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ORIGINAL

BEHAVIOUR OF CHOLINESTERASES AFTER FATIGUE CONDITIONS IN ENDURANCE RUNNERS

COMPORTAMIENTO DE LAS COLINESTERASAS TRAS CONDICIONES DE FATIGA EN CORREDORES DE FONDO

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ABSTRACT

The objective of the present study was to evaluate the effect of intense training in endurance athletes on the behaviour of cholinesterases (ChE) after fatigue conditions and its relationship with other internal load markers. 18 male athletes specialized in endurance events participated. ChEs and two index of heart rate variability were evaluated at three different moments, before the study protocol (BASAL), 15 minutes after (FINAL) and the day after finishing the training (24H). A one-way ANOVA with post-hoc Tukey HSD was used to compare means. Significant differences were found in the variables analysed ($p < .001$), with very large effect sizes ($d > 0.9$) between BASAL, FINAL and 24H and moderate correlations between ChE and LnRMSSD and SS ($p < .001$). The behaviour of the ChEs showed a significant change ($p < .001$) after exercise and relationship with other internal training load indicators. Our results indicate that ChEs are related to fatigue in the studied athletes and may be a measure for training load determination.

KEY WORDS: Acetylcholinesterase, Butyrylcholinesterase, Recovery, Internal load markers, Heart rate variability

RESUMEN

El objetivo del presente estudio fue evaluar el efecto de un entrenamiento intenso en atletas de resistencia sobre el comportamiento de las colinesterasas (ChE) tras condiciones de fatiga y su relación con otros marcadores de carga interna. Participaron 18 atletas de sexo masculino especialistas en pruebas de resistencia. Se evaluó las ChE y dos índices de variabilidad de la frecuencia cardiaca en tres momentos diferentes, previo al protocolo (BASAL), 15 minutos posterior al protocolo (FINAL) y 24 horas después del entrenamiento (24H). Un ANOVA de una vía con post-hoc de Tukey HSD se utilizó para comparar las medias. Se encontraron cambios significativos en las variables analizadas ($p < .001$) con tamaños de efecto muy grandes ($d > 0.9$) en los diferentes momentos y correlaciones moderadas entre variables ($p < .001$). El comportamiento de las ChE muestra un cambio significativo ($p < .001$) posterior al ejercicio y una relación con otros indicadores de carga interna. Nuestros resultados indican que las ChE tienen relación con la fatiga en el caso de los deportistas estudiados, pudiendo ser una medida para determinar la carga de entrenamiento.

PALABRAS CLAVE: Acetilcolinesterasa, Butirilcolinesterasa, Recuperación, Marcadores de carga interna, Variabilidad de la frecuencia cardiaca

INTRODUCTION

Quantifying the training load is considered of utmost importance because of its use in the control of workloads during exercise [1]. This is why it is fundamental to quantify the stress generated by the exercise known as the internal load [2] since it allows to determine if the stimulus caused by the external load [3] facilitates increase in athletes' performance with optimal recovery to meet training adaptations [2].

Lack of load control and inadequate training density can cause fatigue induced by exercise or intense physical activity, making athletes unable to produce maximum voluntary muscle strength [4]. This can be central or peripheral fatigue, where one of its principal components is the change in the mechanisms found after the neuromuscular junctions [5-7]. This type of fatigue alters the neural action potential mechanism and the muscular action potential by diverse factors; among these, neurotransmission deregulation, and a decrease in the sensitivity of cholinergic receptors, among others [7].

It is known that physiological and metabolic changes seem to be the cause of this fatigue [8,9]. In response, there are several methods for internal training load control and measure; among these, heart rate variability (HRV), which has been described as a stress, fatigue, recovery, and training adaptation indicator through activation of the autonomous nervous system and its interaction with the heart [10-12]. One of the most widely used HRV index is the square root of the sum of the mean squared difference of all successive heartbeat-to-heartbeat intervals (RMSSD) as a measure of parasympathetic activity, and to a greater extent, the Naperian logarithm of the RMSSD (LnRMSSD), because of its greater sensitivity [13,14]. On the other hand, Naranjo et al. [15] have proposed a parameter to measure sympathetic activity called the stress score (SS), which is the inverse of the SD2 index of the Poincaré plot and represents a directly proportional value to sympathetic activity.

Despite its importance, very few works have focused on neurotransmission, and specifically on cholinesterases (ChE) and their possible role in fatigue [16]. The ChE (acetylcholinesterase and butyrylcholinesterase) are enzymes in charge of hydrolyzing the neurotransmitter acetylcholine (ACh) and they have been widely studied in clinical research. However, these enzymes seem to have additional biological functions that are not completely known [17,18]. Acetylcholinesterase (AChE) is found mainly in the heart, brain, and skeletal muscle modulating ACh in the synaptic cleft. Butyrylcholinesterase (BChE) predominates in the liver and blood serum hydrolyzing circulating ACh and possibly replacing the action of AChE [18].

