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ORIGINAL

STRESS SCORE AND LnrMSSD AS INTERNAL LOAD PARAMETERS DURING COMPETITION

STRESS SCORE Y LnrRMSSD COMO PARÁMETROS DE CARGA INTERNA DURANTE UNA COMPETICIÓN

Miranda-Mendoza, J.¹; Reynoso-Sánchez, L.F.²; Hoyos-Flores, J.R.³; Quezada-Chacón, J.T.⁴; Naranjo, J.⁵; Rangel-Colmenero, B.⁶ y Hernández-Cruz, G.⁷

¹ PhD in Physical Culture Sciences. Professor at the School of Sports Organization, Autonomous University of Nuevo Leon. Nuevo Leon (Mexico) mmj5-7_12@hotmail.com

² PhD in Physical Culture Sciences. Physical education and sport science program, Autonomous University of Occident. Sinaloa (Mexico) felipe.reynoso@udo.mx

³ Mastery in Physical Activity and Sport with High Performance guidance. Professor at the School of Sports Organization, Autonomous University of Nuevo Leon. Nuevo Leon (Mexico) raul9991NBP@hotmail.com

⁴ PhD in Physical Culture Sciences. Sports training program, Biomedical Sciences Institute, Autonomous University of Ciudad Juarez. Chihuahua (Mexico) minaquezada21@gmail.com

⁵ PhD in Medicine. Department of Sports and Informatics. Pablo de Olavide University. Seville (Spain) jonaore@gmail.com

⁶ PhD in Morphology. School of Sports Organization, Autonomous University of Nuevo Leon. Nuevo Leon (Mexico) blanca.rangelc@uanl.mx

⁷ PhD in Physical Activity and Quality of Life. School of Sports Organization, Autonomous University of Nuevo Leon. Nuevo Leon (Mexico) german.hernandezcrz@uanl.edu.mx

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ABSTRACT

The aim of this study was to analyse the behaviour of the stress score (SS) and the Neperian logarithm of the Root Mean Square of Successive R-R Interval differences (LnrMSSD) of heart rate variability (HRV) as indicators of internal load throughout sympathetic and parasympathetic modulation, supported by biochemical parameters of internal load. 14 handball university athletes (age 22.30 ± 1.83 years) were evaluated. Six times of HRV and biochemical markers were collected. Each variable were analyzed by conventional statistics and using the Cohen's d , and Hopkins magnitude for the sample size effect. It was analyzed the Pearson correlations between variables. The LnrMSSD, SS and cortisol presented significant changes ($p < .05$). Correlations were found between HRV (SS and LnrMSSD) and CK respectively. Results of this study shows that SS can be a reliable method for the evaluation of internal load during competition.

KEY WORDS: Physiological stress, Autonomic nervous system, Heart rate variability, Biological markers, Internal load, Handball.

RESUMEN

El objetivo del estudio fue analizar el comportamiento del stress score (SS) y el logaritmo neperiano de la media de la raíz cuadrada de las diferencias de los intervalos sucesivos R-R (LnrMSSD) de la variabilidad de la frecuencia cardíaca (VFC) como indicadores de la carga interna mediante la modulación simpática y parasimpática, apoyado con parámetros bioquímicos de carga interna. Se evaluaron 14 atletas universitarios de balonmano (edad 22.30 ± 1.83 años). Se monitoreó la VFC y marcadores bioquímicos en seis momentos. Se analizaron las diferencias entre las tomas de cada variable mediante estadística descriptiva convencional y el tamaño del efecto con la d de Cohen y la magnitud de Hopkins. Se examinaron las correlaciones de Pearson entre variables. El LnrMSSD, SS y cortisol presentaron cambios significativos ($p < .05$). Se encontraron correlaciones entre los parámetros de la VFC (SS y LnrMSSD) con creatin kinasa (CK) respectivamente. Los resultados del estudio muestran que el SS puede ser una metodología fiable para la evaluación de la carga interna durante una competición.

PALABRAS CLAVE: Estrés fisiológico, Sistema nervioso autónomo, Variabilidad de la frecuencia cardíaca, Marcadores biológicos, Carga interna, Balonmano.

INTRODUCTION

In the sport scope, control of the internal load is essential to maximize positive training adaptations, that help to an adequate planning of competition and training [1]. Furthermore, useful information can be obtained to determine which athletes are prepared for the competition demands [2]; thus, the quantification of internal load is considered a relevant topic in sports [3].

