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# Oxidative Stress, Testosterone, Cortisol, and Vitamin D: Differences in Professional Soccer Players of African and Caucasian Origin

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### Highlights of the Study

- Racial differences (Africans vs. Caucasians) in the behavior of oxidative stress, testosterone (T), cortisol, and vitamin D (Vit D) in professional soccer players are not associated with variations in athletic performance.
- This finding can be explained by the higher levels of T in Africans which counteract lower levels of Vit D and higher levels of oxidative stress.
- Individualized training programs and supplementation of antioxidants and Vit D are suggested.

**Keywords**
Cortisol · Oxidative stress · Soccer · Testosterone · Vitamin D

**Abstract**
***Background:*** Under conditions of intense exercise, the production of free radicals and cortisol increases, whereas blood levels of testosterone and vitamin D decrease. The aim of the study was to evaluate the behavior of these parameters, ethnic differences, and their relationships with overtraining. ***Materials and Methods:*** Fifty professional soccer players were studied. Oxidative stress, testosterone, cortisol, and vitamin D were collected in pre- and mid-competitive season, and their differences in Africans and Caucasians were evaluated. ***Results:*** An increase in oxidative stress was observed in mid-season in both groups, but this was more significant in Africans (386 ± 162.6 vs. 277.8 ± 106.9 UCarr, $p$ = 0.005; 2,965.4 ± 815.8 vs. 2,560.6 ± 608.1 BAP, $p$ = 0.035). Levels of testosterone and vitamin D were higher in August compared to February in all participants; in both months, testosterone levels were higher in Africans (11.5 ± 2.4 vs. 9.1 ± 2.6, $p$ = 0.004; 10.3 ± 1.6 vs. 7.7 ± 2.3, $p$ = 0.000), whereas vitamin D levels were higher in Caucasians (39.4 ± 11.1 vs. 33.4 ± 9.7, $p$ = 0.048; 31.8 ± 9.7 vs. 27.4 ± 9.4, in August and February, respectively). Insufficient/deficient levels of vitamin D were more frequently observed in Africans, but the difference was close to significance only in August. ***Conclusions:*** Although lower levels of vitamin D and higher levels of cortisol and oxidative stress in mid-season in Africans could have a negative influence on performance, no symptoms of overtraining were observed, probably due to higher levels of testosterone which enable homeostatic balance.



## Introduction

The practice of soccer at a professional level is very demanding in terms of physical resources and requires important physiological adaptations. Indeed, under con-

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ditions of strenuous exercise, changes in plasma levels of several biological mediators have been reported. In such conditions, the production of free radicals (FRs) in skeletal muscles increases significantly. Within a physiologic limit, FRs have positive effects, enhancing muscle contraction, cell growth, immune function, and upregulating the expression of antioxidant substances [1, 2]. However, prolonged and intense exercise which is not followed by an adequate rest period can lead to excessive production of FRs which, if not counteracted by the antioxidant system, can cause oxidative damage and can lead to muscle fatigue and overreaching/overtraining situations [1, 2]. At the same time, a decrease in levels of testosterone (T) and an increase in plasma levels of cortisol (C) have also been observed. Given that T and C play a significant role in protein and carbohydrate metabolism, a greater than 30% decrease in their ratio has been considered a marker of overtraining [3, 4]. Plasma levels of vitamin D (Vit D) have been also investigated because this vitamin is necessary for the maintenance of musculoskeletal performance and has been found to be insufficient in 30–40% of soccer players during the winter. Recent studies have shown relationships between FRs, T, C, and Vit D in professional soccer players which can be summarized as follows: an inverse relationship between Vit D and FRs [5]; a positive relationship between Vit D and T during summer and between FRs and C in winter (i.e., mid-competitive season) [6, 7]. In recent years, more and more African athletes have been recruited by professional soccer teams, due to expectation of higher levels of performance, related to greater muscular strength and/or sprint velocity of African athletes. It is not known whether such differences in performance are associated with some biological mediators. After adjustment for age and other confounding factors (i.e., socioeconomic status and lifestyle behaviors) [8], the general population of Africans compared to Caucasians show higher levels of markers of oxidative stress after exercise [9, 10], significantly higher free T levels [11, 12], lower morning levels, and higher evening C levels; taking into account the circadian variations of this hormone, the Africans show a flatter diurnal C slope [8, 13]. Africans have also been reported to have lower serum levels of Vit D serum, probably due to ethnic differences in polymorphisms in Vit D-binding protein [14, 15]. On the basis of these observations, and considering the lack of data on the ethnic differences in athletes, the aim of the present study on professional soccer players was twofold: first, to evaluate the behavior of these biological mediators, and their relationships, in two different periods of the competition season (at the beginning of the season and mid-season); and second, to study variations related to different ethnic origins and their possible relationships with the potential onset of symptoms of overtraining.

