Active recovery training does not affect the antioxidant response to soccer games in elite female players

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ABSTRACT

Changes in plasma endogenous and dietary antioxidants and oxidative stress markers were studied following two 90-min elite female soccer games separated by 72 h of either active or passive recovery. The active recovery group (n=8) trained for one hour at 22 and 46 h after the first game (low-intensity cycling and resistance training) while the passive group rested (n=8). Blood samples were taken before, immediately after, 21, 45 and 69 h after the first and immediately after the second game. The oxidative stress markers and antioxidants were not affected by active recovery. The oxidative stress marker oxidized glutathione increased by the same extent after both games, while the lipid peroxidation marker diacrons reactive-oxygen metabolites remained unchanged. The endogenous antioxidants total glutathione, uric acid and ferric reducing/antioxidant power assay increased immediately after both games with the same amplitude, while increases in cysteine, cysteine-glycine and total thiols reached significant levels only after the second game. The changes in dietary antioxidants after the first game were either rapid and persistent (tocopherols, ascorbic acid increased; polyphenols decreased) or delayed (carotenoids). This resulted in high pre-second game levels of tocopherols, ascorbic acid and carotenoids. Polyphenols returned to baseline at 69 h and were not affected by the second game. In conclusion, the soccer-associated dietary but not endogenous antioxidant defence is persistent. Similar acute oxidative stress and endogenous antioxidant responses and dissimilar dietary antioxidant reactions occur during two repeated female soccer games. Finally, the complex antioxidant response to soccer is not affected by active recovery training.

KEY WORDS: INTERMITTENT EXERCISE, TRAINING, RECOVERY, FREE RADICALS, FOOTBALL

INTRODUCTION

Strenuous intermittent exercise increases the production of reactive oxygen species (ROS) which subsequently activates antioxidant defence mechanisms in order to maintain homeostasis [1]. Disturbances in the balance between ROS production and the capacity in the antioxidant defence in favour of ROS production may lead to oxidative stress [2]. Excessive exercise induced oxidative stress is believed to be a factor leading to fatigue [3] and may thereby contribute to decreased performance during exercise and possibly to a delayed recovery process.

The intensive physical work performed during a soccer game relies heavily on both aerobic and anaerobic energy production [4]. Several performance parameters, such as sprint, jump ability and isokinetic strength are reported to be impaired following a soccer game in both male [5-6] and female players [7]. Additionally, increased oxidative stress has been reported following a single soccer game and a soccer specific running protocol in male players [5-6,8,9]. However, we have previously shown that a single soccer game in elite female players induces a parallel elevation of oxidized glutathione and several antioxidant compounds without changes in lipid peroxidation [10]. Whether the robust antioxidant response that occurred after a single soccer game in female players is maintained during repeated games is unknown. This issue is important to address given the fact that international women soccer tournaments only allow for two days of recovery between games. Such a short recovery period between two games underlies the growing interest in developing effective recovery strategies to improve performance.

Various strategies including massage, contrast-water immersion and active recovery training are used by elite athletes [11]. There are currently no studies available evaluating the effectiveness of active recovery training one day after a competitive soccer game. The theoretical benefits of active recovery training include an acceleration of the recovery time of neuromuscular and blood markers of physical stress as well as a quicker restoration of the redox balance and the inflammatory reaction. Accordingly, an efficient recovery would promote optimal competitive performance and may help elite players to cope with high training and game loads [11, 12]. In this respect, we have previously reported that active-recovery does not accelerate neuromuscular parameters [7] or affects the inflammatory mediators after elite female soccer games [13]. The effects of active-recovery training on oxidative stress markers and antioxidants during repeated elite soccer games remain unknown.

Antioxidants in repeated female soccer games

We have previously established the acute changes in plasma antioxidants and oxidative stress markers following a single soccer game. The aim of the present study was to compare the oxidative stress reaction and the response of endogenous and dietary antioxidants following two repeated female soccer games separated by 72 h of either passive or active recovery.

METHODS

Subjects. Twenty-two elite female soccer players from the highest division in Sweden and Norway played two 90-min games separated by 72 h. Three players were not available on the recovery days between the matches and one player was taking medication (insulin). Moreover, as the physical loading of goalkeepers differs from that of field players they were not included in the analysis. Thus, in total, blood samples from sixteen field players (height: 167±5 cm, weight: 64±2 kg, age: 22±3 yrs, maximal oxygen consumption 54±3 ml·kg^{-1}·min^{-1}) were used for the analysis of oxidative stress markers and antioxidant levels. The games were conducted in the middle of the soccer season when the players are accustomed to playing games. Prior to the commencement of the study the players had rested at least 3 days from games and two days from soccer training. None of the players were smokers. None of the players reported the use of anti-inflammatory drugs or antioxidant supplements. Approximately half of the players reported the use of oral contraceptives. The players who were not taking contraceptives had regular menstrual cycles. The menstrual cycle phase of participants in this study was not assessed. According to Akova et al. [14] the phase of the menstrual cycle might influence some of the antioxidant/pro-oxidant markers. It is interesting to note that Chung et al. [15] investigated the role of oestrogens in exercise-induced oxidative stress and found minimal differences in oxidative stress levels between the luteal and follicular phases during exercise.

