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Effect of Phlebodium Decumanum on muscle damage, inflammation and cortisol during a precompetitive mesocycle in a National Volleyball Team

Efecto del Phlebodium Decumanum sobre el daño muscular, inflamación y cortisol durante un mesociclo precompetitivo en Selección Nacional de Voleibol

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Abstract. The severe training load of a competitive mesocycle may not be assimilated correctly by athletes, and can provoke endocrine imbalance, muscle damage and inflammation. Supplementation with antioxidants such as Phlebodium Decumanum (PHL) helps protect against such imbalances. The aim of this study is to analyse the effect of PHL on the fatigue during a precompetitive mesocycle in volleyball players. Twenty-four male volleyball players were divided into two groups (experimental and control). The experimental group was supplemented with three PHL capsules in the morning and three in the afternoon for 20 days. Blood samples were taken before training, at the end of the third week and at the end of the fourth week to evaluate creatine kinase (CK), interleukin 6 (IL-6), tumour necrosis factor-alpha (TNF-α), interleukin 10 (IL-10) and cortisol. The results show the control group, the third Cortisol were significantly increased ($p < .05$). In the experimental group, however, a significant increase ($p < .05$) was only observed in the third IL-10 value. In addition, significant differences ($p < .05$) were observed between the control and experimental groups in the second CK. Furthermore, there were moderate variations (effect size, $ES > 0.5$; 90% CI) in CK, IL-6 and TNF-α. In conclusion, PHL supplementation during a precompetitive mesocycle by volleyball players protects against the muscle damage, inflammatory modulation and cortisol response caused by fatigue.
Keywords: Inflammation, Cortisol, Antioxidants, Inflammatory response, Muscle damage.

Resumen. Altas carga de entrenamiento de un mesociclo competitivo, pueden no ser asimiladas de forma correcta por atletas, pudiendo ocasionar desequilibrio endocrino, daño muscular e inflamación. La suplementación con antioxidantes como el Phlebodium Decumanum (PHL), puede proteger ante estos desequilibrios. El objetivo de este estudio fue analizar el efecto del PHL sobre la fatiga de un mesociclo precompetitivo en voleibolistas. Veinticuatro voleibolistas divididos en dos grupos (experimental y control) participaron en este estudio. El grupo experimental se suplementó con tres capsulas de PHL por la mañana y tres por la tarde por 20 días. Se efectuaron tomas de sangre antes del entrenamiento, al finalizar la tercera semana y al finalizar la cuarta semana para evaluar creatin kinasa (CK), interleucina 6 (IL-6), factor de necrosis tumoral-alfa (TNF-α), interleucina 10 (IL-10) y cortisol. Los resultados mostraron un aumento significativo ($p < .05$) en la tercera toma de Cortisol en el grupo control. El grupo experimental solo se observó un aumento significativo ($p < .05$) en la tercera toma para IL-10. Además, se observó una diferencia significativa ($p < .05$) entre los grupos para CK en la segunda toma, así como cambios moderados ($ES > 0.5$; 90%IC) para CK, IL-6 y TNF-α. En conclusión, la suplementación con PHL durante un mesociclo precompetitivo en jugadores de voleibol mostro efectos protectores beneficiosos sobre el daño muscular, modulación inflamatoria y respuesta del cortisol ocasionado por la fatiga.
Palabras clave: Inflamación, Cortisol, Antioxidantes, Respuesta inflamatoria, Daño muscular.

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Introduction

Many studies have highlighted the importance of controlling physical activity and training in terms of intensity, volume, density, frequency and duration, in order to maintain or improve health status (American College of Sports Medicine, 2018; Halson, 2014; Impellizzeri, Marcora & Coutts, 2019). The goal for athletes is mainly to enhance sports performance, and so their training programmes are usually demanding in terms of intensity and volume, seeking to provoke acute fatigue and thus induce the body to super-compensate, thus increasing performance (Bourdon et al., 2017; Halson, 2014).

