO-6, Villers-lès-Nancy, France) with French (REGAL) and German (SOUCI) tables for food composition. The diet was evaluated during the training and recovery periods by the estimation of food and beverage intake. The subjects were asked to record food intake as precisely as possible in a notebook. All these data were validated using a specific picture book to estimate quantities [14].

The subjects did not take antioxidant supplementation during the training and recovery periods.

Biochemical analyses

Blood was collected from the antecubital vein in EDTA tubes while the subjects were seated. Plasma was obtained by centrifugation of the samples at 1,000*g* for 10 min at 4 °C. Plasma was separated into aliquots and frozen at −80 °C for biochemical analyses.

Oxidized lipids measured by malondialdehydes (MDA), advanced oxidation protein products (AOPP), ferric reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity, lipid-soluble antioxidants (α-tocopherol, β-carotene and lycopene), retinol, triacylglycerols (TG) and total cholesterol (CH) were measured in the plasma in PRE, POST1 and POST14.

Oxidative stress is a well-established mechanism of ROS-induced cellular injury. MDA, resulting from polyunsaturated fatty acids oxidation is commonly used as an indicator of lipid peroxidation [15]. Indeed, the phospholipids of cell membranes contain a large amount of polyunsaturated fatty acids which are highly susceptible to oxidative stress. Despite some relevant methodological limitations [15], MDA is correlated with severity of respiratory disease inducing intermittent hypoxia [12]. Similarly, as protein is known to be sensitive to ROS, AOPP, a by-product of protein oxidation, is a reliable marker for oxidative stress in many inflammation processes [13, 32] as hypoxia exposure [21].

All reagents were purchased from Sigma Chemical Co. (Sigma–Aldrich, St Quentin Fallavier, France).

Concentrations of plasma MDA were determined as thiobarbituric reactive substances as previously described [23]. The pink chromogen was extracted with *n*-butanol (4 ml), and its absorbance was measured at 532 nm by spectrophotometry (Perkin Elmer Cetus, Norwalk, CT, USA). MDA concentration was calculated using 1,1,3,3-tetraethoxypropane as standard. The intra-assay coefficient of variation is 3.4%.

The AOPP were determined in the plasma using the semi-automated method described by Witko-Sarsat et al. [32]. Briefly, AOPP were measured by spectrophotometry on a microplate reader (Benchmark, Bio-Rad laboratories, Hercules, CA, USA) using Microplate Manager Software (Bio-Rad laboratories, Hercules, CA, USA) and were calibrated with chloramine-T solutions that, in the presence of potassium iodide, absorb at 340 nm. In test wells, 200 µl of plasma diluted in phosphate buffered saline (PBS) (1:4, v/v) mixed with 20 µl of acetic acid was placed on a 96-well microliter plate (Nunc, Roskilde, Denmark). The intra-assay coefficient of variation is 2.1%.

Plasma FRAP concentrations were assessed according to the manual Benzie and Strain method [3] and measured by spectrophotometry (Uvikon) at a controlled temperature (37 °C). FRAP concentration was calculated using an aqueous solution of known Fe^{2+} concentration ($FeSO_4$, $7H_2O_2$) as standard at a wavelength of 593 nm. The intra-assay coefficient of variation is 2.7%.

Plasma TEAC was measured at 600 nm using a commercially available kit (Total Antioxidant Status, Randox Laboratories Ltd., San Francisco, CA, USA). The intra-assay coefficient of variation is 1.5%.

α-Tocopherol, retinol β-carotene and lycopene were analyzed by reversed-phase HPLC on a Waters (Milford, MA, USA) apparatus equipped with a 600 pump, a 710 automatic injector, and a 996 diode-array detector and controlled by MILLENNIUM 2.1 (Millipore Waters Chromatography, Millipore, St Quentin, France) [18]. Tocopherol acetate and echinenone (Sigma Chemical Co., St. Louis, MO, USA) were added to the samples as internal standards (for α-tocopherol and, retinol, β-carotene and lycopene, respectively). The intra-assay coefficients of variation for α-tocopherol and, retinol, β-carotene and lycopene are 1.1, 1.3, 1.0 and 0.9%, respectively.

