Effect of post-training and post-match antioxidants on oxidative stress and inflammation in professional soccer players

Efecto de los antioxidantes post-entrenamiento y post-partido sobre el estrés oxidativo y la inflamación en jugadores profesionales de fútbol

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Abstract. The objective of this study was to determine if the addition of post-activity antioxidants is a useful strategy for improving the specific analytical parameters related to oxidative stress and inflammation. The study was carried out in an Italian Serie A soccer team, between October, 2019 and January, 2020. In October, the measurements were carried out on the players without having taken post-activity antioxidant supplementation. The intervention period corresponded from the end of October to the beginning of January, a period in which post-activity antioxidants were supplemented. The results obtained confirmed that the players who took antioxidants during the intervention period in the form of a mixture of a natural pineapple smoothie with a concentrate of tart cherry, pomegranate, black currant and beet in stick form, significantly improved the parameters associated with oxidative stress, although a significant improvement in the parameters related to inflammation was not observed. The use of antioxidants for a period of seventy days is a post-activity intervention strategy that can be considered effective for improving the reduction of parameters related to the oxidative effect derived from the practice of physical exercise at a professional level in soccer, although more studies are needed to determine the anti-inflammatory effect.

Keywords: Homeostasis, exercise, recovery, elite athletes, supplements.

Resumen. El objetivo de este estudio fue determinar si la inclusión de antioxidantes post-actividad es una estrategia útil para mejorar los parámetros analíticos específicos relacionados con el estrés oxidativo y la inflamación. El estudio se llevó a cabo en un equipo de fútbol de la Serie A italiana, entre octubre de 2019 y enero de 2020. En octubre, las mediciones se llevaron a cabo en los jugadores sin haber tomado la suplementación antioxidante post-actividad. El periodo de intervención correspondió desde finales de octubre hasta principios de enero, periodo en el que se suplementaron los antioxidantes post-actividad. Los resultados obtenidos confirmaron que los jugadores que tomaron antioxidantes durante el periodo de intervención en forma de una mezcla de un licuado de piña natural con un concentrado de cereza ácida, granada, grosella negra y remolacha en forma de stick, mejoraron significativamente los parámetros asociados al estrés oxidativo, aunque no se observó una mejora significativa en los parámetros relacionados con la inflamación. El uso de antioxidantes durante un periodo de setenta días es una estrategia de intervención post-actividad que puede considerarse eficaz para mejorar la reducción de los parámetros relacionados con el efecto oxidativo derivado de la práctica de ejercicio físico a nivel profesional en el fútbol, aunque se necesitan más estudios para determinar el efecto antiinflamatorio.

Palabras clave: Homeostasis, ejercicio, recuperación, atletas de élite, suplementos.

Introduction

The physical demands of professional soccer have become more intense in recent seasons (Barnes, Archer, Hogg, Bush & Bradley, 2014), and it is increasingly necessary to monitor and evaluate the player’s physical parameters to optimize performance (Zhou, Gómez & Lorenzo, 2020). The use of biomarkers can more effectively improve the ability of trainers to assess the recovery period after a training session and to set the intensity of subsequent sessions (Palacios, Pedrero-Chamizo, Palacios, Maroto-Sánchez, Aznar & González-Gross, 2015). Among the most commonly used are: tumor necrosis factor (TNF-á), interleukin-6 (IL-6), lactate dehydrogenase (LDH), or C-reactive protein (CRP) (Fernández-Lázaro, Fernandez-Lazaro, Miéglo-Ayuso, Córdova Martínez, Seco-Calvo, & Fernandez-Lazaro, 2020).