Sometimes, however, the training load placed on athletes is not properly assimilated during recovery periods, leading to imbalances in homeostasis and causing exercise-induced fatigue (Edwards, 1981; Halson, 2014; Woods, Garvican-Lewis, Lundy, Rice & Thompson, 2017). These fatigue states can be caused by imbalances in physiological, metabolic and psychological systems (Coutts, Crowcroft & Kempton 2021; Lepers, Maffiuletti, Rochette, Brugniaux & Millet, 2002; Noakes, 2000), provoking muscle damage, endocrine imbalance, immune response and/or inflammation or oxidative stress (Coutts, Crowcroft & Kempton 2021; Thorpe, Atkinson, Drust & Gregson, 2017), and raising levels of creatine kinase (CK), cortisol, inflammatory cytokines such as interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α), and anti-inflammatory cytokines such as interleukin 10 (IL-10) (Banfi, Colombini, Lombardi & Lubkowska, 2012; Brancaccio, Lippi & Maffulli; 2010; Chamera et al., 2016; Chazaud, 2016; Pedersen & Toft, 2000).

Volleyball being a team sport with high intensity intermittent characteristics (Sheppard et al., 2007), requires the assessment of the training load to understand the training process (McLaren et al., 2018) and thus by measuring

the internal load, monitor recovery and improve it (Gabbett et al., 2017; West et al., 2021). It is for this reason that the recovery period is sometimes sought to be optimized (Soligard et al., 2016). To this end, some reviews have evidenced the most commonly used methods for recovery to decrease muscle damage and the inflammatory process (Crowther, Sealey, Crowe, Edwards, & Halson, 2017; Dupuy, Douzi, Theurot, Bosquet & Dugué, 2018; Nieman, Stear, Castell, & Burke, 2010). However, a great deal of attention has recently been presented to the use of antioxidants (Antonioni, Fantini, Dimauro, & Caporossi, 2019), where their beneficial effect has been demonstrated (Nocella et al., 2019).

The most commonly used methods are the use of vitamin C, beta-carotenes, polyphenols, Phlebodium Decumanum (PHL) and Coenzyme Q_{10} (Antonioni, Fantini, Dimauro, & Caporossi, 2019; Díaz-Castro et al., 2012; Fernández et al., 2005; Nieman, Stear, Castell & Burke, 2010; Nocella et al., 2019). However, recently a study by Martín-Pozo et al. (2019), in which he presents some studies within the literature that speak of the anti-inflammatory and immunomodulatory potential, showed the complete phytochemical characterization of PHL, leading to its study and its beneficial effect. It has been reported that during training, PHL is an immunomodulator that helps prevent the above-mentioned alterations caused by high training loads and also promotes medium-term recovery (Díaz-Castro et al., 2012; Fernández et al., 2005). PHL is a small spore from a type of fern called Polypodium leucotomos, which has been patented (US 6.228.366). Its formulation is obtained from a water-soluble frond fraction (EXPLY-37®) (Gatusso, Cortadi & Gatusso, 2008), purified and standardised by hydroalcoholic extraction. It has been used in several research studies on the prevention of oxidative and muscle damage and of immune and inflammatory imbalances. PHL protects against catabolism and reduces CK, myoglobin, IL-6 and TNF-α, and heightens the antioxidant activity of enzymes such as catalase and glutathione reductase. Finally, it is known to increase aerobic, anaerobic and strength performance. Research studies of PHL have been conducted with experimental groups of medium to high-level athletes performing physical exercise (Díaz-Castro et al., 2012; Fernández et al., 2005; García Verazaluce et al., 2015).

