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Original Article

Effect of two complex training protocols of back squats in blood indicators of muscular damage in military athletes

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Abstract. [Purpose] The aim of this study was to determine the variations in the blood muscular damage indicators post application of two complex training programs for back squats. [Subjects and Methods] Seven military athletes were the subjects of this study. The study had a quasi-experimental cross-over intra-subject design. Two complex training protocols were applied, and the variables to be measured were cortisol, metabolic creatine kinase, and total creatine kinase. For the statistical analysis, Student's t-test was used. [Results] Twenty-four hours post effort, a significant decrease in cortisol level was shown for both protocols; however, the metabolic creatine kinase and total creatine kinase levels showed a significant increase. [Conclusion] Both protocols lowered the indicator of main muscular damage in the blood supply (cortisol). This proved that the work weight did not generate significant muscular damage in the 24-hour post-exercise period.

Key words: Complex training, Muscular damage indicators, Post-activation potentiation

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INTRODUCTION

Currently, training methods help to increase sports performance, but they also generate variations in the indicators of muscular damage post effort[1–5)], which produces negative organic alterations in athletes. The foregoing is further evidenced when applying training protocols in subjects with a low training level; these subjects, higher muscular damage is produced when applying the same training load[6, 7)]. Therefore, in addition to evaluating the improvement in their physical capabilities, it is also necessary to analyze blood muscular damage and fatigue indexes, since it seems that most of the training methods used produced metabolic alterations increasing muscular damage post effort.

Testing general fatigue, local fatigue, and/or organic alterations through blood indicators such as creatin kinase and/or cortisol is an essential element of sports training[8)]. Several of the studies consulted for this study focused on the analysis of muscular damage indicators with protocols of constant resistance[9–12)] or intermittent sprint training[10, 13)]. Unfortunately, there is little evidence regarding protocols of complex training in power zones (from 0.6 m/s to 0.9 m/s for the vertical speed of the bar)[11, 14–16)].

Currently, modern training methods are being used to achieve post-activation potentiation (PAP) in athletes, and complex training protocols have had a leading role in several studies[15, 16)]. These training methods correspond to a wide spectrum of

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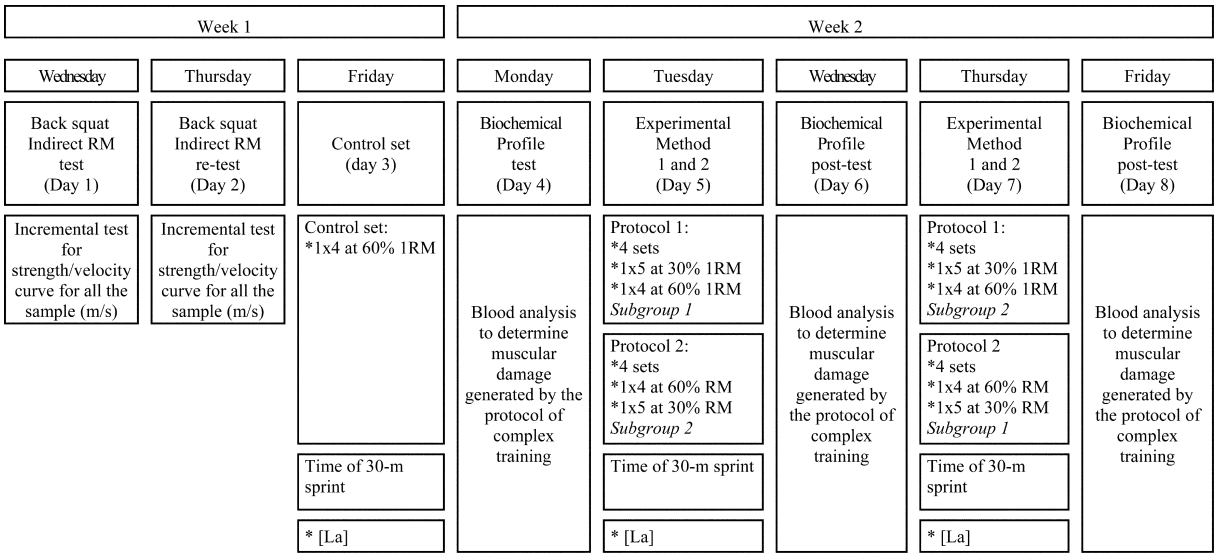

Fig. 1. Experimental design of complex training for back squats
RM: repetition maximum; [La]: concentrations of lactate

protocols varying the intensity during training sessions, but there are no studies on complex training protocols in power zones measuring the variations of blood muscular damage indicators post effort. Working in power zones would allow athletes to activate the central nervous system and the muscles involved, generating post-activation potentiation and a later increase in the sports performance in athletes[17].