The players were informed about the experimental procedures and possible discomforts associated with the study and gave their written informed consent to participate. The study was conducted according to policy statement set fourth in the Declaration of Helsinki and approved by the Regional Ethics Committee of Uppsala, Sweden.

Experimental Design. Two games were played during a period of four days. The players performed a standardised 20 min soccer-specific warm-up on the field prior to both games. The games were separated by two days of either active or passive recovery. A randomized blocked design was used to assign the players into an active recovery group (n=8) or a passive

recovery group (n=8). The groups were matched for age (23±4 yrs active, 22±3 yrs passive) height (167±6 cm active, 167±3 cm passive), weight (63.3±7.1 kg active, 65.0±4.9 kg passive), maximal oxygen consumption (55.4±3.6 ml·min^{-1}·kg^{-1} active, 53.8±2.4 ml·min^{-1}·kg^{-1} passive) and playing position. Blood was sampled prior (-3 h), within 15-20 min (0h), 21 h, 45 h, and 69 h after the first game and within 15-20 min after the second game (74h). A standardised breakfast was planed before the blood sampling. The players had breakfast 1 h prior to the blood sampling. We noted similar weather conditions during both games (light rain and ~12ºC). We have previously reported that the amount of high intensity running (1.09 (SE 0.2) vs 1.11 (SE 0.1) km), plasma CK levels (323 (SE 36) vs 376 (SE 53) U·L^{-1}), neuromuscular changes and fluid intake were similar in both games [7]. We have also previously reported that the mean heart rate was slightly higher ($p < 0.05$) in the second game (168 (SE 2) bpm) compared to the first game (162 (SE 2) bpm) [13].

Diet. The food intake was standardised during the study period, starting on the evening before the game. Intake of carbohydrate (CHO), protein and fat were adjusted 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/body weight protein) [16]. 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 (See [10] for more details).

Active recovery training. The active recovery training consisted of one hour of low-intensity exercise performed between the two games (22 h and 46 h after the first game). The training consisted of 30 min sub-maximal cycling (60% of HF*peak*) and 30 min low intensity resistance training (<50 % of 1 repetition maximum). During the period between the two games, all players were allowed to perform their habitual daily activities with the exception of participating in any kind of exercise. During the one-hour period when the active recovery group performed the low-intensity exercise, the control group was instructed to rest [7].

Biochemical measures for oxidative stress makers and antioxidant levels.

All analyses were performed on blood samples collected from the antecubital vein. Plasma samples were analysed for reduced (GSH) and oxidized (GSSG) glutathione as markers of oxidative stress and GSH:GSSG ratio as an indicator of plasma pro- and anti-oxidant redox status [17]. 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). The CV for the method was below 7%. The ratio of GSH:GSSG was calculated by dividing GSH with GSSG. The Diacrons reactive oxygen metabolites (d-ROMs) test was used to assess lipid peroxidation [18] and was performed according to the manufacturer's instructions (Diacron International, Grossetto, Italy). The analysis was fully automated, using a Technicon RA 1000 system (Technicon Instruments Corporation, NY, USA). The specificity and sensitivity of the d-ROMs test to measure oxidative stress have been questioned in sports medicine [19,20].The d-ROMs test has, however, been validated by EPR spectrometry, which is considered as the golden standard method to measure oxidation-reduction phenomena in vitro [21].Moreover, the d-ROMs test has also 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 [22-24].

In the present study, we only measured non-enzymatic endogenous antioxidants. The term 'endogenous defence system' in the present study only refers to the response of uric acid, glutathione (TGSH), cysteine, homocysteine and cysteine-glycine. The thiols, including glutathione (TGSH), cysteine, homocysteine and cysteine-glycine, were analysed in plasma with the use of chemical reduction and according the "homocysteine by HPLC" kit provided by Biorad Laboratories GmbH (Munich, Germany). Total thiols represent the sum of glutathione, cysteine, homocysteine and cysteine-glycine. The endogenous antioxidant uric acid was analysed in plasma with standard routine measurements with the use of a Modular P® Analyzer (Hitachi, Tokyo, Japan). Ferric reducing/antioxidant power assay (FRAP) were determined in plasma as described elsewhere [25]. For the determination of α-tocopherol and total 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 were used for analysis [26]. 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 the determination of ascorbic acid in plasma, heparinised 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 HPLC [27, 28] with a CV below 5%. Plasma calibrators quantified against the NIST 970 SRM served as standards. For analysis of total polyphenols, 50 µL heparinised plasma were mixed with 150 µL ethanol for 2 min, before centrifugation at 3000 g at 4 °C for 15 min. Fifty µL of the clear supernatant were used for the Folin-Ciocalteu method as previously described [29]. Quercetin prepared in

ethanol served as standard solution, and the results are given as µmol/L quercetin equivalents (QE) with a CV below 10%. Total carotenoids is the sum of lutein, zeaxanthin, β-krytpoxanthin, 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 µL of the clear supernatant were 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 analyses. A two-way repeated-measures ANOVA for normally distributed data was used to study changes in several parameters over time in the two groups and to compare the amplitude of changes before and after the first and second games. Where significant time differences were found, a Dunnett Post Hoc test (to compare data at individual time points to baseline values) or Tukey's Post Hoc were applied. Data that were not normally distributed were analysed using non-parametric Friedmans test with Dunn Post Hoc. P values below 0.05 were considered statistically significant. Data are presented as means and standard error of the mean (SE). 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

Effects of active recovery training. No differences were observed between the active and passive recovery groups in the levels of endogenous and dietary antioxidants or oxidative stress markers. There was, however a significant time effect for the response of both endogenous and dietary antioxidants as well as in oxidative stress markers. Therefore, changes observed after the games are presented as a mean for all 16 players in figure 1 (a-c) and figure 2 (a-d).