Triacylglycerols (TG) and cholesterol (CH) were assayed in plasma by using enzymatic colorimetric methods with commercial kits (Biomerieux, Craponne, France). The concentrations were measured spectrophotometrically (Hitachi U 2001; Hitachi Instruments, Inc, San Jose, CA,

USA) at 505 and 500 nm for triacylglycerols and cholesterol, respectively. The intra-assay coefficient of variation is 4.0%.

Statistics

Statistical analyses were performed using Statview software (5.0, SAS Institute Inc©, USA). The values are expressed as means (±SD) or percentage. A 2-way repeated-measure ANOVA was used (two groups: CG and HG, three times: PRE, POST1 and POST14) followed by post hoc Tukey test. A value of $P < 0.05$ was considered significant.

Results

Subjects characteristics

Age, height, body mass and $\dot{V}O_{2max}$ were 21 ± 2 years, 174 ± 5 cm, 64 ± 6 kg and 58.6 ± 8.7 ml min^{-1} kg^{-1} for CG; and 23 ± 4 years, 175 ± 6 cm, 67 ± 9 kg and 59.7 ± 6.3 ml min^{-1} kg^{-1} for HG, respectively. Training data and complete results regarding performance have been published elsewhere [26].

Briefly, the mean training profile in the two groups of skiers was divided as follow: 87% of training at or below the onset of blood lactate accumulation (OBLA) (intensity I and II), 8% of training above OBLA (mostly intensity III and IV) and 5% of speed/weight-lifting exercises.

$\dot{V}O_{2max}$ remained unchanged between PRE and POST measurements for both CG (58.6 ± 8.7 vs. 57.1 ± 6.8 ml min^{-1} kg^{-1}) and HG (61.7 ± 4.4 vs. 59.3 ± 2.0 ml min^{-1} kg^{-1}).

Mean nocturnal SaO_2 for a given altitude significantly decreased from 93.6 ± 0.5% at 2,500 m to 91.7 ± 1.1% at 3,000 m ($P < 0.05$) and to 89.8 ± 0.5% at 3,500 m ($P < 0.05$) [4].

Plasma biochemical values

Values regarding MDA, AOPP, FRAP, TEAC, and lipids-soluble antioxidants and retinol are detailed in Table 1 for CG and in Table 2 for HG.

Oxidative stress

In HG, AOPP increased in POST1 (+130%, $P < 0.01$). AOPP did not significantly change in CG. MDA did not change regardless the groups and periods.

Global antioxidant markers

FRAP was decreased significantly in POST1 regardless of the group (−17%, $P < 0.05$ for CG and −20%, $P < 0.01$ for HG). In POST14, FRAP came back to PRE values for CG, whereas it remained lower for HG (−12%, $P = 0.05$).

Similar to FRAP, TEAC was significantly decreased in POST1 regardless of the group (−13%, $P < 0.01$ for CG and −40%, $P < 0.01$ for HG). In POST14, TEAC returned

Table 1 Advanced oxidation protein products (AOPP), malondialdehydes (MDA), ferric reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC), selected lipid-soluble non-enzymatic antioxidants, retinol, triacylglycerols (TG) and cholesterol (CH) assessed at rest before (PRE), immediately after (POST1) and 14 days after (POST14) a "living low-training low" (control group) stimulus

	PRE		POST1		POST14	
	Mean	SD	Mean	SD	Mean	SD
MDA (μmol/L)	1.23	0.19	1.27	0.09	1.33	0.10
AOPP (μmol/L)	124.3	44.9	144.0	43.9	52.8	13.5
FRAP (μmol/L)[a]	852	25	708*	189	795	114
TEAC (mmol/L)[a]	1.07	0.09	0.97**	0.09	1.06	0.07
CH (mmol/L)	4.04	0.60	4.31	0.58	3.86	1.43
TG (mmol/L)	0.78	0.37	0.54	0.11	1.15*	1.11
α-Tocopherol (μmol/L)[a]	23.9	3.7	26.5	2.9	20.3	4.0
α-Tocopherol (μmol/mmol of TG and CH)	4.96	1.71	5.46	1.49	4.07	1.98
Retinol (μmol/L)[a]	0.56	0.06	0.66	0.14	0.65	0.15
Lycopene (μmol/L)	0.39	0.09	0.33	0.11	0.37	0.09
β–Carotene (μmol/L)	0.11	0.05	0.10	0.05	0.15	0.09