Although it is known that the use of antioxidants has a positive immunomodulatory and anti-inflammatory effect, there are still discrepancies between studies (Antonioni, Fantini, Dimauro, & Caporossi, 2019; Taherkhani, Suzuki & Castell, 2020), possibly due to the hormesis of the supplementation protocols (Slattery, Bentley, & Coutts, 2015). It has been shown that the effect of a high dose of PHL has a beneficial effect on these indicators (Díaz-Castro et al., 2012). However, the need for long-term antioxidant supplementation protocols is also known (Bentley, Ackerman, Clifford, & Slattery, 2015; Nocella et al., 2019). This effect has been demonstrated in the medium term with PHL at a lower dose, so it is considered that with a slightly higher dose the effect of hormesis could be greater in the medium term.

Moreover, to our knowledge, no such study has been made of the effects of PHL during a pre-competitive period, although this training phase is characterised by high workloads aimed at improving athletic performance (Coutts & Reaburn, 2008; Coutts, Reaburn, Piva & Murphy, 2007). It has been hypothesized that PHL will have a positive effect on markers of muscle, endocrine, immune and inflammatory damage justifying the need to control the imbalances caused by the accumulation of fatigue and the effects that this could cause during a precompetitive period. Therefore, the objective of this research is to analyze the effect of supplementation with Phlebodium Decumanum on muscle damage, endocrine response, immune and inflammatory modulation caused by fatigue in a pre-competitive mesocycle during a selective concentration of the Mexican National Volleyball Team prior to its participation in the Central American and Caribbean Games.

Materials and methods

Design

This longitudinal quasi-experimental study was carried out with two block-randomised groups during a four-week pre-competitive mesocycle to evaluate the effects of PHL supplementation on endocrine and enzyme indicators of muscle damage and on immune and inflammatory response.

Participants

Twenty-four members of the Mexican male volleyball team were randomly allocated to one of two study groups to be of equal size, with 12 athletes in each: an experimental group (EG) (age 27.0 ± 4.06 years, weight 89.28 ± 8.13 Kg, height 192.57 ± 4.91 cm, fat mass 23.03 ± 2.39%, muscle mass 49.82 ± 2.16% and VO_2max 50.21 ± 2.18 mL · Kg^{-1} · min^{-1}); and a control group (CG) (age 22.3 ± 3.0 years, weight 86.7 ± 8.5 Kg, height 191.7 ± 8.0 cm, fat mass 24.0 ± 3.0%, muscle mass 48.9 ± 2.6% and VO_2max 49.7 ± 1.9 mL · Kg^{-1} · min^{-1}). The equality of the groups was established by the balance in the physical characteristics presented (weight, height, % muscle mass, VO_2max, etc.). The details of the study were explained at an informative meeting, after which all 24 gave their signed informed consent to take part in the study. This research was carried out in accordance with the indications of the Faculty Bioethics Committee (RCB-11/2010) and ethical standards expressed in the Helsinki Declaration (World Medical Association, 2013).

Procedure

The research protocol was applied during a training camp to select the Mexican male volleyball team prior to its participation in the Central American and Caribbean Games. During the days immediately preceding the start of this training session, every player underwent a medical

examination, and a full medical history was obtained, to ensure there was no pathology, injury or consumption of medications or supplements that might interfere with the results of the study.

At the first time point (T1), a blood sample was taken, a stress test (30-15 Intermittent Fitness Test, IFT) (Buchheit, 2008) was conducted and a kinanthropometric body composition analysis was performed (based on the full profile stipulated by the International Society for the Advancement of Kinanthropometry, ISAK). The training programme, consisting of four microcycles, then began. Each microcycle was composed of four training days followed by one rest day. Each day consisted of two three-hour training sessions: the first, in the morning (at 7 a.m.) was focused on strength or resistance training, and the second (at 4 p.m.) on technical aspects of volleyball. Both groups performed the same training schedule (Table 1). Finally, another two blood samples were taken: one at 24 hours after the third microcycle (T2) and another at 24 hours after the fourth (T3).