**Table 1.** Demographic and anthropometric characteristics of participants

| | Africans (*n* = 12) | Caucasians (*n* = 38) | *p* value |
|---|---|---|---|
| Age, years | 25.1±4.2 | 26.6±4.6 | 0.1 |
| Height, cm | 179.5±4.6 | 177.4±6.2 | 0.1 |
| Weight, kg | 77.5±5.6 | 75.3±5.6 | 0.1 |
| BMI | 24±1.1 | 23.9±1.8 | 0.4 |

## Materials and Methods

This study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. All participants were informed about the purpose and test procedures and gave their written consent. Institutional Ethics Committee approval was not required because of the observational nature of the study [16]. The athletes enrolled in the study were male football players (starting lines or substitutes), with at least 5 years of experience in soccer either at a professional or semi-professional (juvenile) level. All the athletes belonged to a team of the Second Italian Division (Serie B) and were studied during three competitive seasons (2018–2021).

The exclusion criteria were the following: (a) any medical or endocrine disorder that could affect endogenous hormonal production, (b) no regular supplementation of Vit D, (c) intestinal disorders that could impair absorption (e.g., celiac disease, inflammatory bowel disease, or irritable bowel syndrome), (d) suspicion of the use of drugs, (d) contract ending before the conclusion of the study, and (d) serious injuries with a loss of more than 20% training sessions and matches. All athletes were evaluated in two different periods, at the beginning of the competitive season (August) and mid-season (February). The preseason period starts in late July, and the competitive season starts in the third or fourth week of August and ends in May; the off-season period takes place in June during which training is reduced. February represents the middle part of the competitive season, when training and matches are most frequently practiced. Indeed, during the competition season the athletes enrolled in this study took part weekly in 5 training sessions (approximately 90 min each). The intensity, volume, and methods of training were similar for all athletes. However, goalkeepers and occasionally other players in the team were given specific individual training sessions. Moreover, they took part in one official game (occasionally 2 games) per week. Clinical examinations were performed regularly. In particular, symptoms of possible overtraining (fatigue, muscle pain, sleep disturbances, and depressed mood) were carefully and regularly checked. A qualified dietitian provided nutrition counseling to the players, their coaches, and trainers, guiding them in the correct interpretation of food labels and in the choice of well-selected food. The training diet was made up of 55–65% carbohydrate, 12–15% protein, and less than



