NUTRITIONAL PROFILE AND OXIDATIVE STRESS IN ADOLESCENT SOCCER PLAYERS

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Abstract High-intensity exercise increases reactive oxygen species formation, which in excess may cause oxidative stress. We assessed nutritional status and exercise-induced oxidative stress in 20 adolescent male soccer players (age: 15–17). Participants were divided into two teams for a 60-minute friendly match and evaluated immediately before (Pre-match), 30 minutes after (Post-match I) and 24 hours after (Post-match II) the game. All players recorded a 3-day dietary intake. Biochemical tests were performed for lipid profile, muscle damage (creatinine and creatinine kinase [CK]) and oxidative stress (thiobarbituric acid-reactive substances [TBARS], protein carbonyls [PC], reduced glutathione [GSH], and vitamins E, C, and A). CK and creatinine were significantly elevated at Post-match I ($p < 0.01$), returning to baseline at Post-match II. Vitamins E, C and A were significantly elevated at Post-match I ($p < 0.01$), but only vitamins E and A remained high at Post-match II. TBARS showed no significant changes. GSH showed a significant decrease ($p < 0.01$) and PC showed a slight but significant increase ($p < 0.01$) at Post-match II. The recruitment of non-enzymatic antioxidants prevented lipid peroxidation, but dietary and especially endogenous defence responses were insufficient to prevent protein oxidation. Proper nutrition is essential to improve the activity of the antioxidant defence system, preventing exercise-induced oxidative stress.

Key words adolescent, exercise, nutritional status, oxidative stress, soccer, football

Introduction

In recent years there has been growing interest in evaluating the physical, physiological and psychological characteristics of soccer players in order to contribute to the early identification of talented athletes (Nikolaidis, Vassilios Karydis, 2011; Tahara et al., 2006). There is no doubt that a diet tailored to suit the needs of players has a positive effect on their sports performance (Hernandez, Nahas, 2009; Karakilcik, Halat, Zerin, Celik, Nazligul, 2014; Eskici, 2016).

Overall, the rapid growth and development in adolescence cause an increase in energy and nutrient demand (Boisseau, Vermorel, Rance, Duché, Patureau-Mirand, 2007). As a result, adolescent athletes have special nutritional needs due to the additional demands associated with training and competition (Iglesias-Gutiérrez et al., 2005; Russell, Pennock, 2011; Karakilcik et al., 2014; Eskici, 2016). However, energetic and metabolic demands associated with soccer activities vary according to the level of competition and the individual characteristics of the players (González, Cobos, Molina, 2010; Nikolaidis, Vassilios Karydis, 2011).

The high-intensity training routine associated with non-oriented or bad eating habits may overwhelm the endogenous and exogenous antioxidant systems, causing oxidative stress (Bloomer, Goldfarb, McKenzie, 2006; Nieman, Bishop, 2006; Shing et al., 2007; Powers, Radak, Ji, 2016). Oxidative stress is associated with tissue inflammation, fatigue, muscle injury, impaired recovery after high-intensity exercise, and reduced immune function (Michailidis et al., 2007; Nieman, Bishop, 2006; Shing et al., 2007; Zanella, Souza, Godoy, 2007; Hadžović-Džuvo et al., 2014; Silva et al., 2014), all which may impair athlete performance. Indeed, a monitored and well-balanced diet will provide the antioxidants needed to battle oxidative stress induced by high-intensity training routine in athletes (Karakilcik et al., 2014; Eskici, 2016). It has been hypothesized that oxidative stress is responsible for increased muscle soreness and decreased strength at hours or days after exercise, even in athletes with a well-balanced diet. Despite this, the literature lacks studies that concomitantly evaluate nutritional status and exercise-induced oxidative stress, especially with regard to adolescent soccer players. This study aimed to assess nutritional status and exercise-induced oxidative stress in adolescent soccer players.

Methods

Participants

Twenty adolescent male soccer players aged 15 to 17 years were randomly selected from the under-17 category of a professional soccer club located in the city of Ribeirão Preto, state of São Paulo, south-eastern Brazil. All participants were non-smokers, had no history of alcohol consumption, had been playing soccer for at least 2 years (2.6 ±2.0 years), played in any position but goalkeeper, and were participating in the regular season league competition, with training sessions 5 days a week (mean of 4 hours daily) and at least one official game per week. The use of anti-inflammatory drugs or antioxidant supplements within 3 months prior to the study trial was an exclusion criterion.

