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Research article

Cell damage, antioxidant status, and cortisol levels related to nutrition in ski mountaineering during a two-day race

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Abstract
The aim of this study was to measure the effect of nutrition on cell damage, antioxidant enzymes, and cortisol during a two-day ski mountaineering competition. Twenty-one male skiers participated in the study. Creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (AP), cortisol and C-reactive protein (CRP), glutathione peroxidase (GPx) and reductase activities (GR) and C-reactive protein (CRP) levels, total antioxidant status, and cortisol levels were measured in serum the day before and immediately after the race. Their diet was also analysed during the competition. Enzymes and cortisol levels significantly increased after the competition. CK and LDH and cortisol levels were negatively correlated to total energy, protein, and fat intake. Intake of vitamin A, B_1, B_2, B_6 and niacin was negatively correlated to LDH and AP. A negative correlation was also found between CK activity and Na, Fe, and Zn intake. Cortisol levels were negatively correlated to the intake of vitamins C, B_1 and B_2, and niacin. A positive correlation was found between serum GPx and intake of energy, carbohydrates, proteins, A and B vitamins, and folic acid. Skiers with the lowest nutrient intake during the competition were the ones who showed greater cell damage and lower antioxidant enzyme activity and cortisol levels, which may impair performance and also cause injuries and accidents. Particularly, skiers should have high intakes of total energy, macronutrients, vitamins A and B, Na, Zn, and Fe in order to decrease the deleterious effect of strenuous exercise.

Key words: Energy intake, micronutrients, muscle damage, antioxidant enzymes, skiing.

Introduction

Strenuous exercise, particularly eccentric exercise (in which muscles contract and lengthen), causes muscle cell damage, the so-called exercise-induced muscle damage (EIMD). Serum creatine kinase (CK) and lactate dehydrogenase (LDH) activities have been used as markers of muscle cell damage because they are released into blood when a disruption occurs in the sarcomere. High blood levels of these enzymes have been observed in many sports. The highest levels have been found in marathon (Kobayashi et al., 2005), ultramarathon (Fallon et al., 1999), ironman (Neubauer et al., 2008), and cross-country skiing (Ronsen et al., 2004).

Although exercise-induced muscle damage is necessary for training progress, excess damage may impair performance. Thus, the damaging effect of eccentric muscle contractions, though necessary for adaptive remodelling, can effect subsequent exercise sessions due to residual muscle pain, restriction of movement, and a reduced capacity to exercise at a beneficial intensity. In fact, muscle-damaging exercise may lead to an impaired muscle function, mainly occurring as a loss of torque and range of motion (Paschalis et al., 2007; Radák et al., 1999).

Cells are continuously producing reactive oxygen species (ROS) due to their metabolic processes (Urso and Clarkson, 2003). ROS may damage DNA, cytosolic molecules, and cell membranes (lipoperoxidation), causing cell damage and also death. Strenuous exercise has been shown to be able to accelerate ROS production (Nikolaidis et al., 2008). The effects of ROS are opposed by a complex of antioxidant molecules. These antioxidants may be enzymatic (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase) and non-enzymatic [vitamins A, E, and C, glutathione, ubiquinone, flavonoids, trace elements (selenium, copper, zinc, manganese), uric acid, albumin, ferritin, bilirubin].

The term "oxidative stress" refers to an imbalance between ROS production and the antioxidant defence. Oxidative stress may therefore result from an increased production of ROS (e.g. after strenuous exercise) and/or decreased levels of antioxidants (Nikolaidis et al., 2008). Oxidative stress is associated with muscle fatigue during contraction and with muscle damage and suffering after exercise (Finaud et al., 2006).

Thus, both muscle damage and oxidative stress may impair muscle function, which is particularly important in sports performance, and crucial in high risk disciplines, like skiing, where functional muscle impairment may cause injuries and even accidents that may be fatal.

The effect of nutrition on muscle damage (Bloomer, 2007; Howatson and Van Sormeren, 2008) and oxidative stress (Finaud et al., 2006) has been discussed in extensive reviews. Most studies have measured the relationship between intake of nutritional supplements such as vitamin C, vitamin E, vitamin A, ß-hydroxy-ß-methylbutyrate, coenzyme Q_{10}, n-acetylcysteine, and a combination of antioxidants. Because of the differences in dose, supplementation period, and exercise interventions, conclusions regarding this subject are difficult to make. However, studies suggest that long-term supplementation with vitamin C and/or E may decrease the signs and symptoms of EIMD through ROS reduction following

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muscle damage (Bloomer, 2007; Howatson and Van Sormeren, 2008). By contrast, Urso and Clarkson (2003) stated that a wise recommendation for athletes would be to take a diet rich in antioxidants rather than supplements.

