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Relation between oxidative stress markers and antioxidant endogenous defences during exhaustive exercise

ANTONI SUREDA[1], PEDRO TAULER[1], ANTONI AGUILÓ[1], NURIA CASES[1], EMILIA FUENTESPINA[2], ALFREDO CÓRDOVA[3], JOSEP A. TUR[1], & ANTONI PONS

[1]*Laboratori de Ciències de l'Activitat Física, Departament de Biologia Fonamental i Ciències de la Salut, Universitat de les Illes Balears, Crtra. Valldemossa Km 7.5. E-07122-Palma de Mallorca, Illes Balears, Spain,* [2]*Laboratori del Carme, Hospital Son Dureta, INSALUD, Palma de Mallorca, Illes Balears, Spain, and* [3]*Escuela de Fisioterapia de Soria, Universidad de Valladolid, Soria, Spain*

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Abstract

Hydrogen peroxide (H_2O_2) could induce oxidative damage at long distance from its generation site and it is also an important signalling molecule that induces some genes related to oxidative stress. Our objective was to study the plasma and blood cells capability to detoxify H_2O_2 after intense exercise and its correlation with oxidative damage. Blood samples were taken from nine professional cycling, participating in a mountain stage, under basal conditions and 3 h after the competition. Catalase and glutathione peroxidase activities decreased (40 and 50% respectively) in neutrophils after the cycling stage, while glutathione peroxidase increased (87%) in lymphocytes. Catalase protein levels and catalase specific activity maintained basal values after the stage in plasma. Catalase protein levels decreased (48%) in neutrophils and its specific activity increased up to plasma values after exercise. Myeloperoxidase (MPO) increased (39%) in neutrophils after the cycling stage. Exercise-induced hemolysis and lymphopenia inversely correlated with cellular markers of oxidative stress. Plasma malondialdehyde (MDA) directly correlated with neutrophil MPO activity and erythrocytes MDA. Intense exercise induces oxidative damage in blood cells as erythrocytes and lymphocytes, but not in neutrophils.

Keywords: *Oxidative stress, antioxidants, exercise, hydrogen peroxide, catalase, glutathione peroxidase*

Abbreviations: *ANOVA, analysis of variance; CAT, catalase; DNPH, 2,4-dinitrophenylhydrazine; GP, glutathione peroxidase; H_2O_2, hydrogen peroxide; HOCl, hypochlorite; MDA, malondialdehyde; MPO, myeloperoxidase; O_2^-, superoxide anion; OH·, hidroxil radical; ROS, reactive oxygen species*

Introduction

Hydrogen peroxide (H_2O_2) is a central oxygen metabolite, produced as a result of the superoxide anion (O_2^-) dismutation as the result of the activity of some enzymes as glucose oxidase. Under normal physiological conditions, the majority of reactive oxygen species (ROS) is produced in the mitochondrial electron transport [1,2]. However, ROS are produced in other biochemical pathways such as the respiratory burst of neutrophils or xanthine oxidase activity [2]. H_2O_2 is an active ROS and can be the source of other ROS, such as the hydroxyl radical. H_2O_2 half life is longer than the one of other ROS as hydroxyl radical (OH·). H_2O_2 present similar characteristics to H_2O and it can diffuse out of the generation locus. Then, it is possible to find H_2O_2 at sites far from

Correspondence: A. Pons, Laboratori de Ciències de l'Activitat Física, Universitat de les Illes Balears, Crtra. Valldemossa, km 7.5 E-07122-Palma de Mallorca, Balears, Spain. Tel: 34 971173171. Fax: 34 971173184. E-mail: dbsapb0@uib.es

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its generation place. H_2O_2 initiates the oxidation of several target molecules [3], or induces molecular injury [4,5]. Extracellular H_2O_2 has recently gained much attention as a possible signalling molecule [6]. The control of H_2O_2 levels is important to avoid the potential damaging effects, and to allow the signalling function. The antioxidant enzymes catalase (CAT) and glutathione peroxidase (GP) are the most important enzymes detoxifying H_2O_2. GP also participates in the detoxification of lipid hydroperoxides using glutathione [7]. Both enzymes are distributed among blood cells (erythrocytes, lymphocytes and neutrophils) and plasma; however, no studies have been carried out to establish the role of these enzymes in equilibrating the blood oxidative status. The enzyme myeloperoxidase (MPO) also uses H_2O_2 as substrate to generate hypochlorite (HOCl). This enzyme is specific of phagocytes and it is secreted to plasma during the acute phase immune response.