The study was approved by the Research Ethics Committee of our institution and was conducted in accordance with the provisions of the Declaration of Helsinki. Written informed consent was obtained from all participants and their parents or legal guardians prior to their inclusion in the study.

Study trial

The players were randomly divided into two teams for a simulated match of 60 minutes in length, with no halftime interval. Two goalkeepers were asked to participate in the match, but they were not included in the study analysis due to the large difference in physical behaviour between goalkeepers and players in all other positions during a soccer match, which could invalidate our results. Participants were asked to refrain from high-intensity physical activities 48 hours prior to the game.

The match started at 3 pm at a temperature of 26°C and relative humidity around 55%. After approximately 30 minutes of playing time we collected 10-µL blood samples by index finger-prick from all players for measurement of blood lactate concentration (Accusport/Accutrend Lactate Analyzer; Boehringer Mannheim, Castle Hill, Australia) in order to determine exercise intensity during the match. Mean blood lactate concentration was 5.1 ±2.0 mmol/L, characterizing a game of moderate to high intensity effort.

In the week before the study trial, all athletes underwent anthropometric measurements and completed a food record at home for dietary assessment. Blood samples for biochemical analysis were collected at three time points: immediately before the start of the game (Pre-match); 30 minutes after the end of the game (Post-match I); and 24 hours after the end of the game (Post-match II).

Anthropometric assessment

Anthropometric measurements were performed using the techniques proposed by D.B. Jelliffe (1966). Percentage body fat (% BF) was estimated using the four skin-fold (triceps, subscapular, suprailiac, abdominal) equation proposed by Faulkner (1968). Body composition was also assessed by tetrapolar bioelectrical impedance analysis (BIA) (Biodynamics®, BIA 310E, Seattle, USA). Height was measured to the nearest 0.1 cm using a calibrated stadiometer (Alturaexata®, TBW, São Paulo, SP, Brazil), and weight was measured with a digital scale (Filizola®, São Paulo, SP, Brazil) to a precision of 50 g. Participants were measured and weighed barefoot and wearing their sporting gear. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$.

Dietary assessment

Three-day food records were completed by the participants in the week prior to the match on three alternate days (two weekdays and one weekend day) and were used to collect dietary data. The intakes of energy, macronutrients and antioxidant vitamins A, C and E were quantified using the NutWin® software (Universidade Federal de São Paulo/UNIFESP, São Paulo, SP, Brazil) and compared with the Dietary References Intakes (DRIs) and the nutrient intakes recommended by the Brazilian Society for Sports Medicine for healthy adult and adolescent athletes (Hernandez, Nahas, 2009).

Blood biochemistry

A 5 mL blood sample was collected at each time point (Pre-match, Post-match I, and Post-match II) by venipuncture into sterile vacuum tubes containing clot activator. The samples were centrifuged at 3,500 rpm for 10 minutes at room temperature. The supernatant (serum) was separated, transferred to an Eppendorf tube and stored at –30°C, and the pellet was discarded.

For lipid profile analysis, total cholesterol, triglyceride and high-density lipoprotein (HDL-c) concentrations were determined using commercial laboratory kits (Labtest®; Labtest Diagnóstica, Lagoa Santa, MG, Brazil). Low-density lipoprotein (LDL-c) concentration was calculated using the Friedewald equation for triglycerides <400 mg/dL: LDL-c = total cholesterol – HDL-c – triglycerides/5 (Santos et al., 2001).

For the assessment of muscle damage, creatinine and creatinine kinase (CK) levels were measured using commercial laboratory kits (Labtest®).

Several blood components were measured for the assessment of oxidative stress. Serum lipid peroxidation was quantified by TBARS and determined by colorimetric method, according to A.L. Spirlandeli, R. Deminice and

A.A. Jordao (2014). Protein Carbonils were determined according to R.L. Levine, J.A. Williams, E.R. Stadtman and E. Shacter (1994). Reduced glutathione (GSH) was measured using the method described by J. Sedlak and R.H. Lindsay (1968). Ascorbic acid was determined by colorimetric reaction with 2,4-DNPH, as previously described by O.A. Bessey (1960). Vitamin A and E concentrations were determined by high-performance liquid chromatography (Shimadzu Co., Kyoto, Japan) according to J. Arnaud, I. Fortis, S. Blachier, D. Kia and A. Favier (1991).