There are various research papers on the physiology and biochemistry of cross-country skiing (Ronsen et al., 2004), less for alpine skiing (Seifert et al., 2005), and only a few for ski mountaineering (Tosi et al., 2009). Ski mountaineering is a sport that combines the skiing and mountaineering techniques. While using skiing techniques for much of the time, ski mountaineers climb otherwise inaccessible or dangerous slopes on foot using a range of mountaineering equipment. To move uphill skiers may put climbing skins on the base of their skies, which are removed one they get to the top.

It is a well recognized sport practised in many mountainous countries, particularly in those with a long skiing tradition. National and World championships have been held in the past years. Combined ski mountaineering and shooting was an official event of the 1924 Winter Olympics, followed by demonstration events in 1928, 1936 and 1948. Efforts are made to make ski mountaineering by itself part of the 2018 Olympic Games.

Thus, the aim of this study was to measure the muscle cell damage and changes in antioxidant status caused by a two-day ski mountaineering competition and to ascertain its relationship with nutrition, since little research has been done on the relationship between daily diet and these parameters; even more, there is no information about food and drink intake during a competition and muscle cell damage and oxidative stress in this particular sport.

Methods

This study was conducted in the ski mountaineering official race. The race started at 1847 m of altitude. In this two-day competition, skiers covered 12 km with a drop of 1350 m on the first day, and 7 km with a drop 850 m on the second day (see the race course in Figure 1). Temperature ranged from 0ºC and 10ºC, and humidity was around 65% and 75%. In this race, skiers competed in teams of two. Both skiers had to reach the finishing line together on both days of the competition, otherwise they would be penalised or disqualified.

Twenty-one male skiers (mean age 37.3 ± 7.0 years) participated in the study (physical characteristics are shown in Table 1). They all signed an Informed

