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Physical performance in women´s football preseason. Body composition, heart rate and biomarkers, are they important?

Rendimiento físico durante la pretemporada en fútbol femenino. Composición corporal, frecuencia cardíaca y biomarcadores, ¿son importantes?

José María Izquierdo Velasco[1,2]; Ana María de Benito Trigueros[2]; Elvira Delgado Márquez[3], & Juan Carlos Redondo Castán[2]

1.Valoración del rendimiento deportivo, actividad física y salud y lesiones deportivas (REDAFLED), University of Valladolid, Spain.
2.Department of Physical Education and Sports, University of León, Spain.
3.Department of Economics and Statistics, University of León, Spain.

Abstract

A well-designed football pre-season is crucial for the subsequent physical performance of the players. This study aimed to analyse, in this period, the effects of body composition, heart rate variability and physiological biomarkers on female football players' physical performance. To this end, 22 amateur female football players (23.68 ± 3.69 years) of the same team participated in the study. Physical performance factors (acceleration capacity and jumping ability), body composition, perceived exertion load, heart rate variability and some physiological biomarkers (salivary testosterone and cortisol) were evaluated weekly during a 5-week pre-season period (from T0 to T5). Wilks' Lambda indicated a significant F-value in all variables except the high and low heart frequency ratio (LFtoHF). Scheffe's post-hoc identified differences between T0 and all weeks in anthropometry variables, and between T0 and T5 in all other variables. According to the regression analyses, it was revealed a negative impact between percentage of body fat and physical capacities, particularly with 20-m (-13.77) and 40-m (-14.46) when the exertion is measured by the logarithm of the root mean square of successive R-R interval differences (RMSSD). Therefore, the present research suggests that amateur female football players who start the training season with a lower body fat percentage are able to achieve a better fitness level in a short period of time.

Keywords: Soccer; monitoring; amateur status, quantification; physical capacities; physiological state.

Resumen

Una pretemporada de fútbol bien diseñada es fundamental para el posterior rendimiento físico del jugador. Este estudio tuvo como objetivo analizar, en este período, los efectos de composición corporal, variabilidad de la frecuencia cardíaca y biomarcadores fisiológicos en el rendimiento físico en jugadoras de fútbol. Para ello, participaron en el estudio 22 jugadoras amateur (23,68 ± 3,69 años) del mismo equipo. La capacidad de aceleración y de salto, la composición corporal, la carga de esfuerzo percibida, la variabilidad de la frecuencia cardíaca y los niveles de testosterona y cortisol se evaluaron semanalmente durante un período de pretemporada de 5 semanas (de T0 a T5). Lambda de Wilks indicó un valor F significativo en todas las variables excepto el índice de frecuencia cardíaca alta y baja (LF-HF). El post-hoc de Scheffe identificó diferencias entre T0 y todas las semanas en variables antropométricas, y entre T0 y T5 en todas las demás variables. De acuerdo con los análisis de regresión, se reveló un impacto negativo entre el porcentaje de grasa corporal y las capacidades físicas, particularmente con 20 m (-13,77) y 40 m (-14,46) cuando el esfuerzo se mide por el logaritmo de la raíz cuadrada de la media de la diferencia al cuadrado entre latidos adyacentes (RMSSD). Por lo tanto, la presente investigación sugiere que las jugadoras de fútbol amateur que comienzan la temporada de entrenamiento con un porcentaje de grasa corporal más bajo pueden lograr un mejor nivel de condición física en un corto período de tiempo.

Palabras clave: fútbol; monitorización; amateur; cuantificación; capacidades físicas; estado fisiológico.

Correspondence/correspondencia: José María Izquierdo Velasco
Valoración del rendimiento deportivo, actividad física y salud y lesiones deportivas (REDAFLED), Universidad de Valladolid, España
Email: josemaria.izquierdo@uva.es

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Introduction

Exploring a system to know the football performance is not merely of interest in terms of tactical aspects and technical skills (Carpita et al., 2019), but is also hugely significant in terms of physical performance. Football players are expected to have a high fitness level to successfully play a 90-minute football match. Aerobic metabolism dominates the energy delivery for the activities involved in this kind of sports competition, but for short sprints, jumps, tackles and duels, anaerobic energy release is critical for who runs fastest or jumps highest (Stølen et al., 2005). Therefore, both aerobic and anaerobic capacities are essential for football performance, and optimal load periodization is needed for athletes to play at their best.