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**Table 2.** FRs (expressed in UCarr), T, C, and Vit D values in August and February in African and Caucasian soccer players

| | Africans | | | Caucasians | | |
|---|---|---|---|---|---|---|
| | August | February | *p* value | August | February | *p* value |
| FRs | 219.9±35.1 | 386±162.6 | **0.001** | 211.4±49.2 | 277.8±106.9 | **0.000** |
| >300 | 0/12 | 3/12 | | 1/38 | 8/38 | **0.001** |
| >500 | 0/12 | 3/12 | **0.004** | 0/38 | 3/38 | |
| BAP | 2,239.3±256.6 | 2,965.4±815.8 | **0.003** | 2,224.5±343.9 | 2,560.6±608.1 | **0.002** |
| BAP/F | 10.4±2.3 | 8.9±4.5 | 0.1 | 10.9±2.6 | 9.6±1.7 | **0.008** |
| Free T | 11.5±2.4 | 10.3±1.6 | 0.088 | 9.1±2.6 | 7.7±2.3 | **0.007** |
| C | 8.2±1.6 | 9.6±1.7 | **0.024** | 8.6±2.3 | 10.9±4.4 | **0.002** |
| T/C | 1.46±0.4 | 1.08±0.1 | **0.007** | 1.1±0.3 | 0.81±0.3 | **0.000** |
| T/C < 0.75 | 1/12 | 2/12 | 0.537 | 3/38 | 12/38 | **0.009** |
| Vit D | 33.4±9.7 | 27.4±9.4 | 0.07 | 39.4±11.1 | 31.8±9.7 | **0.001** |
| >30, *n/N* (%) | 6/12 (50) | 4/12 (33.3) | 0.4 | 30/38 (78.9) | 21/38 (55.2) | |
| 20–30, *n/N* (%) | 5/12 (41.6) | 4/12 (33.3) | | 8/38 (21) | 14/38 (36.8) | |
| <20, *n/N* (%) | 1/12 (8.3) | 4/12 (33.3) | | 0/38 | 3/38 (7.8) | **0.028** |

30% fat, with an adequate intake of vitamins and minerals. In the off-season (holiday period), no dietary control was performed. Before each experimental session, players were instructed to avoid any supplement that could modify blood parameters. Body weight and height were measured, and BMI was calculated. Blood samples were collected in the morning (around 8–9 a.m.) from the antecubital vein while in a seated position, after the athletes had been fasting overnight and at least 48 h after the last training session. Care was taken to test the players in the same temporal order to avoid any circadian variation in the measured variables. Blood samples were centrifuged for 10 min at a speed of 3,500 rpm, and serum samples then were stored at −20°C prior to analysis.

FRs were evaluated using the Free Radical Analytical System (Diacron, Grosseto, Italy) [16, 17]. Briefly, in a pipette, 25 μL of the serum sample was mixed with an acetic acid buffered solution (pH 4.8), and chromogenic substrate was then added to the mixture. The mixture was centrifuged and then incubated in the thermostatic block of the system. Absorbance was recorded at 505 nm. The measurement is expressed in Carratelli units (UCarr), where 1 UCarr corresponds to 0.08 mg/dL $H_2O_2$. The suggested upper limit for FRs is >300 UCarr. A value >500 UCarr is considered indicative of severe oxidative stress.

The biological antioxidant activity (BAP) of plasma was measured using a colored solution containing ferric $Fe^{3+}$ ions bound to a chromogenic substrate which is decolorized on reduction of $Fe^{3+}$ to $Fe^{2+}$ ions by the reducing power of the antioxidant activity of plasma added to the reaction solution. The intensity of decoloration can be measured photometrically at the wavelength of 505 nm. The normal value for BAP in healthy subjects is >2,200 μmol/L.

Free T and C were analyzed with assay kits (DRG Diagnostics International Inc, New York, USA). The intra- and interassay coefficients of variation (CVs) for T were 3.7% and 5.6%, respectively. The intra- and interassay CVs for C were 4% and 5.7%, respectively. All assays were performed in duplicate. The range of normal values is 2.6–8.7 ng/mL for T and 6.4–21 μg/dL for C. A T/C ratio <0.75 is considered a risk factor of overtraining [3]. Vit D was evaluated using DiaSorin 25 hydroxyvitamin D (DiaSorin Inc S.p.A, Italy); repeatability CV = 3–6% and reproducibility CV = 6–11%, according to standard operating procedures. Vit D levels >30 ng/dL are considered as sufficient, between 20 and 30 ng/ml as insufficient, and between 10 and 20 ng/ml as deficient.