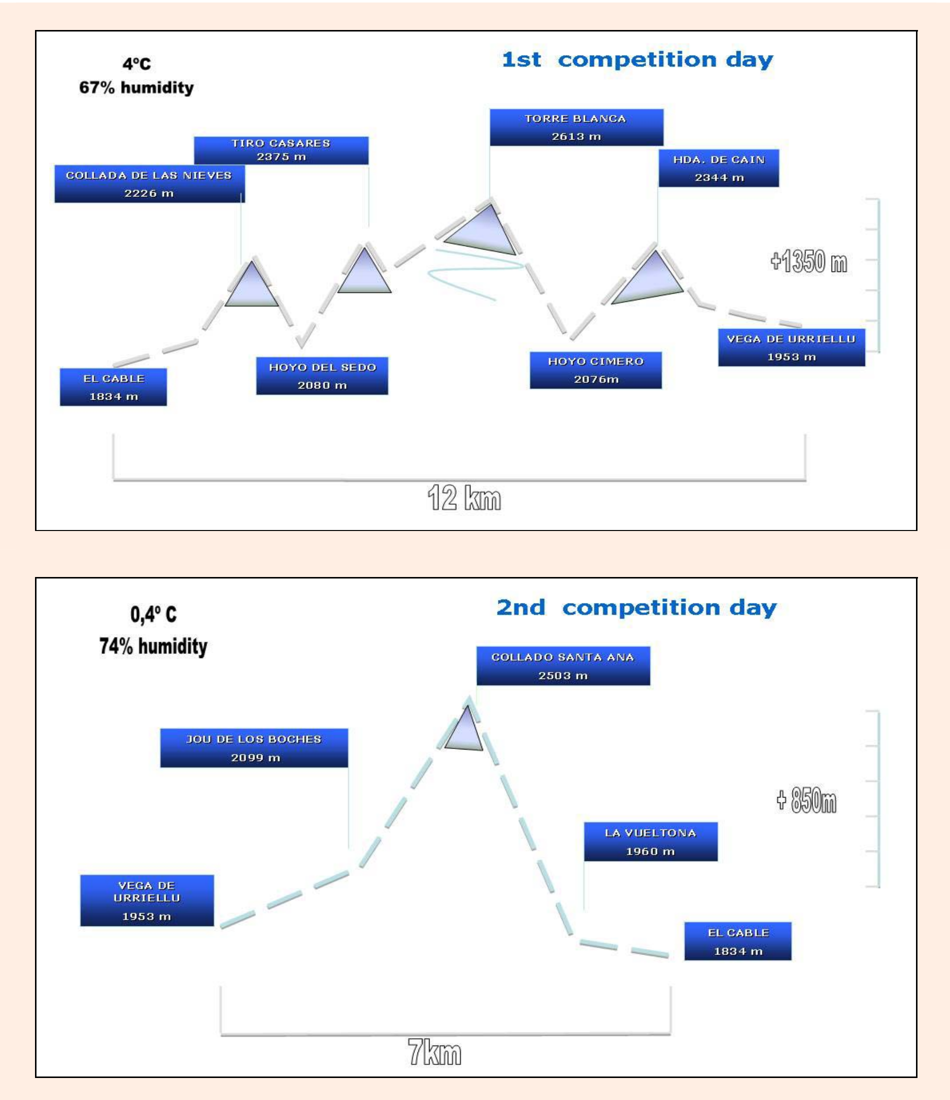

Figure 1. Race course.

Consent, and the Institutional Ethics Committee approved the study.

Table 1. Physical characteristics of the participants (n = 21). Values are means (±SD).

Age (years)	37.3 (7)
Weight (kg)	73.5 (8)
Height (m)	175 (9)
BMI (kg·m^{-2})	23.9 (2)
Endomorphy	2.8 (0.9)
Mesomorphy	4.5 (0.9)
Ectomorphy	2.1 (1.0)
Body Fat (%)	12.8% (2)
Body Muscle (%)	48.2% (4)
6 skinfolds (mm)	67.7 (20)

BMI = Body Mass Index. 6 skinfolds: Sum of skinfold thicknesses measured at six sites: triceps, subscapular, abdominal, suprailial, thigh, and lower leg.

All skiers underwent an anthropometric assessment including height, weight, body mass index (BMI), and measurement of skinfold thickness at six sites (triceps, subscapular, abdominal, suprailial, thigh, and lower leg) using a skinfold caliper (Harpenden, UK).

Circumferences of the upper arm, thigh, and lower leg were measured, as well as the following four diameters: biepicondylar humerus (elbow), bistyloid at the wrist, biepicondylar femur (knee), and bimalleolar in the ankle. All measurements were done by a level 3 ISAK anthropometrist following the guidelines outlined by the International Society for the Advancement of Kinanthropometry (ISAK). Weights and percentages of fat, bone, and muscle were subsequently calculated in order to evaluate body composition, as it has been published elsewhere (Gil et al., 2007). The endomorphic, mesomorphic, and ectomorphic components of the somatotype were also calculated (Heath and Carter, 1967).

Nutrient analysis

Food diaries from the competition days were used to assess the intake of skiers. In order to properly record the food taken, each subject was provided with a notebook to record the type and quantity of food eaten during the race. Detailed information was given to the skiers. Subjects were requested to record every single intake (meal and drink) during these days (the day before the competition and the two days of the race).

Dietary analysis was performed using Nutritionist III software (version 7.0, N-squared Computing, San Bruno, CA), which evaluates micronutrients, macronutrients, and minerals. These evaluations were analysed by the same trained person. This analysis provided a detailed information about the intake of calories, proteins, carbohydrates (total and simple), fats (saturated, monounsaturated, and polyunsaturated), vitamins, and minerals. The selected software calculates the absolute measure of the amount of each nutrient (in grams, milligrams, or micrograms) and the corresponding percentages.

Blood analysis

The day before and at the end of the competition blood samples were drawn from the antecubital vein with subjects in the seated position. Three ml of blood were collected into two vacutainer tubes containing EDTA for antioxidants enzyme determination, and 7 ml were placed in Vacutainer tubes containing gelose for biochemical analysis. For antioxidant enzyme analysis, blood samples were centrifuged and the serum was stored at -80ºC until analysis.

Creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (AP), cortisol and C-reactive protein (CRP) were measured using a CN-Cobas, Integra 400 Plus (Roche, Alemania/Japan). Hormones were measured using a Modular Analytics SWA (Roche, Germany/Japan) analyzer. These measurents were performed in the Laboratory of Haematology, Hospital de Basurto, Bilbao.

Enzymatic determinations, such as plasma levels of Total Antioxidant Status (TAS), Glutathione Reductase (GR) and Glutathione Peroxidase (GPx) were performed in the Laboratory of Cellular Physiology, Department of Physiology, Faculty of Medicine and Odontology (University of the Basque Country). TAS was determined using the NX2332 kit from Randox laboratories (Antrim, UK). GR was measured photometrically using the RS 2368 (Randox, Antrim, UK) kit and GPx with the Glutathione Peroxidase Assay kit (Cayman chemical, USA).

