

Original Research

Exploratory study of seasonal changes in body composition, bone parameters, testosterone, and cortisol in Spanish male footballers.

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Abstract: Body composition (BC) and bone quality are critical factors in the reduction of sport-related injuries and in the improvement of physical performance. Furthermore, testosterone and cortisol may influence these parameters. The aim of this work was to examine changes in total and regional bone mineral density (BMD), bone mineral content (BMC), lean mass (LM), fat mass (FM), total cortisol (tC) and total testosterone (tT) levels of professional soccer players of the Spanish professional league over 2 consecutive seasons in the following stages: start-preseason, start-season, mid-season, and end-season. An observational study was conducted with twenty-six male players (age = 26.9 ± 4.5 years). Statistical analyses included repeated-measures ANOVA to assess changes across the four time points, with post hoc tests corrected using the Bonferroni method. Pearson's correlation coefficient (r) was used to evaluate associations between variables. FM was more responsive to physical activity across a soccer season than the other parameters analyzed. Results revealed an association between tT variations and changes of trunk FM and LM ($p < 0.05$). Therefore, soccer season leads to variations in BC and serum biomarkers of the players that must be considered to improve players performance and prevent injuries.

Keywords: body parameters; bone remodeling; testosterone; cortisol; soccer season.



1. Introduction

Professional soccer season includes preseason, in-season, and off-season periods. The preseason being a relatively short period, it is characterized by high-volume and high-intensity training to improve technical and physical performance, as well as facilitate the adaptation of players after the off-season (Calleja-Gonzalez et al., 2020). The off-season is typically a period of reduced training or complete rest, which can lead to detraining effects, including declines in cardiovascular fitness, muscular strength, and technical skills (Mujika & Padilla, 2000). The extent of these losses depends on the duration of inactivity and the strategies implemented to maintain fitness levels, such as individualized training programs or minimal activity sessions (Silva et al., 2016). The in-season period is mainly focused on technical and tactical sessions, but intense exercises are also included during matches (Bangsbo et al., 2006). Thus, the characteristics of different season training programs and match performance influence the physical demands placed on players, highlighting the importance of structured off-season programs to minimize performance losses.

Therefore, optimizing body composition not only enhances physical performance but also contributes to overall musculoskeletal health, allowing players to better cope with the high physiological demands of soccer competition and maintain long-term athletic performance. In addition, during soccer competition, physical demands and physiological changes are related to player success (Gomez-Piqueras et al., 2019). Body composition has been

identified as a critical factor in the improvement of physical condition and in the reduction of sport-related injuries (Iaia et al., 2009; Mills et al., 2017). Several studies have demonstrated that reduced body fat and increased muscle mass improve physical performance (Anwar & M Noohu, 2016; Silvestre et al., 2006). Additionally, bone remodeling also plays a key role in the physical fitness status of soccer players. Bone and muscle interactions are bidirectional (Brotto & Bonewald, 2015) and it has been demonstrated that muscle forces improve bone homeostasis (Avin et al., 2015), while impaired bone quality is related to reduced muscle strength in athletes (Schnackenburg et al., 2011). Moreover, different authors have shown that high-impact sports, such as soccer, increase bone mineral density (BMD) in comparison to low-impact sports, reducing risk for fractures (Ubago-Guisado et al., 2015; Vlachopoulos et al., 2018).

The season requirements also induce other physiological alterations that can be detected analyzing different biochemical markers (Lee et al., 2017). Given the crucial role of testosterone and cortisol in regulating physiological adaptations, their influence extends beyond muscle strength and metabolism to bone health. Testosterone plays a key role in bone formation by stimulating osteoblast activity and enhancing bone mineralization, which may help counteract the mechanical stress imposed by high-impact activities like soccer (Khosla & Monroe, 2018). Conversely, elevated cortisol levels, particularly during periods of intense training or accumulated fatigue, can accelerate bone resorption by increasing

osteoclast activity and inhibiting calcium absorption, ultimately leading to reduced bone mineral density (Weaver et al., 2016). Furthermore, it has been suggested that testosterone may protect against bone loss (Van Den Beld et al., 2000) and increase fat-free mass and muscle strength in men (Van Den Beld et al., 2000; Varanoske et al., 2020), while elevated plasma cortisol has been associated with BMD loss in the lumbar spine (Reynolds et al., 2005). However, the different published studies shown controversial results that could be related to the diverse experimental conditions and to the heterogeneous participants included in them (Molina-López et al., 2022).

Thus, an increased knowledge about physiological changes during soccer season may help to improve players conditions and performance. Therefore, the aim of this study was to analyze changes in body composition, bone density, cortisol and testosterone levels

in Spanish elite soccer players over a season to identify potential fluctuations and trends.

2. Materials and Methods

Participants - Adult male soccer players playing in the professional soccer championship in Spain were recruited. Players that did not participate in the whole season (from start-preseason to end-season) were excluded. Initially, 58 soccer players were accepted to participate in the study but, due to the injuries and the changes that occur in the transfer market, 26 players (age = 26.9 ± 4.5 years; height = 181 ± 5.4 cm; weight = 75.7 ± 5.1 kg) were included (Figure 1). All participants gave voluntary, written informed consent prior to participation in the study in accordance with the Helsinki Declaration and institutional ethics approval (CEI19/088).