Exercise-induced muscle damage occurs after a session that is characterized by high-intensity, particularly eccentric, muscle contractions (Clarkson & Hubal, 2002). Moreover, the inclusion of an eccentric-overload training program is usual in soccer due to it has shown the optimization of the specific physical condition of soccer players (Raya-González, Suárez-Arrones, Rísquez Bretones & Sáez de Villareal, 2017). Following this structural damage, there is a marked reduction in the control of calcium ion release from the sarcoplasmic reticulum resulting from this initial damage, leading to further muscle fiber damage and apoptosis (Gissel & Clausen, 2001).
Degeneration of muscle fibers and concomitant acute inflammation begin in the first few hours after injury. Immediately after muscle damage, the sarcolemma ruptures and myofibers undergo necrosis, which is reflected in increased plasma levels of muscle proteins (i.e. creatine kinase, ...). Muscle necrosis activates resident mast cells, which in turn secrete cytokines (i.e. IL-1α, IL-6, TNFα) to recruit circulating inflammatory cells from the surrounding vasculature (Kozakowska, Pietraszek-Gremplewicz, Jozkowicz, & Dulak, 2015). The result is the formation of reactive oxygen species or free radicals (Power, Kavazis & Jackson, 2011), which can further damage proteins (Kozakowska, et al., 2015), and the cell membranes when produced in excess, and contribute to skeletal muscle damage (Fernández-Lázaro, Fernandez-Lazaro, Mielgo-Ayuso, Navascués, Córdova Martínez, & Seco-Calvo, 2020). This damage includes reductions in muscle strength, marked muscle soreness, and elevated biomarker levels of oxidative stress, inflammation and muscle damage (Thomas, Dent, Howatson, & Goodall, 2017), a process that ultimately overwhelms the body’s antioxidant capacity, resulting in the body adapting and attempting to respond appropriately to restore homeostatic balance (Spanidis, et al., 2018).

Specific analytical parameters indicative of oxidative stress include Biological Antioxidant Potential (BAP), Reactive Oxygen Metabolites (ROM), BAP/ROM ratio, selenium concentration, zinc concentration, arachidonic acid/ eicosapentaenoic acid ratio (AA/EPA), arachidonic acid/ docosahexaenoic acid ratio (AA/DHA), and adrenocorticotropin hormone (ACTH). Free radicals, which can be found as nitrogen derivatives or ROM, have a rather high reactivity and a short lifetime (Haida & Hakiman, 2019).

BAP plays a prominent role as a plasma barrier against oxidative stress, represented by enzymes or endogenous substances introduced through diet (Takam, et al., 2016). Omi et al. (2019) provided the BAP/ROM index as a measure to express the antioxidant status of athletes.

Selenium, a naturally occurring trace mineral, plays an important role in endogenous antioxidant defense as an essential component of selenoproteins, and conditions the activity of the glutathione peroxidase enzyme. The properties of this trace mineral focus on improving sports performance and recovery from training (Fernández-Lázaro, et al., 2020).

Zinc is recognized as a redox-inert metal, and functions as an antioxidant through the catalytic action of copper/zinc superoxide dismutase, stabilization of membrane structure, protection of sulphydryl protein groups, and positive regulation of metallothionein expression, and it also suppresses anti-inflammatory responses that would otherwise increase oxidative stress. Zinc deficiency and excess have been shown to cause cellular oxidative stress (Lee, 2018). Also, some studies have found that the body’s antioxidant potential was not sufficiently potent to neutralize the pro-oxidant potential of training and matches, so supplementation may be of interest (Takam, et al., 2016).

Vitamin C is an antioxidant that directly removes superoxide, hydroxyl and lipid hydroperoxide radicals, and plays an important role in the recycling of vitamin E generated in membranes during oxidative stress (Kojo, 2004). In the case of vitamin E, it is the main antioxidant that acts on cell membranes and other lipid-rich structures such as mitochondria or the sarcoplasmic reticulum (Ji, Gómez-Cabrera & Vina, 2006). Both vitamins have shown to decrease the IL-6 response to exercise (Zimmermann, 2003), while high doses maintained over time have been shown to hinder certain adaptations (Morrison, et al., 2015; Paulsen, et al., 2014).