A football season, from a training point of view, is divided into four phases: pre-season, early game, late game and transition phase (Mara et al., 2015). Pre-season training lasts only 6-8 weeks for professional teams, but it is a crucial period because players often return in a significantly detrained state as a result of the summer intermission period (Brito de Souza et al., 2021). Pre-season training loads for elite football players are the highest of the season (Jeong et al., 2011). Due to this large training load, coaches often use monitoring strategies to obtain information about their players' fitness, trying to avoid overexertion or overtraining syndrome (Impellizzeri et al., 2004). Furthermore, it must be taken into account that adaptations to pre-season training stimuli do not occur uniformly in all players. Individual features such as physical fitness, external load, age, or body composition determine the physiological stress athletes can withstand, so pre-season training results may vary (Impellizzeri et al., 2005). For all these reasons, it is recommended to assess body composition (Mala et al., 2015), physical performance factors and biological, hormonal and psychological markers to periodically monitor both daily training load and fatigue levels of players (Halson, 2014). According to Komsis et al. (2018), a 4-week transition phase of detraining leads to a reduced 20 and 30m sprint performance in male amateur football players. Therefore, pre-season training pursues developing players' physical capacities to prepare them for the competitive season requirements and protect them against in-season injuries (Ekstrand et al., 2020).

The session rating of perceived exertion (RPE) and the heart rate variability (HRV) are the noninvasive most used tools for quantifying training load adaptations (Eston & Parfitt, 2018). Flatt et al. (2017) found that female football players' HRV is related to RPE under different training loads. HRV is also used to monitor physiological adaptations and fatigue (Flatt et al., 2017; Ravé & Fortrat, 2016). The vagus index of HRV, such as the root mean square logarithm of the continuous RR interval difference (lnRMSSD) reflects the parasympathetic modulation of the heart. It is sensitive to fatigue and useful for assessing individual training adaptability of players (Flatt & Esco, 2016). Therefore, cardiac parasympathetic activity is considered a 'global' sign of homeostasis, which provides information on some aspects of recovery status and may explain why planning high-intensity training when HRV is at or above baseline can help improve endurance performance (Stanley et al., 2013).

On the other hand, salivary testosterone (T) and cortisol concentration (C) have also been suggested as indicators of psychophysiological monitoring in football (Vilamitjana et al., 2017; Botelho et al., 2020). It has been shown that high-intensity training or periods of competition can lead to an elevated salivary cortisol status and an improved T and C ratio (TtoC) accompanied by positive emotional state, especially among female athletes (Maya et al., 2016). However, the relationship between TtoC, inflammation or muscle damage, and HRV is still unclear.

In addition, according to physical capacities, sprint tests have also become very popular among football researchers, since outputs of this skill were significantly correlated with the vertical and horizontal jump performance of the athletes (Loturco et al., 2018). Tracking this information can help coaches design and stage training loads that could be used to achieve optimal fitness levels: the main aim of the pre-season.

Although the literature shows important advances in the analysis of physical factors and physiological stress markers for monitoring the training loads of male football players, the behaviour and relevance of these variables during the pre-season of amateur female players are still unknown. Bridging this knowledge gap may be important to help coaches and athletes better understand pre-season physiological stress and plan appropriate training strategies to improve athletic performance without risk of injury or overtraining. Then, the purpose of this study was to investigate the best indicators of amateur female football players' physical performance (sprint and jump capacities) during a 5-week pre-season period, through the analysis of the magnitude of changes in body composition and physiological stress biomarkers. It is based on the hypothesis that as pre-season is characterized by a high training load, it promotes: an improvement in physical performance, an imbalance in the TtoC, and the reduction in the parasympathetic regulation of the athlete's heart. Hence, salivary testosterone and HRV are also expected to decrease, as are the high correlations between cortisol and HRV.

Methods

Study design

This work was a single-group descriptive study, where players performed a 5-week period of 3 training sessions per week: Tuesday, Wednesday, and Friday. Each training session lasted 120 minutes and included 15 minutes of warm-up exercises, 45 minutes of physical fitness preparation, and 60 minutes of technical and tactical practice. All training sessions started at 19:00. In addition, 4 friendly matches were played on Sundays.

