

# Aerobic and anaerobic training sessions promote antioxidant changes in young male soccer players

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**OBJECTIVE:** The aim of this study was to investigate the effect of aerobic vs. anaerobic intense training sessions on biomarkers of oxidative stress.

**METHODS:** The included sample comprised 18 junior male soccer players (18-21 years) during the intermediate season. Blood samples were obtained before (baseline) and after aerobic or anaerobic training sessions and the following substances were assayed: (i) the biomarkers of cellular damage Thiobarbituric Acid-Reactive Substances and Oxidized Glutathione; (ii) the non-enzymatic antioxidants Reduced Glutathione and Total-Glutathione, (iii) the antioxidant enzymes Superoxide Dismutase, Catalase, Glutathione Reductase, Glutathione Peroxidase and Glutathione S-Transferase.

**RESULTS:** (a) the contents of Thiobarbituric Acid-Reactive Substances and Oxidized Glutathione showed no significant differences before vs. after aerobic or anaerobic training sessions. (b) After aerobic training sessions, the activity of Superoxide Dismutase, Glutathione Reductase, and the contents of Reduced Glutathione and Total Glutathione were decreased; the activity of Glutathione S-transferase and Glutathione Peroxidase were increased while Catalase activity remained unaltered. (c) After anaerobic training sessions, Catalase activity decreased; Glutathione-Peroxidase increased; Superoxide Dismutase, Glutathione Reductase, and Reduced, Oxidized and Total Glutathione showed no significant differences.

**CONCLUSION:** These results provide evidence of a more pronounced systemic oxidative stress after the aerobic as compared to the anaerobic training session in young soccer players.

**KEYWORDS:** Reactive oxygen species, Oxidative stress, Antioxidants, Aerobic and anaerobic sessions, Soccer players.

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## INTRODUCTION

Soccer is an intermittent sport that encompasses brief bouts of high-intensity running and longer periods of low-intensity exercise, plus changes of space, intensity, flexibility, direction, acceleration capability and basic speed.<sup>1,2</sup> The structure of a training program induces muscle changes; thus an endurance protocol produces major adaptations in aerobic metabolism, while sprint training increases the concentration of energetic substrates and the activity of anaerobic-metabolism-related enzymes.<sup>3</sup>

The routine normally used for the physical training of soccer players improves aerobic capacity, resulting in an increase in oxygen consumption, particularly in skeletal muscle and heart;<sup>4</sup> this increase in O<sub>2</sub> consumption is associated with increased production of reactive oxygen species (ROS) at levels that may overwhelm the antioxidant defenses.<sup>5</sup>

Exercise-induced aerobic bioenergetic reactions in mitochondria and cytosol increase the production of ROS,<sup>5</sup> which are molecules that have one or more unpaired electrons in their outer orbitals.<sup>5</sup> Excess of ROS can be scavenged by enzymatic as well as non-enzymatic antioxidants to protect against deleterious oxidative stress,<sup>6</sup> which in turn can be defined as an imbalance between

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oxidants and antioxidants in favor of the former.<sup>5</sup> Antioxidants are substances that delay or prevent the oxidation of a substrate; they can act by blocking the formation of ROS or by interacting with them, turning them into inactive and electrically stable compounds, thus decreasing their capacity to damage important biomolecules.<sup>5</sup>

As every tissue, muscle fibers also contain both enzymatic and non-enzymatic antioxidants that work as a complex, continuous and concerted ROS detoxification system; each of these antioxidants is responsible for the reduction of different ROS, and they are located in distinct cellular compartments.<sup>5</sup> These antioxidants protect muscle fibers from oxidative injury during periods of increased oxidant production (e.g., intense or prolonged exercise).<sup>7</sup>

Several reports on oxidative stress regarding different types of exercise have shown a consequent increase in ROS generation;<sup>8,9</sup> soccer is one of these sports,<sup>10-13</sup> which thus promotes systemic oxidative stress, especially in the absence of a previous adaptation through regular training.<sup>12,14,15</sup>

As mentioned above, relatively few studies on oxidative stress related to soccer players have been reported so far and no previous study was carried out comparing the effect of aerobic vs. anaerobic training sessions on the antioxidant defenses and biomarkers of oxidative stress in soccer players.