Oxidative stress markers. GSSG significantly increased with a similar magnitude after the first (30 (SE 9) %) and second games (42 (SE 9) %) (Fig. 1a), whereas GSH remained unchanged after both games. However, GSH decreased at 45 h (-25 (SE 5)%) and 69 h (-20 (SE 8) %) after the first game and thus the pre-second game GSH levels were lower than the

pre-first game levels (Table 3). As a result of increased GSSG and unchanged GSH the GSH:GSSG ratio decreased after both games. However, the decrease in GSH:GSSG ratio was statistically significant only after the first game (-13 (SE 7)%). The GSH:GSSG ratio returned to baseline 21 h after the first game and significantly decreased below baseline at 45 h and 69 h. Consequently, the GSH:GSSG ratio remained unchanged after the second game, possibly due to low pre-game values of GSH (Table 3). Lipid peroxidation measured by d-ROMs was not altered acutely during the games and remained unchanged at all time points except at 69 h when it was 7 (SE 2) % lower than baseline values (Table 3).

Endogenous (non-enzymatic) antioxidant compounds. Figure 1 (b-c) shows changes in TGSH and total thiols during the first and second games. The general response in the endogenous antioxidants was characterised by a robust increase immediately after both games. More specifically, both TGSH (Fig. 1b) and UA increased with similar magnitude after both games. FRAP also increased similarly after both games (Table 1). Although the magnitude of change for cysteine, cysteine-glycine, and total thiols was comparable following both games, the increase in these compounds reached a statistically significant level only after the second game (Table 1).

Dietary antioxidants. Figure 2 (a-d) shows acute changes in total tocopherols, total polyphenols, total carotenoids and AA before and after the games. The dietary antioxidant response pattern was characterized by either a rapid and persistent increase (α-tocopherol, total tocopherol and AA), decrease (polyphenols) or a delayed increase (carotenoids) after the first game. Alpha- tocopherol, total tocopherols and AA (Fig. 2a,d) increased immediately after the first game and remained elevated at all time points, except for a temporary normalisation of AA at 21 h. Consequently, the pre-second game levels of the dietary antioxidants AA, α-tocopherol and total tocopherol were significantly higher than pre-first game levels. Immediately after the second game, there were no further significant increases in total tocopherols and AA (Fig. 2a and 2d), whereas α-tocopherol slightly decreased (Table 2). The total carotenoid compounds are not involved in the acute antioxidant response to exercise, as there were no immediate changes in carotenoids after both the first and the second games (Fig. 2c). The increase in carotenoids was delayed and occurred several hours after the first game: lutein, zeaxanthin, and β-crytpoxanthin increased at 21 h and lycopene, α-carotene, and β-carotene increased only at 69 h after the first game (Table 2). Consequently, the pre-second

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game levels of the carotenoids lutein, β-crytpoxanthin, α-carotene, lycopene, total carotenoids were significantly higher than pre-first game levels. Finally, the level of total polyphenols significantly decreased immediately after the first game, remained reduced until 69 h and was not affected by the second game (Fig. 2b).

DISCUSSION

In the present study we established for the first time the response pattern of oxidative stress markers and antioxidant compounds during two repeated elite female soccer games separated by 72 h active or passive recovery. The main findings were that similar acute oxidative stress and endogenous antioxidant responses and dissimilar dietary antioxidant responses occurred after two repeated elite female soccer games. Furthermore, the dietary but not the endogenous antioxidant response to a female soccer game is persistent. Combined endogenous and dietary antioxidant defences seem to prevent lipid peroxidation during two repeated elite female soccer games. Finally, our findings do not support the beneficial role of active recovery training on the antioxidant response in elite female soccer players.

The active recovery training consisting of cycling (60% HRpeak) and resistance training (< 50% 1 RM) has no influence on the recovery of oxidative stress markers or antioxidant levels during repeated soccer games in elite female players. Our findings are in accordance with our previous data suggesting the lack of effects of active recovery training on the recovery pattern of specific neuromuscular fatigue parameters, blood markers of physical stress markers [7] and inflammatory markers [13]. In accordance with our results, recovery strategies as active recovery or cold water immersion do not accelerate the recovery of several neuromuscular or biochemical markers following intermittent exercise [30,31,32,33]. Furthermore, the game-performance (measured as mean HR and time > 85 HR*peak*) was similar in both games and did not differ between the players in the active or passive recovery group. Regardless of the recovery regime, the players were thus able to perform at a high intensity during the second game. This further supports the finding of a lack of effect of active recovery training on any biological marker used in this study.

