Antioxidant response to oxidative stress induced by exhaustive exercise

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Abstract

The aim of this work was to demonstrate the occurrence of oxidative stress during exhaustive exercise and to determine the antioxidant response. Eight voluntary male subjects participated in this study. The exercise was a cycling mountain stage (171 km) and the cyclists took a mean±S.E.M. time of 270±12 min to complete it. Blood samples were taken before the cycling stage, immediately after the stage, 3 h after finishing the stage and on the morning of the following day. We determined the activities of erythrocyte antioxidant enzymes, blood levels of oxidised glutathione, plasma levels of antioxidant vitamins and carotenoids, and the serum lipid and cholesterol profile. The mountain cycling stage induced significant increases in catalase and glutathione reductase activities. Significant decreases in glutathione peroxidase activity, both determined with hydrogen peroxide and with cumene hydroperoxide as substrates, were observed. Blood oxidised glutathione and serum uric acid rose after the stage. Plasma vitamin E increased after the stage but dropped to below basal values after 3 h of recovery. Triglycerides and VLDL-cholesterol increased significantly after the stage and remained high 3 h after the cycling stage. The mountain cycling stage induced oxidative stress, as was evidenced by the increases in blood GSSG and in serum urate concentrations and by the pattern of change of erythrocyte antioxidant enzyme activities. A specific utilisation of \textit{a}-tocopherol against oxidative stress during recovery was evidenced.

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1. Introduction

Exhaustive exercise is associated with accelerated generation of reactive oxygen species (ROS) that results in oxidative stress \cite{1,2}, which can induce adverse effects on health and well being. Even moderate exercise may increase ROS production exceeding the capacity of antioxidant defences \cite{3,4}. Specific sources of ROS during exercise include leakage of electrons from the mitochondrial electron transport chain, xanthine oxidase reaction, haemoglobin oxidation and activated neutrophils \cite{1,2}. The ROS has been shown to induce damage in all cellular macromolecules, such as lipids, proteins and DNA \cite{5}.

Erythrocytes are susceptible to oxidative damage as a result of the high polyunsaturated free fatty acid content of their membrane and the high cellular concentrations of oxygen and haemoglobin, a potentially powerful promoter of oxidative processes \cite{6}. Erythrocytes are exposed to ROS that are constantly generated from both internal and external sources even under normal conditions, and they may be targeted for oxidative damage during exercise. However, erythrocytes, as well as the whole body, contain an elaborate antioxidant defence system that includes antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase and non-enzymatic anti-
oxidants such as tocopherols, ascorbate, urate and glutathione (GSH).

Alpha-tocopherol acts to protect polyunsaturated fatty acids in biological membranes against lipid peroxidation [7]. The effects of vitamin E supplementation on athletic performance and endurance [8, 9] and lipid peroxidation [10, 11] have already been investigated. However, the exercise studies have varied in the intensity, duration and mode of activity chosen for the study model. Additionally, variations in the fitness levels of subjects and assays used to assess oxidative damage have contributed to the inconsistent findings.

If exercise increases oxidative stress, greater amounts of vitamin E should be oxidised and plasma α-tocopherol levels should be diminished. Moreover, it has been demonstrated that exhaustive exercise in healthy subjects results in blood GSH oxidation [12]. Hence, it could be appropriate to study the existence of changes in blood glutathione disulphide (GSSG) and also in α-tocopherol as well as other plasma antioxidant levels.

In order to demonstrate oxidative stress during the cycling stage, we determined GSSG, as a marker of radical induced oxidation of molecules, as well as non-enzymatic (α-tocopherol, carotenoids, urate) and enzymatic antioxidants (catalase, superoxide dismutase, glutathione peroxidase). Since oxidative stress induces alterations in cholesterol handling (13), we also determined the effects of the cycling stage on triglyceride and cholesterol serum profile.

2. Materials and methods

2.1. Subjects and exercise

Eight voluntary male subjects participated in this study. They were all professional cyclists participating in the “Setmana Catalana 2000”, a 5-day competition for professional cyclists held near Barcelona (Spain). Subjects were informed of the purpose of this study and the possible risks involved before giving their oral consent to participate. The study protocol was in accordance with the Declaration of Helsinki and was approved by the local ethics committee (Ethical Committee of ‘Hospital Son Dureta’).