The use of antioxidants in athletes is a controversial topic (de la Cruz Sánchez, Pino Ortega, Moreno Conteras, Cañadas Alonso, & Ruiz-Risueño Abab, 2015). Although exercise initially appears to be counterproductive to membrane integrity (Perez, Cabral de Oliveira, Estevez, Molina, Prieto, & Alvarez, 2003), over time, overcompensation occurs with increased intracellular water, causing membrane quality to improve (Issurin, 2009).

Studies in young elite soccer players have concluded that after soccer training or matches there is an excessive production of free radicals and therefore oxidative stress, which could decrease the efficiency of the body’s antioxidant system (de la Cruz Sánchez, et al., 2015; Djordjevic, et al., 2012). Thus, a diet high in antioxidants and rich in fruits and vegetables would be recommended from a preventive point of view (de la Cruz Sánchez, et al., 2015; Jayedi, Rashidy-Pour, Parohan, Zargar, & Shab-Bidar, 2018) and the strategies related to diet, food supplements and performance monitoring were the most suitable to improve the performance and the recovery (Nogueira, Salguero del Valle, Molinero González, & Márquez Rosa, 2021), together with a correct body composition for the direct influence on the physical performance of athletes (Ceballos-Gurrola, et al., 2020).

The supply of antioxidants in the diet may be insufficient in athletes, as established by Yavari et al. in
2015. Although a balanced diet rich in antioxidants has been proposed as a dietary recommendation to improve endogenous antioxidant capacity and reduce exercise-induced oxidative stress, there is insufficient evidence to support this hypothesis in high-performance athletes (Trapp, Knez & Sinclair, 2010). Furthermore, soccer players show a higher level of oxidative stress than other athletes. Therefore, we can affirm that food intake is probably insufficient in this population, and they would probably benefit from supplementation (Siquerier-Coll, Muñoz-Marín & Grijota-Pérez, 2019). Therefore, an additional supply could be needed. This supplementation is gaining more and more interest due to its properties against inflammation, muscle damage and oxidative stress (Rojoano-Ortega, Molina-López, Moya-Amaya & Berral-de la Rosa, 2021). The aim of this study is to evaluate the antioxidant and anti-inflammatory effect of a specific supplementation after training and matches on professional soccer players.

Material And Methods

Participants

The total sample was composed of 19 professional soccer players belonging to a first division Italian team, aged between 19 and 33 years-old. The mean age was 25.89 ± 3.04 years; the mean weight was 85.44 ± 7.69 kg, and the mean height was 186.26 ± 4.49 cm.

The sample of players was heterogeneous, with players from different continents, races and playing positions on the field. The athletes signed a consent form informing them of the procedure to be carried out in the study and the risks and benefits of their participation. All the players gave written informed consent according to the Declaration of Helsinki. The study was approved by the Virgen Macarena-Virgen del Rocio University Hospitals Research Ethics Committee.

Experimental design

The season began in July 2019, with the first evaluation of the athletes taking place at the end of October, four months after the pre-season and start of competition took place, without having taken any specific post-activity antioxidant supplementation. Throughout the research period, the athletes were given general nutritional supplements based on food intake, after training and matches, in the form of a shake containing 250 mL of oat or rice drink, 30 g of protein isolate, 5 g of creatine, 5 g of creatine and 5 g of protein isolate, 5 g of creatine and 5 g of glutamine, in addition to daily polyunsaturated fatty acids, at doses of 700 mg of eicosapentaenoic acid (EPA) and 240 mg of docosahexaenoic acid (DHA), a daily dose of 2000 IU of vitamin D. Before training the athletes were given mineral salts, whose composition was 310mg of sodium, 54.3mg of potassium, 8.14mg of calcium and 5.89mg of magnesium. To ensure compliance with the intake of these basic supplements, an automated personalized dosing system was incorporated using the TIMEDI JV- DEN blister machine for the preparation of single doses with individualized supplementation per player and time of intake.