Performance

Total race time was measured to determine the performance of the skiers in the competition. As this was a two-day competition, total race time was made up of the sum of the time of the first day and that of the second day.

Statistical analysis

Data were statistically analysed using software SPSS® 13.0. A Student's *t* test was used to analyse pre- and post-competition differences. Pearson's R was also calculated to evaluate the correlation between the different parameters.

Results

Table 1 shows the age and physical characteristics of participants. Total energy intake of skiers was 3089 ± 606 kcal/day (Table 2). Of this energy, 44.5% came from carbohydrates, 36.5% from fat, and 19 % from protein.

Table 2. Quantities of protein, fat, and carbohydrate consumed by skiers and their percent contribution to the total energy intake. Values are means (±SD) .

	Per day	Per weight	Energy ratio
Energy (kcal·day^{-1})	3089 (606)	41.5 (10)	
Carbohydrate (g·day^{-1})	350 (91)	4.69 (1.4)	44.5%
Fat (g·day^{-1})	128 (30)	1.72 (0.5)	36.5 %
Protein (g·day^{-1})	111 (24)	1.49 (0.4)	19 %

Table 3. Mean daily vitamin and mineral intake and corresponding percentage of the Recommended Dietary Allowances (RDA[a]) or Adequate Intakes (AI[b]) of the studied skiers. Values are means (±SD).

	Per day	RDA[a] / AI[b]	RDA/AI Percentage
Vitamin A (μg·day^{-1})	1289 (284)	900[a]	144 %
Vitamin B_1 (mg)	1.95 (0.59)	1.2[a]	163 %
Vitamin B_2 (mg)	2.68 (0.67)	1.3[a]	206 %
Niacin (mg)	33.9 (8.87)	16[a]	212 %
Vitamin B6 (mg)	2.9 (0.84)	1.3[a]	222 %
Folate (μg)	309 (68.0)	400[a]	77 %
Vitamin B12 (μg)	17.3 (6.53)	2.4[a]	721 %
Vitamin C (mg)	138 (81.4)	90[a]	166 %
Vitamin D (IU)	3.28 (2.44)	5[b]	66 %
α-tocopherol (mg)	21.3 (6.43)	15[a]	141 %
Sodium (mg)	2351 (821)	1500[a]	157 %
Potassium (mg)	3742 (691)	4700[a]	80 %
Calcium (mg)	1077 (252)	1000[b]	108 %
Phosphorus (mg)	1596 (265)	700[a]	228 %
Magnesium (mg)	406 (72.8)	420[a]	90 %
Iron (mg)	23.4 (5.78)	8[a]	293 %
Zinc (mg)	10.7 (2.39)	11[a]	97 %
Iodine (μg)	48.9 (16.2)	150[b]	33 %

Vitamin B_1 = Thiamine, Riboflavin = Vitamin B_2

Skiers met many of the recommended vitamin and mineral requirements (Table 3). However, their intake was far below the RDA/AI for folate, vitamin D, potassium, magnesium, zinc, and particularly iodine.

As shown in Table 4, activities of enzymes related to cell damage, such as AST, LDH, and CK, significantly increased after the competition. C-reactive protein (CRP) and cortisol levels were also increased ($p < 0.01$).

In relation to antioxidant status, glutathione peroxidase activity significantly increased after the competition. Glutathione reductase activity slightly decreased (non significant), while TAS remained unchanged.

A negative correlation was seen between muscle cell damage (LDH and CK) and total energy, protein, and fat intake (Table 5). CK-MB activity was also negatively correlated to fat intake ($p < 0.05$). Besides, blood cortisol levels were higher in skiers with the lowest carbohydrate and protein intake and the total of calories ingested ($p < 0.01$). In terms of oxidative stress, a positive correlation was found between glutathione peroxidase activity in serum and total energy, carbohydrate, and protein intake.

When the effect of intake of the different vitamins and the biochemical parameters were examined (Table 6), a statistically significant negative correlation was found between alkaline phosphatase activity and vitamin B6 and niacin intake and also between LDH and the intake of different vitamins (A, B_1, B_2, B_6, and niacin). CRP levels were positively correlated to vitamin B_{12} intake. Blood cortisol levels were higher in participants with the lowest intakes of vitamins C, B_1, B_2, B_6, and niacin ($p < 0.01$).

A positive correlation was seen between glutathione peroxidase activity and the amount of vitamins A, B_1, B_6, B_{12} ($p < 0.05$), and niacin ($p < 0.01$) in diet.