## METHODS

### Subjects

Eighteen men (average age  $18.27 \pm 0.21$  years; average weight  $73.29 \pm 1.83$  kg; average height  $180 \pm 0.1$  cm and average Body Mass Index  $22.73 \pm 0.26$  kg/m<sup>2</sup>) participated in the present study, all of them junior soccer players with the "Aval" club (main division of the state of Santa Catarina), located in the city of Florianopolis, Southern Brazil, during the intermediate season.

Participants were healthy, non-smokers and did not frequently consume caffeine or alcoholic beverages. The objectives and procedures of this research were explained to the participants before they were asked to sign a written consent. This study was approved by the Ethics Committee on Human Research of the Federal University of Santa Catarina (CEUA case number 014/2001).

### Experimental design

Blood samples (3-5 ml) were collected from each group just before and immediately after an aerobic or anaerobic training session for analysis. Diet (quality and quantity) was controlled by the team's medical staff, and the consumed amounts regarding antioxidant content were similar among the athletes (fruit, vegetables, etc.).

Early in the morning (7 AM), while fasting, blood samples were harvested from each group (see below). Two groups were set up for the aerobic or the anaerobic intense training sessions; these were supervised by the coach and his staff; The groups were randomly selected as follows: X1 - Aerobic Group comprising 9 subjects, focused on endurance session: all participants in the group performed one supervised session of aerobic training, consisting of a run lasting 45 min, according to pre-established heart rate (65-75% of their maximal heart rate), covering a distance of ca. 6,780 m; X2 - anaerobic group comprising 9 subjects, focused on sprints, namely high intensity short-duration intermittent exercises; all participants in the group performed a supervised session of anaerobic training, consisting of a total run of 15/20 min, subdivided into eight sprints of maximal 40 seconds, with break intervals of 2 min between sprints, according to a pre-established heart rate (85% of their maximal heart rates). The total covered distance per sprint was 250 m.

For both groups, the time interval from blood samples harvested early in the morning (7 AM) to those harvested at the end of each training session was 4 hours (11 AM).

Sample preparation: Sample preparation has been described elsewhere.<sup>13</sup> The content of thiobarbituric acid-reactive substances (TBARS) was determined in plasma, while the content of reduced glutathione, total glutathione, and oxidized glutathione in whole blood extracts. Samples were not frozen to avoid enhanced lipid auto-oxidation by butylhydroxytoluene, and also because reduced glutathione is rapidly oxidized at relatively low temperatures.<sup>16</sup>

### TBARS assay in plasma

Determination of TBARS was used to assay endogenous lipid oxidation according to Ohkawa<sup>17</sup> and Bird and Draper<sup>18</sup>, which were expressed as nmol TBARS/ml ( $\epsilon_{535} = 153 \text{ mM}^{-1} \text{ cm}^{-1}$ ).

### Enzymatic and non-enzymatic antioxidants in blood

Superoxide Dismutase activity was measured at 550 nm according to the method of cytochrome-c reduction.<sup>19</sup> Catalase activity was determined by measuring the decrease in a freshly prepared solution of hydrogen peroxide (10 mM) concentration at 240 nm.<sup>20</sup> Glutathione Peroxidase was measured at 340 nm through the Glutathione/NADPH/Glutathione Reductase system, by the dismutation of *tert*-butylhydroperoxide.<sup>21</sup> Glutathione Reductase was measured at 340 nm through the oxidation rate of NADPH, in a reaction medium containing buffered DPTA (diethylenetriaminepentacetic acid) and 1 mM Oxidized Glutathione.<sup>22</sup> Glutathione S-Transferase was measured at 340 nm according to Habig,<sup>23</sup> using CDNB (1-chloro-2,4-dinitrobenzene) as substrate and 0.15 M GSH concentration. All activities are expressed per milliliter of whole blood.

## Glutathione assay

Reduced glutathione was measured according to Beutler,<sup>24</sup> using Elmann's reagent (2-dithionitrobenzoic acid, DTNB). Briefly, acid extracts were obtained by the addition of trichloroacetic acid, followed by centrifugation. Supernatants from the acid extracts were added to DTNB in Na-PO<sub>4</sub>; the formation of thiolate anion was determined at 412 nm. TG was also measured at 412 nm according to the method of Tietze,<sup>25</sup> and Oxidized Glutathione was calculated in equivalents of reduced glutathione.