Values are mean ± SD for 5 subjects

* $P < 0.05$; ** $P < 0.01$ vs. PRE

[a] Reference values for healthy adults: FRAP, 673 μmol/L; TEAC: 0.88 mmol/L; α-tocopherol: 30 μmol/L [1]; retinol: 1.5 μmol/L [1]

Table 2 Advanced oxidation protein products (AOPP), malondialdehydes (MDA), ferric reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC), selected lipid-soluble non-enzymatic antioxidants, retinol, triacylglycerols (TG) and cholesterol (CH) assessed at rest before (PRE), immediately after (POST1) and 14 days after (POST14) a "living high-training low" (hypoxic group) stimulus

	PRE		POST1		POST14	
	Mean	SD	Mean	SD	Mean	SD
MDA (μmol/L)	1.18	0.15	1.26	0.19	1.22	0.19
AOPP (μmol/L)	71.5	8.4	164.2*	24.8	81.1	15.1
FRAP (μmol/L)[a]	1,047	217	837**	157	920*	121
TEAC (mmol/L)[a]	1.14	0.15	0.93**	0.22	1.09	0.09
CH (mmol/L)	4.58	0.59	4.83	1.27	4.89	1.24
TG (mmol/L)	0.69	0.30	0.84	0.39	1.17	0.44*
α-Tocopherol (μmol/L)[a]	27.2	3.5	26.3	6.3	25.6	8.7
α-Tocopherol (μmol/mmol of TG and CH)	5.16	0.98	4.63	1.67	4.22	1.81
Retinol (μmol/L)[a]	0.81	0.12	0.61	0.11	0.73	0.24
Lycopene (μmol/L)	0.45	0.10	0.32*	0.13	0.29*	0.11
β-Carotene (μmol/L)	0.12	0.03	0.09*	0.04	0.08*	0.03

Values are means ± SD for 6 subjects

* $P < 0.05$; ** $P < 0.01$ vs. PRE

[a] Reference values for healthy adults: FRAP, 673 μmol/L [9]; TEAC: 0.88 mmol/L [9]; α-tocopherol 30 μmol/L [1]; retinol: 1.5 μmol/L [1]

to PRE values for both groups despite a trend toward lower values for HG (−7%, $P = 0.10$).

Throughout the study (11 subjects × 3 time points), FRAP and TEAC were strongly positively correlated ($r = 0.63$, $P < 0.001$). Additionally, MDA was negatively correlated with both global antioxidant markers ($r = -0.48$, $P < 0.05$ with FRAP and $r = -0.45$, $P < 0.05$ with TEAC).

Lipid-soluble antioxidants and retinol

Regardless of the group (CG and HG), α-tocopherol expressed over blood lipids content (i.e., TG and CH) remained unchanged throughout the study.

HG exhibited significantly reduced β-carotene in POST1 and POST14 (−25%, $P < 0.05$ and −33%, $P < 0.05$, respectively), whereas CG values were not affected.

As for β-carotene, only HG had decreased lycopene in POST1 and POST14 (−29%, $P < 0.05$ in POST1 and −36%, $P < 0.05$ in POST14). Between PRE and POST1 and between PRE and POST14, the lycopene decrease was significantly stronger in HG than for CG (−29 vs. −9%, $P < 0.05$ and −36 vs. +35%, $P < 0.01$).

Retinol concentrations were not modified regardless the groups and periods.