Strenuous physical activity increases oxygen consusmption and induces oxidative stress as result of increased free radical production [8]. It may also initiate reactions that resemble the acute phase immune response to infection [9]. Exercise has been shown to induce inflammatory-like changes in immune cell counts and acute phase protein release [10]. Whereas circulating neutrophils increase after exhaustive exercise, lymphocyte count rapidly decrease. This changes in the cell counts could remain for several hours after exercise [11]. Moderate training enhances the immune function, but exhaustive exercise might cause perturbations to the immune system, increasing the risk for upper respiratory tract infections [12]. It is estimated that 2–5% of the total mitochondrial electron flux leaks to form O_2^-. Increased electron flux in the active muscle may lead to an enhancement of ROS production [2]. High-intensity exercise also activates xanthine oxidase pathway giving a rise of O_2^- [13]. Blood cells present different contribution to the H_2O_2 generation. Erythrocytes can produce H_2O_2 as a result of the oxygen induced heme oxidation. In a first step the heme oxidation produces superoxide anion that is dismutated to H_2O_2 and O_2 [14]. Neutrophils produce superoxide anion when they are activated by several stimulus and the NADPH-oxidase is assembled. NADPH-oxidase generate superoxide anion which is dismutated to H_2O_2. This H_2O_2 is used by the neutrophil MPO to produce HOCl. All these reactions are produced in the phagosome [15]. Lymphocytes can generate H_2O_2 as a result of the mitochondrial respiration [16].

All the cells are sensitive to the increased H_2O_2 [6]. However blood cells respond in a different way to exercise. Intense exercise induces neutrophil priming for oxidative activity and activates acute phase protein release [17]. The neutrophil increased capabilities to synthesize ROS coexist with decreased neutrophil antioxidant defences [18]. The low antioxidant enzyme activities in primed neutrophils after exhaustive exercise magnify the importance of other antioxidant defences in these cells. Antioxidant enzyme activities show a great adaptation to oxidative stress increasing in lymphocytes as a consequence of physical activity [19]. Exercise-accompanied hormonal changes and ROS generation could inhibit lymphocyte proliferation, and oxidative damage could activate apoptotic processes [20]. Erythrocytes are very sensitive to oxidative stress, because they are unable to repair damaged components as proteins by re-synthesis, and their membranes are vulnerable to peroxidative damage [21].

It is interesting to study the cellular and plasmatic capabilities to deactivate the H_2O_2 during a physiological situation that increases its production. This study describes the differential response of erythrocytes, neutrophils and lymphocytes and their antioxidant defences against H_2O_2 during the acute phase immune response induced by exhaustive exercise. The correlations between blood cells and plasma antioxidant enzymes, and indicators of oxidative damage were also established.

Materials and methods

Subjects

Nine voluntary male subjects participated in this study. They were all professional cyclists participating in the "Challenge Volta a Mallorca 2002", a five-day competition for professional cyclists. Subjects were informed of the purpose of this study and the possible risks involved before giving their written consent to participate. The exercise was a mountain stage (171.8 Km). The cyclists took a mean of 275 min to complete this stage.

Experimental procedure

Venous blood samples were taken from the antecubital vein with suitable vacutainers with EDTA as anticoagulant. Samples were taken the morning of the cycling stage day, after overnight fasting, and immediately after the stage.

Blood samples were used to purify neutrophils, lymphocytes, erythrocytes and to obtain plasma. Blood cells were quantified in an automatic flow cytometer analyser Technicon H*2 (Bayer) VCS system. CAT and GP enzyme activities and oxidative damage markers were determined in all erythrocytes,

lymphocytes, neutrophils and plasma. MPO activity was determined in neutrophils. We also determined the CAT protein levels in neutrophils and plasma.