## Statistical analysis

Data analysis was descriptive: means and standard deviations were calculated for all measured variables, using SPSS (IBM SPSS Statistics 18) software. Both groups (aerobic and anaerobic training session) were checked for normality using the Shapiro-Wilk (if n < 50) and the Kolmogorov-Smirnov (if n > 50) statistical tests. Depending on the normality of these data, the paired Student-*t* test (parametric data) was used. The minimal accepted level of significance was p < 0.05.

## RESULTS

Plasma concentrations of TBARS showed no significant changes before or after the aerobic and anaerobic sessions, as shown in Table 1. Similarly, no statistical differences were found for the contents of different forms of glutathione before and after the anaerobic training session compared to the moment just before starting each training session (Table 1).

Neither were there any differences in the contents of Oxidized Glutathione; however significant decreases in Total and Reduced Glutathione values measured in whole blood were obtained in samples after vs. before the aerobic training session, as shown in Table 1.

The erythrocytic activity of Catalase and Glutathione Reductase show no significant changes when compared between the two groups as shown in Table 2.

In the Aerobic Training Group, significant increases in Glutathione S-Transferase and Glutathione Peroxidase activities, and a significant decrease in Superoxide Dismutase activity were detected after vs before aerobic exercise.

In the Anaerobic Training Group, no differences were found in the activity of Superoxide Dismutase and Glutathione S-Transferase, while a significant increase in Glutathione Peroxidase and a significant decrease in Catalase activity were observed as shown in Table 2.

## DISCUSSION

Glutathione is an important endogenous protection system against cell damage caused by ROS. It is present in high concentrations in mammalian cells, including humans, in its reduced form (~99%), together with much smaller amounts of the oxidized form (~1%).<sup>5</sup> This ubiquitous tripeptide is continuously restored in all cellular compartments, acting relatively rapidly against ROS formation, especially in blood, after aerobic or anaerobic training.<sup>13</sup> It should be noted that relatively small increases (2-3%) in the cellular content of Oxidized Glutathione can promote severe oxidative stress damage.<sup>5</sup>

In the present study Total Glutathione levels were significantly lower after the aerobic training session, similarly to findings in soccer players after a complete daily routine of combined aerobic and anaerobic exercises.<sup>13</sup> In contrast, Vider et al.<sup>47</sup> showed enhanced Total Glutathione levels after a treadmill performance in endurance athletes; Sahlin et al.<sup>43</sup> also found enhanced Total Glutathione levels after cycling performances in trained athletes compared to sedentary subjects; this may reflect such glutathione mobilization and synthesis. The duration of 8 and 4h of the study of Schwingel et al.<sup>13</sup> and the present study, respectively, were probably not sufficient to allow for the detection of either the hepatic mobilization or the use of glutathione in other tissues such as muscles, or the *de novo* synthesis in liver of this endogenous antioxidant.

Because ROS generation is enhanced during or after an aerobic exercise, a parallel increase in peroxidative damage to lipids would also be expected.<sup>9</sup> Several studies indicate that such lipid oxidation byproducts (usually measured as TBARS or malondialdehyde levels) are increased after acute intensive exercise.<sup>30-35</sup> In our study TBARS levels showed no significant variation after the aerobic or anaerobic training sessions. Other reports

**Table 1** – Plasma levels of TBARS and whole blood levels of GSSG, GSH and TG in soccer players before and after aerobic and anaerobic training session

	Aerobic session		Anaerobic session	
	before	after	before	after
TBARS (nmol ml <sup>-1</sup> )	8.68 ± 0.35	7.84 ± 0.92	9.48 ± 0.64	10.24 ± 1.5
GSSG (µM)	0.40 ± 0.08	0.24 ± 0.07	0.39 ± 0.08	0.24 ± 0.06
GSH (µM)	0.98 ± 0.03	0.80 ± 0.02 <sup>b</sup>	0.93 ± 0.04	0.90 ± 0.03
TG (µM)	1.31 ± 0.09	1.04 ± 0.06 <sup>b</sup>	1.26 ± 0.09	1.09 ± 0.07