*Statistical Analysis*

We compared FRs, BAP, BAP/FRs ratio, T, C, and Vit D values, collected in preseason and mid-season in African and Caucasian athletes. A comparison between African and Caucasians in each experimental session was performed. Data are reported as mean ± standard deviation for continuous variables, whereas categorical and dichotomous variables are reported as frequencies and percentage. The two-sample Student's *t* test was used to compare continuous variables, when the distribution of data was normal; the Wilcoxon's rank sum test was used otherwise. The $\chi^2$ test was used to evaluate associations between categorical data. $p < 0.05$ was considered significant. The Pearson coefficient was used to assess the relationships between Vit D, FRs, T, C, and T/C ratio. When an athlete was a member of the club for two or three consecutive seasons, the average of values was computed.

## Results

Out of 62 athletes initially enrolled in the study, 12 were excluded from the analysis. Three were goalkeepers, who performed a milder training program; 4 were transferred to another club; and 5 were injured during the season and did not participate for a long time in training and games. Therefore, only 50 athletes were evaluated; out of them, 38 were Caucasians, and 12 were Africans or with African ancestors. We did not observe any differences in demographic and anthropometric characteristics of participants (Table 1).

**Table 3.** FRs (expressed in UCarr), BAP, T, C, and Vit D values in African and Caucasian soccer players, compared in different periods of the competitive season

| | July | | | February | | |
|---|---|---|---|---|---|---|
| | Africans | Caucasians | *p* value | Africans | Caucasians | *p* value |
| FRs | 219.9±35.1 | 211.4±49.2 | 0.2 | 386±162.6 | 277.8±106.9 | **0.005** |
| >300, *n*/*N* (%) | 0/12 | 1/38 (2.6) | 0.5 | 6/12 (50) | 11/38 (28.9) | 0.1 |
| BAP | 2,239.3±256.6 | 2,224.5±343.9 | 0.4 | 2,965.4±815.8 | 2,560.6±608.1 | **0.035** |
| BAP/FRs | 10.4±2.3 | 10.9±2.6 | 0.2 | 8.1±1.7 | 9.6±1.7 | **0.006** |
| Free T | 11.5±2.4 | 9.1±2.6 | **0.004** | 10.3±1.6 | 7.7±2.3 | **0.000** |
| C | 8.2±1.6 | 8.6±2.3 | 0.2 | 9.6±1.7 | 10.9±4.4 | 0.1 |
| T/C | 1.46±0.4 | 1.1±0.3 | **0.002** | 1.08±0.1 | 0.81±0.3 | **0.018** |
| T/C < 0.75, *n*/*N* (%) | 1/12 (8.3) | 3/38 (7.8) | 0.9 | 2/12 (16.6) | 12/38 (31.5) | 0.3 |
| Vit D | 33.4±9.7 | 39.4±11.1 | **0.048** | 27.4±9.4 | 31.8±9.7 | 0.08 |
| >30, *n*/*N* (%) | 6/12 (50) | 30/38 (78.9) | 0.05 | 4/12 (33.3) | 21/38 (55.2) | 0.1 |
| 20–30, *n*/*N* (%) | 5/12 (41.6) | 8/38 (21.1) | 0.05 | 4/12 (33.3) | 14/38 (36.8) | 0.1 |
| <20, *n*/*N* (%) | 1/12 (8.3) | 0/38 | | 4/12 (33.3) | 3/38 (7.8) | |