The exercise was a mountain stage (171 km) of the “Setmana Catalana 2000”. This mountain cycling stage was the third stage of the “Setmana Catalana 2000”. The two prior stages were shorter, 120 and 150 km, and did not include mountainous terrain. The sportsmen’s mean (± S.E.M.) age was 23.8 ± 0.9 years, height 180 ± 2 cm and weight 70.0 ± 1.5 kg. The values were in the range of normal subjects with the same age. Their VO2 max was 80.2 ± 1.6 ml·kg⁻¹·min⁻¹, which was higher than that of normal subjects. The cyclists took a mean ± S.E.M. time of 270 ± 12 min to complete this stage.

2.2. Experimental procedure

Venous blood samples were taken from the antecubital vein with suitable vacutainers. The basal venous blood was obtained from the subjects participating in this study on the morning of the cycling stage day, after 12 h overnight fasted conditions, immediately after the stage and 3 h after finishing the stage. A blood sample was also obtained on the morning of the following day, once again after 12 h overnight fasted conditions, which represents approximately 15 h of recovery. We determined antioxidant enzyme activities in erythrocytes, blood levels of oxidised glutathione and plasma levels of carotenoids, retinol and α-tocopherol. We also determined uric acid and the lipid and cholesterol profile in serum. These determinations in serum were not carried out on the morning of the following day.

2.3. Plasma vitamin and carotenoid determination

Plasma was obtained after centrifugation at 1000 × g of the blood samples obtained as above and was stored at −80 °C until use. The deep-frozen plasma was thawed and mixed to disperse possible precipitates. The extraction of liposoluble vitamins and carotenoids was carried out using n-hexane after deproteinization with ethanol [14]. Liposoluble vitamins and carotenoids were determined by HPLC in the n-hexane extract of plasma after drying in a nitrogen current and redissolving in methanol. The mobile phase consisted of 550:370:80 acetonitrile/tetrahydrofuran/H2O. The HPLC was a Shimadzu with a diode array detector and the column was a Nova Pak, C18, 3.9 × 150 mm. Retinol and α-tocopherol were determined at 330 and 290 nm, respectively. Criptoxanthine, β-carotene and lycopene were determined at 460 nm; luteine/zeaxanthine was determined at 450 nm.

2.4. Enzymatic determinations

We determined the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in erythrocytes. All activities were determined with a Shimadzu UV-2100 spectrophotometer at 37 °C.

Catalase activity was measured by the spectrophotometric method of Aebi [15] based on the decomposition of H2O2.

Glutathione reductase activity was measured by a modification of the Goldberg and Spooner [16] spectrophotometric method. This assay required oxidised glutathione as the substrate.

Glutathione peroxidase-1 activity was measured using an adaptation of the spectrophotometric method of Flohé and Gunzler [17]. This assay required H2O2 as a substrate and glutathione reductase and NADPH as an enzyme indicator.

Glutathione peroxidase-2 activity was determined as for glutathione peroxidase-1 but the substrate was cumene hydroperoxide.
Superoxide dismutase (SOD) activity was measured in erythrocytes by an adaptation of the method of McCord and Fridovich [18]. The xanthine/xanthine oxidase system was used to generate the superoxide anion. This anion produced the reduction of cytochrome c, which was monitored at 550 nm. The superoxide dismutase of the sample removed the anion superoxide and produced an inhibition of the reduction. The value of this reduction was used as a measure of the enzyme activity.

2.5. Blood GSSG determination

GSSG was determined in blood by an adaptation of the method described by Winters et al. [19]. This method determines glutathione and other thiols by reverse-phase high performance liquid chromatography by forming fluorescent derivatives with N-(1-pyrenyl)maleimide (NPM). Blood was deproteinized with 30% trichloroacetic acid containing 2mM EDTA. An aliquot of 250 µl of the acidic supernatant was taken and neutralised with KOH/NaHCO₃ 2 N. In order to determine GSSG, 2-vinylpiridine was added to the neutralised sample and the mixture was incubated at room temperature for 60 min to block free GSH. Then, GSSG was reduced enzymatically to GSH by addition of NADPH and glutathione reductase in a very fast process. This aliquot was derivatized by the addition of NPM. Derivatization was completed within 5 min and the fluorescent derivatives were then stabilised by acidification. At this point the sample was injected into the HPLC system.

