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Plasma antioxidant responses and oxidative stress following a soccer game in elite female players

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We aimed to investigate markers of oxidative stress and levels of endogenous and dietary antioxidants in 16 elite female soccer players in response to a 90-min game (average intensity $82 \pm 3\%$ HR_{peak}). Blood samples were taken before, immediately and 21 h after the game. Plasma-oxidized glutathione, the ratio of reduced to oxidized glutathione (GSH:GSSG) and lipid peroxidation measured by d-ROMs were used as markers of oxidative stress. Plasma endogenous [uric acid, total glutathione (TGS)] and dietary antioxidants (α -tocopherol, ascorbic acid, total carotenoids and polyphenols) were analyzed using liquid chromatography and the Folin–Ciocalteu method. Exercise induced an acute increase ($P < 0.05$) in GSSG, uric acid,

TGS, α -tocopherol, and ascorbic acid. In parallel, the GSH:GSSG ratio and polyphenols decreased ($P < 0.05$). GSSG, GSH:GSSG ratio, uric acid, TGS, and ascorbic acid returned to baseline at 21 h, while polyphenols and α -tocopherol remained altered. Total carotenoids increased above baseline only at 21 h ($P < 0.05$). Lipid peroxidation, measured by d-ROMs, remained unchanged throughout the study. Thus, intermittent exercise in well-trained female athletes induces a transient increase in GSSG and a decrease in the GSH:GSSG ratio, which is effectively balanced by the recruitment of both endogenous and dietary antioxidants, resulting in the absence of lipid peroxidation measured by d-ROMs.

An exercise stimulus has been shown to induce the production of free radicals, which may lead to oxidative stress and cause damage to cellular tissue (Davies et al., 1982). During exercise, whole-body oxygen consumption may increase up to 20-fold (Saltin & Åstrand, 1967), while oxygen consumption in active muscles may reach 100 times the resting level (Davies et al., 1982). Most of the oxygen consumption in skeletal muscle is used to form ATP and water ($\sim 95\%$), while the remaining 2–5% undergoes a reduction to produce superoxide radicals (Jackson, 1998). Under normal physiological conditions, the quantity of reactive oxygen and nitrogen species (ROS/RNS) is in a fine-tuned equilibrium with the antioxidant defense system, consisting of endogenous and dietary antioxidant compounds. Imbalances may be mediated by increased ROS/RNS production or depletion in the antioxidant defense system. Imbalances in favor of increased ROS/RNS may be followed by oxidative damage. Oxidative damage to cellular targets is characterized by a progressive change or degradation of biomolecules as lipids, proteins, and DNA, and the recruitment of defense mechanisms may prevent or limit these changes (Djordjević, 2004; Blomhoff, 2005).

Exhaustive exercise has been shown to produce excessive amounts of ROS/RNS, leading to oxidative stress (Sastre et al., 1992). However, in well-trained athletes, only limited production of ROS/RNS may occur. This may be explained by a well-adapted antioxidant defense system in athletes (Brites et al., 1999). Whether a balance between the production of ROS/RNS and the recruitment of antioxidant defense systems prevents the occurrence of lipid peroxidation in response to exercise in well-trained athletes is, however, not fully understood. Moreover, most studies on oxidative stress and antioxidant responses to exercise have been performed using prolonged endurance exercise protocols (Aguiló et al., 2005; Tauler et al., 2005). In this respect, the physiological load of intermittent exercise, such as soccer games, differs from a continuous steady-state exercise. Consequently, extrapolation of data from a continuous steady-state exercise to intermittent exercise should be made with caution. During a soccer game, the aerobic energy system is highly taxed (Bangsbo, 1994; Krstrup et al., 2005) and a high load is exerted on active muscles (Andersson et al., 2008). In this respect, increases in markers of oxidative stress in plasma following soccer games and soccer-specific

running protocols in male players have been suggested to occur (Kingsley et al., 2005; Ascensão et al., 2008; Ispirlidis et al., 2008; Tauler et al., 2008). Increased oxidative stress caused by training and games may compromise the exercise performance of players during a long season and especially during times with the most intensive match schedule (Ascensão et al., 2008). Understanding how oxidative stress and the antioxidant defense system respond following games in female players may therefore provide new information on the physiological effects of soccer games. It may also provide valuable information on the recovery processes following games, which is specifically important for women soccer players as there are only 2 days of recovery between games in international tournaments. Furthermore, it may also yield new information that can be used to scientifically address the issue related to the usage of antioxidant supplements by elite female players. Evidences in favor of increased antioxidant defense in plasma following soccer games in males have, in fact, been supported by some (Kingsley et al., 2005; Ascensão et al., 2008) but not all studies (Kingsley et al., 2005; Tauler et al., 2008). To our knowledge, the responses of antioxidant compounds and oxidative stress markers immediately and 21 h after an elite female soccer game have not been documented previously.