Increases in GSSG are associated with exercise intensity [34] and the low-intensity recovery training in the time period after the first game did not affect the accumulation of GSSG. Importantly, although the effects of active recovery training have been evaluated using a large battery of physiological and biological parameters [7,13], the possibility that this

recovery strategy might have affected other parameters not evaluated in this study can not be excluded.

A main finding from this study was that the exercise intensity of both games mediated oxidation of GSH, thereby causing increased GSSG levels. The oxidation of GSH to GSSG is a sensitive marker of free radical production since GSH donates a pair of hydrogen ions to neutralise peroxides [35]. When the oxidation of GSH to GSSG exceeds the enzymatic reducing capacity GSSG is exported from the muscle cell (as well as other cells such as liver cells) to maintain GSH:GSSG ratio in plasma. Thus, the increased GSSG and reduced GSH:GSSG ratio observed after both games implies that free radical production was enhanced. The increase in GSSG is similar to what has been observed in several studies following exercise [36-37]. It has been suggested that increased levels of free radicals during exercise may cause reduced force production [17]. We have previously shown that several neuromuscular parameters were reduced with similar amplitude after both games [7]. Thus, the increased GSSG may be related to the observed neuromuscular fatigue after the games. We did not, however, find a significant correlation between changes in CK levels and GSSG after the games.

Interestingly, we observed an increase in GSSG occurring despite unchanged GSH levels. This result can be explained by adequate GSH availability and possible replenishment from other compartments such as the liver. Similarly, an increase in blood GSSG without a concomitant decrease in GSH has previously been shown following exercise [34,38]. Although GSSG increased, lipid peroxidation measured by d-ROMs remained unchanged after the two games. This finding implies that antioxidant defence systems in well-trained female players can prevent lipid peroxidation during two repeated soccer games separated by 72-h recovery. Nevertheless, it is important to highlight the difficulties to detect reactive intermediates directly in vivo because of their short half-lives [39]. The d-ROMs test has been questioned [19, 20] and as other methods it has its advantages and disadvantages, which is the reason why no single measurement can adequately describe oxidative damage [2]. It is therefore suggested that the use of a battery of measurements is important to reliably monitor changes in oxidative stress [40]. The use of several biomarkers allowed us to highlight the fact that despite the lack of change in lipid peroxidation measured by d-ROMs increases in GSSG, decreases GSSG:GSH ratio together with increases in antioxidant levels occurred.

We observed that the soccer-induced increase in ROS was accompanied by a robust response in several antioxidant compounds after the games. In general, there was a similar acute response pattern of endogenous antioxidants following both games. This implies that

the involvement of the endogenous antioxidants in the early line of defence against ROS is maintained during two repeated soccer games. Our hypothesis is supported by the fact that UA and TGSH, both regarded as important plasma antioxidants, increased similarly following both games. We also observed increases in cysteine and cysteine-glycine after the games. The majority of GSH is synthesised in the liver and its release during exercise is promoted by increased levels of catecholamines, glucagon and vasopressin [41,42]. In the kidney, GSH is metabolized to glutamate, cysteine and glycine, which are subsequently released into the circulation [43]. Thus, the increase in cysteine and cysteine-glycine after both games further implies increased liver GSH turnover. However, although cysteine, cysteine-glycine and total thiols increased following both games, the amplitude of changes was statistically significant only in response to the second game. This might be explained by the lower pre-second game levels of thiols. Reduced thiols levels can be related to the progressive elevation in dietary antioxidants after the first game, which might have strengthened the total antioxidant defence capacity. In these circumstances the contribution of thiols to the total antioxidant defence capacity would be less important.

In general, compared to the endogenous antioxidants, the dietary antioxidants showed either a rapid and persistent increase (α-tocopherol, total tocopherols and AA), decrease (polyphenols) or a delayed increase (carotenoids) after the first game. The acute responses of the dietary antioxidants tocopherols, AA and polyphenols were dissimilar following the two repeated soccer games as the acute increase in tocopherols and AA and decrease in polyphenols seen after the first game did not occur after the second game. The acute increase of the tocopherols and AA and decrease of polyphenols after the first game indicate a role of dietary antioxidants in the early line of defence against increased ROS production. However, contrary to the rapid normalisation of the endogenous antioxidants, there was a persistent elevation of AA and tocopherols and reduction of polyphenols after the first game. AA and tocopherols compounds are provided by diet and stored in adipose tissue and only moderate amounts of the total body pool are found in the circulation. The delayed increase of these compounds following the first game may suggest that the soccer-induced elevation of ROS mediates their relocation in order to strengthen the endogenous antioxidant defence and restore the redox balance. This response occurred without alteration of the intake of the antioxidants during the study period.