The HPLC was a Shimadzu with a fluorescent detector operating at an excitation wavelength of 330 nm and an emission wavelength of 380 nm. The column was a Waters Spherisorb 5 µm ODS2, 34.6×150 mm. Mobile phase consisted of 650:350 acetonitrile/H₂O acidified with 1 ml of acetic acid per litre and 1 ml of ortho-phosphoric acid per litre.

2.6. Determination of serum lipid and cholesterol parameters

Serum triglycerides, total cholesterol and HDL-cholesterol were determined by colorimetric methods using autoanalyzer DAX-72 (Technicon, Bayer). Triglycerides were hydrolyzed to glycerol and fatty acids using lipoprotein lipase. Glycerol, in a reaction catalyzed by glycerol kinase, is converted to glycerol-3-phosphate. In a third reaction, glycerol-3-phosphate is oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. Finally, in a reaction catalyzed by peroxidase, hydrogen peroxide reacts with 4-aminoantipyrine and N-ethyl-N-sulfohydroxypropyl-m-toluidine sodium salt (TOOS) to form a chinoneimide whose absorbance can be measured at 524 nm [20]. To obtain total cholesterol, serum samples were hydrolyzed with cholesterol esterase. Then, in the presence of oxygen, cholesterol oxidase oxidizes free cholesterol to cholest-4-ene-3-one and hydrogen peroxide. The colour reaction using peroxidase is the same as above. Cholesterol bound to high density plasma lipoproteins (HDL-cholesterol) was measured as the total cholesterol after immuno-inhibition of LDL, VLDL and chylomicrons with anti-β-lipoproteins, as antibody to avoid the binding of lipoproteins to the enzymes used [21].

VLDL-cholesterol and LDL-cholesterol were calculated according to the Friedewald equations [20]: [VLDL-cholesterol=triglycerides/5] and [LDL-cholesterol=total cholesterol−(HDL-cholesterol+VLDL-cholesterol)].

2.7. Uric acid determination

Uric acid was determined by an enzymatic method based on the specific uricase-catalysed oxidation of uric acid to allantoin and hydrogen peroxide [22]. The reaction of hydrogen peroxide with 4-aminoantipyrine and 2-hydroxy-3,5-dichlorobenzene sulfonate catalysed by peroxidase produces a red chromophore. The red colour is directly proportional to the uric acid concentration in the sample, and is quantified by an endpoint measurement at 524 nm. This determination was made in an autoanalyser Technicon DAX® System.

2.8. Statistical analysis

Statistical analysis was carried out using a statistical package for social sciences (SPSS 9 for windows). All the data were tested for their normal distribution. Results are expressed as means±S.E.M. and p<0.05 was considered statistically significant. ANOVA for repeated measures was used to determine the significance of the differences in all the parameters determined during the stage and the recovery. The absolute values were used for ANOVA analysis. When a significant ANOVA was found, a Student’s t-test for paired data was used to determine the differences between the involved groups.

3. Results

Table 1 shows the erythrocyte antioxidant enzyme activities expressed per blood volume. The ANOVA analysis revealed significant differences for CAT (F=7.696, p=0.001), glutathione peroxidase-1 (F=6.565, p=0.006), glutathione peroxidase-2 (F=9.334, p=0.001) and glutathione reductase (F=4.481, p=0.011) erythrocyte activities. The mountain cycling stage induced a significant increase (30%) in catalase activity. This activity returned to basal values after 3 h of recovery. Glutathione peroxidase activity, both determined with hydrogen peroxide and with cumene hydroperoxide as substrates, decreased significantly—11.3% and 17.6%, respectively—after the stage, but returned to basal levels after the 3 h of recovery. No changes were observed in SOD activity.