Given the above discussion, the aim of this study was to investigate the responses of plasma markers of oxidative stress [oxidized glutathione, ratio of reduced and oxidized glutathione (GSH:GSSG), lipid peroxidation measured by d-ROMs] and levels of endogenous [uric acid, total glutathione (TGSH)] and dietary (ascorbic acid, α -tocopherol, total polyphenols, and total carotenoids) antioxidants immediately and 21 h after intermittent exercise consisting of an international 90-min friendly soccer game.

Methods

Subjects

Twenty-two elite female soccer players played an international 90-min friendly game. The players participating in the study played in teams of the highest division in Sweden and Norway. Owing to economical restrictions, blood samples from 16 field players (height: 167 ± 5 cm, weight: 64 ± 2 kg, age: 22 ± 3 years, maximal oxygen consumption 54 ± 3 mL/kg/min) were randomly chosen for the analyses of markers of oxidative stress and antioxidant levels. The game was conducted in the middle of the soccer season, when the players are accustomed to playing games. Before the commencement of the study, the players had rested at least 3 days from games and 2 days from soccer training. The players were informed about the experimental procedures and possible discomforts associated with the study, and a written informed consent was obtained. The study was conducted according to the Declaration of Helsinki and approved by the Regional Ethic Committee of Uppsala, Sweden (pnr 2004: m-364).

Experimental design

Baseline values for oxidative stress markers and antioxidant levels were sampled at 10.00 hours, i.e., 1 h before the start of the soccer game (baseline). Blood samples were collected within 15–20 min (0 h) and 21 h (after breakfast at 10.00 hours the following morning) after the game. Plasma samples were analyzed for oxidized glutathione (GSSG) as a marker of oxidative stress, GSH:GSSG ratio as an indicator of redox status (Powers & Jackson, 2008) and the d-ROMs test as a marker of lipid peroxidation (Cesarone et al., 1999). Additionally, two endogenous antioxidants (uric acid and TGSH) and several dietary antioxidants (ascorbic acid-AA-, α -tocopherol, polyphenols and carotenoid compounds) were analyzed in plasma. We only measured two endogenous antioxidants in the present study. However, the activity of the enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are major constituents of the endogenous antioxidant defense system (Ji, 2000). Therefore, the term “endogenous defence system” in the present study only refers to the response of TGSH and uric acid. Two days before the commencement of the study the players performed a maximal oxygen uptake test running on a treadmill. Throughout the study period, the players were supervised in order to standardize the physical activities and diet.

Diet

The food intake was standardized for all players during the study period, starting on the evening before the game. All players were given a meal plan formulated by a nutritionist. The players ate each meal together. The players were given a dinner on the night before the game, breakfast before the game, lunch and dinner after the game and breakfast on the following morning. The composition of the meals was developed using a national food database (“Food on data” 4.3 LKH, Norway). Carbohydrate (CHO) and protein intake were adjusted according to the players’ body weight ($55/60/65/70$ kg, respectively) to meet the recommendations for daily recovery (intake of ≥ 6 g/kg body weight CHO and ≥ 1.2 g/kg body weight protein) (Maughan et al., 2004). The food was chosen to replicate the player’s normal diet as much as possible and did not contain any food items with known high antioxidant levels. The meal plan included a variation of bread, cereals, milk/yoghurts, meat, pasta/rice, fruit, and vegetables to ensure adequate intake of macro- and micronutrients. In addition, the players were instructed to drink a sports drink during the game providing approximately 30–60 g CHO/h (Maxim Energy™). The sports drink did not contain vitamin C. After the blood sample was taken at the end of the game, each player had a CHO intake of 1 g/kg body weight within 30 min to ensure optimal recovery (banana, yoghurt, and sports drink) (Maughan et al., 2004).

Evaluation of aerobic work load during the game

Heart rate was recorded to evaluate the aerobic work load during the game. The players wore a heart rate monitor around their chest, and data were continuously collected every 5 s (Polar Team System, Polar Electro OY, Kempele, Finland).