Thus, the long-lasting changes of the dietary antioxidant compounds suggest that they are involved in the stabilisation and maintenance of homeostasis in the pro- and anti-oxidant redox balance several days following the first soccer game. These persistent changes might

explain the fact that these compounds did not further increase immediately after the second game and suggest sufficient levels of available dietary antioxidants in the circulation during the second soccer game. We also report the lack of acute changes in carotenoids following the soccer games. This is similar to the findings previously reported after intermittent exercise in males subjects [9] suggesting that carotenoids are not involved in the acute response to increased ROS after exercise. However, we observed a delayed increase of carotenoids starting 21 h after the first game. This recruitment pattern suggests that carotenoids are slowly mobilized from adipose tissue possibly to strengthen the antioxidant defence capacity in response to the soccer game.

We have previously shown that the two repeated soccer games, with comparable workload, induce similar reductions in sprint ability, isokinetic knee strength, and elevations in CK, urea and perceived muscle soreness [7]. The present study reveals that the endogenous antioxidants are similarly recruited during repeated soccer games whereas there are dissimilarities in the acute response of dietary antioxidants. Data on changes in oxidative stress and antioxidant markers in response to repeated soccer games in male and female players are scarce. However, in line with our findings, two repeated cycling exercise bouts in males showed similar changes in blood GSH, GSSG, plasma AA, CK, perceived exertion and lipid peroxidation [44,45]. In contrast, following repeated eccentric exercise sessions in untrained females, blunted CK, oxidative stress and antioxidant responses occurred during the second exercise session, a phenomenon labeled as the "repeated bout effect" [46]. Inconsistencies between these studies can be due to differences in the exercise protocols and the training status of the subjects [38].

In conclusion, the present study established for the first time the time course of changes in circulating oxidative stress and antioxidant systems during two repeated elite female soccer games. Combined endogenous and dietary antioxidant defences seem to prevent lipid peroxidation during two repeated elite female soccer games. The use of active recovery training in the period between two elite female soccer games has no impact on the oxidative stress and antioxidant systems.
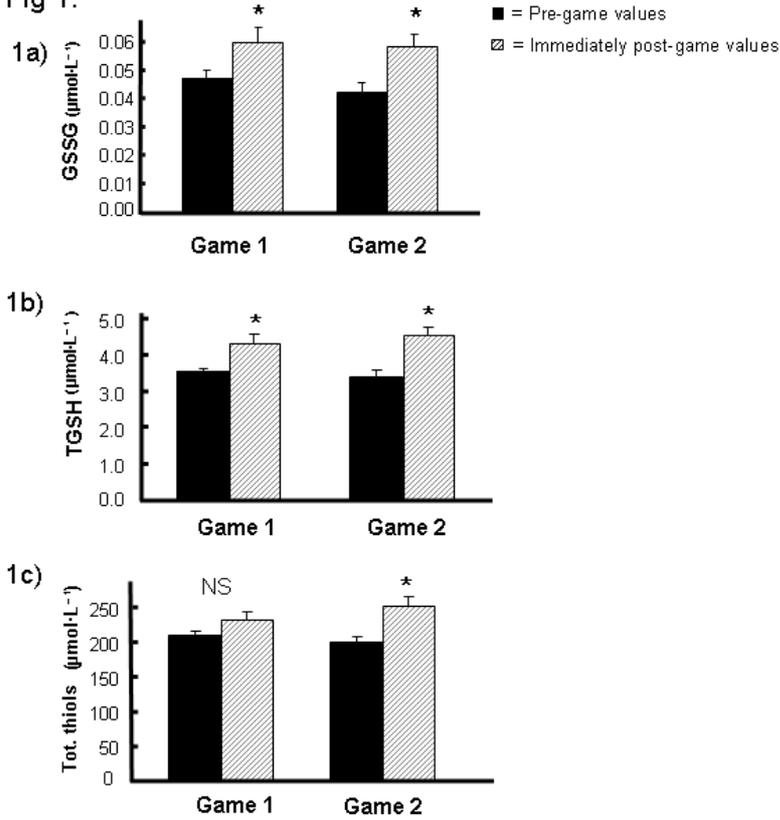
Figure 1 a-c. Pre- and immediately post- game values for GSSG, TGSH, and total thiols following two soccer games separated by 72 h in elite female players (n =16). * significantly higher than pre-game values, NS; no significant changes.
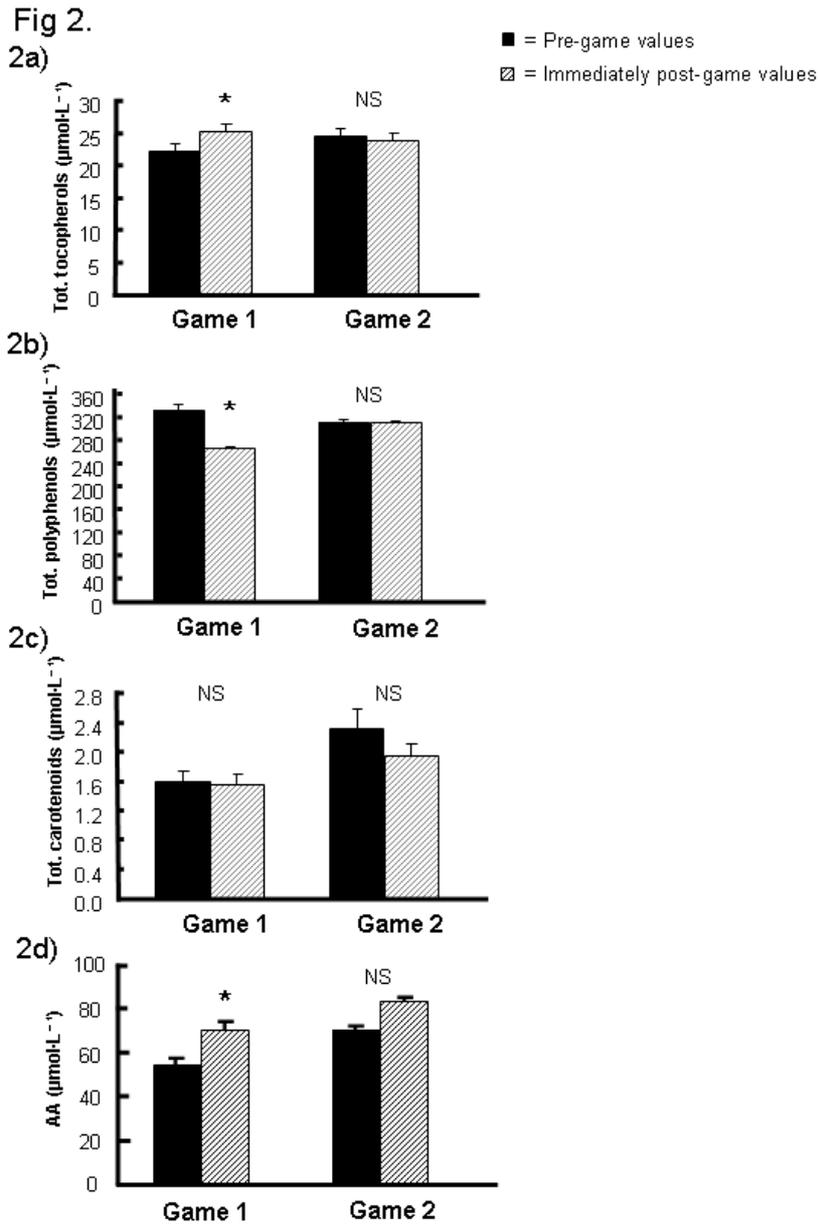
Figure 2 a-d. Pre- and immediately post- game values for the dietary antioxidants total tocopherols, total polyphenols, total carotenoids and AA following two soccer games separated by 72 h in elite female players (n =16). * significant changes compared to pre-game values, NS; no significant changes.