To evaluate the internal load, different methods have been proposed. Some research show a relationship between creatine kinase (CK) and urea with intensity and volume of exercise parameters [4]. CK can be a determinant in the individual load of training in intermittent sports, in which elevated loads and eccentric contraction components exist [5]. On the other hand, urea indicates protein catabolism, which reflects that the volume of the training session has been high [5]. It has also been suggested for the training control and to avoiding possible negative adaptations to the physical loads both in training and competition [4, 6]. Cortisol is another indicator of internal load, which is one of the main glucocorticoids that prepare the organism to respond to an internal or external stimulus [7]. These stimuli can be caused by psychophysiological stressors that occur because of strong and continuous efforts, that response have been observed before training or competition as an indicator of an anticipatory stress response [8, 9]. The cortisol response to stress and ther fluctuations can function or not synchronously, in relationship with the degree of psychophysiological stress that occurs, and at the same time, on the relationship with the autonomous nervous system [10, 11].

These biochemical evaluations (CK, urea and cortisol) have been used in many research with success; however, they are invasive methods, implicating special care in the handling of blood samples and high-cost specialized equipment [12]. For this reason, currently, other non-invasive methods are being used, such as heart rate variability (HRV), proposed as a tool for the autonomic nervous system evaluation through the interaction of the sympathetic and parasympathetic systems, which show a different cardiac response due to the physiological demands [13]. In this way, HRV provides information of the training adaptation [14], fatigue and stress generated by the high physical demands in conditions of training and competition [15].

HRV is a sensitive indicator that measures the alterations caused by physiological and psychological stress, since during exercise, sympathetic activity increases, whereas during recovery there is a parasympathetic activity reactivation. This balance reflects the restoration of cardiovascular homeostasis, which is important in overall recovery [16]. In practice, the most used indexes for monitoring training loads are the root mean square of the successive differences of RR intervals (rMSSD) and the cross-sectional diameter of the Poincaré dispersion diagram (SD1) which reflect parasympathetic activity [17], and the Napierian logarithm of the rMSSD (LnrMSSD), which at present is used with great frequency in team [1, 18]. With regard to sympathetic activity, the parameter that approaches a possible interpretation is the longitudinal diameter of the Poincaré dispersion diagram (SD2), which is the inverse of sympathetic activity. To avoid this inverse character, Naranjo, et al. [13] proposed in its place two indicators for the interpretation of the Poincaré plot: the stress score (SS) with the purpose of obtaining a value directly proportional to the sympathetic activity and the sympathetic-parasympathetic ratio (S: PS ratio), with the purpose of obtaining a clear relationship between the

sympathetic and parasympathetic activity that reflects the autonomic balance. The authors concluded that these indexes can serve as a tool for assessing the assimilation of training and competition loads in professional soccer players [17]. However, although there are studies on the application of the LnrMSSD in other sports, to date, there are no data on the application of SS in other sports.

On the other hand, in team sports, where samples are usually heterogeneous and of small size, it has been suggested to use another type of statistical approach that is more useful than p values for assessing significant changes in a contrast of hypothesis [19], such as the smallest worthwhile change (SWC) of Hopkins, Marshall, Batterham, & Hanin [20] and Cohen's effect size (d).

The purpose of our study was to analyse the behaviour of SS and LnrMSSD as indicators of sympathetic and parasympathetic physiological stress and its relationship with biochemical markers during a national competition in handball players and to see the contribution of Cohen's and Hopkins statistics in the analysis of these variables.

MATERIAL AND METHODS

SUBJECTS

Fourteen university handball players (age: 22.30 ± 1.83 years; height: 180.74 ± 6.59 cm; weight: 83.86 ± 14.80 kg; percentage of body fat: 18.51 ± 8.22 %) who belong to the representative team of the Autonomous University of Nuevo Leon, all with experience in national competitions, participated voluntarily and were previously informed of the procedure, and signed a consent letter with the approval of the Bioethics Committee in Health Science Research, COBICIS (COBICIS-801/2015/124-01HCG).

PROCEDURE

Before the study all subjects underwent a complete physical examination that included a medical history, examination and body composition using dual-energy x-ray absorptiometry (DEXA) with the aim of discard any disease that could affect the purpose of the research.

All evaluations were performed during the national university championship, which is the team's most important in their annual cycle of training. The LnrMSSD, SS, CK, urea and cortisol were evaluated in the following stages: one week before the competition (REST); the second, the day before the start of the competition (PRE); the third, after the last match of the competition (END), which lasted one week accumulating six games including the final, one game per day; the fourth was performed the morning after the competition (24H), the fifth on the second day (48H), and the sixth on the third day after the competition (72H).