Biochemistry

Blood samples were collected from the antecubital vein at all time points (baseline, immediately and 21 h post game). Uric acid was analyzed using standard routine measurements in a

Modular P[®] Analyzer (Hitachi, Tokyo, Japan) with a CV of <5%. The Diacron reactive oxygen metabolite (d-ROMs) test was used to assess lipid peroxidation and was performed according to the manufacturer's instructions (Diacron International, Grosseto, Italy). The d-ROMs test monitors a rather persistent radical cation formed in the reaction of alkoxy and peroxy radicals derived from the hydroperoxides with a suitable additive *N, N*-diethyl-*para*-phenylenediamine (DEPPD). Thus, the d-ROMs level is proportional to the serum hydroperoxide concentration in the plasma (Alberti et al., 2000). Hydroperoxides are products of the peroxidation of proteins, peptides, amino acids, lipids and fatty acids (Nakayama et al., 2007). The analysis was fully automated using a Technicon RA 1000 system (Technicon Instruments Corporation, New York). The test was carried out in the kinetic mode. Results are expressed in arbitrary units (CARR Units) (Cesarone et al., 1999) with a CV <10%. According to the manufacturer's instructions, a normal value for d-ROMs is between 250 and 300 CARR U in healthy individuals. In trained individuals, lower basal values have been reported (<250 CARR U) (Banfi et al., 2006). A value between 301 and 320 CARR U indicates a borderline condition of oxidative stress status, while a value above 320 CARR U indicates oxidative stress (Diacron International).

TGSH in the plasma was analyzed with the use of chemical reduction and according to the "homocysteine by HPLC" kit provided by Biorad Laboratories GmbH (Munich, Germany). Standard solutions prepared in PBS served as calibrators with a CV <5%. In comparison with whole-blood glutathione, plasma contains <1% glutathione (Ji, 2000). However, plasma glutathione can be used as a model for intracellular glutathione, as it is assumed that plasma glutathione reflects intracellular whole-blood glutathione. Reduced plasma glutathione constitutes about 50–70% of the total plasma glutathione while GSSG constitutes about 5–10% of total glutathione. Moreover, the fraction of TGSH in our study includes components other than pure glutathione, such as adducts of plasma proteins, while GSH and GSSG is free plasma glutathione. Hence, the observed value of TGSH is higher compared with GSH and GSSG. Quantification of endogenous oxidized glutathione (GSSG) and reduced glutathione (GSH) was performed using a two-dimensional chromatographic system with parallel Hypercarb columns coupled with dual fluorescence detectors (FLD). After sample preparation as described previously (Sakhi et al., 2006), 10 µL of the supernatant was injected into the chromatographic system. Derivatization of GSSG was performed using monobromobimane (MBB) and ortho-phthalaldehyde (OPA). The CV for the method was below 7%. The ratio of GSH:GSSG was calculated by dividing GSH with GSSG.

For the determination of ascorbic acid in plasma, heparinized plasma was immediately acidified using an equal volume of 10% MPA and stored at –70 °C until analysis within 3 months. Samples were analyzed by high-performance liquid chromatography (HPLC) (Karlsen et al., 2005, 2007) with a CV below 5%. Plasma calibrators quantified against the NIST 970 SRM served as standards. For the determination of α -tocopherol by HPLC, proteins were precipitated by the addition of 3 volumes of isopropanol, followed by centrifugation at 3000 g at 4 °C for 15 min. The internal standard tocol was added with the isopropanol and 5 µL of the clear supernatant was used for analysis (Richheimer et al., 1994). A fluorescence detector operated at 295 (ex) and 330 (em) was used for the detection with a CV below 5%. Standards prepared in 1% bovine serum albumin in phosphate-buffered saline were used for quantification. For analysis of total polyphenols, 50 µL heparinized plasma were mixed with 150 µL ethanol for 2 min, before centrifugation at

3000 g at 4 °C for 15 min. Fifty microliters of the clear supernatant was used for the Folin–Ciocalteu method as described previously (Maskarinec et al., 1999). Quercetin prepared in ethanol served as a standard solution, and the results for polyphenols are given as µmol/L quercetin equivalents (QE) with a CV below 10%. The total carotenoid is the sum of lutein, zeaxanthin, β -cryptoxanthin, α -carotene, β -carotene, and lycopene. They were determined in plasma by HPLC. Proteins were precipitated and removed by the addition of a 4.5 volume of isopropanol, followed by centrifugation at 3000 g at 4 °C for 15 min. The internal standard astaxanthin was added in the isopropanol. Twenty-five microliters of the clear supernatant was used for analysis. The mobile phases consisted of A: 20% water and 24% acetone in ethanol and B: acetone. The gradient conditions were as follows: from 2% to 100% B within 20 min, followed by 100% B for 15 min. Detection was performed at 453 nm using a variable wavelength detector. Plasma calibrators quantified against the NIST 968c SRM were used as standards and the CV for the method was below 5%.