TBARS: Thiobarbituric Acid-Reactive Substances; GSH: Reduced Glutathione; TG: Total Glutathione; GSSG: Oxidized Glutathione Values are means ± SD (both groups n = 09). \*p ≤ 0.05 and <sup>b</sup>p ≤ 0.01 means significant differences between before versus after aerobic or anaerobic training session of the same treatment group

**Table 2** – Contents of erythrocytic antioxidant enzymes (CAT, SOD, GST, GR and GPx)

	Aerobic session		Anaerobic session	
	before	after	before	after
CAT (mmol min <sup>-1</sup> ml <sup>-1</sup> )	2.21 ± 0.20	2.27 ± 0.24	2.92 ± 0.38	1.80 ± 0.15 <sup>a</sup>
SOD (U SOD ml <sup>-1</sup> )	80.15 ± 2.05	67.20 ± 2.70 <sup>b</sup>	70.73 ± 2.42	70.73 ± 2.81
GST (μmol min <sup>-1</sup> ml <sup>-1</sup> )	72.08 ± 5.46	108.23 ± 11.82 <sup>a</sup>	51.42 ± 4.49	55.00 ± 3.99
GR (μmol min <sup>-1</sup> ml <sup>-1</sup> )	49.58 ± 2.79	52.57 ± 3.88	103.10 ± 7.33	109.35 ± 9.91
GPx (μmol min <sup>-1</sup> ml <sup>-1</sup> )	96.79 ± 9.93	463.02 ± 42.21 <sup>b</sup>	63.03 ± 6.11	331.82 ± 23.10 <sup>b</sup>

CAT: Catalase; SOD: Superoxide Dismutase; GST: Glutathione S-Transferase; GR: Glutathione Reductase; GPx: Glutathione Peroxidase. Values are means ± SD (both groups n = 09). ap ≤ 0.05 and bp ≤ 0.001 means significant differences between before versus after aerobic or anaerobic training session of the same treatment group.

on different types of aerobic exercise also revealed unchanged<sup>26-29</sup> or even decreased TBARS levels,<sup>27</sup> depending on the duration and type of exercise. Zoppi et al.<sup>36</sup> examined young male soccer players in a pre-season scenario, who performed uniform training loads during a three-month period (aerobic, strength and anaerobic). They found significant increases in plasma TBARS contents in a control group (non supplemented), but lower contents in a test group, supplemented with vitamins E and C. Similarly, Maleki et al.<sup>8</sup> evaluating male road cyclists after a 2 h match also detected enhanced malondialdehyde (the main product of the lipoperoxidation process) formation in plasma. Similar results have been reported in children, after moderate swimming,<sup>37</sup> as well as in several other types of exercise.<sup>14,15,38</sup>

A previous study from our laboratory<sup>13</sup> showed elevated levels of TBARS levels in the plasma of soccer players after daily 8 hr. routine training sessions which combined aerobic and anaerobic types exercises in alternation. The discrepancy vis-à-vis our present study is probably attributable to the double duration of the previous training session. Such discrepancies had already been reported in other related studies measuring other oxidative stress parameters, which also comprised many different experimental designs, intensity, conditions of sample harvesting and type of exercise, among others.<sup>26,27,29,36,40</sup> In this context, Gravina et al.<sup>40</sup> examining female soccer players showed that the three important antioxidant enzymes, namely Superoxide Dismutase, Glutathione Peroxidase and Reductase, increased their activity when comparing pre- and post-match moments (18 hours apart).

In the present study Oxidized Glutathione levels showed no significant differences after aerobic or anaerobic training session. However, some other studies showed elevated lipoperoxidation markers, suggesting that both short-term and prolonged exercises (anaerobic, aerobic or severe chronic aerobic performances), favor the conversion of Reduced to Oxidized Glutathione.<sup>42,43</sup> Andersson et al.<sup>10</sup> investigated markers of oxidative stress in elite female soccer players in response to a 90-min game, and found that this exertion induced a significant acute increase in Oxidized Glutathione concomitant to a decrease in