The quantification of CK, urea, and cortisol was performed by collecting morning blood samples (8:00 a.m. to 9:00 a.m. with the exception of the END sample which was performed at 4:00 p.m. because of the competition schedule). Samples were obtained by venepuncture in 4 mL tubes with EDTA as an anticoagulant (BD Vacutainer K2E/K2 EDTA) according to the CLSI (Clinical and Laboratory Standards Institute, 2007) procedure; samples were centrifuged at 3000 rpm for 5 minutes to separate the plasma and were stored at -70°C until processing.

CK AND UREA ANALYSIS.

Test strips were used for CK and urea (Roche, Rotkreuz, Switzerland) using the Reflotron Plus analyser (Reflotron Plus Roche, Rotkreuz, Switzerland).

CORTISOL ANALYSIS.

The ELISA was used with a Cortisol Human ELISA Kit (abcam ab108665-Cortisol Human ELISA Kit). For this analysis, the kit protocol was used. The calibration curve was prepared and placed together with the control and the plasma samples in specific wells of the ELISA plate. The cortisol results have been previously presented in a study that analysed the relationship between stress-psychological recovery and cortisol levels [21].

HEART RATE VARIABILITY

HRV monitoring was performed in a controlled environment using Polar Team 2 (Polar Electro OY, Kempele, Finland) for 15 minutes in a supine position in the morning, controlling the consumption of food or medications that could alter HRV. The obtained data were examined with KUBIOS software (University of Eastern Finland, Kuopio, Finland) using the LnrMSSD (as a time domain variable) and the axes SD1 and SD2 of the Poincaré scatter plot. SS was calculated with the following equation: $1000 \times 1 / \text{SD2}$, following the procedure proposed by Naranjo et al. [13].

STATISTICAL ANALYSIS

For statistical analysis of the data IBM SPSS statistics version 21 (SPSS Inc., Armonk, NY, USA) was used. The data are presented as means (M) and standard deviations (SD); Shapiro-Wilk test of normality was used. For the comparison of means analysis, the ANOVA test with Tukey's post-hoc test was used and Pearson's correlation analysis was performed to determine the interrelations between variables. For all statistical analyses, a $p < .05$ was considered significant.

Effect size (ES) was measured by Cohen's d , considering the following thresholds according to Hopkins et al. [20]: 0.1, small; 0.3 medium; 0.5 large; 0.7, very large

and 0.9, extremely large change. According to Hopkins et al. [20]: a $d=0.2$ was considered the Smallest Worthwhile Change.

RESULTS

Table 1. Means and standard deviation of the results of variables measured at different moments.

	LnrMSSD	SS	CK	Urea	Cortisol
REST	4.22* ± 0.40	7.51** ± 3.22	202.88 ± 119.32	24.57 ± 4.32	189.48 ± 72.90
PRE	4.00 ± 0.31	10.74 ± 3.17	283.55 ± 157.23	29.22 ± 9.06	242.94 ± 37.80
END	3.62 ± 0.61	14.08 ± 4.87	788.50 ± 706.68	32.37 ± 6.51	162.22Δ ± 69.83
24H	4.16* ± 0.40	9.46* ± 3.07	641.31 ± 560.50	28.0 ± 5.3	150.09ΔΔ ± 53.05
48H	4.20* ± 0.50	9.02* ± 4.01	428.89 ± 302.94	30.48 ± 9.87	198.42 ± 66.92
72H	4.30** ± 0.49	8.89* ± 4.34	284.57 ± 114.94	28.38 ± 8.77	175.18 ± 64.10

Note: Means (*M*) and standard deviation (*SD*) of all analyzed variables. CK = Creatine kinase; REST = at rest; PRE = pre-competition; END = end of competition; 24H = 24 hours after end of competition; 48H = 48 hours after end of competition; 72H = 72 hours after end of competition. * = $p < .05$, ** = $p < .01$ significant differences in relation to END measurement. Δ = $p < .05$. ΔΔ = $p < .01$ significant differences in relation to PRE measurement.

Table 2. Effect size using Cohen's *d* in the different measurements of the analyzed variables.