Neutrophil and lymphocyte purification

The neutrophil fraction was purified following an adaptation of the method described by Boyum [22]. Blood was carefully introduced on Ficoll in a proportion of 1.5:1 and was then centrifuged at 900*g*, 18°C for 30 min. The lymphocyte layer was carefully removed. The precipitate containing the erythrocytes and neutrophils were incubated at 4°C with 0.15 M ammonium chloride to haemolyse the erythrocytes. The suspension was centrifuged at 750*g*, 4°C for 15 min and the supernatant was then discarded. The neutrophil phase at the bottom was washed first with ammonium chloride and then with phosphate buffer saline, pH 7.4. Finally, the neutrophils were lysed with distilled water.

The lymphocyte slurry was then washed twice with PBS and centrifuged for 10 min at 1000*g*, 4°C. The cellular precipitated of lymphocytes was lysed with distilled water.

Erythrocyte and plasma purification

Blood samples were centrifuged at 900*g*, 4°C for 30 min. The plasma was recovered, and the erythrocyte phase at the bottom was washed with PBS and centrifuged as above. Erythrocytes were reconstituted and haemolysed with distilled water in the same volume as plasma.

Enzymatic determinations

CAT activity was measured by the spectrophotometric method of Aebi [23] based on the decomposition of H_2O_2. GP activity was measured using an adaptation of the spectrophotometric method of Flohé and Gunzler [24]. This assay required H_2O_2 as a substrate and GR and NADPH as enzyme indicators. Neutrophil MPO activity was measured by guaiacol oxidation [25]. The reaction mixture contained sodium phosphate buffer pH 7 and 13.5 mM guaiacol. The reaction was initiated by adding 300 μM H_2O_2, and changes at 470 nm were monitored. All activities were determined with a Shimadzu UV-2100 spectrophotometer at 37°C.

Protein catalase levels

CAT levels were determined by ELISA using polyclonal antibody Anti-CAT (Calbiochem) which recognises human CAT. We followed an adaptation of the method to determine the SOD protein levels [18].

Suitable dilutions of the neutrophil suspensions, plasma or standard dilutions of CAT (50 μl) were placed in each well of the plate per duplicate (Polystyrene Assay Plate, Costar). The plate was then incubated (37°C for 3 h) in order to adsorb the sample proteins to the surface of each well. A solution of 1% bovine albumin was added into each well and the plate was incubated (37°C for 3 h) in order to saturate all binding protein sites of the plate. Then, the plate was washed four times with NaCl 0.9%-Tween 20. The commercial antibody (1:1000) was placed into each well and the plate was newly incubated for 3 h at 37°C. The plate was then washed as above. The secondary antibody against the IgG chain, conjugated to alkaline phosphatase (1:500) was placed into each well and the plate was incubated in the same conditions as above. The wells were newly washed and the phosphatase substrate solution (*p*-nitroanilide) was added. Finally, the absorbance was measured at 405 nm.

MDA levels

MDA as a marker of lipid peroxidation was analyzed in plasma and erythrocytes by a colorimetric assay kit (Calbiochem, San Diego, CA, USA).

Protein carbonyl derivates determination

Protein carbonyl derivates were measured by an adaptation of the method of Levine [26] using the precipitates of deproteinised samples. Precipitates were re-suspended with 2,4-dinitrophenylhydrazine (DNPH) 10 mM, and incubated for 60 min at 37°C. Then, samples were precipitated with 20% trichloroacetic acid, and centrifuged for 10 min at 1000*g* at 4°C. The precipitate was washed twice with ethanol:ethyl acetate (1:1) to remove free DNPH. 6 M Guanidine in 2 mM phosphate buffer, pH 2.3, was added to the precipitate, and samples were incubated for 40 min at 37°C. Finally, samples were centrifuged for 5 min at 3000*g* at 4°C to clarify the supernatant and absorbance was measured at 360 nm. The molar absorption of 22,000 $M^{-1} \cdot cm^{-1}$ was used to quantify the protein carbonyl levels. Samples were analysed against a blank of guanidine solution.

Statistical analysis

Statistical analysis was carried out using a statistical package for social sciences (SPSS 11.0 for Windows). Results are expressed as the mean ± SEM and $P < 0.05$ was considered statistically significant.