Table 1.
Training load imposed during each microcycle

		Microcycle 1	Microcycle 2	Microcycle 3	Microcycle 4
		Exe/Vol/Int	Exe/Vol/Int	Exe/Vol/Int	Exe/Vol/Int
Session 1	Squats	4/3s x 3r/75%	4/3s x 3r/85%	4/3s x 3r/85%	4/3s x 3r/85%
	Barbell jumps	3s x 6r/30%	3s x 6r/50%	3s x 6r/50%	3s x 6r/50%
	Back	6/3s x 12r/65%	6/3s x 12r/75%	6/3s x 8r/85%	6/3s x 8r/85%
	Triceps	6/3s x 12r/65%	6/3s x 12r/75%	6/3s x 8r/85%	6/3s x 8r/85%
	Chest	3/3s x 16r/65%	3/3s x 16r/65%	3/3s x 12r/75%	3/3s x 8r/85%
	Biceps	3/3s x 16r/65%	3/3s x 16r/65%	3/3s x 12r/75%	3/3s x 8r/85%
	Shoulders	3/3s x 4r/65%	3/3s x 4r/75%	3/3s x 4r/75%	3/3s x 4r/85%
	Snatch	5/3s x 3r/70%	5/3s x 3r/70%	5/3s x 3r/70%	5/3s x 3r/85%
	Deadlift	5/3s x 3r/70%	5/3s x 3r/70%	5/3s x 3r/70%	4/3s x 3r/70%
	Clean/Jerk				
		Vol/Int (%HR)	Vol/Int (%HR)	Vol/Int (%HR)	Vol/Int (%HR)
Session 2	Group 1 vs Group 2 Intersquads by task and position (3vs3, 4vs4, 6vs6) of one serve plus three balls	45/55	40/60	35/65	30/70

Note: Exe = Number of exercises; HR = Heart rate, as % of maximum heart rate in the stress test; Int = Relative intensity, from individual one-repetition maximum (1RM); Vol = Volume, calculated as number of series (s) multiplied by repetitions (r).

Supplementation protocol

The players in the EG received the PHL supplementation, at a dose of six capsules per day, three at 7 a.m. before the morning training session and another three at 4 p.m. before the afternoon session, for 20 days (the duration of the training mesocycle). This supplementation began after the T1 blood sample, the 30-15 IFT and the kinanthropometry. Each 400 mg capsule contained 250mg of water-soluble fraction of the PHL extract and 150mg of rhizome powder, prepared according to the procedure described by US patent No. 6.228.366 and marketed as EXPLY-37®. The CG did not receive any type of placebo intervention.

Blood sampling

Venous blood samples were taken by puncturing the median ulnar vein, using a double-bevel needle, after first sterilising the area. The samples were collected in 6mL tubes with EDTA anticoagulant (BD Vacutainer, K2 EDTA) and subsequently placed in a Solbat J-40 centrifuge (SOLBAT S.A. de C.V., Puebla), operated at 3000 rpm for seven minutes. Finally, the plasma was collected in 1mL Eppendorf microtubes and stored at -20 °C until required.

Pro-inflammatory and anti-inflammatory markers

The IL-6, IL-10 and TNF-α cytokines were analysed by a sandwich ELISA immunoassay, using the Human IL-6 Uncoated ELISA, Human IL-10 Uncoated ELISA and Human TNF-α Uncoated ELISA kits (Invitrogen, Thermo Fisher Scientific). The regents were prepared following the manufacturer's instructions, using a 96-well microplate, in which each well was covered by the capture antibody. The corresponding standards, the calibration curve, the samples and the detection antibody were then prepared. The results obtained were read on a BIO-RAD iMark spectrophotometer (Bio-Rad Laboratories Inc, Hercules, California) at 450 nm.

Cortisol

Cortisol was analysed by competitive ELISA immunoassay using a cortisol ELISA kit (Immuno-Biological Laboratories, America), according to the kit instructions. A solid phase of the capture antibody was placed in each well of a 96-well microplate. The standards, the calibration curve, the samples and the conjugated enzyme were then prepared. The plate was read using a BIO-RAD iMark spectrophotometer (Bio-Rad Laboratories Inc, Hercules, USA) at 450nm.