Variable		REST	PRE	END	24H	48H
CK	PRE	-0.58				
	END	-1.42	-1.17			
	24H	-1.29	-1.00	0.23		
	48H	-1.07	-0.63	0.71	0.49	
	72H	-0.70	-0.01	1.23	1.06	0.69
UREA	PRE	-0.70				
	END	-1.44	-0.40			
	24H	-0.71	0.17	0.74		
	48H	-0.83	-0.13	0.23	-0.33	
	72H	-0.58	0.09	0.52	-0.05	0.23
CORTISOL	PRE	-0.97				
	END	0.38	1.50			
	24H	0.63	2.04	0.20		
	48H	-0.13	0.85	-0.53	-0.43	
	72H	0.21	1.33	-0.19	-0.43	0.35

Note: REST = at rest; PRE = pre-competition; END = end of competition; 24H = 24 hours after end of competition; 48H = 48 hours after end of competition; 72H = 72 hours after end of competition. 0.1 = small effect; 0.3 = medium effect; 0.5 = large effect; 0.7 = very large effect; 0.9 = extremely large effect.

Table 1 shows the descriptive data from the variables when the different samples were taken, including means (*M*), standard deviation (*SD*) and their respective significance, finding changes mainly in the LnrMSSD and SS variables, with a greater decrease and increase, respectively with the END measurement. With regard to cortisol differences were seen in the END and 24H measurement; in the PRE, the highest peak for this parameter was seen. Figure 1 shows the graphic behavior of LnrMSSD and SS during the recordings.

Table 2 shows the effect size of the analyzed variables in all samples taken (CK, urea and cortisol). In CK, changes are observed in the effect size in all measurements; however, the most relevant changes with extremely large effect sizes (<0.9) that were present in the END, 24H and 48H measurements with regard to the REST measurements. While in urea the most relevant change was in the END measurement with regard to the REST measurement with an effect size of 1.44, which is considered to be extremely large. Finally with regard to the variable cortisol, this showed different change in effect size with extremely large changes in the PRE measurement with regard to the REST, and in the END and 24H measurement with regard to the PRE. On the other hand, in Table 3, we also find the effect size with regard to the HRV parameters (LnrMSSD and SS). The LnrMSSD showed extremely large changes (<0.9) in the END measurement with regard to the REST measurement and at 24H, 48H and 72H with regard to the END measurement. The SS showed the biggest changes in the PRE and END measurements with regard to the REST measurement, and the 24H, 48H and 72H measurements with regard to the END measurement.

Significant correlations of CK with SS ($r = 0.265$; $p = .001$) and LnrMSSD ($r = 0.329$; $p = .002$) were found. The rest of the variables did not have any significant correlation.

Table 3. Show the effect size using Cohen's d in the different measurements of the HRV variables (SS and LnrMSSD).

Variable		REST	PRE	END	24H	48H
LnrMSSD	PRE	0.62				
	END	1.19	0.83			
	24H	0.15	-0.44	-1.06		
	48H	0.04	-0.49	-1.05	-0.09	
	72H	0.04	-0.75	-1.24	-0.31	-0.20
SS	PRE	-1.01				
	END	-1.62	-0.83			
	24H	-0.62	0.41	1.16		
	48H	-0.42	0.48	1.14	0.12	
	72H	-0.37	0.49	1.13	0.15	0.03

Note: REST = at rest; PRE = pre-competition; END = end of competition; 24H = 24 hours after end of competition; 48H = 48 hours after end of competition; 72H = 72 hours after end of competition. 0.1 = small effect; 0.3 = medium effect; 0.5 = large effect; 0.7 = very large effect; 0.9 = extremely large effect.