Between the ingested amount of minerals and muscle damage (Table 7), a negative relationship was found between production of CK and CK-MB and sodium, iron, and zinc intake. Also, higher intakes of potassium, calcium, phosphorus, magnesium, iron, and zinc resulted in lower cortisol levels, but in higher levels of glutathione peroxidase.

Correlations between macronutrient intake and oxidative stress markers and performance (race time) are shown in Table 8. There was a statistically significant negative correlation between the ingestion of carbohydrates and the duration of the competition ($p < 0.05$). In contrast, oxidative stress markers and other blood parameters (not shown) were not correlated to performance time.

Table 4. Biochemical parameters related to oxidative stress and cell damage before and immediately after the race. Values are means (±SD). Reference values are also shown.

	Pre-race	Post-race	Reference values
AST (U·L^{-1})	25.7 (1.4)	43.0 (3.2) **	5.0-37
ALT (U·L^{-1})	21.3 (1.3)	23.9 (2.7)	5.0-41
GGT (U·L^{-1})	18.3 (1.1)	17.1 (1.4)	11.0-50.0
AP (U·L^{-1})	67.4 (3.1)	64.2 (3.1)	40.0-129
LDH (U·L^{-1})	305 (12)	402 (14) **	240-480
CK (U·L^{-1})	173 (20)	664 (74) **	0-195
CK-MB (U·L^{-1})	2.59 (.4)	23.4 (2.5) **	6.0-25
CRP (g·L^{-1})(10^{-3})	.10 (.02)	.65 (.04) **	0-0.5
Cortisol (μg·dL^{-1})	6.6 (.8)	16.8 (1.0) **	5.00-25.0
GPx (U·L^{-1})	68.13 (26.23)	117.70 (29.58) **	27.5-73.6
GR (U·L^{-1})	101.57 (36.48)	85.62 (19.50)	33-73
TAS (mol·L^{-1}) (10^{-3})	.26 (.03)	.29 (.06)	1.3-1.7

** $p < 0.01$. AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: γ-glutamyl transpeptidase, AP: alkaline phosphatase, LDH: lactate dehydrogenase, CK: creatine kinase (all subunits), CK-MB: creatine kinase, B subunit; CRP: C-reactive protein, GPx: glutathione peroxidase, GR: glutathione reductase, TAS: total antioxidant status.

Table 5. Correlations between biochemical parameters related to cell damage, cortisol, and oxidative stress at the end of the race and the intake of energy, carbohydrate, protein, and fat.

	Energy ($Kcal \cdot kg^{-1}$)	CHO ($g \cdot kg^{-1}$)	Protein ($g \cdot kg^{-1}$)	Fat ($g \cdot kg^{-1}$)
AST ($U \cdot L^{-1}$)	-.333	-.163	-.353	-.467
ALT ($U \cdot L^{-1}$)	-.291	-.180	-.286	-.455
GGT ($U \cdot L^{-1}$)	-.292	-.291	-.423	-.346
AP ($U \cdot L^{-1}$)	-.092	.019	-.111	-.085
LDH ($U \cdot L^{-1}$)	-.450	-.297	-.487*	-.504*
CK ($U \cdot L^{-1}$)	-.472*	-.363	-.528*	-.535*
CK-MB ($U \cdot L^{-1}$)	-.397	-.303	-.393	-.476*
CRP ($g \cdot L^{-1}$)(10^{-3})	.034	.174	.073	-.047
Cortisol ($\mu g \cdot dL^{-1}$)	-.387	-.460	-.372	-.343
GPx ($U \cdot L^{-1}$)	-.628**	-.691**	-.495*	-.432
GR ($U \cdot L^{-1}$)	.606*	.692**	.546*	.529
TAS ($mol \cdot L^{-1}$) (10^{-3})	-.323	-.283	.071	-.328

* $p < 0.05$, ** $p < 0.01$. CHO: carbohydrates, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: γ-glutamyl transpeptidase, AP: alkaline phosphatase, LDH: lactate dehydrogenase, CK: creatine kinase (all subunits), CK-MB: creatine kinase, B subunit; CRP: C-reactive protein, GPx: glutathione peroxidase, GR: glutathione reductase, TAS: total antioxidant status.

Discussion

The results of this study are relevant because it was conducted during a real competition where skiers had to carry their food in their backpacks, and food supplies were therefore limited. It should also be noted that the measurement of the intake of all nutrients is given per kg body weight of the skier instead of as the total amount ingested, thus providing a more accurate picture of their macro- but particularly micronutrient intake.