Currently, the dynamics of blood muscular damage indicators in relation to complex training are unclear. However, investigation of the muscular damage that could potentially occur in muscles involved in back squats is a viable means of determining appropriate methodologies for sports training.

The main focus of this study was to determine the variations in blood indicators of muscular damage after the application of two complex training protocols for squats with military athletes. The second aim of this study was to determine the acute effect of two complex training protocols for back squats on time taken in 30-m sprints.

SUBJECTS AND METHODS

Developing interventions with elite athletes is a privilege only a few researchers have experienced, but it entails some methodological issues. It is known that elite athletes represent a very small sample; fortunately, there are experimental designs that provide a solution for reduced and specific samples. Using the same subjects for the control and experimental conditions is one of the most common strategies that enables researchers to increase the number of participants in investigations on humans. Seven male athletes participated in this study. They were subjected to two protocols of complex training with back squats. Specific blood muscular damage indexes were evaluated, before and after the application of the complex training protocols, for every subject. A quasi-experimental crossover intra-subject design with pre-test and post-test evaluations was used for application of the protocols.

Before beginning of the study, the weight, size, and skinfolds of each subject were measured. The subjects were required to restrain from ingesting caffeine, drugs, or any substance that could increase their metabolism during the course of the experiment. After the preliminary assessment of blood muscular damage indexes (baseline), the group of seven athletes was subdivided into two. During the first experimental session, *subgroup 1* received an intervention consisting of Protocol 1 (P1) and *subgroup 2* received an intervention consisting of Protocol 2 (P2); in the second experimental session, *subgroup 1* received an intervention consisting P2 and *subgroup 2* received an intervention consisting P1. Analyses of blood indexes for muscular damage were carried out 24 hours after the application of the complex training protocols (Fig. 1).

Seven male military athletes from the Chilean Navy (age, 25.0 ± 2.6 years; weight, 67.1 ± 2.0 kg; height, 172.7 ± 3.6 cm; body mass index, 22.5 ± 1.0 kg/m^2; body fat percentage, 12.0 ± 2.6%) were enrolled in the study (Table 1). Each athlete and their coach was informed about the aim of the study and the possible risks of the experiment, and they all signed an informed consent form before the application of the protocols. The consent form and the study were approved by the Human Research Ethics Committee of the Universidad de Granada, Spain (registration no. 933).

For sample characterization, weight and height were measured with a Health o meter® Professional scale and stadiometer. Skinfolds were measured with a F.A.G.A.® caliper. Using the methods Durnin & Womersley[18], the skinfolds of the biceps,

Table 1. Characteristics of the sample (mean ± SD)

	Experimental group (n=7)
Age (years)	25.0 ± 2.6
Height (cm)	172.7 ± 3.6
Weight (kg)	67.1 ± 2.0
BMI (kg/m^2)	22.5 ± 1.0
Fat percentage	12.0 ± 2.6

BMI: body mass index; SD: standard deviation

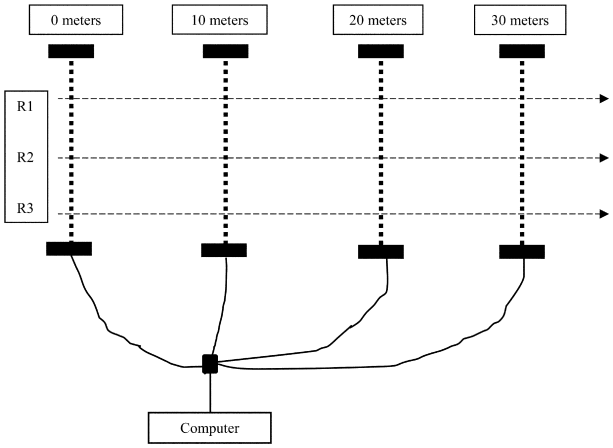

Fig. 2. Sequence of 30-m sprint in the experimental protocol
R1: first 30-m sprint; R2: second 30-m sprint; R3: third 30-m sprint; ▮---▮, photocell.

triceps, subscapularis, and supraspinatus were measured to determine the fat percentage.

Standard warm-up: For evaluation of one repetition maximum (1 RM), the control series, and the complex training protocols, the standard warm-up consisted of a 10-minute jog. During the first 5 minutes, subject performed a low-intensity run, and during the subsequent 5 minutes, they added some ballistic movements to the lower limbs (hip adductions, abductions, flexion and extension, and knee and ankle flexion and extension).