The training warm-up consisted of submaximal aerobic activity, dynamic stretching, and football-specific dynamic exercises (Bisciotti et al., 2020). According to Botelho et al. (2020), physical fitness preparation was divided into 3 stages focused on conditioning abilities: cardiorespiratory fitness and aerobic endurance (stage A, 2 weeks); anaerobic endurance (stage B, 2 weeks); and neuromuscular fitness, such as muscle strength (stage C, 1 week). Technical and tactical preparation included action skills with the ball (dribbling, passing, kicking, and heading), game simulation and playing in a reduced field. A typical weekly schedule of the pre-season training program is presented in Table 1. The coach and physical trainer were responsible for training prescription, monitoring, and overall control of the sessions. Players were encouraged to maintain the highest level of performance at every stage of their training. The warm-up for each friendly match lasted 20 minutes, and each subject played a total of 150 – 220 minutes (184.56 ± 35.83 minutes).

Table 1. A typical weekly schedule of training

<table>
<tr><th>Monday</th><th>Tuesday</th><th>Wednesday</th><th>Thursday</th><th>Friday</th><th>Saturday</th><th>Sunday</th></tr>
<tr><td rowspan="7">Rest</td><td>Assessment</td><td>Training session warm-up</td><td rowspan="7">Rest</td><td>Training session warm-up</td><td rowspan="7">Rest</td><td>Match warm-up</td></tr>
<tr><td rowspan="2">Training session warm-up</td><td rowspan="2">AE/AN/ST 45´</td><td>AE/AN/ST 25´</td><td rowspan="5">Friendly match</td></tr>
<tr><td>SG 20´</td></tr>
<tr><td>AE/AN/ST 30´</td><td>TeT 15´</td><td rowspan="2">TaT 40´</td></tr>
<tr><td>SG 15´</td><td>TaT 15´</td></tr>
<tr><td>TeT 60´</td><td>SM 30´</td><td>SM 20´</td></tr>
<tr><td>RPE</td><td>RPE</td><td>RPE</td><td>RPE</td></tr>
</table>

AE, Aerobic endurance training; AN, Anaerobic endurance training; RPE, rate of perceived exertion; SG, Small-side games; SM, Simulation match; ST, Strength training; TeT, Technical training; TaT, Tactical training.

To standardise the procedure, all tests were carried out under the same protocol and in the same order. All assessments were taken 1 hour before the first weekly training session (Tuesday). Thus, the first assessments (T0) correspond to the initial level of the players, while the following assessments (T1-T5) measure the cumulative effect of the previous training week, including the match. In order to minimise the learning effect in the physical tests, players underwent a familiarisation session 24 hours before the T0 assessment. RPE was daily assessed 20-30 minutes after each training session and match. Figure 1 shows the experimental design.

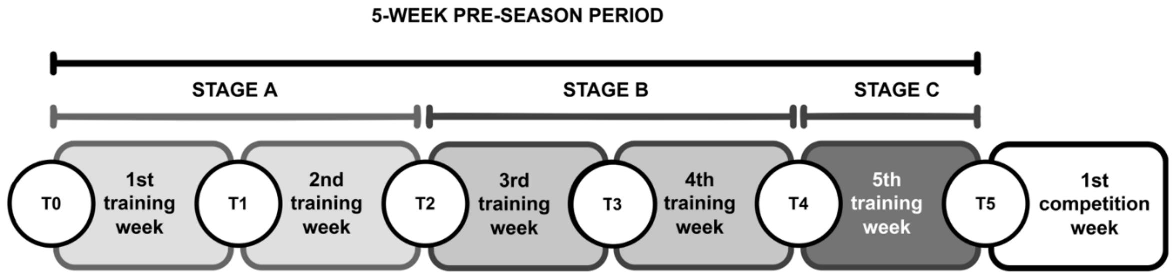

Figure 1. Experimental design. T0, first assessment; T5, final assessment. Stage A: cardiorespiratory fitness and aerobic endurance; stage B: anaerobic endurance; stage C: neuromuscular fitness.

Sample

Twenty-two amateur female football players from the Castilla y León Regional League in Spain voluntarily participated in this study. Regarding the positions of the players, the group was composed of 3 goalkeepers, 4 defenders, 3 fullbacks, 6 midfielders and 6 forwards. Demographic data of the players are described in Table 2.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the ethics committee of the local university. All participants gave written informed consent prior to data collection.