The department of nutrition developed nutritional guidelines for the athletes and supervised more than 20 weekly intakes. The estimated calorie distribution in the supervised meals was: 50% of carbohydrates, 25% of proteins and 25% of lipids, an average intake of 5-7g of carbohydrates/kg of Body Weight (BW), and 1.6-1.8g of protein/kg of BW, with special emphasis on the biological value of the intakes and on the post-training and post-match recoveries.

This was an experimental study with an intervention lasting ten weeks, carried out from the 27th of October, 2019 to the 5th of January, 2020, in which ten official competition matches were played and athletes were given a specific supplementation, in addition to the general one already mentioned, based on antioxidants after training and matches, by means of a mixture of 250 mL of natural pineapple smoothie and a 10 mL stick of a concentrate rich in polyphenols (Table 1).

The athletes underwent a pre- and post-intervention fasting blood draw to analyze the following specific analytical parameters: BAP, ROM, AA/EPA, AA/DHA, ACTH, Zinc and Selenium, related to oxidative stress and inflammation, and measured by a specialized laboratory (Table 2).
The statistical analysis was carried out with the SPSS software package for Windows, v. 25.0 (SPSS Inc., USA). The means and standard deviations of all variables were calculated. The Shapiro-Wilk test was applied for testing data normality. When this condition was fulfilled, Student’s t-tests were performed to determine significant differences between pre- and post-test, otherwise, Wilcoxon tests were performed. Pearson’s r and Spearman’s rho tests were performed to evaluate the correlation between different parameters using a simple linear regression model. Descriptive statistics are presented as means ± standard deviations (SD). The following criteria were adopted to explain the level of correlations: 0.1 < r ≤ 0.3, small; 0.3 < r ≤ 0.5, moderate; 0.5 < r ≤ 0.7, large; 0.7 < r ≤ 0.9, very large; and r > 0.9, almost perfect (Witz, Hinkle, Wiersma & Jurs, 1990).

Results

The results of the parameters studied are shown in table 3. In the period analyzed, a significant decrease in ROM values was observed, while both BAP and serum Selenium levels were significantly reduced. The BAP/Rom ratio significantly increased between the two periods. The values of the AA/DHA and AA/EPA ratios decreased, although this was not significant. ACTH slightly increased, although this was not statistically significant. The concentration of Zinc decreased, but not significantly between the two assessments.

![Table 3. Mean values of the analyzed parameters.](image)

**Figure 1. BAP/Rom Index**

**Discussion**

The purpose of this study was to evaluate the antioxidant and anti-inflammatory effect of a specific supplementation after training and matches on professional soccer players; and the results confirmed that the intake of antioxidants can be considered effective to improve the reduction of parameters related to the oxidative stress derived from the practice of physical exercise.

Professional soccer players have a higher oxidative stress as compared to the normal population; thus, some authors have discussed the suitability of including antioxidant supplementation (Takam, et al., 2016; Jayedi, et al., 2018; Tavari, et al., 2015; de la Cruz Sánchez, et al., 2015). Oxidative stress is the consequence of an imbalance between ROM production and BAP. This stress can both cause damage to cellular components and have detrimental effects in physiological conditions, during physical exercise, or in diseases (Kruk, Aboul-Enein, KBadna & Bowser, 2019).

Omi et al. (2019) administered whey protein antioxidants to American football players after training for four months, and observed an improvement in the oxidative stress expressed by the BAP/Rom index, relating this improvement to an increase in muscle mass. In addition, they observed a decrease in mean ROM values and mean BAP values, a trend that was also found in our study, with a significant decrease in the ROM values must be underlined. Figure 1 shows the individual values of the BAP/Rom index for the October and January measurements.
significant improvement in total antioxidant status.

Following our nutritional intervention, we observed a decrease in BAP. This decrease did not correspond to the increase observed by Bolner et al. who found a proportional increase in BAP and ROM in athletes subjected to high muscle activity loads. The authors considered that this excess was an adaptation to training, which triggered the body’s own antioxidant mechanism.