Statistical analysis

A repeated-measure ANOVA for normally distributed data was used to test for possible significant time differences. When a significant time difference was identified, a Tukey's *post hoc* test was used. The d-ROMs data were not normally distributed, and were log-10 transformed before ANOVA. *P*-values below 0.05 were considered statistically significant. Data are presented as means and standard error of the mean (mean $\pm$ SEM), except for heart rate data, which are reported as means and standard deviation (mean $\pm$ SD). The Statistical Package for the Social Sciences (SPSS Inc., version 12.0) and Statistica (StatSoft Inc., version 7.0) were used for the analyses.

Results

Aerobic work load during the game

The average heart rate during the game was 163 ± 2 b.p.m., which corresponds to $82 \pm 3\%$ of HR_{peak}. The players spent on average 40 ± 4 min (Table 1) above 85% of HR_{peak} during the game, defined as a high-intensity work load (Bangsbo, 1994).

Markers of oxidative stress and antioxidant levels immediately after the game

The absolute GSSG concentration significantly increased immediately after the game ($30 \pm 9\%$, $P < 0.05$). No changes were observed in GSH,

Table 1. Amount of time (min) spent in different heart rate zones during the soccer game

HR zone (% of HR _{peak})	0–60	60–75	75–85	85–90	90–95	95–100
Total minutes (min)	3 ± 1	13 ± 2	35 ± 2	25 ± 2	13 ± 3	2 ± 1

Data are presented as mean $\pm$ SD.

Table 2. The response of oxidative stress markers, endogenous, and dietary antioxidant levels following an elite female soccer game

<i>n</i> = 16	Baseline values	Immediately post game	21 h post game
<i>Oxidative stress markers</i>			
GSSG ($\mu\text{mol/L}$)	0.047 ± 0.003	$0.056 \pm 0.004^*$	0.051 ± 0.003
GSH:GSSG	41.4 ± 3.1	$35.1 \pm 2.9^\#$	37 ± 2.7
d-ROMs (CARR U)	245 ± 25	249 ± 25	244 ± 24
<i>Endogenous antioxidant levels</i>			
Uric acid ($\mu\text{mol/L}$)	251 ± 14	$277 \pm 15^*$	254 ± 14
TGSH ($\mu\text{mol/L}$)	3.5 ± 0.1	$4.3 \pm 0.2^*$	3.9 ± 0.1
GSH ($\mu\text{mol/L}$)	1.9 ± 0.1	2.0 ± 0.2	2.0 ± 0.2
<i>Dietary antioxidant levels</i>			
Total polyphenols ($\mu\text{mol/L QE}$)	332 ± 10	$264 \pm 4^\#$	$245 \pm 12^\#$
Ascorbic Acid ($\mu\text{mol/L}$)	54 ± 3	$70 \pm 4^*$	58 ± 3
α -tocopherol ($\mu\text{mol/L}$)	20 ± 1	$23 \pm 1^*$	$22 \pm 1^*$
Lutein ($\mu\text{mol/L}$)	0.18 ± 0.02	0.19 ± 0.02	$0.23 \pm 0.03^*$
Zeaxanthin ($\mu\text{mol/L}$)	0.04 ± 0.00	0.04 ± 0.00	$0.06 \pm 0.01^*$
β -cryptoxanthin ($\mu\text{mol/L}$)	0.18 ± 0.03	0.19 ± 0.04	$0.23 \pm 0.06^*$
α -carotene ($\mu\text{mol/L}$)	0.13 ± 0.02	0.12 ± 0.02	0.15 ± 0.04
β -carotene ($\mu\text{mol/L}$)	0.51 ± 0.06	0.49 ± 0.06	0.58 ± 0.10
Lycopene ($\mu\text{mol/L}$)	0.56 ± 0.04	0.53 ± 0.04	0.65 ± 0.08
Total carotenoids ($\mu\text{mol/L}$)	1.6 ± 0.1	1.6 ± 0.1	$1.9 \pm 0.3^*$

Data are presented as mean $\pm$ SE.