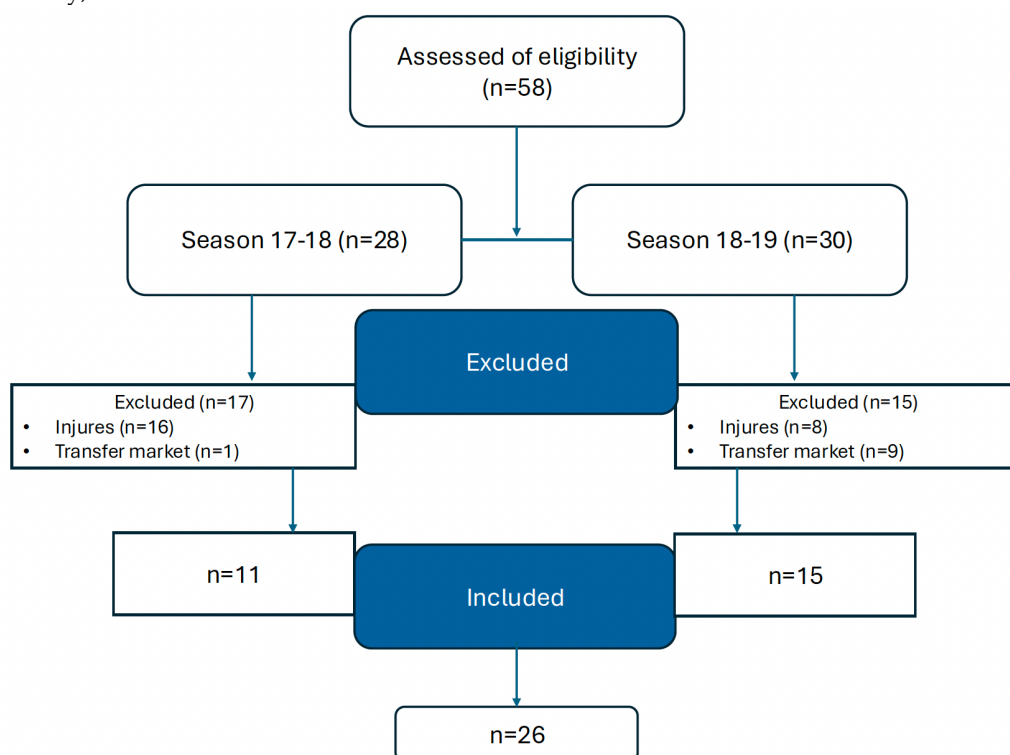


Figure 1. Selection of the study participants.

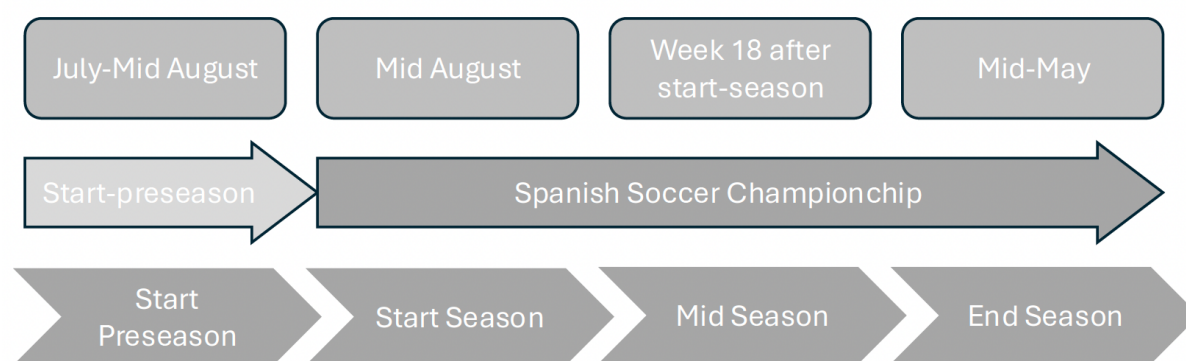


Figure 2. Timeline of the study's phases.

Study Design - The investigation was developed as an exploratory observational study (trial registration number: NCT04839172, 04/07/2021, retrospectively registered). Longitudinal changes in body composition were examined over 2 consecutive seasons (2017-2018 and 2018-2019), 4 times per season in the following stages: start-preseason, start-season, mid-season, and end-season (Figure 2). The preseason covers the period from the beginning of July until mid-August, while the Spanish soccer championship covers the period from mid-August until mid-May. Mid-season measures were taken at week 18 after the start of the season.

Body and bone parameters monitoring - At each testing time, data were obtained from routine testing by the medical services of the team.

Total and regional (legs, arms and trunk) BMD, bone mineral content (BMC), lean mass (LM) and fat mass (FM) were obtained using a dual-energy X-ray Absorptiometry scan (DEXA LUNAR iDXA SERIES X, GE Healthcare, USA) in accordance with standard procedures. Briefly, players lay down in a supine position on the scanning bed, with feet slightly apart

and hands next to hips with palms facing forward. Moreover, body mass index (BMI) was calculated as $\text{weight}/\text{height}^2$.

Fasting blood samples were collected at 8:00 am to determine total cortisol (tC) and total testosterone (tT) levels by chemiluminescence (ATELLICATM IM 1300/1600 Analyzer, Siemens Healthcare Diagnostics Inc, USA). The samples were collected in a fasted state, and the extraction was carried out by nurses sent by the external laboratory contracted by the football club for this task. Once collected, the samples were transported and safeguarded, ensuring the transport chain was maintained to guarantee the samples were kept in optimal conditions for analysis. After processing, it was the laboratory that sent the results to the club.