The statistical significance of the CAT and GP was assessed by a two-way analysis of variance (ANOVA). The statistical factors analysed were the cell type (Cll) and the exercise (E). When significant effects were found, a one-way ANOVA was used to determine the differences between the groups involved. One-way ANOVA was also used to determine differences in the cell number, enzymes activities, CAT protein levels and oxidative damage. The possible bivariate correlations between the different parameters were also analysed.

Results

The cycling stage significantly influenced the blood counts of neutrophils, lymphocytes and erythrocytes (Figure 1). The number of circulating neutrophils significantly increased (325%) after the cycling stage. Lymphocyte number decreased about 38% after exercise, and the cycling stage produced slight haemolysis decreasing significantly the erythrocyte number about 9%.

Oxidative damage markers in blood cells and plasma are presented in Table I. MDA levels increased in plasma and erythrocytes after the cycling stage. The increase in MDA was higher in plasma than in erythrocytes. Protein carbonyl derivates increased in plasma, erythrocytes and lymphocytes, while neutrophils maintained basal levels. The most susceptible compartment to protein oxidation was the lymphocyte fraction as it increased the protein carbonyl derivates about 40%, while the increase in plasma and erythrocytes was about 20%.

Table II shows the CAT and GP activities in blood cells and plasma, and the neutrophil MPO activity before and after the cycling stage. Basal CAT activity expressed per cell number was different depending on cell type and also, exercise influenced CAT activity in a different way in each cell type. Erythrocyte CAT activity was lower than the neutrophil and lymphocyte ones, which were similar at baseline. CAT activity maintained the basal values in lymphocytes and erythrocytes but it decreased about 40% in neutrophils after exercise. GP activity was influenced by the cell type and by the exercise and a significant interaction between them was found. Neutrophil GP activity decreased significantly after the cycling stage (−50%). Lymphocyte GP activity showed an important increase (+87%), and erythrocytes maintained the basal GP activity after exercise. The erythrocyte GP basal activity was lower than the one in neutrophils and lymphocytes. Erythrocyte and neutrophil post-exercise GP activities were not significantly different, whereas lymphocytes showed significant higher activity than other cell types.

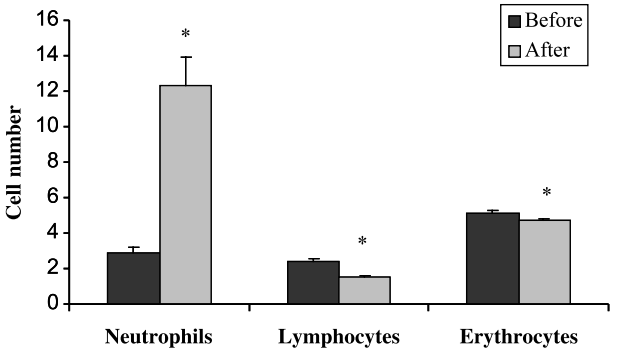

Figure 1. Changes in the number of circulating cells before and after the cycling stage. Neutrophil and lymphocyte numbers are expressed as 10^3 cells/μl blood, and erythrocytes as 10^6 cells/μl blood. (*) Indicate significant differences, $p < 0.05$.

Table I. Oxidative damage in blood cells and plasma before and after the cycling stage.

	Before	After
MDA levels		
Plasma (μmol/l plasma)	4.75 ± 0.80	8.04 ± 1.08*
Erythrocytes (nmol/10^9 cells)	18.9 ± 0.8	25.8 ± 1.1*
Protein carbonyl derivates		
Plasma (μmol/l plasma)	297 ± 18	361 ± 20*
Neutrophils (μmol/10^9 cells)	4.59 ± 0.62	4.55 ± 0.78
Lymphocytes (μmol/10^9 cells)	4.90 ± 0.46	6.85 ± 0.66*
Erythrocytes (μmol/10^{12} cells)	55.8 ± 3.2	67.3 ± 4.1*

Changes in the levels of MDA and protein carbonyl derivates. (*) Indicate significant differences using one-way ANOVA, $p < 0.05$. Means ± SEM, $n = 9$.