Statistical analysis

All results were expressed as mean with standard deviation. Because data related to biochemical variables were collected and analyzed at three different time points for each individual, comparisons of means over time were performed using linear regression mixed-effects models. The assumption of normality of residuals was verified graphically using a normal plot. Data showing atypical values were log transformed to achieve normal distribution and expressed as the geometric mean and standard deviation. Data were analysed using the statistical program SAS® version 9.0 (SAS Institute Inc., Cary, NC, USA). The level of significance was set at 5%.

Results

A total of 20 adolescent male soccer players participated in the study, mean age was 16.6 ±0.4 years. The athletes had a mean body weight of 66.7 ±7.4 kg, height of 1.70 ±0.1 m, and BMI of 21.9 ±1.5 kg/m^2, within the normal range for age. Both methods used to estimate % BF yielded similar results, 11.5% by the four-skin-fold predictive equation and 11.4% by BIA.

Dietary intake values of all participants and recommended ranges of calories, macronutrients and antioxidant vitamins are described in Table 1. The athletes had a mean energy intake of 37.6 kcal/kg, which is within the recommended dietary intake range. However, the distribution of macronutrients was unbalanced, showing a high-protein (1.62 g/kg), high-fat (1.12 g/kg), normal to low-carbohydrate (5.28 g/kg) diet. None of the athletes met the recommended daily intake levels for vitamins E and A, but 65% met the recommended dietary vitamin C intake (Table 1).

Table 1. Dietary intake of calories, macronutrients and antioxidant vitamins in adolescent male soccer players (n = 20)

Variables	Mean ±SD	Recommendations	Percent adequate athletes
Total calories (kcal)	2476.6 ±470.1	–	–
Calories/kg body weight	37.6 ±8.5	30–50 kcal/kg BW/day[a]	80
Proteins/BW (g/kg BW)	1.62 ±0.39	1.2–1.6 g/kg BW/day[a]	30
HBV proteins (%)	58.0 ±9.4	Minimum of 65% [b]	20
Carbohydrates/BW (g/kg BW)	5.28 ±1.38	5–8 g/kg BW/day[a]	45
Lipids/BW (g/kg BW)	1.12 ±0.28	1 g/kg BW/day[a]	40
Saturated fatty acid (%)	9.1 ±1.9	10%[a]	40
Monounsaturated fatty acid (%)	8.7 ±1.7	10%[a]	25
Polyunsaturated fatty acid (%)	4.4 ±0.8	10%[a]	0
Vitamin E (mg)	6.19 ±2.45	12 mg/day [c]	0
Vitamin C (mg)	261.08 ±496.06	63 mg/day [c]	65
Vitamin A (ug)	345.13 ±147.77	630 ug/day [d]	0

BW − body weight; HBV − high biological value; SD − standard deviation.

Source: [a] Hernandez, Nahas (2009); [b] Regulamento Técnico... (1998); [c] Estimated Average Requirement (2000); [d] Estimated Average Requirement (2001).

The variables analysed at the three experimental time points (Pre-match, Post-match I, and Post-match II) are compared in Table 2. The lipid profile showed no changes after match-induced physical stress. CK and creatinine levels were significantly elevated at Post-match I (34.4% and 25.4%, respectively) ($p < 0.01$), indicating muscle damage; however, these values returned to baseline at Post-match II. Vitamins A, C and E were also significantly elevated at Post-match I (26.3%, 8.6%, and 14.0%, respectively) ($p < 0.01$), but only vitamin E and A levels remained high at Post-match II. TBARS values showed no significant changes at any time point, which indicates the absence of lipid peroxidation. At Post-match II, while GSH levels showed a significant decrease (38.6%) ($p < 0.01$), PC levels showed a slight but significant increase (10.0%) ($p < 0.01$), indicating protein-induced oxidative stress (Table 2).

Table 2. Comparison of variables analysed in adolescent male soccer players (n = 20) at three different time points – immediately before the start of the game (Pre-match), 30 minutes after the end of the game (Post-match I), and 24 hours after the end of the game (Post-match II)