Statistical Analysis - A repeated-measures ANOVA was conducted to compare changes in body composition, bone parameters, and serum biomarkers across four time points in the season: start-preseason (SP), start-season (SS), mid-season (MS), and end-season (ES). The within-subjects factor 'Time' included these four levels. Subsequently, when ANOVA displayed a significant result, a post hoc pairwise t-test was performed to compare

means with the p-value corrected by the Bonferroni method. The prerequisites for normality and sphericity were evaluated using the Shapiro-Wilk test and the Mauchly test, respectively (Field, 2013, p.543). In case of breach of the normality condition, the non-parametric Friedman test was performed followed by the Nemenyi post hoc test (Demšar, 2006). In case of breach of the sphericity condition, the Greenhouse-Geisser correction for epsilon values < 0.75 and the Huynh-Feldt correction for epsilon values ≥ 0.75 were used as recommended by Barcikowski and Robe (1984). The effect size will be expressed using the Generalized Eta-Squared (η^2G) index for the repeated-measures ANOVA and the Kendall's W index for the Friedman test to assess the magnitude of the effects in both statistical approaches. To analyze the relationships between changes in body composition, bone parameters, and serum biomarkers, Pearson's correlation coefficient (r) was calculated. The statistical significance of r was assessed using a t-test with $n-2$ degrees of freedom to test the null hypothesis that $r = 0$ (Field, 2013, p.266). Interpretation of r values followed conventional thresholds: weak (0.1–0.3), moderate (0.3–0.5), and strong (>0.5) (Cohen, 1988, p.79). The significance level was set at $p < 0.05$. Data are presented as mean $\pm$ SD.

For the available sample size ($n=26$) and considering an effect size of $f = 0.4$ (Cohen, 1988, p.287), a significance of $\alpha = 0.05$ and conservative values of the nonsphericity correction coefficient (0.70) and the correlation among measures coefficient (0.5), a power of 99% was obtained in the ANOVA test for repeated measures.

3. Results

The study found significant seasonal changes in body composition and hormonal levels in elite soccer players. Bone mineral content (BMC) showed the greatest increase in the whole body and legs, while fat mass (FM) and lean mass (LM) varied throughout the season. Testosterone levels peaked at mid-season, while cortisol levels fluctuated, reaching their lowest at mid-season. Changes in FM and LM were associated with testosterone and cortisol variations.

Changes in body composition - Table 1 shows seasonal changes in BMC, BMD, LM, FM, and BMI. As indicated, we observed significant changes in BMC of the whole body, legs, and trunk across the season. The greatest significant increase in BMC was found at whole body when start-season was compared with mid-season. A similar increase was observed in the BMC of the legs at mid-season, in comparison to start-preseason. On the contrary, trunk BMC was significantly reduced at start-season. Unexpectedly, BMD variability across the season was low and the only significant seasonal change was found in the trunk. The smallest BMD of the trunk was observed at start-season, similar to BMC, and it was significantly lower compared to the start-preseason or to the mid-season values. Despite this changes, no significant differences were found in BMC and BMD at end-season.

FM (kg) and %FM seem to be the more responsive variable to physical activity (Table 1). These variables were significantly reduced at start-season compared with start-preseason. Similar decreased was observed at end-season when compared with start-preseason, except for arms. Concerning LM, our results showed that absolute LM (kg) is relatively stable across the season, observing only a substantial increase in the trunk at mid-season in comparison to start-preseason.

Table 1. Seasonal changes of the body composition and bone.

Body variable	Mean $\pm$ SD				Post hoc test					
	SP	SS	MS	ES	SP vs SS <i>p</i> -value	SP vs MS <i>p</i> -value	SP vs ES <i>p</i> -value	SS vs MS <i>p</i> -value	SS vs ES <i>p</i> -value	MS vs ES <i>p</i> -value
BMC (g)										
Whole body	3662 $\pm$ 263	3650 $\pm$ 265	3675 $\pm$ 255	3665 $\pm$ 254	ns	ns	ns	0.002	ns	ns
Legs ^b	1488 $\pm$ 112	1495 $\pm$ 115	1502 $\pm$ 110	1495 $\pm$ 111	ns	0.025	ns	ns	ns	ns
Arms ^d	494 $\pm$ 50	490 $\pm$ 46	495 $\pm$ 51	494 $\pm$ 51	ns	ns	ns	ns	ns	ns
Trunk ^d	1082 $\pm$ 100	1068 $\pm$ 104	1078 $\pm$ 97	1078 $\pm$ 99	0.023	ns	ns	ns	ns	ns
BMD (g/cm²)										
Whole body	1.43 $\pm$ 0.07	1.42 $\pm$ 0.08	1.43 $\pm$ 0.07	1.42 $\pm$ 0.08	ns	ns	ns	ns	ns	ns
Legs ^c	1.66 $\pm$ 0.09	1.65 $\pm$ 0.09	1.66 $\pm$ 0.09	1.66 $\pm$ 0.10	ns	ns	ns	ns	ns	ns
Arms ^d	0.92 $\pm$ 0.11	0.91 $\pm$ 0.11	0.92 $\pm$ 0.12	0.91 $\pm$ 0.11	ns	ns	ns	ns	ns	ns
Trunk ^b	1.22 $\pm$ 0.08	1.21 $\pm$ 0.08	1.22 $\pm$ 0.07	1.22 $\pm$ 0.08	0.005	ns	ns	0.005	ns	ns
FM (kg)										
Whole body	10.08 $\pm$ 1.78	8.95 $\pm$ 1.48	9.40 $\pm$ 1.87	9.07 $\pm$ 1.46	<0.001	ns	0.002	ns	ns	ns
Legs ^a	3.34 $\pm$ 0.71	2.99 $\pm$ 0.60	3.17 $\pm$ 0.67	3.00 $\pm$ 0.53	<0.001	ns	<0.001	ns	ns	ns
Arms ^d	1.15 $\pm$ 0.18	1.03 $\pm$ 0.17	1.11 $\pm$ 0.21	1.07 $\pm$ 0.18	<0.001	ns	0.036	ns	ns	ns
Trunk ^a	4.69 $\pm$ 1.06	4.02 $\pm$ 0.90	4.22 $\pm$ 1.13	4.10 $\pm$ 0.92	<0.001	0.041	0.009	ns	ns	ns
FM (%)										
Whole body	13.0 $\pm$ 2.1	11.7 $\pm$ 1.8	12.2 $\pm$ 2.2	11.9 $\pm$ 1.7	<0.001	ns	0.001	ns	ns	ns
Legs ^a	12.2 $\pm$ 2.3	11.0 $\pm$ 1.9	11.7 $\pm$ 2.3	11.1 $\pm$ 1.8	<0.001	ns	0.001	0.047	ns	ns
Arms ^d	11.9 $\pm$ 1.7	10.9 $\pm$ 1.9	11.7 $\pm$ 2.0	11.3 $\pm$ 1.7	0.010	ns	ns	ns	ns	ns
Trunk ^a	13.3 $\pm$ 2.9	11.5 $\pm$ 2.4	12.0 $\pm$ 3.0	11.7 $\pm$ 2.5	<0.001	0.043	0.007	ns	ns	ns
LM (kg)										
Whole body	63.48 $\pm$ 4.59	64.02 $\pm$ 4.33	63.79 $\pm$ 4.38	63.75 $\pm$ 4.37	ns	ns	ns	ns	ns	ns
Legs ^c	22.38 $\pm$ 1.83	22.62 $\pm$ 1.71	22.41 $\pm$ 1.85	22.44 $\pm$ 1.80	ns	ns	ns	ns	ns	ns
Arms ^a	8.02 $\pm$ 0.80	7.97 $\pm$ 0.85	7.90 $\pm$ 0.85	7.91 $\pm$ 0.84	ns	ns	ns	ns	ns	ns
Trunk ^a	29.60 $\pm$ 2.40	29.99 $\pm$ 2.26	30.02 $\pm$ 2.13	29.95 $\pm$ 2.25	ns	0.027	ns	ns	ns	ns
LM (%)										
Whole body	82.2 $\pm$ 2.1	83.6 $\pm$ 1.8	83.1 $\pm$ 2.1	83.3 $\pm$ 1.8	<0.001	0.030	0.003	0.038	ns	ns
Legs ^a	82.3 $\pm$ 2.2	83.6 $\pm$ 2.1	82.8 $\pm$ 2.2	83.3 $\pm$ 1.8	<0.001	ns	0.002	ns	ns	ns
Arms ^a	82.8 $\pm$ 1.9	83.7 $\pm$ 2.2	83.1 $\pm$ 2.6	83.5 $\pm$ 1.8	ns	ns	ns	ns	ns	ns
Trunk ^c	83.7 $\pm$ 2.9	85.4 $\pm$ 2.5	85.4 $\pm$ 2.5	85.3 $\pm$ 2.5	<0.001	0.003	0.007	ns	ns	ns
BMI (-)^a	23.55 $\pm$ 0.97	23.38 $\pm$ 0.97	23.42 $\pm$ 0.95	23.32 $\pm$ 0.98	ns	ns	0.029**	ns	ns	ns