According to eligibility criteria, athletes had to be correctly registered in the Spanish Football Federation, answer a self-information health questionnaire, and sign a consent form. Exclusion criteria included the use of drugs that may impair the results (antibiotics, anti-inflammatory drugs or anabolic drugs), any injuries or medical conditions that could limit physical activity and not attending the training sessions for a full week (including follow-ups with the coach and the physical trainer) (Botelho et al., 2020).

Table 2. Demographic data of the players (mean ± SD).

	N = 22
Age (years)	23.68 ± 3.69
Height (cm)	165.54 ± 5.53
Body mass (kg)	67.18 ± 7.12
Body Mass Index (kg·m^{-2})	24.77 ± 1.86
Training Experience (years)	8.72 ± 2.47
Menstrual cycle phase (T0 and T5)	Follicular†

BMI, body mass index.; †81.82% (18 players) of the players were evaluated during the follicular phase of the menstrual cycle in the 2 moments (T0 and final-T5-), and 9,09% (2 players) made use of oral contraceptives.

Procedures

Set of self-reporting questions. This questionnaire was designed *ad hoc* and was used to obtain information about athletes' illnesses, menstrual cycle stage, drug consumption, and injury and/or surgical history.

Training Load (TL). The perceived training load (TL) was daily calculated as the product of the training session duration (in minutes) and the training intensity indicated by the RPE (0–10 scale) according to Foster et al. (2001). TL was recorded separately for each week.

Heart Rate Variability (HRV). The interval between consecutive heartbeats (R-R) was obtained between 16:00 and 18:00 in the evening. Using a heart rate monitor (POLAR®, model S810, Finland), data was continuously recorded in the locker room for 10 minutes, while the subject was resting in a supine position, at 20-25º temperature controlled and silent. R-R interval was calculated from the middle 6 minutes (from the second to the eighth minute). Kubios® HRV software (Tarvainen et al., 2014) was used to perform HRV analysis. The following metrics were used: logarithm of the root mean square of successive R-R interval differences (RMSSD), standard deviation of all normal R-R intervals (SDNN), and the high and low frequency ratio (LFtoHF).

Anthropometric Assessment. Body mass (BM) was measured to the nearest 0.1 kg with participants clothed in shorts and T-shirts using a digital scale (Healthometer, Neosho,MO, USA). Height was measured to the nearest 0.1 cm with participants barefoot using a wall-mounted stadiometer (Heightronics, QuickMedical, Issaquah, WA, USA). Body fat percentage (BF%) was determined according to the Bioelectrical impedance analysis guidelines (Kyle et al., 2004), using a tetrapolar bioelectrical impedance (BIA 310e, BIODYNAMICS, Seattle, Washington). Body mass index (BMI, kg·m^{-2}) was calculated.

Salivary Cortisol (C) and Testosterone (T) Concentrations. The samples were collected in other locker room before the first training session of the week, following the standards established by the Saliva Collection Guidelines of Salimetrics (Salimetrics, 2015), and according to the protocol by Botelho et al. (2020) for participants and samples tested. Saliva samples were collected centrifuged at 3,000 rpm, for 20 minutes, at 4° C and were subsequently stored at -20° C. The average in-analysis coefficients of variation for T and C analyses were 3.3% (3.1-3.5) and 3.8% (3.5-4.1), respectively. T and C ratio (TtoC) was used as a biomarker variable for further analyses.

Sprint capacity. A 40-m (with 20-m split time) sprint test was conducted on the same surface where training sessions and friendly matches took place (artificial field-turf pitch) and timed using a single beam photocell system (DSD Laser System®, DSD Inc., Spain). Players started the sprint test half a meter behind the first two photocells (Izquierdo et al., 2020). A visual stimulus was used to indicate to players when to start, and players then sprinted 40m at maximum intensity. Players conducted 3 trials and the best one was registered.

Jump capacity. Players' vertical jump capacity was measured using a laser platform (SportJUMP System PRO®, DSD Inc., Spain) sited in the pitch. Players carried out 3 countermovement jumps (CMJ), with 45-60 seconds' rest between jumps (Warr et al., 2020). The best jump height record in centimeters was analysed for the CMJ variable.