Values are means ± S.E.M. of eight subjects. Symbols were used to indicate significant differences (paired Student’s t-test) when ANOVA analysis for repeated measures was significant: *p<0.05 as compared to before stage values.

Table 1
Changes in erythrocyte antioxidant enzyme activities during cycling stage and recovery

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Before stage</th>
<th>After stage</th>
<th>Short recovery</th>
<th>Long recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase K/ml blood</td>
<td>40.3±2.2</td>
<td>52.5±2.7*</td>
<td>35.8±1.3#</td>
<td>41.9±3.4#</td>
</tr>
<tr>
<td>G. peroxidase-1 nkat/ml blood</td>
<td>70.6±3.3</td>
<td>62.6±2.2*</td>
<td>67.6±3.4</td>
<td>67.8±1.7</td>
</tr>
<tr>
<td>G. peroxidase-2 nkat/ml blood</td>
<td>29.5±1.8</td>
<td>24.3±0.8*</td>
<td>26.7±0.9</td>
<td>31.6±1.2#</td>
</tr>
<tr>
<td>G. reductase nkat/ml blood</td>
<td>25.6±2.2</td>
<td>31.1±2.2*</td>
<td>24.4±1.8*</td>
<td>27.0±2.1#</td>
</tr>
<tr>
<td>SOD pkat/ml blood</td>
<td>27.0±0.4</td>
<td>26.8±0.3</td>
<td>27.1±0.2</td>
<td>26.6±0.2</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. of eight subjects. Symbols were used to indicate significant differences (paired Student’s t-test) when ANOVA analysis for repeated measures was significant: *p<0.05 as compared to before stage values, #p<0.05 as compared to after stage values and &p<0.05 as compared to short recovery values.

The changes in the blood GSSG levels during the stage and recovery are shown in Fig. 1. The ANOVA analysis revealed significant differences (F=3.587, p=0.026). Blood GSSG rose about 27.5% after the mountain cycling stage (p<0.001), remained high after 3 h of recovery and returned to basal levels on the morning of the following day.

Table 2 shows the changes in retinol, α-tocopherol and carotenoids in plasma. The cycling stage did not change the plasma retinol levels. Nonetheless, the ANOVA analysis of α-tocopherol showed significant differences during the stage and recovery (F=5.71, p=0.04). α-Tocopherol increased about 4.3% (p=0.022) after the stage in plasma but decreased below basal values (9.8%) 3 h after the end of the stage, returning to basal values on the morning of the following day. No changes were observed in plasma carotenoid levels during the stage or the recovery.

The changes in serum lipid and cholesterol profile are shown in Table 3. Plasma total cholesterol, HDL-cholesterol and LDL-cholesterol did not change throughout the period studied. However, the ANOVA analysis revealed significant differences during the study in the plasmatic levels of triglycerides (F=9.945, p=0.001) and VLDL-cholesterol (F=9.855, p=0.001). Triglycerides and VLDL-cholesterol increased significantly after the stage and remained high 3 h after the cycling stage.

Finally, the changes in the plasmatic concentrations of uric acid were analysed and a significant ANOVA was found (F=5.562, p=0.011). The cycling stage induced a significant increase (24.7%) in the plasma concentration of uric acid. This greater uric acid concentration was maintained 3 h after the stage (Fig. 2).

4. Discussion

Previous studies developed with the same subjects participating in a similar cycling stage have shown that no changes in haematocrit are produced [13]. Thus, we can assume that variations in blood and plasma parameters

Table 2
Changes in plasma carotenoids and lipophylic vitamins during cycling stage and recovery