Notes: ^a Repeated measures ANOVA; ^b Repeated measures ANOVA with Greenhouse-Geisser correction; ^c Repeated measures ANOVA with Huynh-Feldt correction; ^d Friedman test. SP = start-preseason; SS = start-season; MS = mid-season; ES = end-season. BMC = bone mineral content; BMD = bone mineral density; FM = fat mass; LM = lean mass; BMI: body mass index

However, the analysis of the relative lean component (LM%) showed significant differences between the testing times. For the whole body, legs, and trunk, the LM% was markedly increased at start-season in comparison to start-preseason and remained unchanged until the end of the study. Similar variability was observed in LM% of arms, but no significant differences were found across the season. These alterations in body composition may have contributed to the significant reduction in BMI observed across the season ($p < 0.05$).

Variations of serum testosterone and cortisol

- As showed in Figure 3, hormonal values of all set of samples remained within the normal ranges (total testosterone = 3-10 ng/mL; total cortisol = 5-25 $\mu\text{g/dL}$). The highest concentration of total testosterone (tT) was observed at mid-season ($7.38 \pm 2.26 \text{ ng/mL}$) and it was significantly increased in comparison to end-season ($6.30 \pm 1.87 \text{ ng/mL}$) (Fig. 3A). Total cortisol (tC) exhibited significant variation across the season ($\chi^2(3) = 27.47$, $p < 0.001$), with a large effect size ($W = 1.057$), whereas total testosterone (tT) showed variation, but it did not reach statistical significance ($F(3, 75) = 2.54$, $p = 0.088$), and the effect size was small ($\eta^2G = 0.022$). The smallest tC concentration was observed at mid-season ($13.12 \pm 2.09 \mu\text{g/dL}$) and it was significantly lower compared with start-preseason ($14.73 \pm 1.89 \mu\text{g/dL}$), start-season ($14.32 \pm 1.98 \mu\text{g/dL}$) and end-season ($16.07 \pm 2.56 \mu\text{g/dL}$). The data recorded at end-season was the highest tC concentration observed in our study, but no significant differences were obtained in comparison to start-preseason or to start-season (Fig. 2B).

As shown in Fig. 3C, the testosterone/cortisol ratio (tT/tC) exhibited significant variation across the season ($\chi^2(3) = 24.09$, $p < 0.001$), with a moderate effect size ($W = 0.927$). We observed the highest value of tT/tC at mid-season (0.056 ± 0.019), which was significantly increased in comparison to the tT/tC at start-season (0.046 ± 0.019).

However, tT/tC returned to similar start-season levels at end-season (0.042 ± 0.014).