$P < 0.05$.

*Significantly higher and

$^\#$ Significantly lower compared with baseline values.

whereas the GSH:GSSG ratio decreased ($13 \pm 7\%$, $P < 0.05$). No changes in d-ROMs levels were detected after the game (Table 2). This indicates that oxidized glutathione increases, and GSH:GSSG decreases immediately after the game without increases in the peroxidation levels measured by d-ROMs. The antioxidants TGSH ($23 \pm 7\%$), uric acid ($11 \pm 2\%$), AA ($33 \pm 7\%$), and α -tocopherol ($15 \pm 2\%$) significantly increased immediately after the game whereas total polyphenols significantly decreased ($18 \pm 3\%$) (Table 2). In contrast, the carotenoid compounds (lutein, zeaxanthin, β -cryptoxanthin, α -carotene, β -carotene, and lycopene) remained unchanged from baseline values (Table 2). This indicates an acute recruitment of both endogenous antioxidants and the majority of dietary antioxidants after the game.

Markers of oxidative stress and antioxidant levels at 21 h

The GSSG concentration and GSH:GSSG ratio returned to baseline levels at 21 h [Fig. 1(a)]. The d-ROMs levels remained unchanged. The endogenous antioxidant compounds uric acid and TGSH and the dietary antioxidant AA returned to baseline levels 21 h after the game [Fig. 1(b) and (c)]. In contrast, the total polyphenols remained significantly reduced whereas α -tocopherol remained elevated [Fig. 1(b)]. Total carotenoids significantly increased above baseline levels only at 21 h after the game, mainly due to

increases in lutein, zeaxanthin and β -cryptoxanthin (Table 2).

Discussion

The present study shows for the first time that 90 min of intermittent exercise in well-trained female soccer players induces a mobilization of both endogenous and dietary antioxidants in response to a transient increase in GSSG and a decrease in the GSH:GSSG ratio. The recruitment of the antioxidant defense seemed to prevent lipid peroxidation measured by d-ROMs. Furthermore, our results indicate that the dietary antioxidant polyphenols may play an important role in the dietary antioxidant defense during intermittent exercise.

Changes in plasma thiols, especially oxidized glutathione and GSH:GSSG ratio, have been used as markers of oxidative stress status in biological systems (Ji, 1999). Hence, the significant increase in GSSG and decrease in the GSH:GSSG ratio observed immediately after the game indicate increased free-radical production. Considering that 2–5% of the total $\dot{V}O_2$ results in the formation of ROS/RNS (Jackson, 1998) and that the players worked at an average intensity level of 82% of HR_{peak} (approximately 67% of $\dot{V}O_{2 peak}$), an increased production of ROS/RNS was expected during the soccer game. The observed increases in GSSG and decrease in the GSH:GSSG ratio are in agreement with data

Fig. 1. Changes in (a) GSSG, GSH:GSSG and GSH (b) dietary antioxidants (AA, α -tocopherol, carotenoids and polyphenols), and (c) endogenous antioxidants [uric acid and total glutathione (TGS)] immediately post and 21 h after the soccer game. Data represent percentage change from baseline values. *Significantly higher than baseline values, $P < 0.05$. #Significantly lower than baseline values, $P < 0.05$.

reported during various exercise protocols (Viguie et al., 1993; Chung et al., 1999; Watson et al., 2005). The changes in the GSSG levels and GSH:GSSG ratio were brief and returned to baseline within 21 h following the game. In parallel with the increase in GSSG and decreased GSH:GSSG ratio, an immediate mobilization of the antioxidant defense system occurred. To our knowledge, we report for the first time a significant decrease in total polyphenols immediately after exercise. This suggests that plasma polyphenols are instantly utilized to eliminate the increased levels of ROS/RNS. Polyphenols represent a large group of aromatic compounds and exist in most food items. Studies on polyphenols suggest that they have powerful antioxidant activities and are able

to scavenge a diversity of ROS species or even inhibit their formation (Halliwell et al., 2005; Morillas-Ruiz et al., 2006). Polyphenols are available in the circulation but are not known to be stored in peripheral tissues (Rechner et al., 2002). Based on our results, it is reasonable to suggest that polyphenols are part of the first line of defense against oxidative stress in well-trained athletes. However, the question of whether polyphenols are mobilized in sedentary subjects following intermittent exercise remains unknown.