Creatine kinase

Creatine kinase was analysed by automatic reflection photometry with CK Reflotron test strips (Roche Diagnostics) exposed to 30µL of plasma and subsequently inserted into the Reflotron Plus analyser (Roche Diagnostics, Rotkreuz, Switzerland).

Statistical analysis

Statistical analysis was performed using SPSS v.21 (SPSS Inc., Chicago, USA), assuming a level of significance of $p < .05$ for all tests.

All data are presented as means and standard deviations. The normality of the distribution was verified using the Shapiro-Wilk test. Homoscedasticity was confirmed using the Levene test. The comparisons by time point, for each group, were compared by the repeated measures ANOVA test and comparisons between groups were performed using 2x3 repeated measures ANOVA.

To avoid multiple inferences and type I and II errors, the inter-group changes were determined using the progressive statistic approach described by Hopkins, Marshall, Batterham, and Hanin (2009), based on the smallest worthwhile change (SWC) and the standardized difference in effect size (*ES*, 90% CI). The thresholds for the change *ES* were evaluated using modified units of Cohen's principle, whereby > 0.2 represents a small degree of change, > 0.6 a moderate change, > 1.2 a large change and > 2.0 a very large change. The qualitative possibilities of change were evaluated as follows: <1%, most likely no; 1-5%, very unlikely; 6-25%, unlikely; 26-75%, possible; 76-95%, likely; 96-99%, very likely; >99% the most likely (Hopkins et al., 2009).

Results

Table 2 shows the descriptive data for T1, T2 and T3 for the CG, for all the variables analysed, as means and standard deviations. It also presents the results of the intergroup comparison by the repeated measures ANOVA test for each time point. For Cortisol, the values obtained at T3 were significantly ($p < .05$) higher than those obtained at T1.

Table 2.
Descriptive statistics and intragroup comparison of the concentrations of the variables analysed for the control group

CG		T1	T2	T3
		M ± SD	M ± SD	M ± SD
CK (U/L)	$F_2 = 0.41$; $p > .05$; $np^2 = .036$; 1-β = .10	191.3 ± 162.1	239.2 ± 188.7	242.3 ± 200.8
IL-6 (pg/mL)	$F_2 = 1.78$; $p > .05$; $np^2 = .14$; 1-β = .33	1.2 ± 1.0	1.3 ± 1.0	1.6 ± 1.3
TNF-α (pg/mL)	$F_2 = 2.21$; $p > .05$; $np^2 = .16$; 1-β = .40	8.9 ± 2.3	10.4 ± 2.7	9.7 ± 2.2
IL-10 (pg/mL)	$F_2 = 4.58$; $p < .05$; $np^2 = .29$; 1-β = .71	6.1 ± 1.8	5.8 ± 1.6	7.7 ± 2.9
Cortisol (ng/mL)	$F_{1.23} = 8.34$; $p < .001$; $np^2 = .43$; 1-β = .81	216.8 ± 76.7	279.6 ± 44.7	288.2 ± 49.4*

Note: The mean (M) and standard deviation (SD) are presented for each variable and time point.
* $p < .05$ with respect to T1.

Table 3 shows the means and standard deviations for the descriptive data (all variables) and the intergroup comparison, as analysed by the repeated measures ANOVA test for the EG. In this group, a statistically significant increase ($p < .05$) was only measured for IL-10 at T3 compared to T1.

Figure 1 shows the results obtained by the 2x3 repeated measures ANOVA for the CG and the EG at each time point and overall. In this case, the EG presented a significant decrease ($p < .05$) in CK at T2.