Effect of sex

The decrease in FRAP after training (PRE minus POST1) was lower for women compared to men (−154 vs. −213 μmol/L, $P < 0.05$). After 14 days of recovery (PRE − POST14), the changes in FRAP (−59 vs. −141 μmol/L, $P < 0.05$) and TEAC (−0.96 vs. +0.01 mmol/L, $P < 0.01$) were lower for women compared to men. On the contrary, there was not significant sex-effect (i.e., men vs. women) regarding the changes in MDA, AOPP and lipids soluble antioxidant either after training (PRE − POST1) nor after recovery (PRE − POST2).

Vitamins A, C and E intakes

The daily vitamin and energy intakes are presented in Table 3. During the training period, the daily intake of vitamin E represented 53 and 85% of the sedentary recommended daily allowance (RDA) for HG and CG, respectively. During the recovery period, the daily intakes represented 73 and 71% of the sedentary RDA for HG and CG, respectively. During the training, HG intakes of vitamin E were significantly lower ($P < 0.05$) than those of CG. Regardless of the time (i.e., training or recovery) and group (i.e., HG or CG), vitamin A and C intakes, as by the sum of retinol and β-carotene was close to the RDA for sedentary person.

Discussion

We previously reported that 18 days of LHTL in elite runners weakened the plasma antioxidant status [24], whereas the same parameters were not affected when the same training was performed without hypoxic exposures. The findings of the present study confirm that LHTL

Table 3 Daily vitamins A, E and C intakes and percentage of lipids, carbohydrates and proteins in the energy contribution of hypoxic (HG, $n = 6$) and control (CG, $n = 5$) groups

	Training period				Recovery period				RDA	CNI
	HG		CG		HG		CG			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Vitamin A (RE/day)	689	104	796	184	619	100	719	79	600	200
Vitamin E (mg/day)	6.4	0.6	10.2*	2.3	8.7	1.0	8.5	0.9	12	12
Vitamin C (mg/day)	108	10	110	7	111	13	109	6	110	100
Daily energy intake (kJ\day)	12,737	1,024	12,680	1,003	12,067	985	11,985	873	–	–
Lipids (%)	23	2	28*	4	26	2	27	3	30	–
Carbohydrates (%)	58	3	54	3	54	6	56	4	50	–
Proteins (%)	19	2	18	2	20	3	17	2	20	–

Values are mean ± SD. RDA is calculated on the basis of a daily energetic expenditure of 9,196 kJ/day. CNI have to be added to RDA for each 4,800 kJ/day spent above 9,196 kJ/day

RE retinol equivalent, *RDA* recommended daily allowances and *CNI* complementary nutritional intakes [1]

HG vs. CG: * $P < 0.05$

impairs plasma antioxidant status in elite endurance athletes as illustrated by the decreases in FRAP, TEAC, lycopene and β-carotene. However, this is the first study to show that the antioxidant status was not fully restored within 2 weeks after such LHTL training. Indeed, although both groups (i.e., HG and CG) decreased their antioxidant status in POST1, the values of CG returned to baseline levels after 14 days of normoxic recovery, whereas those of HG remained lower, suggesting that LHTL affects the mid-term recovery of the antioxidant status.

Impairment of antioxidant status has been well described in the literature following a variety of stimuli among which overtraining periods [20] and various hypoxic training models [24]. Training at moderate altitude in cross-country skiers has also been shown to alter antioxidant status [29]. However, no information is available as to the long-term recovery of the athlete's antioxidant capacity after such training. Indeed, although the improvement of performance as previously described [5] do not seem to suffer from the plasma antioxidant drop observed immediately after LHTL [24], a chronic deficiency in antioxidant could alter the long-term performance [28]. We should also note that the performance was not augmented after LHTL in the present study. Here, we provide evidence that antioxidant parameters did not return to baseline values 2 weeks after 18 days of LHTL, as evidenced by the persistently decreased plasma FRAP and carotenoids in POST14, contrary to what happened following the same training program but conducted with resting periods in normoxia (for CG). In this context, Rousseau et al. [27] reported that the plasma concentration in carotenoids was negatively associated with oxidative stress markers in competitive athletes.