Enzymes activities expressed per ml of cell volume and plasma allows the comparison between the blood cell compartments and plasma. Using this expression neutrophils and lymphocytes showed similar basal CAT and GP activities, whereas erythrocyte CAT activity was lower. After exercise, lymphocytes increased the antioxidant enzyme activities, mainly GP activity, while erythrocytes increased CAT activity. Neutrophil CAT and GP activities decreased about 50%. Plasma presented the lowest CAT and GP activities expressed in comparable values, without significant differences before and after exercise. CAT and GP activities expressed per blood volume (data not shown) were around 1000 times higher in erythrocyte compartment than in neutrophil, lymphocyte and plasma compartments when their contribution to the whole blood was compared.

Neutrophil MPO activity increased (39%) after the cycling stage when it was expressed per cell number and also when it was expressed per ml of blood (data not shown). Post-exercise MPO activity expressed per cell number increased two folds after the stage versus basal values.

Table II. Catalase and glutathione peroxidase activity in blood cells and plasma before and after the cycling stage.

	Before	After	E	Cll	ExCll
Catalase (K/10^9 cells)			×	×	
Neutrophils	18.1 ± 2.1^{a}	$10.8 \pm 1.8^{b,\star}$			
Lymphocytes	$23.6 \pm 3.3^{a,c}$	28.5 ± 3.9^{c}			
Erythrocytes	4.40 ± 0.37^{b}	5.47 ± 0.44^{b}			
Glutathione peroxidase (nKat/10^9 cells)			×	×	×
Neutrophils	63.1 ± 11.5^{a}	$31.2 \pm 3.0^{c,\star}$			
Lymphocytes	85.3 ± 5.6^{b}	$160 \pm 14^{d,\star}$			
Erythrocytes	13.9 ± 1.0^{c}	14.4 ± 1.1^{c}			
Catalase (K/ml cell)			×	×	
Neutrophils	60.4 ± 7.2^{a}	$36.0 \pm 5.9^{b,\star}$			
Lymphocytes	$78.8 \pm 11.2^{a,c}$	95.1 ± 13.0^{c}			
Erythrocytes	48.6 ± 3.8^{b}	$62.3 \pm 4.7^{a,\star}$			
Plasma (K/ml plasma)	0.36 ± 0.02^{d}	0.38 ± 0.05^{d}			
Glutathione peroxidase (nKat/ml cell)			×	×	×
Neutrophils	$210 \pm 38^{a,d}$	$104 \pm 10^{b,\star}$			
Lymphocytes	284 ± 18^{a}	$533 \pm 48^{c,\star}$			
Erythrocytes	$155 \pm 11^{a,b}$	$164 \pm 12^{b,d}$			
Plasma (nKat/ml plasma)	0.82 ± 0.07^{e}	0.74 ± 0.03^{e}			
MPO (nKat/10^6 cells)					
Neutrophils	108 ± 7	$151 \pm 18^{\star}$			

CAT and GP activities expressed per number and volume of cells. Neutrophil and lymphocytes CAT and GP activities were calculated taking into account the cell volume of 300 μl/10^9 cells. The erythrocyte enzyme activities expressed per cell volume were calculated taking into account the hematocrit of $46.6 \pm 0.6\%$ in basal conditions and $41.0 \pm 0.6\%$ after exercise together with the red cell counts. Two-way ANOVA. (×) Indicates significant effects (E or Cll) or significant interaction (E⋆Cll) between the two ANOVA factors. Different letters indicate different values. (⋆) Indicates significant differences before vs. after exercise, $p < 0.05$. Means ± SEM, $n = 9$.

CAT protein concentration and specific activity in neutrophils and plasma are shown in Table III. Plasma CAT concentration and the specific CAT activity after the cycling stage maintained basal values. Neutrophil CAT concentration maintained basal values expressed per blood volume, but its levels significantly decreased (48%) when it was expressed per cell number. The basal specific activity in neutrophils was significantly lower than plasma basal specific activity, however, after the cycling stage the neutrophil CAT specific activity increased until it reached similar plasma values.