Table 3.
Descriptive statistics and intragroup comparison of the concentrations of the variables analysed for the experimental group

EG		T1	T2	T3
		M ± SD	M ± SD	M ± SD
CK (U/L)	$F_{1.37} = 2.88$; $p > .05$; $np^2 = .20$; 1-β = .40	157.9 ± 181.6	113.5 ± 56.2	197.6 ± 93.7
IL-6 (pg/mL)	$F_{1.33} = 2.32$; $p > .05$; $np^2 = .17$; 1-β = .33	0.6 ± 0.4	1.0 ± 1.0	0.9 ± 0.4
TNF-α (pg/mL)	$F_2 = 0.60$; $p > .05$; $np^2 = .052$; 1-β = .13	8.4 ± 3.3	9.0 ± 3.3	8.5 ± 2.7
IL-10 (pg/mL)	$F_2 = 4.73$; $p < .05$; $np^2 = .30$; 1-β = .73	5.4 ± 2.0	5.6 ± 1.4	6.9 ± 2.1*
Cortisol (ng/mL)	$F_2 = 3.76$; $p < .05$; $np^2 = .25$; 1-β = .62	214.0 ± 94.6	265.1 ± 70.1	264.9 ± 63.1

Note: The mean (M) and standard deviation (SD) are presented for each variable and time point.
* $p < .05$ with respect to T2.

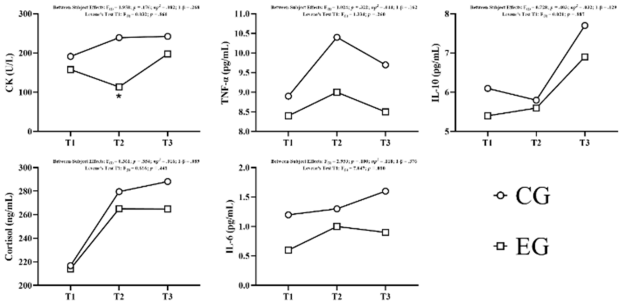

Figure 1. Tests of inter-group differences for the study variables * Differences statistically significant at $p < .05$.

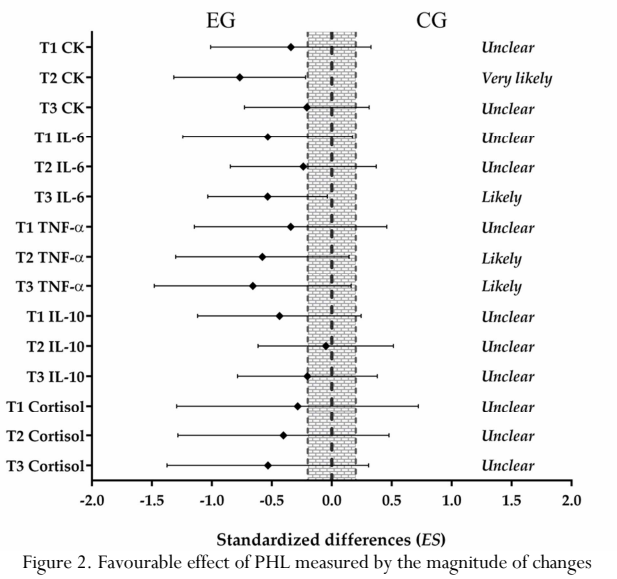

Figure 2. Favourable effect of PHL measured by the magnitude of changes between groups according to the standardized difference in effect size.

Figure 2 shows the favourable effect of PHL via the magnitude of the changes between the experimental and control groups, for each time point, and reveals a moderate and very probable change at T2 for CK with *ES* = -

0.76 (90% CI = -1.31; -0.21). For the proinflammatory cytokines, there was a moderate and possible change at T3 for IL-6, with *ES* = -0.53 (90% CI = -1.03; -0.03), as well as moderate and possible changes at T2 and T3 for TNF-α, with *ES* = -0.57 (90% CI = -1.30; 0.14) and *ES* = -0.65 (90% CI = -1.47; 0.16), respectively.

Discussion

As its main contribution, this paper describes the modulating effect of PHL supplementation in preventing and alleviating the muscle damage, inflammatory modulation and cortisol response that may be caused during a precompetitive training mesocycle.