*Comparison between Preseason and Mid-Season in Caucasian and African Athletes*

FRs were significantly higher in February and exceeded the suggested upper limit of 300 UCarr in 3/12 Africans (25%) and in 8/38 (21%) Caucasian athletes; UCarr values >500 were registered in 3/12 Africans (25%) and in 3/38 (7.8%) Caucasian players (Table 2). The BAP values were also significantly increased in February in both groups but proportionally less than FRs; thus, the BAP/FRs ratio was reduced in this month. Free T values were higher in the warm season, the difference being statistically significant in Caucasians and near to significance in Africans. On the contrary, the values of C were higher in February, the difference being significant in both groups. Therefore, the T/C ratio was significantly reduced in February. The African athletes with a T/C ratio <0.75 were 1/12 (8.3%) in August and 2/12 in February (16.6%) and the Caucasian athletes 3/38 (7.8%) in August and 12/38 in February (31.5%). Vit. D mean values were higher in August and lower in February in all athletes, but the difference was significant only for Caucasians. Accordingly, the percentage of athletes with insufficient/deficient levels was higher in February only in this group (17 vs. 8; $p = 0.028$).

*Comparison between Africans and Caucasians Preseason and Mid-Season*

Analyzing the differences between Africans and Caucasians, in preseason (August) and mid-season (February), respectively (Table 3), we found that FRs values in July were similar in both groups, whereas in February, the increase was significantly higher in Africans (386 ± 162.6 vs. 277.8 ± 106.9). Similarly, the BAP was increased in February more in Africans (2,865.4 ± 815.8 vs. 2,560.6 ± 608.1). The BAP/FRs ratio decreased in February in both groups, possibly due to incomplete antioxidant responses. However, the decrease was more evident in African compared to Caucasian soccer players (8.1 ± 1.7 vs. 9.6 ± 1.7), showing that the production of antioxidant substances in these athletes were proportionally inferior. T levels were higher in Africans, both in August and February. On the contrary, C levels were higher in Caucasians, but the difference did not reach the significance level. Thus, the T/C ratio was higher in African athletes. No significant difference in the percentage of T/C ratio <0.75 was observed. Plasma levels of Vit D were higher in Caucasians (significantly only in August).

*Relationships between Biochemical Parameters*

A strong relationship between Vit D and T was observed in August in Caucasians ($R = 0.611$, $p = 0.000$) and between C and FRs in February in both groups ($R = 0.769$, $p = 0.003$ in Africans; $R = 0.654$, $p = 0.000$ in Caucasians). Moreover, a positive relationship was found between BAP and FRs, both in August and February in Caucasians ($R = 0.364$, $p = 0.024$; $R = 0.838$, $p = 0.000$, respectively) but not in Africans. No other significant relationships between the biological parameters were found. None of the athletes during the season developed symptoms related to overtraining or overreaching (e.g., fatigue, muscle pain, sleep disturbances, and depressed mood).