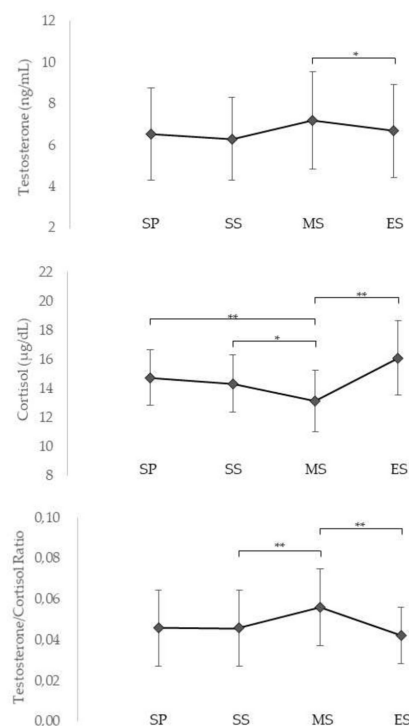


Figure 3. Serum biomarkers evolution across the season. (a) T testosterone levels; (b) Total cortisol levels; (c) Total testosterone/total cortisol ratio. SP = start-preseason; SS = start-season; MS = mid-season; ES = end-season. The graphs represent the mean $\pm$ SD ($n = 26$). * $p < 0.05$; ** $p < 0.01$.

Relationship between body composition and serum biomarkers - To determine if changes in body composition were related to the serum biomarkers alterations, individual variations of the parameters studied (tC, tT, total and regional BMD, BMC, LM and FM) from start-preseason to start-season, start-season to mid-season and mid-season to end-season were calculated (data not shown). Changes in whole body and trunk FM (kg and %) were positively associated with changes in tT concentrations, whereas changes in whole body (%) and trunk (kg) LM were negatively associated with changes of this hormone (Figure 4a). On the other hand, changes in legs FM (kg and %) and legs LM (%) were negatively and positively associated with variations in tC concentrations, respectively (Figure 4b).

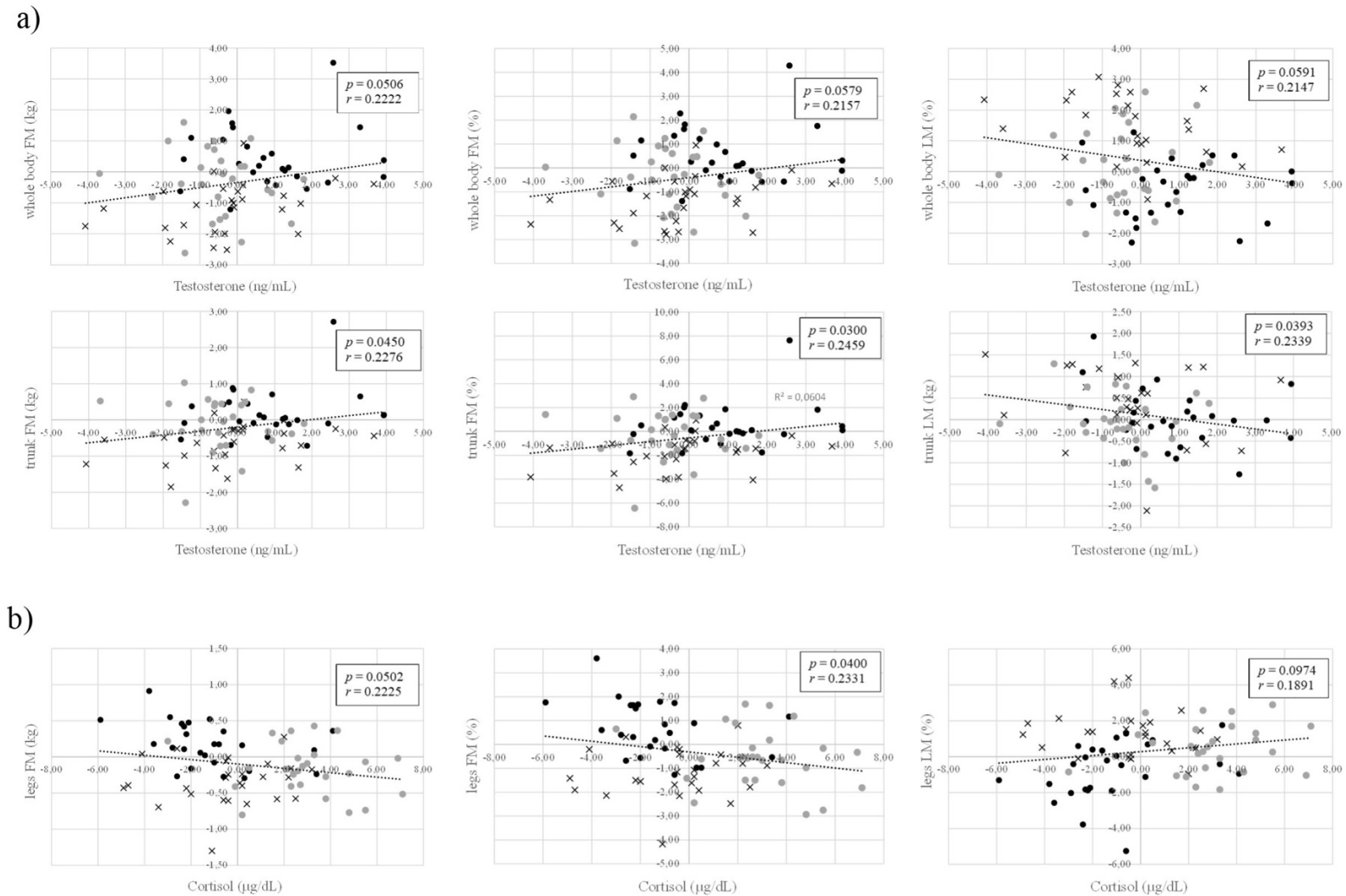


Figure 4. Correlations, across the 4 periods of the season, between variations of (a) body mass components against testosterone, (b) body mass components against cortisol. × Variation from start-preseason to start-season; • Variation from start-season to mid-season; • Variation from mid-season to end-season. R² and *p* values are extracted from linear regression models.