In future studies, it would be interesting to establish whether antioxidant supplementation can produce an imbalance and provoke negative physiological adaptations in the BAP that cannot be compensated for by endogenous antioxidant capacity, or if not, to assess whether a sustained supplementation with antioxidants is more appropriate. Repeated skeletal muscle contractions can generate free radicals and, when prolonged and intense, these can cause oxidative damage to cells (de la Cruz Sánchez, et al., 2015; Pingitore, Lima, Mastorci, Quinones, Iervasi & Vassalle, 2015). Our nutritional intervention led to a significant decrease in ROM, thereby increasing total antioxidant capacity.

Consequently, the question would be, could antioxidant intake during a controlled post-activity period prevent exercise-induced muscle damage according to the data obtained in our research? We found a significant decrease in ROM (p<0.01) in the second measurement, after the intervention period, even though the endogenous antioxidant capacity had decreased, so we could think that there had been a decrease in muscle damage and an improvement in oxidative balance, in general, in most players (Table 4).

On the other hand, selenium is related to endogenous antioxidant capacity (Fernández-Lazaro, et al., 2020), and a decrease in Selenium concentration between the two periods was observed. After analyzing our results, we observed that the ACTH value increased in the second measurement in January with respect to the October measurement, and this was in agreement with the evolution described by Bolner et al. in 2019.

It is unclear whether the protocol studied decreases muscle damage after training and matches, as although a decrease in AA/DHA and AA/EPA ratios was observed, these were not significant. EPA acts competitively against AA for the cyclooxygenase and lipoxygenase enzymes, which are key to reducing inflammation. Consequently, a high value of the AA/EPA ratio could be a marker for chronic inflammation (Nelson & Raskin, 2019).

Tissue availability of polyunsaturated fatty acids (PUFA) depends on several factors, including dietary intake, physical exercise, genetic variation, and metabolic turnover. However, there are few studies that conclude whether a running training activity alone can influence indices associated with PUFA metabolism, such as the omega-3 index and the AA/EPA ratio. According to Davinelli et al. showed in 2019, training can negatively contribute to changes in the omega-3 index and the AA/EPA ratio.

In 2019, Ramos-Campos et al., incorporated supplementation with re-esterified DHA (2100 mg/day) and EPA (240 mg/day) for ten weeks, obtaining lower concentrations of IL6 and decreased values of CPK as muscle damage marker. After observing the values measured in this study, we could not establish a clear relationship between the intervention and the values obtained for the AA/DHA ratio, although the dose of DHA taken by our players was 100 mg of DHA, much lower than in the Ramos-Campos study.

Antioxidants are gaining increasing interest due to their properties related to oxidative stress, inflammation, and muscle damage after exercise (de la Cruz Sánchez, et al., 2015; Jayedi, et al., 2018). Supplementation with tart cherry concentrate in intermittent sports such as soccer, in which inflammatory and oxidative stress markers were measured, was a good tool to optimize and accelerate recovery in athletes (Souglis, Bogdanis, Chryssanthopoulos, Apostolidis, & Geladas, 2018). Other authors also note this potential, claiming that Montmorency cherries improved recovery as well as oxidative stress after high-intensity training (Schneider, Bock, Becker, Moreira, Bello-Klein & Oliveira, 2018; Botwell & Kelly, 2019). Schneider et al. in 2018 conducted an intervention study with two diets with different antioxidant concentrations for fourteen days in triathletes, obtaining an improvement in redox status. In addition, less protein damage (shown by decreased carbonyl levels) and increased antioxidant capacity were observed.

Other authors have claimed to find improvements with antioxidant supplementation in performance, recovery and muscle damage (Botwell & Kelly, 2019); or that dietary supplementation with antioxidant vitamins is beneficial in combating oxidative stress and that exogenous glutathione influences the endurance capacity of athletes (Pingitore et al., 2015). Another authors highlighted the importance of ensuring the intake of antioxidants to improve performance, as well as to improve the redox profile, which would be of interest in order for the subject to reach optimal
antioxidant concentrations during competition (Margaritelis, Paschalis, Theodorou, Kyparos & Nikolaidis, 2018).