Statistical Analyses

To gain an initial understanding of the associations between the different variables of interest, inter-relationships between independent variables and their correlation with the dependent variable were tested. Correlations between variables were calculated using the Pearson correlation coefficient (r). Before the regression analysis, a hypothesis test was performed for the mean with paired samples to identify statistically significant differences between the measurements. Statistical analysis was performed with R 4.0.3. (Lucent Technologies, Georgia-USA). Effects related to weekly TL were assessed using one-way ANOVA (time) with repeated measures. When Wilks' Lambda indicated a significant F-value, Scheffe's *post-hoc* procedures were performed to determine pairwise differences. Partial eta squared (η_p^2) was computed. Statistical significance was set at $p < .05$.

In order to capture the key factors affecting physical performance, this research adopts a panel regression analyses approach to explore the performance during a pre-season in amateur female football players. There are three types of panel data models: pooled regression model, fixed effect model, and random effect model (Greene, 2003).

Here, it was assumed a log-linear relationship (elasticities) between physical performance and variables of interest, and it was built a model in which idiosyncratic characteristics across weeks are taken into consideration. The model's general structure is as follows:

$$Performance_{ijk} = \beta_0 + \beta_1 BF\%_{ijk} + \beta_2 BF\%^2_{ijk} + \beta_3 TtoC_{ijk} + \beta_4 Exertion_{ijk} + \upsilon_i + \varepsilon_{ijk} \quad \upsilon_i \sim N(0, \sigma_\upsilon^2) \quad \varepsilon_{ijk} \sim N(0, \sigma_\varepsilon^2 I)$$

where Performance ∈ {20-m, 40-m, CMJ}, Exertion ∈ {RMSSD, SDNN}, subscript i represents individuals (i=1, …, N players), j the week and k the stage. We dealt with a balanced study design with repeated measures given that each player observed the same number of weeks, and each player is considered as a random effect. Therefore, the β coefficients contain the fixed effects (variables of interest) that change within a player, υ_i (players) is the vector of random effects, and ε_{ij} is the error term. The σ_υ^2 is the variance of random effect and σ_ε^2 is the variance of residual error, and it is assumed that the variances of the errors are normally distributed.

We have included in the regression the BF% and TtoC during pre-season as known control variables. Additionally, the quadratic term of body fat percentage ($BF\%^2$) to capture the fact of diminishing marginal return on BF profit is included. To decide which panel estimator should be given preference, we formally tested the difference between fixed- and random-effects through the Hausman test.

The global descriptive statistics and correlations between variables summarised in Table 3 revealed high correlation (r = .65) between RMSSD and SDNN. These results underscore the need for a jointly consideration of both.

Table 3. Global descriptive statistics and correlations

	BH	BM	BMI	BF (%)	TL	RMSSD	SDNN	LFtoHF	C	T	TtoC	20-m	40-m
BH	1												
BM	.7	1											
BMI	.07	.76	1										
BF(%)	.25	.77	.86	1									
TL	-.02	-.06	-.07	.1	1								
RMSSD	.39	.24	-.04	.06	.2	1							
SDNN	.23	.16	.01	.15	.23	.65	1						
LFtoHF	.20	.62	.7	.77	.04	.04	.15	1					
C	.36	.27	.03	.15	.3	.13	.25	.06	1				
T	.41	.27	.0	.01	.43	.11	.17	.03	.71	1			
TtoC	.39	.36	.146	.26	.28	.41	.27	.13	.06	.13	1		
20-m	.01	.53	.76	.69	.13	-.01	.16	.51	.27	.03	.23	1	
40-m	.16	.56	.78	.70	-.13	.01	.16	.51	-.25	-.05	.22	.99	1
CMJ	.2	.52	.54	.6	.08	-.2	.4	.44	.26	.36	.02	.34	-.35

BH, body height; BM, body mass; BMI, body mass index; BF, body fat; TL, training load; RMSSD, logarithm of the root mean square of successive R-R interval differences; SDNN, SD of all normal R-R intervals; LFtoHF, high and low frequency ratio; C, cortisol; T, testosterone; TtoC, testosterone and cortisol ratio; 20-m, 20 m sprint; 40-m, 40 m sprint; CMJ, countermovement jump.

Results

The mean and standard deviation for the different variables registered throughout the pre-season are reported in Table 4. Wilks' Lambda indicated a significant F-value in all variables (except LFtoHF). Scheffe's *post-hoc* procedures identified differences between T0 and all measurements in anthropometry variables, and between T0 and T5 in all variables.