Exercise-induced muscle damage is a common occurrence following either unaccustomed exercise or an activity of great intensity or duration, and mainly occurs during activities involving eccentric muscle actions. It is hypothesised to be either mechanical or metabolic in nature (Tee et al., 2007). The extent of muscle cell damage can be estimated by measuring a variety of enzymes released by the disrupted cell into blood (Gleeson, 2002). This was a highly demanding competition where skiers, apart from facing hard environmental conditions, had to ski up and down the mountains. Thus, blood levels of some enzymes increased after the race (CK, LDH and AST), demonstrating that muscle damage had occurred in the skiers. CRP (an inflammation marker) and cortisol (indicative of stress and fatigue) levels and glutathione peroxidase (indicative of oxidative stress) activity also increased in blood serum after the competition, suggesting that stress had occurred. It is particularly interesting the fact that the food intake of the competition day had an effect on muscle damage and oxidative stress. Thus, a relevant finding in the study was that muscle cell damage was more marked in skiers with the lowest total energy, protein, and fat intake. Although the mechanism is unknown, some authors have found that addition of protein to a carbohydrate drink attenuates the elevation of CK activity in serum and increases performance (Cockburn et al., 2008; Rowlands et al., 2008).

To the researchers' knowledge, there have been no studies on the influence of dietary fat on muscle damage related to exercise. In the present study it was found that skiers with the lowest fat intake had greater cell damage. An increased fat intake by exercising skiers may perhaps protect them against rupture of the cell membrane, sparing structural lipids of the cell membrane.

Besides, no significant relationship was found between the activity of CK and other enzymes derived from cell rupture in serum and the amount of carbohydrate ingested. Although a high carbohydrate diet is required to increase muscle glycogen and therefore maintain performance, no evidence has been found that it attenuates muscle damage. In fact, previous studies found no differences in muscle soreness and CK activity with high/low-carbohydrate isocaloric diets (Close et al., 2005) or glycogen-depleted/repleted states (Nelson et al., 2004).

In relation to the impact of micronutrient intake on cell damage, it was observed that the higher the intake of vitamins A, B_1, B_2, B_6, and niacin the lower the activity of LDH and alkaline phosphatase. CK activity was also negatively correlated to the intake of sodium, iron, and particularly zinc. Zinc has a significant role in antioxidant cellular defence. For instance, it is a structural element of the superoxide dismutase enzyme, and therefore acts as a cell membrane stabilizer (Micheletti et al., 2001). It should be noted that Zn intake of skiers did not meet the requirements of the general population, and it may be

Table 6. Correlations between biochemical parameters related to cell damage, cortisol, and oxidative stress at the end of the race and mean daily vitamin intake per kg body weight of participants.

	Vit A	Vit B_1	Vit B_2	Vit B_6	Vit B_{12}	Vit C	Vit E	Niac	Folic	Vit D
AST ($U \cdot L^{-1}$)	-.317	-.336	-.294	-.270	-.186	-.214	-.278	-.354	-.121	.027
ALT ($U \cdot L^{-1}$)	-.203	-.230	-.211	-.114	.019	-.161	-.091	-.201	.052	.219
GGT ($U \cdot L^{-1}$)	-.193	-.367	-.330	-.245	.068	-.292	-.058	-.306	-.117	-.032
AP ($U \cdot L^{-1}$)	-.199	-.348	-.446	-.608**	-.273	.105	-.214	-.477*	-.186	-.287
LDH ($U \cdot L^{-1}$)	-.593**	-.552**	-.501**	-.562**	.096	.096	-.346	-.600**	-.380	-.356
CK ($U \cdot L^{-1}$)	-.219	-.344	-.166	-.140	-.235	-.272	-.249	-.285	-.288	-.086
CK-MB ($U \cdot L^{-1}$)	-.402	-.417	-.228	-.312	-.119	-.210	-.233	-.318	-.357	-.329
CK-MB (%)	-.359	-.196	-.027	-.268	.022	.155	-.038	-.029	-.281	-.672**
CRP ($g \cdot L^{-1}$)(10^{-3})	-.208	-.268	-.264	-.010	.531*	-.361	-.097	-.216	-.308	-.161
Cortisol ($\mu g \cdot dL^{-1}$)	-.190	-.630**	-.567 **	-.416	-.342	-.549*	-.317	-.519*	-.446	.151
GPx ($U \cdot L^{-1}$)	.602*	.628*	.329	.540*	.504	.305	.180	.361	.725**	.373
GR ($U \cdot L^{-1}$)	-.118	-.163	.044	.042	.375	.065	.153	.146	-.190	-.111
TAS ($mol \cdot L^{-1}$) (10^{-3})	-.024	.096	.253	.322	.069	-.044	-.044	.204	.191	.074

* $p < 0.05$, ** $p < 0.01$. AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: γ-glutamyl transpeptidase, AP: alkaline phosphatase, LDH: lactate dehydrogenase, CK: creatine kinase (all subunits), CK-MB: creatine kinase, B subunit; CRP: C-reactive protein, GPx: glutathione peroxidase, GR: glutathione reductase, TAS: total antioxidant status. Vit = vitamin, Niac= niacin, Folic = folic acid

Table 7. Correlations between biochemical parameters related to cell damage, cortisol, and oxidative stress at the end of the race and mean daily mineral intake per kg body weight of participants.