Previous studies have reported that intense training, as may occur during a pre-competitive period, frequently imposes severe loads and intensities (Coutts & Reaburn, 2008; Coutts, Reaburn, Piva & Murphy, 2007), provoking metabolic and physiological changes such as fatigue (Lepers et al., 2002; Noakes, 2000) and muscle damage, which in turn raise CK levels (Banfi et al., 2012; Chamera et al., 2015; Coutts, Reaburn, Piva & Murphy, 2007). These outcomes are reflected in our CK results for the CG, which were elevated at T2 and had increased by T3 (Table 2). On the other hand, in the EG, the CK levels remained stable throughout the study period (Table 3). Moreover, the EG and CG presented a significant difference in this respect at T2 (Figure 1), together with a moderate change (*ES* = -0.76; 90% CI = -1.31; -0.21) for the same time point (Figure 2). These results are indicative of a protective effect of PHL against muscle damage, which would corroborate Vargas-Corzo et al. (2014) who studied sedentary subjects and reported that those who received PHL supplementation experienced lower increases in CK, in comparison with a placebo group. In some studies where it has been supplemented with other types of antioxidants, a clear effect of these against muscle damage has not been evidenced (de Jesus Pires de Moraes et al., 2018; García-Cardona, Landázuri, Ayala-Zuluaga & Restrepo Cortes, 2022; García-Dávila et al., 2017), so it is possible that PHL has a greater effect.

When structural damage to the muscle occurs, the resistance of the plasma membrane is reduced, which facilitates the release of CK, as mentioned above, in addition to other substances such as calcium and free radicals (Brancaccio et al., 2010; Montgomery et al., 2008), provoking a transient acute inflammatory response (Nocella et al., 2019). This increases the presence of immune cells and produces an inflammatory effect, raising the levels of pro-inflammatory cytokines such as IL-6 and TNF-α, and consequently those of anti-inflammatory effectors, too, such as IL-10 (Chazaud, 2016). These phenomena are all reflected in our findings, in which the muscle damage presented by the CG, with elevated CK, is very probably related to the elevated IL-6 values observed at T3. This could be due to an effect that has been described in previous research, in which IL-6 participates with monocytes and macrophages to stimulate the immune response and inflammation observed following muscle damage (Brandt & Pedersen, 2010; Pedersen, Akerstrom, Nielsen & Fischer, 2007).

There were no significant changes in the IL-6 values in the EG. However, the inter-group comparison shows that at T3 the values for the CG were higher than those for the EG (Figure 2), with a moderate change (*ES* = -0.53; 90% CI = -1.03; -0.03) reflecting the protective effect of PHL. This finding is in line with the in vitro study in this respect by Punzón, Alcaide, and Fresno (2003) who analysed the anti-inflammatory and immunomodulatory functions of a standardised, purified water-soluble fraction of PHL, and concluded that it produced a partial inhibition of IL-6 and a total inhibition of TNF-α. This modulation effect of IL-6 has also been demonstrated in in vivo studies by García-Verazaluce et al. (2015) and González-Jurado, Pradas, Molina, and de Teresa (2011).

Our data reveal a similar pattern for TNF-α. Overall, there were differences between groups with moderate changes at T2 (ES = -0.57; 90% CI = -1.30, 0.14) and T3 (ES = -0.65; 90% CI = -1.47, 0.16). As this cytokine is produced by macrophages, its function is to intensify the inflammation that results from muscle damage (Brandt & Pedersen, 2010; Finsterer, 2012) and to interact with the type 1 TNF receptor (Leelarungrayub, Khansuwan, Pothongsunun & Klaphajone, 2011). The preventive effect of PHL is apparent in the EG, since the TNF-α values remained stable, as previously reported in two studies, one in vivo (Díaz-Castro et al., 2012) and one in vitro (Punzón et al., 2003), who commented that the PHL increased the release of the soluble TNF2 receptor, which neutralised and inhibited the production and activity of the TNF-α. Unlike previous studies with supplementation of other types of antioxidants where no clear effect has been shown (Nishizawa et al., 2011), PHL seems to show a positive effect.