Table 4. Descriptive data for the variables in all measurements (Mean±SD)

Variables	T0	T1	T2	T3	T4	T5	Time F (*ηp^2*)
TL (a.u.)	n/a	1,975.9±241.7	2,717±301.1	2,928.6±316.43	2,879.5±367.06	2,951.8±370.8	1,545.84* (*.99*)
Anthropometry							
BM (kg)	67.18±7.12	66.93±7.09[a]	66.76±6.97[b]	66.39±6.85[c]	66.22±6.7[d]	66.19±6.56[e]	28.491* (*.58*)
BMI (kg/m^2)	24.47±1.86	24.38±1.85[a]	24.32±1.8[b]	24.19±1.77[c]	24.13±1.73[d]	24.12±1.7[e]	32.092* (*.6*)
BF (%)	26.8±2.68	26.60±2.6[a]	26.36±2.55[b]	26.11±2.63[c]	25.79±2.51[d]	25.17±2.4[e]	189.8* (*.92*)
Heart Rate Variability							
RMSSD (ms)	4.59±.45	4.58±.42	4.52±.39	4.51±.39	4.42±.52	4.33±.42[e]	55.62* (*.77*)
SDNN (ms)	83.55±10.43	79.91±8.2	76.12±7.91[b]	75.21±7.84[c]	72.08±6.8[d]	68.66±7[e]	58.22* (*.77*)
LFtoHF	.86±.2	.90±.21	.91±.21	.89±.2	.87±.2	.88±.21	.76 (*.04*)
Biomarkers							
C(nmol·L^{-1})	6.9±1.4	6.99±1.53	7.19±1.57	8.15±1.75[c]	9.68±1.76[d]	10.29±1.72[e]	344.41* (.95)
T(pmol·L^{-1})	406.67±48.2	451.24±56.52[a]	491.18±57.8[b]	540.31±66.99[c]	630.26±64[d]	651.85±89.7[e]	1,040.67* (.98)
TtoC (nmol)	18.03±1.74	17.92±1.53	17.71±1.75	17.57±1.7	17.10±1.69[d]	16.74±1.72[e]	15.29* (.5)
Physical Capacities							
20-m (s)	3.47±.26	3.43±.25	3.44±.25	3.42±.26	3.38±.25[d]	3.32±.24[e]	30.64* (*.64*)
40-m (s)	6.58±.51	6.5±.49[a]	6.48±.49	6.46±.57	6.39±.48[d]	6.31±.46[e]	30.54* (*.64*)
CMJ (cm)	25.97±1.92	26.2±2.02[a]	26.42±1.95[b]	26.67±2[c]	27.78±2.04[d]	28.28±2[e]	444.95* (*.96*)

** $p< .05$; T0, first assessment; T5, final assessment; RPE, rate of perceived exertion; a.u., arbitrary unit; n/a, not applicable; RMSSD, logarithm of the root mean square of successive heartbeats interval differences; SDNN, SD of all normal heartbeats intervals; LFtoHF, high and low frequency ratio; BM, body mass; BMI, body mass index; BF, body fat; C, cortisol; T, testosterone; TtoC, testosterone and cortisol ratio; 20-m, 20 m sprint; 40-m, 40 m sprint; CMJ, countermovement jump; Scheffe's post-hoc pairwise differences: [a]T1&T0, [b]T2&T0, [c]T3&T0, [d]T4&T0, [e]T5&T0*

Table 5 reports the results of the regression analyses examining the physical performance variables: 20-m, 40-m and CMJ (Models *i* to *iii*), where all the control variables (BF%, BF%^{2} and TtoC) can be considered as significant contributors to the model. These models consider the exertion measures by RMSSD. Results of the Hausman test do not reject the null hypothesis for all models, indicating that random-effects estimators should be considered. The random effect model considers the individuals-specific effects as random variables, and it assumes that individuals-specific effects are normally distributed throughout the population (Greene, 2005).