	Na	K	Ca	P	Mg	Fe	Zn	I/
AST ($U \cdot L^{-1}$)	-.408	-.276	-.116	-.290	-.271	-.336	-.454	-.112
ALT ($U \cdot L^{-1}$)	-.407	-.040	-.031	-.169	-.044	-.135	-.307	-.082
GGT ($U \cdot L^{-1}$)	-.397	-.124	-.241	-.242	-.146	-.115	-.352	-.208
AP ($U \cdot L^{-1}$)	-.009	-.315	-.007	-.071	-.315	-.095	-.238	.063
LDH ($U \cdot L^{-1}$)	-.411	-.447	-.163	-.377	-.263	-.463	-.456	-.253
CK ($U \cdot L^{-1}$)	-.520*	-.418	-.300	-.475	-.407	-.502*	-.612**	-.331
CK-MB ($U \cdot L^{-1}$)	-.476*	-.471*	-.274	-.409	-.393	-.404	-.496*	-.255
CK-MB (%)	.010	-.298	-.118	-.044	-.120	.027	.083	.026
CRP ($g \cdot L^{-1}$)(10^{-3})	-.250	-.257	-.347	-.335	-.220	-.295	-.384	-.413
Cortisol ($\mu g \cdot dL^{-1}$)	-.361	-.490*	-.508*	-.497*	-.595*	-.579*	-.479*	-.195
GPx ($U \cdot L^{-1}$)	.479	.751**	.711**	.778**	.778**	.701**	.561*	.277
GR ($U \cdot L^{-1}$)	-.512	-.130	-.173	-.284	-.084	-.66	-.98	-.283
TAS ($mol \cdot L^{-1}$) (10^{-3})	.111	.438	-.044	.266	.374	.469	.131	-.311

* $p < 0.05$, ** $p < 0.01$. AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: γ-glutamyl transpeptidase, AP: alkaline phosphatase, LDH: lactate dehydrogenase, CK: creatine kinase (all subunits), CK-MB: creatine kinase, B subunit; CRP: C-reactive protein, GPx: glutathione peroxidase, GR: glutathione reductase, TAS: total antioxidant status.

assumed that zinc requirements may be higher during strenuous exercise.

Table 8. Correlations between macronutrient intake (upper half) and oxidative estress markers (lower half), and performance (race time).

	Time	
Carbohydrate ($g \cdot day^{-1}$)	-.490*	
Fat ($g \cdot day^{-1}$)	-.247	
Protein ($g \cdot day^{-1}$)	-.397	
	Pre-race	**Post-race**
GPx	-.272	.171
GR	-.028	-.287
TAS	-.314	.334

* $p < 0.05$. GPx: glutathione peroxidase, GR: glutathione reductase, TAS: total antioxidant status.

Blood cortisol levels were higher in skiers with lower intakes of total energy, carbohydrates, protein, vitamin C, B vitamins, and minerals. Cortisol levels increase after exercise, and carbohydrate intake during strenuous exercise is known to decrease cortisol and catecholamine levels by maintaining glycaemia (Pedersen and Hoffman-Goetz, 2000). It is also known that vitamin C supplementation attenuates this exercise-induced increase in cortisol levels, possibly because of its effect on the adrenal gland or the hypothalamic-pituitary axis (Davison et al., 2007).

On the other hand, a link has been hypothesised between increased oxidative stress and plasma levels of stress hormones. McAnulty et al., in 2007 stated that elevated cortisol levels might deplete cellular glutathione, which is an important substrate in antioxidant defence.

In this group of skiers, antioxidant status, as measured by the GPx enzyme, demonstrated a higher activity related to higher intakes of energy, carbohydrates, protein, folic acid, and particularly minerals. Bloomer et al. (2008) found significant correlations between oxidative stress biomarkers (measured by serum malondialdehyde) and protein, carbohydrate, and vitamin C intake in trained subjects after an exercise protocol.