Variables	Pre-match (mean ±SD)	Post-match I (mean ±SD)	Post-match II (mean ±SD)	*p* (95% CI)
Total cholesterol (mg/dL)	137.85 ±23.59	142.35 ±24.13	139.1 ±23.1	0.08 (−0.5; 9.51)* 0.62 (−3.75; 6.26)† 0.20 (−8.26; 1.75)‡
LDL-c (mg/dL)	71.57 ±25.96	77.28 ±29.19	72.37 ±27.42	0.07 (−0.55; 11.98)* 0.80 (−5.47; 7.07)† 0.12 (−11.18; 1.35)‡
HDL-c (mg/dL)	45.83 ±10.17	47.03 ±13.65	49.05 ±13.82	0.90 (−3.28; 3.70)* 0.21 (−1.26; 5.72)† 0.25 (−1.47; 5.51)‡
Triglycerides (mg/dL)	97.27 ±27.39	90.18 ±28.44	88.41 ±21.26	0.20 (−18.18; 4.01)* 0.11 (−19.95; 2.24)† 0.75 (−12.87; 9.33)‡
CK (U/L)§	317.14 ±2.01	426.27 ±1.88	315.59 ±1.59	<0.01 (55.36; 170.68)* 0.94 (−41.38; 44.03)† <0.01 (−150.50; −65.13)‡
Creatinine (mg/dL)	1.10 ±0.20	1.38 ±0.17	1.08 ±0.15	<0.01 (0.16; 0.38)* 0.71 (−0.12; 0.08)† <0.01 (−0.40; −0.18)‡
GSH (µmol/gProt)	1.84 ±0.24	1.88 ±0.53	1.13 ±0.79	0.83 (−0.30; 0.37)* <0.01 (−1.05; −0.37)† <0.01 (−1.09; 0.41)‡
Vitamin E (µmol/L)	21.91 ±5.86	24.97 ±6.48	25.66 ±5.93	<0.01 (0.99;5.11)* <0.01 (1.68; 5.81)† 0.50 (−1.37; 2.75)‡
Vitamin C (mg/dL)	1.62 ±1.2	1.76 ±1.16	1.68 ±1.16	0.04 (0.005; 0.27)* 0.39 (−0.068; 0.19)† 0.21 (−0.20; 0.05)‡
Vitamin A (µmol/L)	2.66 ±0.48	3.36 ±0.75	3.13 ±0.65	<0.01 (0.45; 0.95)* <0.01 (0.22; 0.71)† 0.06 (−0.47; 0.01)‡
Protein carbonyl (nmol/mg/Prot)	0.10 ±0.02	0.10 ±0.01	0.11 ±0.01	0.76 (−0.008; 0.012)* <0.01 (0.007; 0.028)† <0.01 (0.005; 0.027)‡
TBARS (nmol/mg/Prot)	5.68 ±1.00	5.18 ±0.73	5.23 ±1.04	0.06 (−1.00; 0.01)* 0.08 (−0.96; 0.06)† 0.86 (−0.45; 0.55)‡

95% CI = 95% confidence interval; CK − creatinine kinase; GSH − reduced glutathione; HDL − high-density lipoprotein; LDL − low-density lipoprotein; SD − standard deviation; TBARS − thiobarbituric acid-reactive substances; * Pre × Post I; † Pre × Post II; ‡ Post I × Post II; § Data expressed as geometric mean ±SD.

Discussion

The main findings of the present study were 1) under-17 soccer players have imbalanced macronutrients and antioxidants intake; 2) imbalanced nutrition does not promote impaired circulating blood lipids; 3) however, imbalanced antioxidant intake may exacerbate oxidative stress induced by the training routine.

Although energy intake (37.6 kcal/kg) was within the recommended dietary intake range, there was an imbalanced distribution of macronutrients in the normal diet of players, which is consistent with data from literature reporting a high-protein, high-fat, normal to low carbohydrate diet in soccer players (Iglesias-Gutiérrez et al., 2012; Maughan, Bartagi, Dvorak, Zerguini, 2008; Russell, Pennock, 2011; Galanti et al. 2014). This is a common behaviour among athletes in several sports, including soccer, who attach great importance to proteins and are unaware or disregard the important contribution of carbohydrate intake to sports performance. Conversely, circulating lipids at a normal level were demonstrated in our athletes (Table 2). This seems paradoxical since imbalanced nutrition, especially a high-protein, high-fat diet, may cause elevated circulating triglycerides and cholesterol. Indeed, young athletes have an exercise routine intensity that may control elevated circulating lipids. It may reflect, however, in decreased training performance in the present and future unhealthy perspectives.