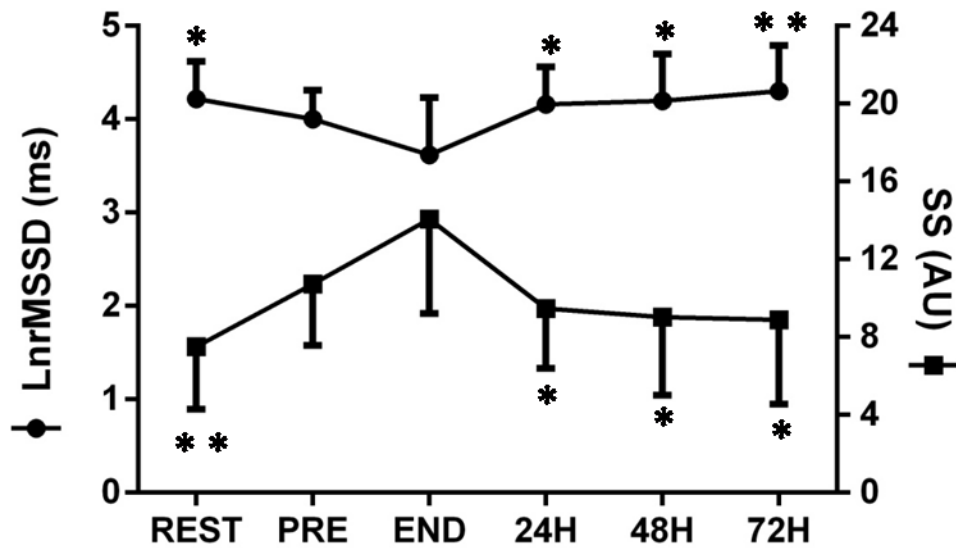


Figure 1. The evolution of the variables during different measurements are shown. Note: LnrMSSD = Napierian logarithm of the root mean square of the successive differences in R-R intervals; SS = stress score. REST = at rest; PRE = pre-competition; END = end of competition; 24H = 24 hours after end of competition; 48H = 48 hours after end of competition; 72H = 72 hours after end of competition. Significant differences observed in any variable with regard to the END measurement are marked with asterisks. * = $p < .05$, ** = $p < .01$

DISCUSSION

The main contributions of this study were a) to present SS and LnrMSSD data in high-level university handball athletes during a competition and b) demonstrate a significant correlation between LnrMSSD and SS with CK.

Regarding SS, we found in the REST measure, values that are within the parameters that Naranjo et al. [13] suggest as normal ($SS < 8$). The highest increase in SS was recorded at the end of competition, which could be considered as the period of greatest physiological stress, probably caused by the accumulation of matches, since as days of recovery passed, the values decreased without reaching the range of normality proposed by Naranjo et al. [13] for soccer players. Although there are no reference values in handball, our data show a behaviour similar to that presented by soccer players who were followed-up with these parameters during a full season, therefore, we consider we are in agreement with Naranjo et al. [17] that the SS can also be an effective indicator in handball for monitoring work load both in competition and training since the stress generated by the physical loads to which athletes are exposed directly influence the behaviour of sympathetic activity.

Regarding LnrMSSD as a parasympathetic indicator, an inverse relationship with SS was found as expected since it reflects opposite activities, noting also that the

lowest values during all measurements were taken at the end of the competition reflecting the lower level of parasympathetic tone. This occurs after the accumulation of six matches in a short period of time including the semi-final and final match, which could reflect the accumulation of fatigue. However, in a single measurement (as in this case), it is not possible to differentiate the stress included by the previous match from the sum of the entire load of competition.

There are reports in the literature that focus on monitoring changes in performance in sports such as soccer and handball using LnrMSSD [22, 23]. We could not find studies of after competition situations that assess the behaviour of parasympathetic activity with this variable that could be reliable for athlete follow up [24]. On the other hand, there are studies that report a decrease in rMSSD after high-level badminton competition because of the accumulation of matches [25], as well as in both active and sedentary subjects after performing a stress test of 80% intensity [26]. Moreover, we consider it important to analyse recovery in a rest period after competition, where the results suggest that 72 hours is sufficient time to return to baseline conditions, coinciding with Carvalho et al. [27], who propose the same recovery time to reduce physiological stress in handball players. Likewise, Saboul et al. [3] recommend measuring rMSSD pre-and post-competition as a method for quantifying loads in real competition conditions. LnrMSSD can provide relevant and different information on the study of recovery after real competition situations, in contrast with studies that focus on the response of the autonomous nervous system in controlled situations [28].

Intense exercise triggers an increase in biological markers; one of the most used in sport for the evaluation of internal load is CK, which is released into plasma when muscle fibres suffer damage caused by repeated and intense contractions [29]. In our study, it was found that the highest mean values (Table 1) were recorded in the sample taken at the end of the competition in accordance with that reported by Chatzinikolaou et al. [30] and de Moura et al. [31] in competitions or in training, although these values showed no significant difference between any of the samples. However, since the measure shows high variability between subjects with a mean variation coefficient (VC) of 79.0%, the information is little useful based on a hypothesis contrast in a small sample with great variability. This problem, which is frequent in team sports, was analysed by Buchheit [19], who suggested the use of the Cohen effect together with the SWC proposed by Hopkins et al. [20].

Using these statistics, we found significant changes in CK, with extremely large effect sizes, in the END, 24H, and 48H samples with regard to the resting sample (Table 2); therefore, we deduced that changes between these samples, irrespective of *p*-values, are relevant and provide useful information on the status of handball athletes at the end of competition and recovery.