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Before stage</th>
<th>After stage</th>
<th>Short recovery</th>
<th>Long recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol (μg/ml)</td>
<td>0.60±0.05</td>
<td>0.60±0.03</td>
<td>0.61±0.04</td>
<td>0.61±0.02</td>
</tr>
<tr>
<td>α-Tocopherol (μg/ml)</td>
<td>25.6±0.6</td>
<td>26.7±0.8*</td>
<td>23.1±0.9*#</td>
<td>25.7±0.7##&amp;</td>
</tr>
<tr>
<td>Lutein/zeaxanthin (μg/l)</td>
<td>95.1±11.2</td>
<td>105±15</td>
<td>98.5±10.6</td>
<td>102±12</td>
</tr>
<tr>
<td>Cryptoxanthin (μg/l)</td>
<td>375±65</td>
<td>353±64</td>
<td>344±69</td>
<td>442±75</td>
</tr>
<tr>
<td>Lycopene (μg/l)</td>
<td>97.8±16.4</td>
<td>95.6±17.0</td>
<td>87.9±16.0</td>
<td>95.0±10.6</td>
</tr>
<tr>
<td>β-Carotene (μg/l)</td>
<td>203±38</td>
<td>188±43</td>
<td>194±43</td>
<td>218±22</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. of eight subjects. Symbols were used to indicate significant differences (paired Student’s t-test) when ANOVA analysis for repeated measures was significant: *p<0.05 as compared to before stage values and &p<0.05 as compared to short recovery values.

Table 3
Serum levels of triglycerides, total cholesterol and lipoprotein-cholesterol during cycling stage and short recovery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before stage</th>
<th>After stage</th>
<th>Short recovery</th>
<th>Long recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>125±11</td>
<td>128±18</td>
<td>136±12</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>46.1±2.8</td>
<td>85.5±9.0*</td>
<td>114±16*</td>
<td></td>
</tr>
<tr>
<td>VLDL-cholesterol (mg/dl)</td>
<td>9.25±0.59</td>
<td>17.1±1.8*</td>
<td>22.8±3.2*</td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>72.6±6.6</td>
<td>71.2±10.6</td>
<td>65.8±7.8</td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>43.4±4.7</td>
<td>45.8±5.5</td>
<td>47.6±4.2</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. of eight subjects. Symbols were used to indicate significant differences (paired Student’s t-test) when ANOVA analysis for repeated measures was significant: *p<0.05 as compared to before stage values.
Oxidation of GSH to GSSG is a sensitive marker of oxidative stress [23]. Some studies have shown that exercise produces a rise in blood GSSG concentration [12,24]. However, it has been reported that only exhaustive exercise induces oxidative stress and glutathione oxidation [12]. Thus, the blood glutathione oxidation presented indicates that this cycling stage could be classified as exhaustive exercise.

The increase in blood GSSG after the cycling stage was produced in spite of the parallel increase in erythrocyte glutathione reductase activity, the enzyme that reduces GSSG to GSH by using NADPH. Almost all of the blood glutathione represents blood cell content, mainly that of the erythrocyte [23]. Thus, erythrocyte glutathione reductase activity is a good parameter to determine the GSSG reduction capacity. The rise in glutathione reductase activity after this cycling stage was in accordance with the findings of other authors [25,26]. These increases in erythrocyte glutathione reductase activity suggest a specific response to the greater anion superoxide production induced by exhaustive exercise. In a previous work, we pointed out a synergistic effect between the activation of catalase and the activation of glutathione reductase [27] and an essential role of the superoxide anion was found [28]. Both enzymes presented a similar pattern of change during the cycling stage. The increased catalase activity could contribute to the rise in glutathione reductase activity when the superoxide anion production overwhelmed the detoxification capacity.

This hypothesis is reinforced because SOD activity was maintained throughout the cycling stage and recovery, in agreement with previously published data [29,30]. However, when erythrocyte SOD activity rises, as after a half-marathon or after lactacidaemic performance, no changes in glutathione reductase activity have been found [31].

The pattern of change in some erythrocyte antioxidant enzyme activities supports the indication that exhaustive exercise induces an imbalance between ROS production and antioxidant defences, which leads to the development of oxidative stress. Glutathione peroxidase activities, both determined with hydrogen peroxide and with cumene hydroperoxide as substrates, decreased their activities after the cycling stage. A similar decrease has been found after a marathon [32]. These decreases are further indications of oxidative stress induced by exercise and could be caused by proteolysis stimulated by oxygen radicals, as occurs in other cells [33]. However, it is more likely that a high production of oxygen radicals can exceed the detoxification capacity of antioxidant enzymes, which results in a changed intracellular redox state and/or modified catalytic centres of antioxidant enzymes, both of which have been associated with enzyme inhibition [34,35]. This second hypothesis is reinforced because the changes found during the cycling stage reverted after recovery.