None of the players met the recommended daily intake levels for vitamins A and E, but 65% met those for vitamin C. Similar to our study, Y. Noda et al. (2009), analysing nutrient intake in male collegiate soccer players, found that their mean intakes of calcium, magnesium, vitamin A, B1, B2, and C were lower than the recommended dietary allowances. In contrast, M. Russell and A. Pennock (2011), in a study of young professional male soccer players, found an adequate intake of almost all nutrients (including vitamins), except for potassium. Several immune processes require the presence of these micronutrients (Hespel, Maughan, Greenhaff, 2006; Nieman, Bishop, 2006), especially when it comes to adolescent players subjected to physical stress. It is widely known that adolescents consume fewer fruits and vegetables, foods rich in these vitamins. This reinforces the importance of the presence of a nutritionist working together with the coaching staff, especially in youth soccer teams.

Vitamins E and A behaved similarly, increasing at Post-match I and remaining elevated at Post-match II, whereas vitamin C had a slight but significant increase at Post-match I, returning to nearly baseline levels at Post-match II. The behaviour of vitamins indicates an acute recruitment of dietary antioxidants after the game, contributing to prevent lipid peroxidation (Roehrs et al., 2009). M. Roehrs et al. (2009), evaluating the influence of plasma retinol levels on oxidative stress biomarkers in haemodialysis patients, found a positive correlation between retinol and MDA. That is, increased blood retinol was directly associated with lipid peroxidation in their patients, which did not occur in the present study, since TBARS, a biomarker of lipid peroxidation, decreased even in the presence of increased vitamin A after the game. In our study, however, the vitamins failed to prevent protein-induced oxidative stress, since PC levels were elevated 24 hours after the game and GSH levels were consequently reduced at this same time point. H. Andersson, A. Karlsen, R. Blomhoff, T. Raastad, and F. Kadi (2010) and P. Tauler et al. (2008) observed an increase in ascorbic acid after a soccer match, compared to baseline levels. However, C.C. Zoppi et al. (2006), investigating antioxidant supplementation, reported that vitamin C and E supplementation produced no effects on the activity of antioxidant enzymes in adolescent soccer players. Thus, literature remains controversial regarding the behaviour of antioxidant vitamins.

The increase in CK levels immediately after the game, together with elevated protein carbonyls and decreased GSH suggests the occurrence of muscle damage and oxidative stress during the match, which is in agreement with observations already reported (Fatouros et al., 2010; Gravina, Ruiz, Lekue, Irazusta, Gil, 2011; Ascensão et al.,

2008). In the study by I.G. Fatouros et al. (2010), CK levels increased significantly after a soccer game, but unlike our players whose levels returned to baseline after 24 hours, CK remained elevated after 48 hours. These authors also demonstrated elevated oxidative stress markers and reduced GSH 24 hours after a soccer match. Another study also observed a decrease in GSH Levels (Mello et al. 2017).

A. Ascensão et al. (2008) demonstrated a soccer match increases the levels of oxidative stress and muscle damage throughout the 72-h recovery period and concluded that redox alterations induced by a soccer match is associated with muscle dysfunction and performance loss. However, studies analysing the effects of nutritional supplementation in soccer players demonstrated antioxidant vitamins promoted a lower increase in CK levels compared to the control group (no supplementation) without performance enhances (Arent, Pellegrino, Williams, Difabio, Greenwood, 2010; Zoppi et al., 2006). In the present study, none of the athletes was receiving dietary supplementation. Another study with football players demonstrated that antioxidant supplementation with vitamin C and E does not attenuate elevated markers of muscle damage but did reduce oxidative stress (de Oliveira, Rosa, Simões-Ambrósio, Jordao, Deminice, 2019).

Micronutrient supplementation is still controversial, but the consumption of a diverse well-balanced diet is a concept that remains largely valid and fully applicable, which may be sufficient to maintain the micronutrient levels required by physical exercise without the need for supplementation. The playing position of the athlete influenced the length of oxidative stress, inflammation and muscle damage markers after an official soccer game (Souglis, Bogdanis, Chryssanthopoulos, Apostolidis, Geladas, 2018), but as this influence was not measured in the actual study, future studies can consider this information.

Conclusion

The present results indicate that the recruitment of non-enzymatic antioxidants, mainly dietary antioxidant vitamins, prevented the occurrence of lipid peroxidation. However, dietary and especially endogenous defence responses were insufficient to prevent protein oxidation. These findings highlight the importance of proper nutrition in sports in order to improve the activity of the antioxidant defence system and hence the metabolic response, thus preventing exercise-induced oxidative stress. We suggest the implementation of nutrition education programs tailored to adolescent soccer players, including nutritional intervention and follow-up, targeting well-balanced food intake that can help counterbalance excessive damage caused by chronic physical exercise.

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