In parallel with the decrease in polyphenols, we observed an increase in the endogenous antioxidant compounds uric acid and TGS. Similar changes in uric acid as well as the total antioxidant status have been shown after soccer games in male players (Bangsbo, 1994; Ascensão et al., 2008). Elevated levels of blood antioxidants at the end of an exercise bout may be explained by either reduced oxidant production at the end of the exercise, up-regulation of endogenous antioxidants and/or the mobilization of antioxidants from tissue stores as a result of increased free-radical production during exercise (Watson et al., 2005). As the up-regulation of uric acid and TGS occurred immediately after the game, these endogenous antioxidants seem to play important roles in the antioxidant defense during the acute phase following intermittent exercise. Uric acid functions as an antioxidant due to its free radical-quenching actions in plasma as well as in skeletal muscle (Hellsten et al., 1997). It has been suggested that the contribution of uric acid in the total plasma antioxidant capacity is approximately 35–65% (Wayner et al., 1987), indicating its importance in the antioxidant defense system. Increased plasma levels of glutathione during exercise have been proposed to originate from the liver, as liver glutathione is shown to decrease during exercise (Sen et al., 1992; Leeuwenburgh & Ji, 1996). The release of glutathione from the liver during exercise may be promoted by increased levels of catecholamines, glucagon, and vasopressin (Ji, 2000). As an antioxidant, glutathione can directly detoxify ROS, serves as a substrate for glutaredoxin to eliminate H_2O_2 and organic hydroperoxides and enhance the functional ability of other crucial antioxidants such as vitamin E and C (Sen & Packer, 2000). The TGS fraction measured in our study also includes other components such as adducts of plasma proteins. This explains the fact that the concentration of TGS is higher than GSSG.

The increased levels of the dietary antioxidants, α -tocopherol and AA observed immediately after the game suggest mobilization of tissue antioxidants into the circulation. The increase in AA is similar to what has been reported previously following 90-min soccer-specific intermittent running protocols in male subjects (Thompson et al., 2001; Kingsley et al.,

2005). It has been suggested that the contribution of AA and α -tocopherol to the total antioxidant capacity in plasma is about 0–24% and 5–10%, respectively (Wayner et al., 1987). Unlike our results, no changes were observed in plasma ascorbate and α -tocopherol immediately after a 60-min game in male players (Tauler et al., 2008). The observation of unchanged total carotenoids immediately after the game is in agreement with previous studies using male subjects after a soccer game and endurance biking (Aguiló et al., 2005; Tauler et al., 2005, 2008), and is likely explained by the slower release of carotenoids from their main storage in adipose tissue. Nevertheless, the contribution of carotenoids to the total antioxidant capacity pool is relatively unknown (Watson et al., 2005).

Interestingly, no significant changes were observed in the d-ROM levels immediately or 21 h after the game. Harma et al. (2006) have questioned the specificity and sensitivity of the d-ROMs test to measure oxidative stress in sports medicine (Harma et al., 2006). More specifically, it has been argued that the d-ROMs test detects ferroxidase activity (Erel, 2005). However, the relationship between d-ROMs test and ferroxidase activity has initially been described by the manufacturer. Indeed, Alberti et al. (2000) reported that the contribution of ceruloplasmin ferroxidase activity is small. First, it has been demonstrated that the oxidase activity of ceruloplasmin is diluted when the serum sample undergoes a 100-fold dilution, which is the case in the d-ROMs test. Second, the oxidase activity of ceruloplasmin is also inhibited by adding sodium azide to the system, thus eliminating the influence of ceruloplasmin on d-ROMs levels (Alberti et al., 2000). Moreover, it has been shown that females have lower levels of ceruloplasmin in the blood compared with males (Alberti et al., 2000), which further eliminates the influence of ceruloplasmin in our samples. Furthermore, the d-ROMs test has been validated by EPR spectrometry, which is considered as the golden standard method to measure oxidation–reduction phenomena *in vitro* (Alberti et al., 2000). Moreover, the d-ROMs test has successfully been validated in large populations of healthy subjects, including athletes, and is regarded as a reproducible method for the quantitative evaluation of the peroxidation of organic compounds (Iamele et al., 2002; Bonina et al., 2005; Banfi et al., 2006). It has also been argued that the non-automated handling of the samples results in imprecision of d-ROMs (Iamele et al., 2002). However, in the present study, the analysis was fully automated. According to the manufacturer's instructions, a value between 301 and 320 CARR U indicates a borderline condition of oxidative stress status, while a value above 320 CARR U indicates oxidative stress (Diacron Inter-

national). In the present study, the average d-ROMs value after the soccer game was 249 ± 25 CARR U, which indicates that no peroxidation had occurred.