On the other hand, CK showed a correlation with HRV parameters (SS and LnrMSSD), which is an important finding because few investigations have focused on analyze these relations, and in those that have been performed, no relation

between HRV and CK was found [6, 32]. This finding does not substitute CK measures for those of HRV, but it shows greater strength in the reliability of the HRV indexes (LnrMSSD and SS) for the control of loads and detecting fatigue.

In this study, it was not possible to evaluate the different parameters used in the research (LnrMSSD, SS, CK urea and cortisol) throughout the competition (match to match), thus it was difficult to know if the fatigue evaluated after the last match was the product of acute fatigue that reflected the stimulus of the final match or chronic fatigue due to the accumulation of matches. Considering this, we looked at urea, which is more related to volume than intensity [33], and which increases its values in blood when intense exercise is prolonged [34].

The results of urea in this study showed a stable behaviour with no significant differences in any of the samples. However, as with CK, the SE showed extremely large changes ($d = 1.44$) in the END sample with regard to the REST. Therefore, if we accept that a relevant increase in urea exists after the final, we can suppose that stress and/or fatigue registered after the final is influenced by the previous accumulation of matches.

Sports competition implies a physical load, which is reflected in athletes as increased stress. This stress affects the organism at the hormone level, causing an imbalance in the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system which can be reflected as an increase in cortisol levels and cardiac response [10, 11]. In the present study, baseline cortisol values were within normal rest values, coinciding with that reported in several studies [8, 35]. Between the REST and PRE samples, there is no significant change, but our results show an extremely large effect size ($d = .97$), possibly related to an anticipatory response to adapt the organism to the demands needed for competition, as reported in tennis, volleyball and basketball players and golfers [7, 9, 36]. This behaviour shown by handball players can be seen associated to the degree of difficulty of the competition, as occurs in rugby players [37].

Cortisol levels significantly decreased in the END sample of the competition ($p < .05$) with an extremely large effect size ($d = 1.50$) with regard to PRE, also with a decrease with a small effect size ($d = 0.38$) with regard to REST. In summary, the REST sample has baseline levels, followed by an increase in the PRE sample ($d = .97$) and then, a decrease in the END sample with regard to REST ($d = 0.38$) and PRE ($p < .05$; $d = 1.50$), which may reflect a variation in the circadian cycle pattern of cortisol secretion, which manifests its maximum values in the morning and decreases constantly during the day [10], since the END sample takes place at a different time from the rest (12:00 pm).

Without considering the sampling time, a decrease in cortisol would not be logical after a match, since players should be under stress caused by the competition. It is necessary, therefore, to correct in some way this effect. If we consider that normal plasma cortisol values at 8:00 am reach 250 ng/dL and at 12:00 pm, 150 ng/dL, we

can see (Table 1) that the mean resting value (REST) is 80% of the normal limit, the PRE is 97% and the END value is 108% of the normal limit. This would indicate that independent of the hour, the END cortisol can be considered qualitatively higher than PRE. This would be coherent with the analysis by Weitzman y colaboradores en 1971 (as cited in Chan & Debono [38] y Maidana, Bruno & Mesch [39]), with the idea that they are not comparable values taken at different hours [40].

On the other hand, the physiological responses through cortisol levels and LnrMSSD and SS parameters did not present a statistically significant correlation between each other, although SS and cortisol behaviour was similar between REST and PRE; this behaviour may be due to an adaptive physiological stress response of both variables [40].

According to McLean, Coutts, Kelly, McGuigan, and Cormack [41], we emphasize the importance of the balance between fatigue and the recovery of athletes to reduce the risk of a decline in sports performance, especially in competition periods. In our post-competition study, physiological stress markers are highly compromised; therefore, it is important to evaluate fatigue markers for internal load monitoring.

The present study was not exempt from limitations, among which the main ones were: (1) The END measurement made at the end of the competition was at a different time from the other measurements; however measuring the status in which the athletes ended was one of the priorities of the study, which is why we assume this limitation; (2) in this study it was not possible to monitor the different parameters in each of the games, due to the characteristics of the competition, where the athletes have little time for analysis.

CONCLUSIONS

SS behaviour can be used as a good indicator for sympathetic physiological stress assessment, whereas LnrMSSD can provide information on recovery through parasympathetic tone activity following national competition in handball players. HRV parameters did not show a correlation with biological markers except CK. Conventional statistical analysis by null hypothesis does not provide relevant information on the significant changes of a competition unlike the statistics of Cohen and Hopkins that provide information on relevant changes.

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