The decrease of the antioxidant parameters during the 18 days of LHTL could be explained by antioxidant intakes lower than the RDA. Indeed, considering that the daily energy expenditure should be greater than the 2,200 kcal from which RDA is calculated, the intake in selected lipid-soluble antioxidant like vitamins A (sum of retinol and β-carotene), E and C for our subjects appear to be insufficient (Table 3) [1]. Although no correlation was found between vitamin intake and neither plasma antioxidant nor oxidative stress markers, the persistent low values of the antioxidant status after the 2 weeks of normoxic recovery point out the deficiency of antioxidant intakes, especially in carotenoids, in the diet of the HG subjects.

While the low intakes in vitamin A are associated with low plasma carotenoids in HG, low intakes in vitamin E are not reflected on the plasma α-tocopherol. Such observation has previously been reported in athletes [27]. These authors suggested that the recommended vitamin E intake for athletes could be overestimated. We can also assume that α-tocopherol body content is more preserved that other antioxidants because its oxidized form is quickly reduced by acid ascorbic. At all events, this decrease in lipid-soluble antioxidants may partially be explained by the relatively low intake in lipids (23 and 27% of the daily energy expenditure [DEE] for HG and CG, respectively) in comparison with the RDA (30% of DEE). It is noteworthy that a low-fat diet is common in endurance athletes [6]. The lower vitamin E intake in HG during the training period is one limitation to the present study. However, since no difference in plasma α-tocopherol was observed between HG and CG, it is unlikely that the differences of plasma antioxidant status within the groups might be explained by this lower vitamin E intake.

Additionally, the AOPP levels measured at rest increased only in the HG confirming that the additional hypoxic exposure is the key factor of imbalance of the prooxidant/antioxidant equilibrium and the subsequent low antioxidant status even 14 days after the training camp. However, this is not confirmed by the second oxidative stress marker, MDA, despite a trend toward higher values in POST1 for the HG. This could be explained by the MDA measurement we used (TBA assay) which is now considered not to be as sensitive as other lipid oxidation markers and not the most specific to oxidative stress [19]. Nevertheless, it has to be noted that MDA is negatively correlated with FRAP ($r = -0.48$) and TEAC ($r = -0.45$) when all the measurements of the study are pooled (11 subjects $\times$ 3 measurement points).

In a previous study we reported a larger oxidative stress increase immediately after 18 days of LHTL elite in runners [24] (i.e., +62% for MDA and +104% for AOPP, respectively) despite exposure to lower altitude. The duration of hypoxic exposure, the intensity of the training and the specificity of each population and activity may explain these different responses to LHTL. First, the runners spent almost 50% more time in hypoxia (16 vs. 11 h per day) [4]. Second, during LHTL the runners spent 46% of their training time above 75% of the maximal intensity [4] versus 27% for the cross country skiers in the present study [26]. Third, the women represent more than half of the athletes on the present study when compared to men, they exhibited a fewer decrease in FRAP immediately after training and the better recovery in FRAP and TEAC in POST14. Fourth, although all the skiers were low-altitude residents, they may be somewhat preconditioned to hypoxia. In fact, they are used to spend times training in altitude during their summer and fall training when they ski on glacier or in moderate altitude station.

Finally, our results regarding the antioxidant capacity are strengthened by the positive correlation between our two antioxidant markers (i.e., FRAP and TEAC; $r = 0.63$; $n = 33$). According to Cao and Prior [7], the correlation between these two markers has never been reported yet. Beyond the methodological considerations, this result points out the marked decrease of the plasma antioxidant capacity of our cross-country skiers in POST1.

In conclusion, the 18 days of LHTL magnified the antioxidant status decrease observed over the normoxic training camp. Furthermore, plasma antioxidant power and carotenoids were not fully restored after 14 days of recovery. In this context, the low intakes in lipid soluble antioxidant observed in elite endurance athletes may be insufficient with respect to the antioxidant needs to recover such LHTL solicitation. Taken together these results suggest that these athletes need to optimize their nutritional intakes during these kinds of training camps. In this context, further studies should determine whether antioxidant supplementation should be prescribed to athletes planning repeated and long LHTL camps during their training season.

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Conflict of interest statement The authors declare no competing financial interests.

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