TABLE 1. Endogenous antioxidants and FRAP response after two soccer games separated by 72 h active or passive recovery.

Endogenous antioxidant compounds (µmol/L)	Baseline (-3 h)		IP game 1 (0 h)		Day 2 (21 h)		Day 3 (45 h)		Pre- game 2 (69 h)		IP game 2 (74h)		ANOVA		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	G	T	G&T
Cysteine (n=16)	180	4	200	10	184	6	183	5	172	8	217 *	12		x	
Homo-cysteine (n=16)	9.1	1.2	9.2	1.0	8.8	1.0	9.0	1.2	8.1	1.0	9.4	1.1		x	
Cysteine-glycine (n=16)	17.3	0.7	19.7	2.0	17.6	0.6	17.4	0.7	15.6	0.9	20.4 *	1.2		x	
Uric acid (n=16)	246	14	273 *	15	259	12	257	11	253	10	288 *	11		x	
FRAP (n=16)	1450	39	1588 *	39	1416	34	1379	31	1360	40	1484 *	48		x	

IP: immediately post game; G: significant effect of recovery group; T: significant time effect; G & T: significant interaction effect between the two ANOVA factors. * significantly higher compared to baseline values and † significantly lower compared to baseline values ($P < 0.05$). Data are presented as mean ± SE.

TABLE 2. Dietary antioxidant response after two soccer games separated by 72 h active or passive recovery

Dietary antioxidant compounds (μmol/L)	Baseline (-3 h)		IP game 1 (0 h)		Day 2 (21 h)		Day 3 (45 h)		Pre- game 2 (69 h)		IP game 2 (74h)		ANOVA		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	G	T	G&T
α-topcopherol (n =16)	20.2	1.1	23.0 *	1.2	22.4 *	1.2	21.8 *	0.9	22.1 *	1.1	21.4	1.1		x	
Lutein (n=16)	0.18	0.02	0.19	0.02	0.23 *	0.03	0.22 *	0.03	0.21*	0.02	0.17	0.02		x	
Zeaxanthin (n=16)	0.04	0.00	0.04	0.00	0.06 *	0.01	0.05	0.00	0.04	0.00	0.03	0.00		x	
Lycopene (n=16)	0.56	0.04	0.53	0.04	0.65	0.08	0.71	0.08	0.98 *	0.12	0.77 *	0.05		x	
β-kryptoxanthin (n=16)	0.18	0.03	0.19	0.04	0.23 *	0.06	0.21	0.04	0.24 *	0.04	0.20	0.03		x	
α-carotene (n=16)	0.13	0.02	0.12	0.02	0.15	0.04	0.16	0.03	0.18 *	0.03	0.14	0.02		x	
β-carotene (n=16)	0.51	0.06	0.49	0.06	0.58	0.10	0.59	0.09	0.67 *	0.10	0.64 *	0.07		x	

IP: immediately post game; G: significant effect of recovery group; T: significant time effect; G & T: significant interaction effect between the two ANOVA factors. * significantly higher compared to baseline values and † significantly lower compared to baseline values ($P < 0.05$). Data are presented as mean ± SE.