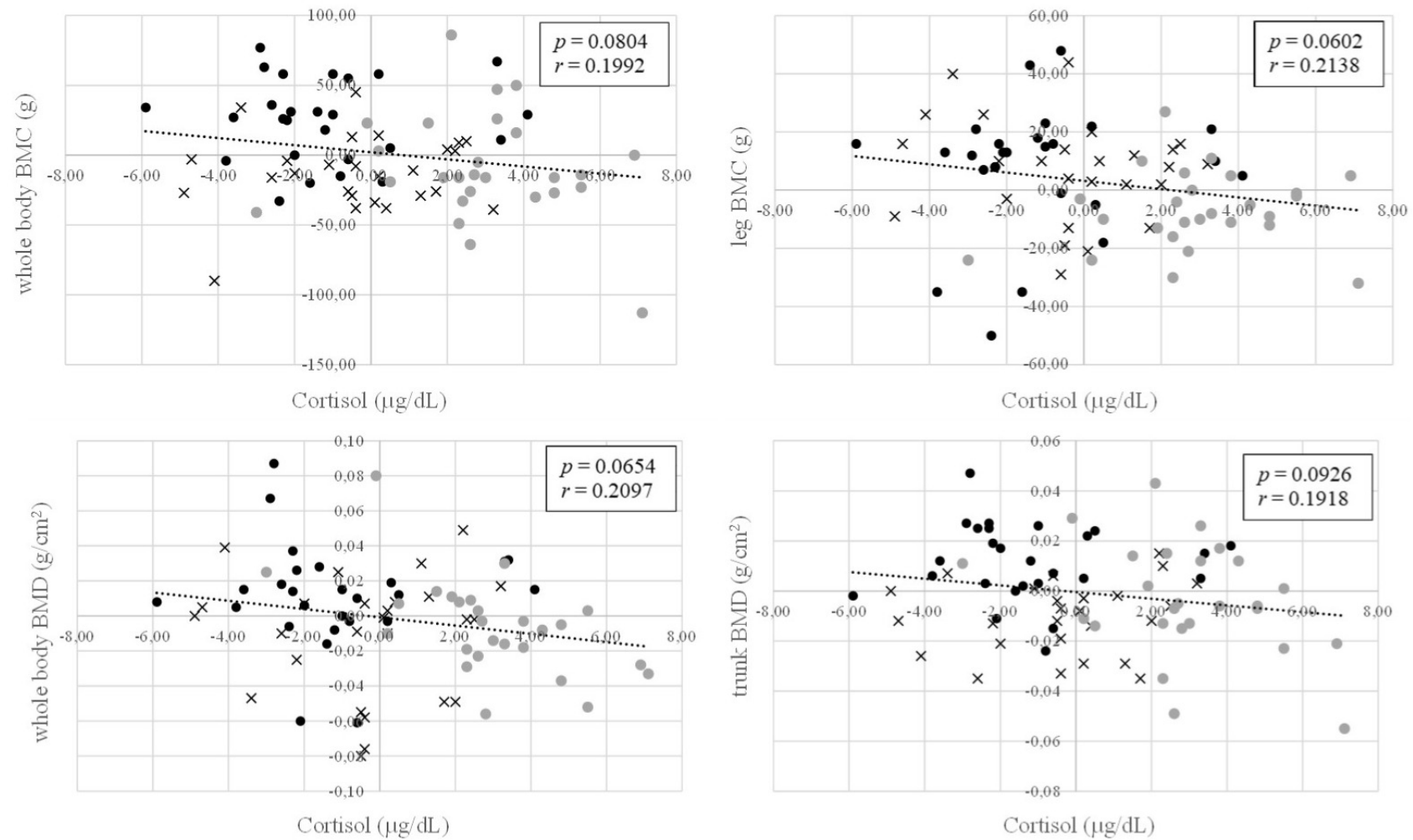


Figure 5. Correlations, across the 4 periods of the season, between variations of bone mineral parameters against cortisol. × Variation from start-preseason to start-season; • Variation from start-season to mid-season; • Variation from mid-season to end-season. R2 and p values are extracted from linear regression models

Variations of whole body and legs BMC, and whole body and trunk BMD showed a negative correlation with tC changes (Figure 5), but no significant correlations were obtained. Lastly, no associations between tT variations and bone changes in the assessed areas were found ($p < 0.1$).

4. Discussion

In this work we have examined the effect of one soccer season on body composition, bone, cortisol and testosterone levels and the association of these parameters. Soccer practice has been related to an enhancement of BMD and BMC (Hagman et al., 2018). However, studies that have analyzed the effect of a soccer season on them have not provided consistent results.

While some authors did not show any difference between the beginning and the end of the study (Milanese et al., 2021; Minett et al., 2017), others have shown an increase on BMC and BMD at the end of season observing different results in the studied regions. Milanese et al (2015) showed an enhancement of the BMD in the pelvis and upper limbs whereas Suarez-Arrones et al (2019) also observed this change in legs. Our study demonstrates that BMC is more variable than BMD across a soccer season. Whole body and legs BMC showed an increase during this period, observing the higher BMC level at mid-season. Opposite of these increases, BMC and BMD were significantly reduced in the trunk at start-season in comparison to the start-preseason, although this change was reversed at end-season. The slight changes obtained in our study may be related to the fact that aerobic sports may not increase bone mass, except in the most exercised anatomical regions (Escalante & Franco-Vicario, 2003). Moreover, variations in the results of the studies that analyze bone remodeling alterations across a soccer season could be associated with the different experimental conditions employed as it has been shown

that differences in the players' levels did not similarly affect bone remodeling process (Karlsson et al., 2003).