Reduced Glutathione contents. Enhanced blood Oxidized Glutathione was also detected after two soccer games with a 72 h recovery interval.<sup>11</sup> The Reduced/Oxidized Glutathione ratio also decreased immediately after a moderate exercise<sup>42</sup> as well as after a run lasting 2-5h.<sup>39</sup> Interestingly, after 3 days of recovery from ergometer cycling (65% of maximal VO<sub>2</sub>) lasting 90 min produced an elevation of Reduced Glutathione levels in blood.<sup>45</sup> A similar result was found after swimming activity lasting for 1, 10 or 60 days.<sup>46</sup>

Regarding the antioxidant enzymes, following the aerobic training session, we found no difference of Catalase activity, while Superoxide Dismutase activity decreased. However, after the anaerobic training session an inverse relation was observed, with Catalase activity significantly decreased, while Superoxide Dismutase activity remained unchanged. Related studies showed a variety of results after different sport practices: increased Catalase activity after aerobic exercise (40 min riding) of moderate intensity (Heart Rate 65-75% of maximum);<sup>44,49</sup> increased Superoxide Dismutase activity after prolonged training aerobic exercises;<sup>33,41,46,50</sup> one study reported that 16 weeks of intensive cycling promoted decreases in the activity of Superoxide Dismutase and Catalase, which remained low after 30 days of recovery;<sup>8</sup> another study reported increased Superoxide Dismutase and unaltered Catalase activity after an anaerobic session of strenuous jumping.<sup>29</sup> A similar study on soccer young players also found a systemic oxidative stress after performing an intermittent high intensity exercise<sup>51</sup>, in which SOD and CAT activity were increased, apparently to compensate the increased levels of lipid (MDA levels) and protein oxidation. Other similar study using a protocol of running test that resembles soccer activity, MDA levels were also enhanced in the early stage of recovery.<sup>52</sup>

We found increased Glutathione S-Transferase activity after the aerobic training, but no difference after the anaerobic test. But after complete and combined daily sessions in soccer players Glutathione S-Transferase activity was decreased.<sup>13</sup> Glutathione S-Transferase is an important dual detoxification enzyme,<sup>5</sup> facilitating the excretion of compounds derived from the xenobiotic biotransformation catalyzed by the superfamily of CYP<sub>450</sub>, and also detoxifying

hydroperoxides generated endogenously in the process of lipid oxidation.<sup>5</sup> As a consequence, an increase in Glutathione S-Transferase activity is expected to occur during and after intense aerobic exercise. Nevertheless, Glutathione S-Transferase continuously uses Reduced Glutathione as a cofactor, and considering that its content is decreased after aerobic exercise, as found in our study, a decrease in the transferase activity is also expected to occur after prolonged periods of exercise, if no induction of this enzyme and no recovery of Reduced Glutathione occurs. Indeed, laboratory animals exhibited enhanced and persistent Glutathione S-Transferase as well as SOD activity during 60 days of continuous exercise, while Catalase and Glutathione Reductase activity remained unaltered together with persistent augmented malondialdehyde levels, thereby revealing the importance and ability of Glutathione S-Transferase to detoxify hydroperoxides.<sup>46</sup>

The recovery of oxidized glutathione back to reduced glutathione is accomplished by Glutathione Reductase; this is an essential step to maintain a cell protection system related to the so-called glutathione cycle. Therefore, an increase in Glutathione Reductase activity would be expected after chronic exercise of long duration. Accordingly, some studies have reported significant increases in Glutathione Reductase (and also in GPx) after exhaustive exercise<sup>15</sup> or at the end of the competitive season,<sup>34</sup> while at the pre-season and middle of the competitive season<sup>44</sup> Glutathione Reductase activity was decreased. In the present study, Glutathione Reductase showed no significant differences between both types of training, while Jenkins<sup>44</sup> showed decreased activity after exercise. Again, the different responses obtained are probably attributable to the duration, pre-training and type of exercise among other aspects already mentioned above.<sup>15</sup>

We detected increased Glutathione Peroxidase after aerobic and anaerobic sessions. These findings are consistent with other related studies.<sup>15,33,38,42,49</sup> As with Glutathione S-Transferase, the Glutathione Peroxidase is also relevant to detoxification of hydroperoxides, also using Reduced Glutathione as a cofactor, therefore the depletion of this important tripeptide affects the responses of both antioxidant enzymes.<sup>5</sup> As found for Glutathione Reductase activity, Glutathione Peroxidase was upregulated only at the end of the competitive season, while it was downregulated at the pre-season and middle of the competitive season.<sup>34</sup>