Supplementation with tart cherry and pomegranate improves markers of inflammation, as reflected in the recovery and performance when ingested days before and several days after a competition (Rojano-Ortega, et al., 2021). In 2014, Bell et al. showed that supplementation with Tart Montmorency cherry concentrate in intermittent sports such as soccer was a good tool for recovery. This same conclusion was repeated in other similar studies, which showed improvements in oxidative stress after high-intensity training (Schneider, et al., 2018; Botwell & Kelly, 2019). Beet juice is another ergogenic aid with high antioxidant capacity, which has been shown to improve muscle pain and the recovery of muscle function (He, Li, Liu, Chuang, Yang & Zuo, 2016). Black currant has also been used, as it has demonstrated an improvement in cycling performance (Murphy, Cook & Willems, 2017).

In the results obtained, we found that after the incorporation of the nutritional intervention with antioxidants during the determined period of ten weeks of competition, there was a significant decrease in the amount of ROM, and a significant increase in the BAP/ROM index, and therefore in the antioxidant capacity, despite the fact that the concentration of BAP decreased.

It is well known that regular exercise can benefit health by improving the body’s antioxidant defenses (de la Cruz Sánchez, et al., 2015). However, intense exercise can generate excess reactive oxygen species (ROS), leading to oxidative stress-related tissue damage and impaired muscle contractility. Interestingly, moderate exposure to ROM is necessary to induce the body’s adaptive responses, such as the activation of antioxidant defense mechanisms. Antioxidant supplementation can reduce ROM levels and muscle fatigue, as well as improve recovery from exercise (He et al., 2016).

According to the data obtained, we could argue that a nutritional intervention with antioxidants after activity produces an increase in antioxidant capacity by reducing the amount of ROS, thereby preventing their excess production and accumulation, which can result in muscle fatigue and contractile dysfunction (Powers, et al., 2011).

Further studies that focus on minimizing oxidative damage and maximizing the exercise-induced adaptive response are essential. Developing promising strategies that combine an effective natural antioxidant diet with personalized exercise within a variety of populations could greatly improve health and quality of life (He et al., 2016).

On the other hand, there is now much evidence that high and sustained doses of antioxidants can hinder certain adaptations (Merry & Ristow, 2016). This study provides data that may point to the protective effect of antioxidants used in post-training and post-match intervention against exercise-induced muscle damage, being important to always refer to the intervention period and dosage established to avoid entering into controversy with possible negative adaptations in other intervention periods or higher dosages of antioxidants, as indicated by Merry et al. (2016).

Our greatest strength is that it provides new evidence on specific supplementation for the reduction of parameters related to oxidative stress derived from physical exercise. The main limitations of this research are the small size of the sample, and the fact that we did not divide the players by positions in the field, nor did we assess whether or not the player played the 10 official matches during the intervention period, and these factors could modify the results. Another limitation is to distinguish which supplementation makes each effect, so it would be interesting in future studies, to perform it in different stages from pre-season.

Conclusions

The intervention with antioxidants post-training and post-match for a period of ten weeks caused significant changes, with a decrease in the ROM values and an improvement in the antioxidant potential of the soccer player expressed by the BAP/ROM index. Therefore, it can be concluded that it is a valid strategy for improving the antioxidant capacity of the soccer player during the competition period.

The improvement in the AA/EPA ratio, although not significant, could be related to anti-inflammatory properties in addition to the antioxidant properties already described.

Practical Applications

Practitioners, trainers and nutritionists could use this concentrated polyphenol-rich supplement to improve the antioxidant capacity of the soccer players during the competition period, in order to optimize recovery and sports performance.

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Conflicts of Interest
The authors declare no conflict of interest.

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