In accordance with our results, several studies have shown that changes in FM (kg) and %FM take place across the soccer season. Suarez-Arrones et al (2019) observed a %FM decrease in the arms and the left leg during the season, while Carling and Orhant (2010) described a decrease of %FM at the first part of the season (mid-season vs. pre-season). Similarly, Milanese et al. (2015) showed a markedly decreased of FM (g) and %FM in whole body, limbs and trunk at mid-season and end-season, in comparison to pre-season. However, they showed a significant increase in FM and %FM from mid-season to end-season. Our results also show a significant decrease in FM and %FM across the season that is more pronounced after the preseason. The disparities observed between our data and results from other works may be explained by differences in the protocols performed. Using a similar protocol than ours, Bilsborough et al (2018) examined changes in Australian football players at 4 different stages (start-preseason, end-preseason, mid-season and end-season). FM and %FM markedly decreased from start-preseason to end of pre-season, similarly to our results. Progressive training loads during preseason play a critical role in reducing fat mass (especially in the trunk and legs) and increasing lean mass. This indicates that a combination of high-volume, progressive resistance training, and high-intensity exercises can optimize body composition for athletes. Silvestre et al. (2006) observed that structured training positively impacts lean mass development in soccer players, reinforcing the importance of preseason as a foundational phase. Mid-season increases in cortisol levels suggest an accumulation of stress and fatigue as a result of sustained physical and psychological demands. Proper load management is essential during this phase to prevent overtraining and burnout. Regular inclusion of active recovery sessions

has been shown to mitigate these effects (Kellmann *et al.*, 2018), promoting balance between performance and health. Changes in bone-related parameters, such as bone mineral content (BMC) and bone mineral density (BMD), particularly in the legs and trunk, suggest that intense training can enhance skeletal health. However, improper load planning could lead to an increased risk of stress-related injuries. Walsh *et al.* (2018) highlighted the importance of monitoring musculoskeletal stress in athletes to reduce injury risks during high-intensity training periods.

We also studied the changes in LM and %LM. We observed that absolute LM remains stable, except for the trunk which increased slightly at mid-season. Nonetheless, %LM markedly increased from pre-season to start-season in whole body, legs and trunk, and it was maintained until the end of the season. Moreover, considering the normal LM values (between 40% and 50%), all the studied areas of the body are above 80%, except the arms. The remarkable enhancement of LM related to the preseason training is supported by other studies that also showed an increase in fat-free mass (FFM) (Bilsborough *et al.*, 2018; Carling, 2010; Milanese *et al.*, 2015) and % FFM (Bilsborough *et al.*, 2018) after this period.

Cortisol secretion is stimulated by exercise and this increase is negatively related to testosterone concentration (Rahimi *et al.*, 2011). However, low intensity exercise results in a reduction of the circulating cortisol levels. When the T/C ratio is studied, a decrease of this ratio by 30% has been described as an indicator of overtraining (Urhausen A, 2002). We observed the highest testosterone level at mid-season which returned to the basal levels at the end-season. On the contrary, the lowest cortisol level was observed at mid-season which was drastically increased at the end-season. Consequently, the T/C ratio increases by 31% at mid-season and decreased by 23% at the end-season. However, we did not find any

significant correlation between the testosterone and cortisol alterations at these stages of the season (data not shown). As described by other authors (Michailidis, 2014; Schelling, 2010), the increase of the testosterone concentration and the T/C ratio at mid-season may be related to an adequate physiological adaptation in players, while the decrease in the ratio and the increase in cortisol levels observed at the end of the season could be connected to the accumulation of fatigue throughout the season, without indicating a state of overreaching.

Preseason training is essential for optimizing body composition and physical preparation in professional soccer players after the rest period (Bangsbo J, Iain FM & Krstrup, 2008). However, the relationship between this training period and the risk of physiological injuries remains a controversial topic. Some studies have indicated an increased risk of injuries during preseason (Agel & Schisel, 2013), while others have not found a significant increase in injury incidence or muscle damage markers during this period (Coppalle *et al.*, 2019).

Factors such as an increase in fat mass (FM) and body mass index (BMI), a decrease in lean mass (LM), and alterations in total testosterone (tT) and total cortisol (tC) levels have been linked to the risk of musculoskeletal injuries (Anwar & M Noohu, 2016; Perroni *et al.*, 2020; Silvestre *et al.*, 2006; Walsh *et al.*, 2018). In our research, the preseason led to a reduction in FM and BMI, as well as an increase in LM. Moreover, tT and tC levels were not affected by this training period.

Although this study did not directly assess the influence of these changes on injury incidence, our findings align with other authors who suggest that the physiological modifications occurring during the preseason may not be associated with an increased risk of injuries (Coppalle *et al.*, 2019). On the contrary, they could contribute to better physical preparation and

injury prevention during the competitive season (Eliakim et al., 2018).

To the best of our knowledge, tT and tC variations across a soccer season have never been associated with body composition and bone changes. In our study, significant differences were obtained when the relationship between the studied hormones and FM and LM was analyzed in the anatomical regions studied. tT was positively associated with variations in trunk FM, and negatively associated with trunk LM. Our results contradict studies that showed that physiological testosterone concentration was negatively associated with FM in elderly men (Van Den Beld et al., 2000) and that FFM was increased with supraphysiologic doses of testosterone (Bhasin et al., 1996). However, a meta-analysis studying the effect of testosterone on FFM indicated that FFM variations may not be only related to testosterone changes (Varanoske et al., 2020). Thus, the conditions of our study may explain these discordant results as we measured variations in physiological tT concentration and in body composition in elite soccer players aged 26.88 ± 4.49 years. Cortisol plays a key role in metabolism regulation and physiological adaptation to training in professional soccer players. An adequate cortisol level is essential for metabolic control, as excessive levels have been associated with metabolic diseases such as obesity. Additionally, it has been suggested that higher cortisol concentrations may influence adipose tissue distribution, promoting visceral fat accumulation (Vegiopoulos & Herzig, 2007) and affecting body composition. In the context of male professional soccer, maintaining a balance in cortisol levels is crucial, as prolonged excess may be linked to a reduction in skeletal muscle mass, especially in the lower limbs, as observed in women with hypercortisolism (Kim et al., 2018). However, the effects of different cortisol concentrations on appendicular skeletal muscle mass (ASMM) do not appear