Table IV shows the correlations between the enzyme activities, the blood cell counts and the markers of oxidative damage. MDA in erythrocytes is directly correlated with plasma MDA and inversely correlated with erythrocyte number. Protein carbonyl derivates are directly correlated with CAT activity and inversely correlated with the erythrocyte number. Neutrophil CAT and GP activities are inversely correlated and MPO directly correlated with plasma MDA. Neutrophil protein carbonyl derivates showed a direct correlation with neutrophil CAT activity. Lymphocyte protein carbonyl derivates are directly correlated with lymphocyte GP activity and inversely correlated with the lymphocyte counts.

Discussion

Intense physical exercise induces a redistribution of immune cells in the body and a release of soluble mediators that resemble an inflammatory response

Table III. Catalase protein levels and specific activity in neutrophils and plasma before and after the cycling stage.

	Before	After	E	Cll	ExCll
ng/μl blood				×	
Neutrophils	0.26 ± 0.02^{a}	0.33 ± 0.05^{a}			
Plasma	0.48 ± 0.04^{b}	0.56 ± 0.05^{b}			
Specific activity (K/mg cat)			×	×	×
Neutrophils	206 ± 10^{a}	462 ± 47^{b}			
Plasma	442 ± 32^{b}	432 ± 48^{b}			
pg catalase/10^3 neutrophil	63.6 ± 6.9	$42.7 \pm 6.8^{\star}$			

Catalase are expressed in neutrophils and plasma per μl blood, and the specific activity as K/mg catalase. Two-way ANOVA. (×) Indicates significant effects (E or Cll) or significant interaction (E⋆Cll) between the two ANOVA factors. Different letters indicate different values. (⋆) Indicates significant differences before vs. after exercise, $p < 0.05$. Means ± SEM, $n = 9$.

Table IV. Correlations between the blood cell types and oxidative damage markers, cell counts and enzyme activities.

	Correlation
Erythrocytes	
MDA vs. plasma MDA	0.568*
MDA vs. erythrocyte number	−0.879**
Protein Carbonyl derivates vs. erythrocyte number	−0.717**
Protein Carbonyl derivates vs. erythrocyte CAT activity	0.525*
Neutrophils	
CAT activity vs. plasma MDA	−0.600**
GP activity vs. plasma MDA	−0.471*
MPO activity vs. plasma MDA	0.486*
Protein Carbonyl derivates vs. neutrophil CAT activity	0.587**
Lymphocytes	
Protein Carbonyl derivates vs. lymphocyte number	−0.760**
Protein Carbonyl derivates vs. lymphocyte GP activity	0.702**

Most significant correlations between the blood cell types and oxidative damage markers, cell counts and antioxidant enzyme activities. Bivariate Correlations. (*) Indicates a correlation at $p < 0.05$. (**) Indicates a correlation at $p < 0.01$.

similar to the one induced by infection [18,27]. We found a great neutrophilia and lymphopenia after the mountain cycling stage. These changes in the circulating immune cell numbers were similar to the ones observed after other types of intense exercises as a duathlon competition [19] or a marathon race [8].

Exhaustive exercise increases ROS production that can induce oxidative damage in blood cells and plasma [8,32,33]. Erythrocytes together with the plasma are the most susceptible fractions to suffer from lipid oxidative damage induced by exercise. The cycling stage decrease the erythrocyte number indicative of a slight haemolysis. Haemolysis could be attributable to the fragility of erythrocyte membranes as a result of the increased lipid peroxidation after exercise [29]. We found an inverse correlation between both the erythrocyte MDA and the carbonyl derivates with the number of circulating erythrocytes ($p < 0.01$). The inverse correlation reinforce the possibility that haemolysis takes place as a result of the oxidative stress induced by the cycling stage. There is also a direct relationship between MDA levels in plasma and erythrocytes. It has been evidenced the existence of lipid exchanges between both compartments [30,31]. These exchanges could be implicated in the similar behaviour of plasma and erythrocyte MDA. The increased oxidative stress could be reflected in a direct activation of some antioxidant enzymes after exercise [29]. Decomposition of H_2O_2 at physiological levels in erythrocytes is mainly due to CAT activity, which depends linearly on H_2O_2 concentration [34]. Erythrocyte antioxidant enzymes are not probably enough to deactivate the increased ROS production after exercise. The excess in ROS production, in spite of increased erythrocyte CAT activity after exercise, can induce erythrocyte membrane peroxidation, increase erythrocyte fragility and therefore facilitate their hemolysis [35].