Table 5. Panel regression tests for performance when exertion is measured by RMSSD: 20-m, 40-m, and CMJ

	Performance					
	20-m		40-m		CMJ	
	i		*ii*		*iii*	
	Coef. (SE)	p-value	Coef. (SE)	p-value	Coef. (SE)	p-value
BF%	-13.77 (1.26)	.0**	-14.46 (1.67)	.0**	3.03 (.92)	.0**
BF%²	2.21 (.19)	.0**	2.32 (.26)	.0**	-.54 (.14)	.0**
TtoC	.006 (.0)	.0**	.007 (.0)	.0**	-.01 (.0)	.0**
RMSSD	-.03 (.0)	.0**	-.033 (.0)	.0**	-.04 (.0)	.0**
constant	22.58 (2.03)	.0**	24.31 (2.68)	.0**	-.82 (1.47)	.576
σ_u	.0		.0		.01	
σ_e	.04		.04		.05	
Observations	66		66		66	

BF%, body fat percentage; BF%^{2}, quadratic term of body fat percentage; TtoC, testosterone and cortisol ratio; CMJ, countermovement jump; RMSSD, logarithm of the root mean square of successive heartbeats interval differences; ** significance at the 1%. Values shown are coefficient estimates and standard errors (SE) from maximum likelihood regressions using a random effect model.

Regression analyses of physical performance when exertion is measured by RMSSD (Table 5) show a positive marginal average impact on 20-m and 40-m of 9.4 percentual points (pp) in both cases. For example, performance increases much more (11pp) with low values of BF% (21%) than with high values (8pp) of BF% (30%). In the case of the CMJ, a negative lower impact is observed.

Regarding the comparison of the percentage change between BF% (T0 to T5) in association with the 20-m and the CMJ, the results represented in Figure 2 highlight the non-linear relationship of the variables over time.

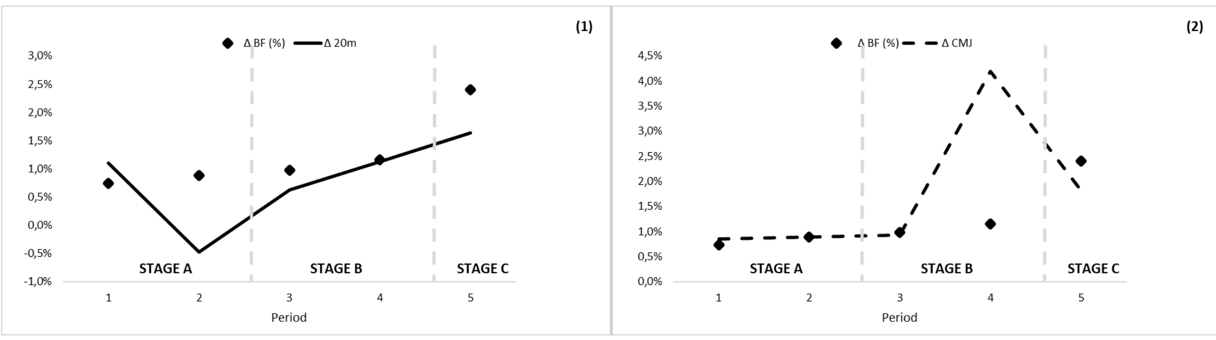

Figure 2. A time series of BF% and 20-m changes (1) and BF% and CMJ changes (2). Stage A, cardiorespiratory fitness and aerobic endurance; stage B, anaerobic endurance; stage C, neuromuscular fitness; Δ BF(%), weekly mean changes in percentage of body fat; Δ 20m, weekly mean changes in 20-m sprint; Δ CMJ, weekly mean changes in countermovement jump.

Discussion

This study aimed to investigate the best indicators of amateur female football players' physical performance during a 5-week pre-season period, through the analysis of the magnitude of changes in body composition, physical, and physiological variables. We mainly found differences in the tendency to improve fitness level after this period. All variables show *near perfect r*-value during the six assessments (except LFtoHF -*very large*). So, we report that 5 weeks of a football training program significantly decreased BF, RMSSD and SDNN; and increased salivary testosterone and cortisol concentrations, and sprint and jump capacities. Despite the values obtained, it is important to remember that a significant variation (i.e., $p < .05$) does not always refer to a meaningful or clinically important change (Botelho et al., 2020). Thus, from a practical point of view, some of these findings may require further careful analyses to avoid potential misinterpretations.