to be conclusive, as some studies have found no significant relationship between serum cortisol levels and ASMM loss (Peeters et al., 2008). In our study, total cortisol (tC) concentration remained within normal ranges, and its variations showed a negative correlation with changes in leg fat mass (FM). This unexpected finding could be explained by differences in the studied population and the variations in tC concentration analyzed. In professional soccer players, it is possible that training intensity and other hormonal factors may modulate this relationship, minimizing the negative impact of cortisol on body composition. These results highlight the importance of further investigating how cortisol levels may influence soccer players' body composition throughout a season, considering not only its impact on fat accumulation but also its role in preserving skeletal muscle mass, which is crucial for athletic performance and injury prevention. Previous research has associated testosterone and cortisol activity with bone remodeling. In elderly men, testosterone levels were positively associated with BMD (Van Den Beld et al., 2000). Moreover, it has recently been demonstrated that serum testosterone measured at the age of 12 years showed a positive correlation with the increase of BMD at the age of 18 years (Tamme et al., 2019). By contrast, bone loss was associated with elevated plasma cortisol in healthy men and women (Varanoske et al., 2020), but no significant association was found in competitive male cyclist (Mathis et al., 2013). In our study, neither changes in BMD nor changes in BMC were related to tT variations (data not shown). However, although variations of whole body and legs BMC and whole body and trunk BMD showed negative correlations with tC changes, these associations did not reach statistical significance ($0.05 < p < 0.1$). Nevertheless, the results suggest a potential inverse trend that warrants further investigation. The lack of significant results points out the need for more studies that confirm the association

between tC and bone turnover in soccer players.

The relationship between cortisol and bone metabolism has been widely discussed in the literature, with evidence suggesting that prolonged exposure to high cortisol levels can negatively impact bone health. Elevated cortisol has been associated with increased bone resorption and reduced bone formation, potentially leading to lower BMD and increased fracture risk (Weaver et al., 2016; Nicks et al., 2019). Cortisol-induced bone loss is particularly relevant in athletes exposed to high training loads, as chronic physical stress and metabolic demands can further influence bone turnover (Moysiuk et al., 2020).

Additionally, previous studies have reported that intense training and competition periods can alter hormonal balance, including cortisol and testosterone fluctuations, which may contribute to changes in bone metabolism (Crewther et al., 2013; Cardinale & Stone, 2006). While some research has found significant associations between testosterone levels and bone parameters, other studies have failed to establish a direct link between these variables, emphasizing the complexity of hormonal regulation in bone homeostasis (Zanker & Cooke, 2004).

In the specific context of soccer players, bone turnover may be influenced by multiple factors, including training intensity, dietary intake, and recovery strategies. Given the high-impact nature of the sport and the variability in training loads across different playing positions, it is crucial to investigate how stress-related hormones like cortisol affect bone health over a competitive season. Our findings, despite not reaching statistical significance, align with previous research suggesting that cortisol fluctuations may be linked to bone health markers. However, further longitudinal studies with larger sample sizes are necessary to clarify this association and determine its potential implications for injury prevention and performance optimization in soccer players.

Our research has some limitations as the study includes a small number of participants due to the dynamism of the soccer player market and players' injuries, our study does not consider the players' position, which may also affect their physical demands and our findings are non-transferable to female professional soccer players.

5. Practical Applications.

Our findings highlight that preseason training has a significant impact on body composition and hormonal levels, reinforcing the importance of a structured and personalized approach during this phase. Preseason training should focus on reducing fat mass (especially in the trunk and legs) and maximizing lean mass development through a combination of progressive strength training and high-intensity exercises. These results suggest that coaches can optimize performance by designing plans that prioritize these objectives.

Regular monitoring of hormonal biomarkers such as testosterone and cortisol is crucial throughout the season, as these hormones show significant correlations with changes in body mass. For instance, increases in testosterone were associated with reductions in trunk fat mass, while variations in cortisol were linked to changes in the legs, both in lean and fat mass. This suggests that coaches can use these measurements to adjust training loads and balance physical and metabolic demands.

Additionally, the testosterone/cortisol ratio was observed to peak mid-season, which could serve as a key indicator for assessing players' physical condition and recovery. Incorporating periodic evaluations of body composition (including FM, LM, BMC, and BMD) and hormonal biomarkers would allow coaches to identify early signs of fatigue or overtraining and adapt programs to maximize performance and prevent injuries.

6. Conclusions

This study demonstrates that professional soccer practice significantly influences body composition, bone structure, and hormonal levels (testosterone and cortisol) in male players of the Spanish soccer championship across a competitive season. Changes in these parameters vary with the season's phases, reflecting the specific physiological demands at each stage. The preseason period is crucial for enhancing physical fitness, leading to reduced fat mass and increased lean mass, particularly in the trunk and legs. Additionally, legs BMC and trunk BMC and BMD are notably impacted. Mid-season hormonal fluctuations, particularly in testosterone and cortisol, likely reflect accumulated stress and physical exertion. Although causal relationships cannot be definitively established due to the observational design, these results emphasize the importance of adapting training and recovery programs to the season's specific demands to optimize both performance and long-term player health.

While negative correlations were observed between changes in cortisol and whole-body/leg BMC and trunk/whole-body BMD, these associations did not reach statistical significance ($p < 0.1$). Although this suggests a potential trend, it does not provide conclusive evidence of a relationship. Further studies with larger sample sizes are needed to better understand these connections.

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