TABLE 3. Oxidative stress markers and GSH response after two soccer games separated by 72 h active or passive recovery

Oxidative stress markers (µmol/L)	Baseline (-3 h)		IP game 1 (0 h)		Day 2 (21 h)		Day 3 (45 h)		Pre- game 2 (69 h)		IP game 2 (72 h)		ANOVA		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	G	T	G&T
GSH (n=16)	1.9	0.1	2.0	0.2	2.0	0.2	1.4 †	0.1	1.5 †	0.1	1.7	0.1		x	
GSH:GSSG ratio (n=16)	41	3	35 †	3	37	3	30 †	2	34 †	2	29 †	2		x	
d-ROMs (CARR. U) (n=16)	245	25	249	25	244	24	246	24	225 †	21	240	22		x	

IP: immediately post game. G: significant effect of recovery group; T: significant time effect; G & T: significant interaction effect between the two ANOVA factors. * significantly higher compared to baseline values and † significantly lower compared to baseline values (P < 0.05). Data are presented as mean ± SE.

REFERENCES

1. Banerjee AK, Mandal A, Chanda D *et al.* (2003) Oxidant, antioxidant and physical exercise. *Mol Cell Biochem* **253**, 307-312.
2. Nikolaidis M, Jamurtas A, Paschalis V, *et al.* (2008) The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress: magnitude and time-course considerations. *Sports Med* **38**, 579-606.
3. Reid M, Khawli F, Moody M (1993) Reactive oxygen in skeletal muscle. III. Contractility of unfatigued muscle. *J Appl Physiol* **75**, 1081-1087.
4. Bangsbo J (1994) Energy demands in competitive soccer. *J Sport Sci* **12**, S5-S12.
5. Ascensão A, Rebelo A, Oliviera E *et al.* (2008) Biochemical impact of a soccer match-analysis of oxidative stress and muscle damage markers throughout recovery. *Clin Biochem* **41**, 841-851.
6. Fatouros I, Chatzinikolaou A, Douroudos I *et al.* (2009) Time-course of changes in oxidative stress and antioxidant status responses following a soccer game. *J Strength Cond Res* **24**, 1389-1398.
7. Andersson H, Raastad T, Nilsson J *et al.* (2008) Neuromuscular fatigue and recovery in elite female soccer: Effects of active recovery. *Med Sci Sports Exerc* **40**, 372-380.
8. Kingsley M, Wadsworth D, Kilduff L *et al.* (2005) Effects of phosphatidylserine on Oxidative Stress following Intermittent running. *Med Sci Sports Exerc* **37**, 1300-1306.
9. Tauler P, Ferrer M, Sureda A *et al.* (2008) Supplementation with an antioxidant cocktail containing coenzyme Q prevents plasma oxidative damage induced by soccer. *Eur J Appl Physiol* **104**, 777-785.
10. Andersson H, Karlsen A, Blomhoff R *et al.* (2009) Plasma antioxidant responses and oxidative stress following a soccer game in elite female players. *Scand J Med Sci Sports* 23, (Epublication ahead of print version).
11. Barnett A (2006) Using Recovery Modalities between Training Sessions in Elite Athletes: Does it help? *Sports Med* **36**, 781-796.
12. Reilly T, Ekblom B (2005) The use of recovery methods post-exercise. *J Sports Sci* **23**, 619-627.
13. Andersson H, Bøhn SK, Raastad T *et al.* (2009) Differences in the inflammatory plasma cytokine response following two elite female soccer games separated by a 72-h recovery. *Scand J Med Sci Sport* **17**, (Epublication ahead of print version).
14. Akova B, Sürmen-Gür E, Gür H *et al.* (2001) Exercise-induced oxidative stress and muscle performance in healthy women: role of vitamin E supplementation and endogenous oestradiol. *Eur J Appl Physiol* **84**, 141-147.
15. Chung S, Goldfarb A, Jamurtas A *et al.* (1999) Effect of exercise during the follicular and luteal phase on indices of oxidative stress in healthy women. *Med Sci Sports Exerc* **31**, 409-413.
16. Maughan R, Burke L, Coyle E (2004) Food, Nutrition and Sports Performance II. In *The International Olympic Committee consensus on sports nutrition*, pp. 24-49, 104-129. New York: Routledge.
17. Powers S, Jackson M (2008) Exercise-Induced Oxidative Stress: Mechanisms and Impact on Muscle Force Production. *Physiol Rev* **88**, 1243-1276.
18. Cesarone M, Belcaro G, Carratelli M *et al.* (1999) A simple test to monitor oxidative stress. *Int Angiol* **18**, 127-130.
19. Harma M, Harma M, Erel O (2006) d-ROMs test detects Ceruoplasmin, not oxidative stress. *Chest* **130**, 1276-1277.
20. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* **38**, 1103-1111.