It has been demonstrated that one of the factors that can influence the activity of ChE is physical exercise, and it is known that after just one session of physical activity, the activity of these enzymes increases considerably in rats [19]. Other

research also carried out in rats, studied the behavior of ChE expression and its relationship with fatigue [16]; however, there is little information regarding the activity of the ChEs in human physical activity. There are two recent studies [20,21], but they do not relate the changes of the ChEs with any effect on fatigue.

We assume, at least theoretically, that one of the possible causes of fatigue could be a decrease in neurotransmission [7], and it can also be inferred that endurance training is a fatigue inducer, probably of neuromuscular origin, although little it is known about this [6]. Therefore, the objective of this study was to evaluate the effect of intense training in endurance athletes on cholinesterase behavior concerning fatigue and with other internal load markers such as LnRMSSD and SS.

MATERIAL AND METHODS

SUBJECTS

Eighteen endurance trained male athletes participated (age: 20.66 ± 2.79 years; height: 173.93 ± 6.17 cm; weight: 63.76 ± 7.63 kg). The participants formed part of a training group; therefore, they all followed the same routine. They voluntarily participated in the study and signed written informed consent. This study was approved by the Ethics Committee of the Universidad Autonoma de Nuevo Leon following the ethical standards of all the principles expressed in the Helsinki Declaration [22] for the conduction of this study.

PROCEDURE

At the start of the study, a medical history and physical examination was conducted in all the athletes to rule out any pathology that would affect research design. The training session was performed on a 400-meter running track of the Universidad Autónoma de Nuevo León at 4:00 p.m. in a moderately hot and humid environment. The training consisted of 100, 200, 300, 400, 800, and 1000-meter intervals at the maximum intensity allowed for each distance with recovery periods of 2 minutes between each interval. Three samples for the variables were obtained: the first before starting the study protocol (BASAL), the second was performed 15 minutes after finishing the training session (FINAL), and the third the day after finishing the training (24H).

The analyzed variables were the LnRMSSD, the SS (non-invasive measures), and ChE (invasive measure) as measures of the internal load to control the physical stress generated by the training. Blood sampling was done by venipuncture and blood was stored in 4-mL tubes with sodium heparin as an anticoagulant (BD Vacutainer sodium heparin) following the procedure of the Clinical and Laboratory Standards Institute [23]. The samples were centrifuged at 3000 rpm for 10 minutes to separate the plasma which was stored at -80° C until processed.

CHOLINESTERASE ANALYSIS

A colorimetric spectrophotometry method was performed using the Acetylcholinesterase Assay Kit ab138871 (Abcam PLC., Cambridge, UK). To obtain the reaction mixture, a standard acetylcholinesterase solution and serial dilutions are prepared for the calibration curve. The standards, samples, and blank controls were placed in the plates. The samples were diluted 1:5 plus 50 μ L of the reaction mixture. The samples are incubated for 30 minutes at room temperature protected from light and are subsequently analyzed in a Bio-Rad microplate absorbance reader at 420 nm (iMark, Bio-Rad Clinical Diagnostics, Hercules, CA) until the result is obtained to subsequently correct the dilution concentrations. Two non-consecutive measurements of the ChEs were made and reliability was calculated by ICC = 0.79 (95% CI = 0.70; 0.86). In this study, both isoforms of ChE, AChE, and BChE, were evaluated.

HEART RATE VARIABILITY

Monitoring of HRV was done using the Polar Team² system (Polar Team², Polar Electro OY, Kempele, Finland) for 10 minutes in a controlled environment and in a supine position. Data were analyzed with Kubios v.2.2 software (Kubios HRV, University of Eastern Finland, Kuopio, Finland) to later calculate the Naperian logarithm of the RMSSD data as a variable of parasympathetic activity. Likewise, with the SD2 values, the SS was calculated as a measure of sympathetic activity, following the protocol proposed by Naranjo et al. [15].

STATISTICAL ANALYSIS

The statistical package SPSS version 25 was used for data analysis (IBM Corp., Armonk, NY) with a significance level of $p < .05$. The Shapiro-Wilk test was used to test normality. ANOVA test was used followed by Tukey HSD post hoc test to compare means. Pearson's correlation was used to define relationships between the variables. The reliability of the cholinesterase measures was assessed by intraclass correlation coefficient analysis.

The magnitude of change per sample was evaluated by effect size (ES) using Cohen's d [24]. The intervals proposed by Hopkins et al. [25] were considered, being 0.1, small change; 0.3 moderate; 0.5 large; 0.7 very large, and 0.9 extremely large.

RESULTS

Table 1 shows the descriptive data of the variables during the different sample moments presented as mean (M) and standard deviation (SD). Also, behavior and changes in analyzed variables are shown. LnRMSSD and SS present a significant change in the FINAL sample, and these two variables together with the ChEs show

a significant change in the 24H sample observing that LnRMSSD and SS have an inverse and similar behavior.

Table 1. Means and standard deviation of the analyzed variables in the three moments of evaluation.