At T1 and T2, IL-10 remained stable in the EG and the CG. However, at T3 it was notably elevated in both groups, although the change was only significant for the EG (Table 3). This finding suggests that IL-10 may function as an anti-inflammatory cytokine, modulating and inhibiting TNF-α (Chernoff et al., 1995; Fatouros & Jamurtas, 2016; Ostrowski, Rohde, Asp, Schjerling & Pedersen, 1999; Peake, Neubauer, Della Gatta, & Nosaka, 2017; Petersen & Pedersen, 2005). This outcome would coincide with the modulation of TNF-α and IL-6 in the EG, possibly due to the natural effect of its incorporation into mononuclear cells and neutrophils stimulated by lipopolysaccharides that suppress the production of cytokines, by inhibiting the transcription of their corresponding genes (Peake, Neubauer, Della Gatta, & Nosaka, 2017; Wang, Wu, Anthes, Siegel, Egan & Billah, 1994; Wang, Wu, Siegel, Egan & Billah, 1994) and probably due to the immunomodulatory effect of PHL. To our knowledge, the present paper is the first to report this hypothesis. The in vitro study by Punzón et al. (2003) also

reported the anti-inflammatory effect of PHL, with increased levels of the soluble TNF2 receptor and of the antagonist receptor of IL-1R (IL-1Ra), which inhibited the pro-inflammatory cytokines TNF-α and IL -1R.

Exercise that is prolonged, strenuous and/or exceeds 60-70% VO_2max can increase the secretion and concentration of cortisol in blood plasma (Budde, Machado, Ribeiro, & Wegner, 2015; Davies & Few, 1973; Raastad, Bjoro & Hallen, 2000). Furthermore, adrenocortical cells can express IL-6 receptors, and so an increased presence of IL-6 can increase that of cortisol, both directly and indirectly (Araujo et al., 2019; Bethin, Vogt & Muclia, 2000; Steensberg, Fischer, Keller, Møller & Pedersen, 2003). This is borne out in our results for the CG, in which levels of cortisol rose significantly after T2, and remained high at T3, coinciding with the increase in IL-6 for this group (Table 2).

However, this effect was not observed in the EG, where levels of cortisol not only remained constant throughout the study. This effect could be due to the immunomodulatory and anti-inflammatory effects of small increases in cortisol in highly-trained individuals under conditions of physical stress (Costello et al., 2018; Galbo, 1985; Pedersen, 1991). Furthermore, in the EG this effect may have been heightened by the PHL supplementation, producing a favourable effect on cortisol, as observed by González-Jurado et al. (2015) who reported that after an exercise session cortisol levels remained constant in an EG given PHL, but increased significantly in a group given a placebo ($p < .05$), a finding that coincides with our own results (González-Jurado, De Teresa, Molina, Guisado & Naranjo, 2009). However, García-Verazaluce et al. (2015) observed a significant decrease in cortisol in a group given PHL and in another given PHL plus Coenzyme Q_{10}, compared to a group given a placebo ($p < .05$ and $p < .001$, respectively).

Our main limitation was that the control group did not receive a placebo with some method of blinded or double-blinded shielding. In addition, we did not performing consecutive measurements and recovery kinetics of inflammatory variables, muscle damage and cortisol after the end of each microcycle. However, we consider that the post-exercise results obtained are the starting point for future research into the behaviour of recovery through PHL supplementation with similar characteristics to this study.

Conclusions

The results obtained in this study show that after a precompetitive training mesocycle, the participants who were given PHL supplementation presented a more favourable profile, in terms of reduced muscle damage, inflammatory modulation and cortisol response, than those in the control group, who were noticeably affected in these respects. For this reason, we conclude that the intake of PHL during a precompetitive mesocycle by elite-level male volleyball players protects against the muscle damage, inflammatory response and heightened presence of cortisol that would otherwise be produced by fatigue.

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Conflicts of interest

The authors report no conflict of interest.

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