Ji et al. (1993) also found that glutathione peroxidase activity in blood was significantly increased with carbohydrate intake but not in the control trial during a cycling test. These authors concluded that CHO supplementation may prevent GSH increase in blood possibly because of its inhibitory effects on hepatic hormone releases, which stimulate GSH output. Thus, it appears that good nutrition practice may help mitigate the oxidative stress caused by an endurance competition.

Skiers had a mean energy intake of approximately 3000 $kcal \cdot day^{-1}$ (41.5 $kcal \cdot kg^{-1}$), which is adequate for an active male to cover daily activities, but not sufficient for this high demanding sport activity. The authors estimated that 4400 ± 392 $kcal \cdot day^{-1}$ were needed for this competition. Energy expenditure is also increased at a high altitude, which should also be taken into account (Wetertep, 2001). Moreover, as it has been recently stated, carrying an additional weight of 1 kg translates in a growth of 2-3% of the energy requirements in ski mountaineering (Tosi et al., 2009). Providing that skiers had to carry food and also clothing for the two-day race, their backpacks alone had a range of 6-8kg whose energetic cost should be added. Taking all this into account, it could be stated that energy needs were not adequately met in this group of skiers. These unmet needs may lead to fatigue and impaired performance, and contribute to an increased number of injuries. In addition, fatigue and tiredness may cause skiing accidents.

Carbohydrate content of the diet was 4.69 ± 1.40 $g \cdot kg^{-1}$, much lower than the recommendations for endurance, high-intensity sports (7-10 $g \cdot kg^{-1}$) (Joint Position Statement, 2000). A diet with low carbohydrate content impairs performance (Joint Position Statement, 2000). Its availability is also inversely related to the rate of exercise protein catabolism (Lemon, 2000). A large proportion of the skiing cycle in ski mountaineering is of an eccentric mode of contraction, and muscle glycogen resynthesis has been shown to be impaired following high-intensity eccentric exercise (Howatson and Van Sorensen, 2008). Restoring depleted glycogen stores is particularly important for an adequate performance during training sessions or competitions on consecutive days (Burke et al., 2006), such as the one discussed here. Therefore, it would be of great importance that skiers increased their carbohydrate intake in order to avoid protein catabolism, replenish muscle glycogen stores, and maintain exercise performance.

In fact, there was a negative correlation between the ingestion of carbohydrates and the race time, that is to say, the fastest skiers had the highest intake of carbohydrates. However, these latter results should be considered carefully for two reasons. Firstly, both skiers from each team had to finish the race together; this makes the individual performance of each skier difficult to assess. Besides, this could also be the reason for not observing correlations between performance and other biochemical parameters, muscle cell rupture and oxidative stress. Secondly, there was an accident on the second race-day; the time of this day was therefore neutralized, meaning that only the time of the first day was available. Despite this, the skiers of the present study finished both stages, and blood for analysis was taken at the end of the second day.

Conclusion

In this sport, apart from having good training background and technical skills, it is mandatory that competitors have an optimal macronutrient and micronutrient intake. Total calorie intake was too low in many skiers, which is particularly important because skiers with the lowest nutrient intake during the competition were the ones who showed greater cell damage and lower antioxidant enzyme activity and cortisol levels, which may impair performance and also cause injuries and accidents.

Perspective for the future

Although many recommendations have been issued in recent years on the importance of optimal nutrition for sports performance, athletes still fail to follow them. In the competition discussed, nutritional needs were particularly difficult to meet due to the fact that, apart from being a highly demanding two-day competition, skiers had to carry the food themselves. Therefore, not only the macronutrient and micronutrient intake, but also the total calorie intake was below the limits. This is very important because the researchers found that the lower the intake was the greater the cell damage, the higher the cortisol levels, and the lower the antioxidant enzyme activities. Further attempts should be made to optimise diet in this group of athletes in order to guarantee their fuel consumption to approach the energy needs.

It would also be interesting to investigate the impact of changes (improvements) in nutrition on the different parameters of muscle cell damage and stress. This could be made by optimising their diets and/or supplementation.

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Key points

- A two-day ski mountaineering race produced muscle cell damage and oxidative stress and an increase in cortisol levels.
- There was a marked insufficient intake of carbohydrates which has been shown to affect performance
- Those skiers with lowest nutrient intake showed greater cell damage, lower antioxidant activity and higher cortisol levels.
- Nutrition should be carefully monitored and assessed in order to minimize the mentioned blood changes to avoid fatigue, injuries and also accidents in this type of sport; particularly when skiers must carry their own food.

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