Previous studies in male players show increased lipid peroxidation (MDA and TBARS) in the plasma following soccer games (Ascensão et al., 2008; Ispirlidis et al., 2008) and a soccer-specific running protocol (Kingsley et al., 2005). The lipid peroxidation levels measured by MDA and TBARS were elevated up to 72 h following the soccer games (Ascensão et al., 2008; Ispirlidis et al., 2008) while hydroperoxide was normalized within 24 h after the soccer-specific running protocol (Kingsley et al., 2005). The lack of lipid peroxidation measured by d-ROMs in our results compared with previous studies may be due to several factors. First, the players in our study were well trained and had a high training volume. Previous studies have demonstrated that regular training is associated with a strengthening of the endogenous antioxidant defense system and thus well-trained athletes probably have improved protection against oxidative insults as compared with sedentary subjects (Brites et al., 1999). Second, differences between our results and previous studies may be due to the fact that all previous studies were performed in males. In this respect, there are indications that estrogen hormones have antioxidant functions (Kendall & Eston, 2002) and may thus contribute to a more efficient antioxidant defense system in women compared with men. This is supported by a previous study in females showing that lipid peroxidation did not occur in response to exercise despite an increase in GSSG (Chung et al., 1999). Third, the observed increase in GSSG was paralleled by a robust antioxidant response. This indicates that free radical production was increased during the game, but was effectively quenched by a robust antioxidant defense system. This may explain the lack of significant increase in peroxidation measured by d-ROMs. Our results suggest a model where the concomitant increase in endogenous and dietary antioxidants in response to the transient increase in GSSG prevented the occurrence of lipid peroxidation measured by d-ROMs in well-trained female players (Fig. 2). It is also suggested that the antioxidant defense systems effectively quenched increased levels of ROS/RNS and hence inhibited excessive lipid peroxidation measured by d-ROMs.

GSSG levels returned to baseline 21 h after the soccer game in well-trained female players. This recovery pattern was paralleled by the recovery of the endogenous antioxidants (uric acid and TGSH). This suggests that the endogenous antioxidants are mainly involved in the early phase of the defense system against increased ROS/RNS. In contrast, uric acid was elevated for more than 72 h following games in male soccer players (Ascensão et al., 2008; Ispiri-

Fig. 2. Hypothetical model describing changes in oxidative stress markers and endogenous and dietary antioxidants following intermittent exercise in well-trained female soccer players.

dis et al., 2008). In the present study, one dietary antioxidant (AA) returned to baseline 21 h after the game while two others (total polyphenols and α -tocopherol) did not. This suggests that the response of the dietary antioxidants may last longer than that of the endogenous antioxidants. The long-term reduction of polyphenols can also be explained by the fact that they are not stored in peripheral tissues and may only be available in the circulation (Rechner et al., 2002). As such, polyphenols may not be replaced in the circulation as fast as other antioxidant compounds. Interestingly, we observed an increase in several carotenoid compounds that occurred 21 h after the game. The delayed increase in carotenoids could be due to their slow release from their main storage site (adipose tissue) following the game. Our findings suggest that in well-trained female athletes, the carotenoid response can serve to strengthen the plasma antioxidant defense system following strenuous exercise.

In conclusion, our results indicate that intermittent exercise in well-trained female soccer players induces a transient increase in oxidized glutathione and a reduced GSH:GSSG ratio accompanied by a robust response of the endogenous and dietary antioxidant defense systems. The orchestrated endogenous and dietary antioxidant responses in well-trained female

athletes helped to prevent the occurrence of lipid peroxidation measured by d-ROMs after a soccer game.

Perspectives

Antioxidant supplements are commonly used in soccer. Our results imply that female elite players have an effective and well-regulated antioxidant defense system. However, as our study has been performed on female players, our conclusion might not be applicable for male soccer. Nevertheless, based on our results, the use of antioxidant supplements in female soccer is questionable.

Key words: d-ROMs, glutathione, lipid peroxidation, polyphenols, intermittent exercise, endurance training.

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