Different studies suggest that apoptosis processes are activated in lymphocytes after intense exercise contributing to the regulation of the immune response [20,28]. In the present study we have evidenced an inverse correlation between lymphocytes carbonyl derivates and lymphocyte number ($p < 0.01$) that may be related to the oxidative damage with lymphopenia and probably with the activation of apoptotic processes. Lymphocyte antioxidant enzymes show a great adaptation to oxidative stress induced by exercise. CAT and GP activities increased in lymphocytes as a consequence of intense exercise. This increase may indicate an activation or a direct induction of the expression of these enzymes by ROS or cytokines so as to increase lymphocyte antioxidant defences [4,36,37]. Several studies observed an upregulation of the antioxidant enzymes SOD and CAT and of Heat Shock Proteins after exercise [38] or in front of low levels of exogenous oxidative stress [39]. However, this increased antioxidant enzyme activities are not enough to completely eliminate the ROS produced after intense exercise. The increase of oxidative damage markers is directly correlated with an increase of H_2O_2 detoxifying enzyme activities as GP in lymphocytes, in order to counteract and reduce the cell damage.

Exercise induces neutrophil priming for oxidative activity [4] as it was evidenced by the increased neutrophil MPO activity. This result points out the importance of MPO as a HOCl source during exhaustive exercise contributing to oxidative stress. There is a direct relationship between neutrophil MPO activity and plasma MDA levels, indicating that neutrophils could be an important source of oxidants in plasma and thereby contributing to oxidative stress. The increase in HOCl production might have negative effects because this molecule would not be consumed in the reaction with bacterial material as occurs during infection, and any enzymatic pathway to deactivate HOCl is known so far. Then, the overproduction of HOCl could be a source of oxidants for lipid peroxidation increasing MDA levels. The response of primed neutrophils to oxidative stress induced by exercise could include the antioxidant enzyme secretion to the extra cellular space in order to increase the plasma antioxidant defences against the oxidative stress induced by exercise [18,19] and the MPO release in order to decrease the capability to produce HOCl. It has been well established that monocytes and macrophages can secrete arginase in

order to increase the availability of intracellular arginine for iNOS and NO synthesis [40]. Neutrophils are capable to release proteins as cytokines and enzymes as MPO and lysozyme to the extracellular space when they are activated [17,41,42]. CAT in neutrophils is located both in the granule fraction and the cytosolic fraction [43], making possible the secretion of CAT by neutrophils in a similar way as the degranulation and secretion of other enzymes. Even though, neutrophils seem to be protected from oxidative damage despite the reduction in their antioxidant enzyme activities, as oxidative markers were not modified in neutrophils after exercise. The neutrophil antioxidant enzyme secretion seems to be not enough to significantly increase antioxidant enzyme levels in the whole plasma, although it could be relevant at local level.

Neutrophil catalase specific activity is lower than plasma activity at baseline, but after exercise increases up to similar plasma. It is suggested that CAT activity could be modified during oxidative stress, possibly by activation through phosphorylation [44] or could be inhibited by interaction with NO. CAT has been shown to be activated in pancreatic β-cells as a result of a decreased NO production in the cell [45]. In this way, neutrophils counteract the lower levels of catalase by increasing its activity, and remain protected from their own ROS production. The direct correlation between CAT activity and protein carbonyls in neutrophils is in accordance with this protective role of the activation of CAT in neutrophils.

In summary, exhaustive exercise induces oxidative stress in blood cells and plasma. Oxidative markers are directly correlated to a decreased erythrocyte and lymphocyte number after exercise. ROS could participate in the lymphopenia and haemolysis induced by intense exercise. The plasma lipid peroxidation during exercise is induced both by the erythrocyte lipid peroxidation and by the neutrophil ROS production. The protein oxidative damage is directly correlated with the GP activity in lymphocytes and with the catalase activity in neutrophils. CAT could be the main enzyme to protect the protein oxidation by hydrogen peroxide in neutrophils and GP in lymphocytes.

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