As a conclusion, our results provide evidence of a more pronounced systemic oxidative stress after the aerobic training session compared to the anaerobic training session in young soccer players. The different types of exercises and sport practice involve different degrees of anaerobic and aerobic metabolism. Therefore, different qualitative as well as quantitative responses regarding oxidative stress and antioxidant adaptations should be expected, which may explain the apparent conflicting reports available in the related literature.

## ■ PRACTICAL APPLICATIONS

During a seasonal training period soccer players as well as other athletes, are facing different physical, physiological and biochemical challenges that can jeopardize the concerted antioxidant capacity of different tissues, leading to a systemic oxidative stress condition. As a perspective, it is here suggested that the training sessions, irrespective of being predominantly aerobic or anaerobic, together with the constitutive antioxidant responses to different types of exercise, should be accompanied by an appropriate intake of dietary and/or supplemented nutritional antioxidants, according to several reports available in the literature.

## ■ CONCLUSION

The results provide evidence of a more pronounced systemic oxidative stress after the aerobic compared to the anaerobic training session in young soccer players.

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## ■ AUTHOR PARTICIPATION

All authors contributed to the study design. Liberali R and Wilhelm Filho D contributed to the execution, data collection, analysis, creation and revision of the manuscript. Petroski ED contributed to revision of the manuscript.

## ■ CONFLICT OF INTEREST

The authors report no conflicts of interest.

## EFEITO SOBRE O ESTRESSE OXIDATIVO PROMOVIDO POR SESSÕES DE TREINAMENTO AERÓBICO COMPARATIVAMENTE A SESSÕES ANAERÓBICAS EM JOGADORES JUVENIS BRASILEIROS DE FUTEBOL

**OBJETIVO:** Investigar o efeito no estresse oxidativo promovido por sessão de treinamento aeróbico comparativamente à sessão anaeróbica em jogadores de futebol juvenis.

**MÉTODOS:** Amostras de sangue de 18 jogadores de futebol juvenis (idade entre 18-21 anos) foram utilizadas. Estas amostras foram obtidas imediatamente antes e após um conjunto de sessão de treinamento

aeróbico comparativamente ao de sessão anaeróbica e biomarcadores de dano celular como conteúdos de Substâncias Reativas a Ácido Tiobarbitúrico (TBARS) no plasma, os conteúdos de defesas antioxidantes não-enzimáticas como a Glutathione Reduzida, Glutathione Oxidada, e Glutathione Total, bem como as atividades de enzimas antioxidantes como Superóxido Dismutase, Catalase, Glutathione Redutase, Glutathione Peroxidase e Glutathione S-Transferase foram avaliadas.

**RESULTADOS:** Os conteúdos de TBARS e Glutathione Oxidada não apresentaram diferenças significativas na comparação entre ambas as sessões. Entretanto, após a sessão de treinamento aeróbico, as atividades da Superóxido Dismutase e Glutathione Redutase, bem como os conteúdos de Glutathione Reduzida e Glutathione Total mostraram diminuições significativas, enquanto que as atividades da Glutathione S-Transferase e Glutathione Peroxidase foram aumentadas e as da Catalase não mostraram diferenças. Por outro lado, após a sessão de treinamento anaeróbico, a atividade da Catalase reduziu-se, a da Glutathione Peroxidase foi significativamente aumentada, enquanto que as da Superóxido Dismutase e Glutathione Redutase, assim como os conteúdos de Glutathione Reduzida, Glutathione Oxidada e Glutathione Total não se alteraram significativamente.

**CONCLUSÃO:** Os resultados evidenciam um estresse oxidativo sistêmico mais acentuado após a sessão de treinamento aeróbico comparativamente à sessão anaeróbica em jogadores de futebol juvenis.

**PALAVRAS-CHAVE:** Espécies reativas de oxigênio; estresse oxidativo; antioxidantes; sessão aeróbica e anaeróbica; jogadores de futebol.

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