## Discussion

The present study confirms previous findings and adds new preliminary information about racial differences on the levels of oxidative stress, T, C, and Vit D in professional soccer players. The dramatic rise of FRs blood levels in February is in agreement with Ferrari et al.'s [5] findings in similar experimental conditions and can be explained by the fact that FRs are formed during the mitochondrial respiration, whose rate is enhanced during exercise or consequently to reperfusion after exercise-induced transient skeletal muscle ischemia [18]. During muscle contraction, the primary sources of FRs are NADPH oxidase, xanthine oxidase, and nitric oxide synthase. Accumulation of lactate, higher body temperature, and secretion of cytokines contribute to increased RFs. Within physiologic limits, FRs produced in skeletal muscle have positive effects, enhancing muscle contraction, cell growth, and immune function; however, when produced in excess, they can interact with the cell membrane lipid bilayer, damaging a range of biological molecules, including not only lipid peroxidation and nucleic acids but also carbohydrates and proteins. Thus, chronic oxidative stress may inhibit physiological adaptations to exercise and contribute to the onset of muscle fatigue and reduction in exercise performance, including the development of overreaching and, in severe cases, overtraining [19]. The increased production of RFs is counteracted, within certain limits, by a contemporary increase of antioxidant substances, which restore the redox state and therefore prevent the decline in performance. It is reasonable that in our athletes, given the absence of symptoms, the activation of the antioxidant system was adequate and efficacious. It is worth noting that the rise of FRs in February was more evident in Africans than in Caucasians, as shown not only by the higher absolute values but also by the observation of pathologic values (>300 UCarr) in a higher percentage of Africans (50%) than in Caucasians (28.9%). This is in agreement with previous findings in vivo and in vitro [9, 10]. Indeed, Fairheller et al. [9, 10] found that African Americans had higher plasma superoxide dismutase, total antioxidant capacity and protein carbonyl levels compared to Caucasians in basal conditions and after a sub-maximal treadmill test. There results were confirmed in vitro in cultured human umbilical vein endothelial cells, which exhibited enhanced oxidative stress and inflammation as evidenced by increased expression of nicotinamide adenine dinucleotide phosphate oxidase, interleukin-6, anti-inducible nitric oxide synthase, anti-endothelial nitric oxide synthase and higher production of nitric oxide end-products. The higher values of blood levels of T found in summer in all the athletes are not surprising, given that the warm season is characterized by a more uninhibited lifestyle and sexual excitement. Similarly, the finding of higher T values in Africans is in agreement with previous research and has been reported to be due to genetic factors [12]. On the contrary, morning C levels were higher in the winter, in the mid-season period, in Caucasian as well in African athletes. This could be due to the adverse climatic conditions and mainly due to physical and psychological stress caused by intensive training and frequent competitions. Our observations are in agreement with results from previous studies [5, 6]. A T/C ratio of <0.75 was observed only in a few athletes at the beginning of the training and more frequently during the competition season (31.5% in Caucasian and 16.6% in Africans).

Given that T is a physiologic anabolic agent, and that C promotes catabolic activities, it is suggested that this ratio could be an expression of the balance between anabolism and catabolism and so when below a threshold, could be assumed as a marker of overtraining [5, 6]. We cannot confirm this suggestion because none of our athletes reported symptoms of overtraining (i.e., fatigue, muscle pain, sleep disturbances, and depressed mood). Indeed, the anabolic and functional status of the muscles is influenced by a number of factors (other hormones, such as IGF-1 and GH, affinity of hormonal receptors, composition of the diet, and neural factors); we suggest that the T/C ratio can be considered as an indirect and rough measure of muscle metabolism and function [5, 6]. Vit D values were higher in August both in Africans and in Caucasians. This observation is not surprising because it is well known that Vit D is mainly synthesized by the skin when exposed to ultraviolet B radiation. Several athletes showed insufficient/deficient levels of the vitamin, mainly in February, i.e., in the middle period of the competition season. In detail, in Caucasians, we found insufficient/deficient Vit D values (8/38 [21%] in September and 17/38 [44.7%] in February), which are close to those reported earlier (42% and 58%, respectively) [5, 6]. In comparison, Vit D values were lower, and the percentage of subjects with insufficient/deficient levels was higher in African athletes September and in February. The reasons of the insufficient/deficient levels in February are not clear. A reduced exposure to sun is unlikely, given that the athletes in the present study and previous studies [5, 6] regularly trained and competed at latitudes with middle/high exposure to the sun. Similarly, a reduced dietary intake is unlikely to be a causative factor. We suggest that the insufficiency may be related to a higher consumption of Vit D linked to a greater muscle activity due to repeated and intense exercise [6, 15]. As for racial differences, it has been hypothesized that the lower levels of Vit D in Africans are due to a genetic polymorphism responsible of a reduced Vit D-binding protein, which results in levels of bioavailable 25-hydroxyvitamin D similar to those in Caucasians. Therefore, these lower levels probably would not indicate a true Vit D deficiency, given that Africans have higher bone mass density, higher calcium levels, and only slightly higher parathyroid hormone levels compared to their Caucasian counterparts [14]. Despite this consideration, and although it is questioned whether Vit D supplementation can really improve the athletic performance, an optimal Vit D level is deemed necessary to maintain the musculoskeletal performance [20]. As for the relationships between the biological parameters under study, we found a significant relationship between Vit D and T in September in Caucasians and between C and FRs in February, both in Caucasian and African soccer players. The positive relationship between Vit D and T could suggest a stimulating effect of Vit D on the secretion of T, given the presence of Vit D receptors in Leydig cells [21]. However, we cannot exclude the possibility that this relationship could be casual or spurious because in the warm season, several factors (climate, uninhibited lifestyle, more sexual excitement, less psychological and physical stress) can also enhance hypothalamic function, stimulating the release of gonadotropin-releasing factors [22]. Therefore, external factors could drive the behavior of both biological parameters in the same direction. Indeed, besides studies which support the hypothesis of a stimulating effect of Vit D on T secretion, others deny this possibility, given that Vit D supplementation has no effect on the hormonal levels [23, 24]. Indeed, we did not observe a direct relationship between Vit D and T in the winter, and this finding does not support the hypothesis of a cause-effect relationship. Similarly, the significant positive relationship observed between FRs and C could suggest that FRs may stimulate the adrenal gland directly and/or via the hypothalamic-adrenal axis [25, 26]. However, as for the connection between Vit D and T, this could be a spurious correlation, due to the activation of different alternative pathways. Indeed, the intense and repetitive exercise stimulates also the autonomic sympathetic system as well as the release of humoral mediators (interleukins, angiotensin II, and beta-endorphins) which could drive both FRs and C in the same direction [22]. Another noteworthy finding is the positive relationship between the FRs and BAP in Caucasians, suggestive of a good homeostatic balance. However, such a relationship was not found in Africans, but this could be due the limited number of enrolled players in this group. The analysis of other relationships provided erratic results with insignificant differences. Thus, we cannot confirm the inverse relationship found by Ferrari et al. [5] between Vit D and FRs, which, in our opinion, could be due to the antioxidant properties of this vitamin, demonstrated in cell cultures and animal models [27, 28].