21. Alberti A, Bolognini L, Macciantelli D *et al.* (2000) The radical cation of N,N-Diethyl-para-phenylendiamine: a possible indicator of oxidative stress in biological samples. *Res Chem Intermed* **26**, 253-267.

22. Banfi G, Malavazos A, Iorio E *et al.* (2006) Plasma oxidative stress biomarkers, nitric oxide and heat shock protein 70 in trained elite soccer players. *Eur J Appl Physiol* **96**, 483-486.

23. Bonina F, Puglia C, Cimino F *et al.* (2005) Oxidative stress in handball players: effect of supplementation with a red orange extract. *Nutr Res* **25**, 917-924.

24. Iamele L, Fiocchi R, Vernocchi A (2002) Evaluation of an Automated Spectrophotometric Assay for Reactive Oxygen Metabolites in Serum. *Clin Chem Labor Med* **40**, 673-676.

25. Benzie I, Strain J (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol* **299**, 15-27.

26. Richheimer S, Kent M, Bernart M (1994) Reversed-phase high-performance liquid chromatographic method using a pentafluorophenyl bonded phase for analysis of tocopherols. *J Chrom A* **677**, 75-80.

27. Karlsen A, Blomhoff R, Gundersen T (2005) High-throughput analysis of Vitamin C in human plasma with the use of HPLC with monolithic column and UV-detection. *J Chromatogr B Analyt Technol Biomed Life Sci* **824**,132-138.

28. Karlsen A, Blomhoff R, Gundersen T (2007) Stability of whole blood and plasma ascorbic acid. *Eur J Clin Nutr* **61**, 1233-1236.

29. Maskarinec G, Chan C, Meng L *et al.* (1999) Exploring the feasibility and effects of a high-fruit and -vegetable diet in healthy women. *Cancer Epidemiol Biomarkers Prev* **8**, 919-924.

30. Gill ND, Beaven CM, Cook C (2006) Effectiveness of post-match recovery strategies in rugby players. *Br J Sports Med* **40**, 260-263.

31. Rowsell G, Coutts A, Reaburn P *et al.* (2009) Effects of cold-water immersion on physical performance between successive matches in high-performance junior male soccer players. *J Sport Sci* **27**, 565-573.

32. Tessitore A, Meeusen R, Pagano R *et al.* (2008) Effectiveness of active versus passive recovery strategies after futsal games. *Strength Cond Res* **22**, 1402-1412.

33. Kinugasa T, Kilding A (2009) A comparison of post-match recovery strategies in youth soccer players. *J Strength Cond Res* **23**, 1402-1407.

34. Ji LL, Fu R, Mitchell EW (1992) Glutathione and antioxidant enzymes in skeletal muscle: effects of fiber type and exercise intensity. *J Appl Physiol* **73**, 1854-1859.

35. Sen CK (1999) Glutathione homeostasis in response to exercise training and nutritional supplements. *Mol Cell Biochem* **196**, 31-42.

36. Goldfarb A, McKenzie M, Bloomer R (2007) Gender comparisons of exercise-induced oxidative stress: influence of antioxidant supplementation. *Appl Physiol Nutr Metab* **32**, 1124-1131.

37. Paschalis V, Nikolaidis MG, Fatouros IG *et al.* (2007) Uniform and Prolonged Changes in Blood Oxidative Stress after Muscle-Damaging Exercise. *In Vivo* **21**, 877-883.

38. Kyparos A, Vrabas IS, Nikolaidis MG *et al.* (2009) Increased Oxidative Stress Blood Markers in Well-Trained Rowers Following Two Thousand-Meter Rowing Ergometer Race. *J Strength Cond Res* **23**, 1418-1426.

39. Urso M, Clarkson P (2003) Oxidative stress, exercise and antioxidant supplementation. *Toxicology* **189**, 41-54.

40. Halliwell B, Whiteman M (2004) Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? *Br J Pharmacol* **142,** 231-255.

41. Lu S, Garcia-Ruiz C, Kulenkamp J *et al.* (1990) Hormonal regulation of glutathione efflux. *J Biol Chem* **265**, 16088-16095.

42. Sies H, Graf P (1985) Hepatic thiol and glutathione efflux under the influence of vasopressin, phenylephrine and adrenaline. *Biochem J* **226**, 545-549.

43. Ji L (2000) Free Radicals and Antioxidants in Exercise and Sports. In *Exercise and Sport Science*, pp. 299-317 [W Garrett and D Kirkendall, editors]. Philadelphia: Lippincott Williams & Wilkins.

44. Viguie CA, Frei B, Shigenaga MK *et al.* (1993) Antioxidant and indexes of oxidative stress during consecutive days of exercise. *J Appl Physiol* **75**, 566-72.

45. Bloomer R, Falvo M, Fry A *et al.* (2006) Oxidative stress response in trained men following repeated squats or sprints. *Med Sci Sports Exerc* **38**, 1436-1442.

46. Nikolaidis M, Paschalis V, Giakas G *et al.* (2007) Decreased blood oxidative stress after repeated muscle-damaging exercise. *Med Sci Sports Exerc* **39**, 1080-1089.