	BASAL M ± SD	FINAL M ± SD	24H M ± SD
ChE (mU/mL)	4195.11 ± 457.84	4354.75 ± 429.58	3104.34 ± 577.08*§
LnRMSSD (UA)	1.84 ± 0.21	0.91 ± 0.26*	1.75 ± 0.13§
SS (UA)	10.42 ± 3.43	26.40 ± 10.42*	10.73 ± 3.92§

Note: M = data means; SD = Standard deviation; BASAL = before training; FINAL = at the end of the training; 24H = one day after training. (*) Significant difference ($p < .001$) with respect to BASAL. (§) Significant difference ($p < .001$) with respect to FINAL.

To reinforce the significant changes found in Table 1, Table 2 shows the effect size values to observe the magnitude of change in the variables and that these changes were not random, where we found that the significant changes assessed showed very large effect sizes in the FINAL sample for LnRMSSD and SS. These two variables together with the ChEs also show very large effect sizes for the FINAL sample.

Table 2. Magnitud of change of the variables by effect size.

Samples	ChE	LnRMSSD	SS
BASAL vs FINAL	0.360	-3.956	2.305
BASAL vs 24H	-2.108	-0.504	0.082
FINAL vs 24H	-2.484	4.323	-2.184

Note: BASAL = before training; FINAL = at the end of the training; 24H = one day after training.

When exploring Pearson's correlation coefficients, moderate and statistically significant correlations were found between ChE and LnRMSSD ($r = -.480$; $p = .001$) and between the ChEs and SS ($r = .419$; $p = .001$).

DISCUSSION

The main contribution of this study was to show the behavior of the ChEs in plasma, as well as their relationship with other internal training load markers before and after a long-distance training session in college runners.

In the literature, it has been described that the ChEs could show a modification of their activity influenced by physical exercise. This has been described in various investigations in both rats [16,19] and humans [20,21]. Also, they have been linked to neuromuscular fatigue in rats after an exercise protocol [16]. However, none of the studies carried out in humans have linked them to their possible role in fatigue. According to our results, the ChE values recorded after the training session in the FINAL sample do not show a significant ($p = 0.851$) or relevant change ($d = 0.36$). These results do not coincide with the increase reported in other studies [20,21], probably due to the exercise protocol used. In both these works, a low-intensity activity is performed: 30 min at 7 km/h [20] and 60 min at 10.6 ± 1.7 km/h [21]. However, in our study, the effect of training at high-intensity intervals is analyzed. This suggests that plasma levels of ChE do not increase due to the high rates of hydrolysis of the neurotransmission mechanism because of the type of exercise performed.

On the other hand, the decrease in ChE that occurs at the 24H sample with regard to BASAL and FINAL samples could be due to several factors; among them, an effect that favors neurotransmission during the recovery process. Another, probably due to internal factors that affect their synthesis [17], such as physical exercise [20] and regularization of acetylcholinesterase (ACHE) gene expression in response to the physiological state caused by external stimuli [18].

The idea of a relationship with neurotransmission would be reinforced by the fact that in the current results an inverse correlation between the ChEs and LnRMSSD (parasympathetic activity) was found, and a direct relationship between the ChEs and SS (sympathetic activity). Parasympathetic activity is known to be mediated by ACh release in the efferent discharge from the vagal nerve [12,26], and in this line, a study by Canaani et al. [27] shows that when AChE activity decreases, heart rate (HR) recovery increases and HRV increases [28]. However, other authors [29] did not find any post-exercise change after inhibition of AchE in healthy subjects, but they did in people with heart disease [30].

The LnRMSSD as expected decreases after exercise and the SS increases immediately in the FINAL sample compared to the BASAL sample due to the almost total suspension of parasympathetic activity and sympathetic elevation, as several studies have reported [31-34]. It has been described in the literature that this could be because the greater the relative intensity of the exercise, the greater the metabolic load (H^+ , inorganic phosphate, epinephrin release) which will affect baroreflex and metaboloreflex stimulation, which would be related to low parasympathetic activity and high sympathetic activity [10,35-37]. Afterward, in recovery, we found a LnRMSSD increase and a SS decrease at 24H with regard to FINAL, reaching values that do not differ from BASAL values. This is mainly due to parasympathetic reactivation, because of regulation of circulating catecholamines, blood pressure, baroreflexes and metaboloreflexes decrease. This, in turn, will have an efferent reflex effect of vagal stimulation that will increase parasympathetic activity and cause a drop in sympathetic stimulation [11,12,38-

40]. On the other hand, normalization of HRV data 24 hours after exercise of these characteristics is well documented in the literature [39,41].

The main limitation of this study was not having access to a direct measure of the neurotransmission processes. On the other hand, and in light of the results obtained, it would have been interesting to observe the behavior of ChE recovery 24 hours after finishing exercise. However, the results found allow us to participate in new perspectives on the role of ChEs in exercise.

CONCLUSIONS

The behavior of cholinesterases could be an indicator of internal load because they present an acceptable relationship with heart rate variability indicators. This could be related to their mediator role in neurotransmission.

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