Several limitations of the present study must be taken into account. First, considering the small sample size of African soccer players, the results need to be considered with caution. Second, the measures of biological parameters were performed only twice in the year. With reference to this, blood C was measured only once in a day, about 60 min after waking up in the morning. Given that Africans, compared to Caucasians, show lower levels of C in the morning but higher levels in the evening with a flatter diurnal slope, it was not possible to appreciate the circadian differences of this hormone. Finally, given that the balance between hormones and vitamins can be modified by diet, exposure to the sun, and training/playing workloads, the validity of results is limited to the specific experimental conditions of our study. Therefore, studies in athletes practicing sports with different training workloads, climatic conditions, and dietary regimens are needed in order to evaluate possible differences in other experimental conditions.

## Conclusions

Our findings are in agreement with previous observations regarding the behavior of FRs, T, C, T/C ratio, and Vit D in different periods of the competitive season. A significant increase of FRs in mid-season, when not properly balanced by the blood antioxidant potential, could be the reason for overtraining/overreaching symptoms. This effect could be limited by individualized training programs and counteracted by a supplement of antioxidants substances. Similarly, a frequent monitoring of the Vit D level, which can influence the performance, is suggested. A novel finding is that some interesting differences between Africans and Caucasians have been observed. Although Africans showed higher FRs and lower Vit D levels than Caucasians, these changes were not associated with remarkable variations in athletic performance. This figure could be an expression of a different homeostatic balance, given that potentially negative factors could be counteracted by higher T levels and more robust antioxidant potential.

## Statement of Ethics

The procedures followed were in accordance with the Declaration of Helsinki, and informed written consent was obtained from each patient.

## Conflict of Interest Statement

None.


## Funding Sources

None.


## Author Contributions

Both authors contributed equally to the research and the manuscript.

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