Regarding anthropometric variables, our results reveal a significant decrease in BF% at T5 assessment, which is in line with Reilly (2006) who suggested that football players can accumulate BF in the off-season and lose more weight during the pre-season period than in other periods. The decrease in BF% after pre-season could be due to the fact that lean body mass increased because players participated in strength and anaerobic training programs during this period (Kalapotharakos et al., 2011). Ostojic (2003) stated that professional male football players' BF significantly drops during the competitive phase, however we have not been able to verify this statement, as it is not part of our study period. Regression analyses identified BF% as the main cause of improvements in physical performance variables. This finding is consistent with Sheppard & Young´s predictive model of team sports players' performance, which proposed that body fat level may play a key role in sprint ability, especially with changes of direction (Sheppard & Young, 2006). It is also in line with the study by Emmonds et al. (2019) which predicted a positive outcome between BF, strength and 10-m and 20-m in professional female football players. Bongiovanni et al. (2021) showed that anthropometric variables are major indicators of sprint ability and aerobic capacity in youth players, but their model reported a minor level of prediction according to jump capacity, suggesting that other factors, including anthropometric variables, may concur to estimate their variation in performance characteristics. Thus, the current research supports the view that the body composition of amateur female football players should be within the normal healthy range, but the BF% should be as low as possible (Osgnach et al., 2010) in order to start the competitive matches. In this sense, it has also been shown that lower BF% is associated with better sprint performance (Osgnach et al., 2010), while fat-free help to generate strength during high-intensity exercise and provide greater absolute strength (Mala et al., 2015). Nonetheless, in order to remain in great physical condition, the ideal BF% should not change significantly throughout the competitive season; and this might require two strength-training sessions per week (Mara et al., 2015). In addition, players who regress significantly should be identified as this could suggest an energy imbalance (Burket et al., 2017).

Our results on HRV variables showed significant differences, according to Scheffe's *post-hoc,* between T0 and T5 in RMSSD and; T0 vs. T2, T3, T4 and T5 in SDNN. These changes in RMSSD could be interpreted as a positive response due to a concomitant improvement in wellness variables (Flatt et al., 2017). Previous investigations have shown that the weekly average of RMSSD (RMSSDmean) can better reflect training status compared to weekly recordings (Plews et al., 2013); and two researchers' groups have found that the RMSSDmean derived from three days a week is sufficient to register the 7-day average (Flatt et al., 2017; Plews et al, 2012), making the implementation of sports teams more convenient. Oliveira et al. (2013) observed that obtaining high aerobic capacity was accompanied by an increase in HRV parameters identifying vagus nerve activation. Furthermore, Botek et al. (2014) found that maintaining high resting HF, T, and RMSSD values in the training process allowed them to effectively develop aerobic capacity.

Current results indicated significant improvements in physical capacities after 5-week pre-season in these athletes. Pardos-Mainer et al., (2020) demonstrated that a 8-week combined strength and power training program increased 40-m speed, which was achieved by our athletes from the fourth week onwards. Mara et al. (2015) reported an improvement by 2.8% in 15-m sprint performance after pre-season training in elite female players. Hence, these results emphasize the importance of developing lower limb strength and explosiveness during the pre-season, which may improve linear sprinting performance.

In light of all this, differences have been reported between professional and amateur female players according to body composition and physical capacities: elite seniors were stronger, faster, more responsive, and had better cardiorespiratory fitness compared to the amateur players (Farley et al., 2021). In this regard, detrimental effects on body composition and physical fitness were observed after both training season cessation and off-season training programs, however off-season training programs seem to ameliorate such harmful effects (Clemente et al., 2021).

Three main limitations of this study must be considered. First, because there is no control group and relatively small sample size, we cannot compare athletes of different age ranges, experience levels or playing positions. Second, different factors may contribute to changing values due to amateur status: energy-intake, hydration status, lifestyle habits, the main job of the players (workers, students), psychological effects, technical qualities of running and jumping, etc. And third, we cannot use heart rate monitors or GPS systems to assess daily/weekly training load. Nevertheless, we consider that this study reflects the real-world situation of many amateur female football teams. Therefore, it brings important knowledge that coaches and physical conditioners may consider in their training programs.

Conclusions

A 5-week pre-season training period, in amateur female football players, was found to improve body composition within decreasing BM, BMI and BF%, heart rate variability within RMSSD and SDNN, biomarkers within testosterone and cortisol, and the physical variables within sprint and jump capacities. Unlike our hypothesis related to the importance of biomarkers and HRV during this period, the analyses would identify the BF% as an important cause of improvements in physical performance variables. For all that, monitoring players' body composition during the pre-season is useful for players, coaches, and physical trainers.

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