Urate is an end product of purine metabolism and has been suggested to function as an antioxidant [36]. The observation that plasma uric acid increased in response to exercise is consistent with the findings of others [36]. In fact, it has been shown that exercise to exhaustion induces a marked rise in plasma urate for a wide range of exercise intensities. The urate increase after exercise has been attributed to a rise in the degradation of adenine nucleotides [36] and a transformation of xanthine dehydrogenase into xanthine oxidase, possibly through the action of proteases or through oxidation of free sulphydryl groups [36]. During exhaustive exercise, such as this mountain cycling stage, there is a greater likelihood that the enzyme responsible for the conversion of xanthine to uric acid will be xanthine oxidase rather than xanthine dehydrogenase. Xanthine oxidase uses molecular oxygen as the electron acceptor, generating the superoxide anion as a by-product [36], contributing to oxidative stress during exercise. Thus, the increase observed in urate indicates a higher anion superoxide production during the cycling stage, which is in accordance with the changes observed in erythrocyte antioxidant enzyme activities.

Basal plasma α-tocopherol levels of the cyclists participating in this study were within the range of well-nourished people [37]. An increase in plasma α-tocopherol levels was observed after the cycling stage. This increase during exercise is consistent with the findings of other studies, where higher plasma α-tocopherol levels after prolonged, intense exercise have also been reported [38,39]. This increase was coincident with the rise in plasma triglycerides and VLDL-cholesterol levels. The mechanisms to produce this increase in α-tocopherol may be due to its mobilisation from tissue storages to the plasma circulation [40,41] via synthesis of VLDL.
The increase in plasma α-tocopherol induced by exercise may be produced after the sportsmen have used and mobilised lipid reserves as a fuel to maintain physical activity, as has been evidenced by the increased triglyceride levels [13]. The mobilisation of serum triglyceride observed after exercise is related to the release of free fatty acids from the periphery, mainly due to considerable energy expenditure during the cycling stage.

An important finding in this study was the decrease observed in plasma α-tocopherol concentration after 3 h of recovery. This decrease was not accompanied by lower levels of triglycerides or VLDL-cholesterol. This finding could indicate an effective consumption of this antioxidant

levels of triglycerides or VLDL-cholesterol. This finding observed in plasma

ture during the cycling stage.

the periphery, mainly due to considerable energy expendi-

rate[42]. However, the maintenance of plasma LDL-

cholerolester, VLDL-cholesterol and triglycerides during recovery allows us to propose that the α-tocopherol decrease was due to the oxidative stress induced by exhaustive exercise. Since α-tocopherol is the antioxidant primarily responsible for scavenging peroxyl radicals [7], an increase in lipid peroxidation could deplete circulating vitamin E.

The lack of changes in plasma LDL concentration is coincident with the maintenance of plasma carotenoid levels. In fact, the lipophilic carotenoids, such as β-carotene, are mainly carried by LDL lipoproteins [43]. Thus, we could suppose that the lack of changes in plasma β-carotene is associated with the maintenance of LDL-cholesterol levels. An association between tocopherols and carotenoids in the protection of LDL against oxidation has been described [43]. The lack of changes in plasma carotenoids and the decreased levels of plasma α-tocopherol are in accordance with the protection role of vitamin E against oxidation in the LDL.

Exercise is associated with oxidative stress, which can induce adverse effects on health and well-being. However, when exercise is combined with a prudent antioxidant supplementation, it can have beneficial effects on health. The existence of this paradox is well known and it has been suggested that the capacity and adaptation of the body’s antioxidant defences may be part of the reason [44].

In summary, a mountain cycling stage induces oxidative stress, as is evidenced by the increases in blood GSSG and in serum urate concentrations and by the pattern of change of erythrocyte antioxidant enzyme activities. Non-enzymatic antioxidant defences are mobilised in order to increase the circulating antioxidant endogenous capabilities. A specific utilisation of α-tocopherol during recovery has been evidenced. In association with the maintenance of cholesterol LDL, plasma carotenoid levels did not change during the stage or recovery.

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References