Blood tests: Before any blood analyses were carried out, there was a 48-hour washout period. Every single analysis was performed under fasting conditions. To quantify variations in the blood indexes of muscular damage, three measurements were used, with the first being the baseline. After this, the group of seven subjects was divided into two random groups (*subgroup 1, n=4; subgroup 2, n=3*). Subgroup 1 received the intervention consisting of P1, while subgroup 2 received the intervention consisting of P2; 24 hours after the application of these protocols, the blood analysis was performed again. On the second day of application of the protocols, subgroup 1 received the intervention consisting of P2, while subgroup 2 received the intervention consisting of P1; 24 hours after the application of these protocols, the third blood analysis was performed (Fig. 1).

The muscular damage indicators measured before and after application of the two complex training programs were cortisol, metabolic creatine kinase (CK-MB), and creatine kinase total (CK-Total). The blood analysis for CK-MB and CK-Total ware performed by enzymatic method. For cortisol, a Chemiluminescent Microparticle Immunoassay was used. Every measurement and analysis was carried out at the Naval Hospital of the Chilean Navy.

Complex training: Prior to application of the complex training protocols, the baseline values for maximum strength of the lower limbs were assessed through performance of squats and speed during a 30-m sprint. For evaluation of 1RM during Squats, a Chronojump Linear Encoder® and the Chronojump software, Version 1.4.6.0®, were used. The 1RM evaluation was performed indirectly through the formula proposed by Sánchez-Medina et al[19]. Twenty-four hours after the assessment of 1RM, a control set was performed that consisted of 4 sets of 1RM 60% squats for the purpose of verifying the vertical speed of the bar for every athlete Bautista et al[20]. Additionally, 30-m sprints were evaluated using a Chronojump Photo Cell® and the Chronojump software, Version 1.4.6.0® (Fig. 2).

The study included two protocols of complex training. The first was defined as P1 and consisted of 4 sets of 5 repetitions at 1RM 30% + 4 repetitions at 1RM 60% + 3 30-m sprints separated by 120 seconds. The second protocol was defined as P2 and consisted of 4 sets of 4 repetitions at 1RM 60% + 5 repetitions at 1RM 30% + 3 30-m sprints separated by 120 seconds (Fig. 3). For the statistical analysis, the best time of the three sprints per set was used (t min).

Data were analyzed as follows and in this order: the cortisol, CK-MB, and CK-Total levels and minimum time required for the 30-m were subjected to the Kolmogorov-Smirnov (K-S) test of normality for both P1 and P2. To compare the behavior of the blood indicators of muscular damage before and after the application of the complex training protocols, Student's t-test was applied. The baseline values were compared with those P1 and those P2 using Student's t-test. The magnitude of the effect for this analysis was calculated using Cohen's d test with the following effect scale: insignificant (d<0.2), small (d=0.2 up to 0.6), moderate (d=0.6 to 1.2), large (d=1.2 to 2.0), and very large (d>2.0).

In order to determine the differences between the control set and the four experimental sets of the complex training protocols, *repeated measures ANOVA* was used. The extent of the effect for this analysis was calculated using the partial eta-squared test. The level of significance for statistical analysis was $p<0.05$. Analysis of the data was carried out using the GraphPad InStat software, Version 3.05.

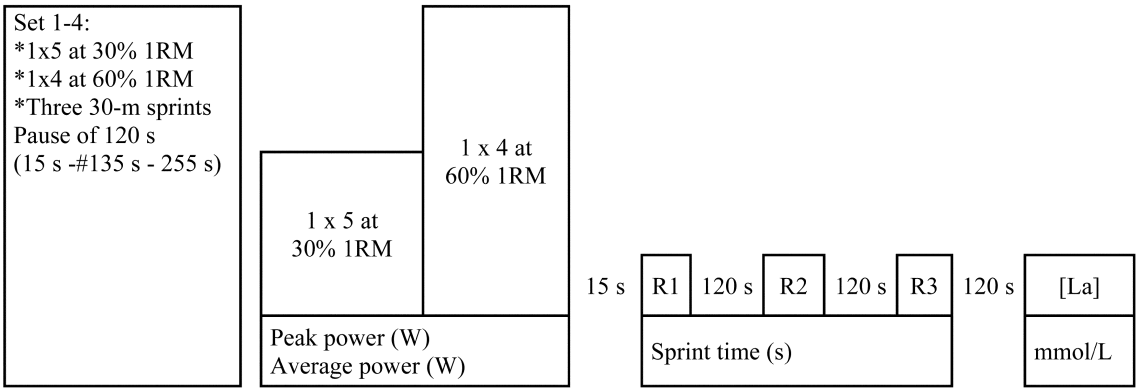

Fig. 3a. Design of experimental protocol 1 with complex training
1RM: 1 Repetition Maximum; 30-m: 30 meters Sprints; R1: first 30-m sprint; R2: second 30-m sprint; R3: third 30-m sprint; [La]: concentration of lactate; mmol/L: milimols per liter

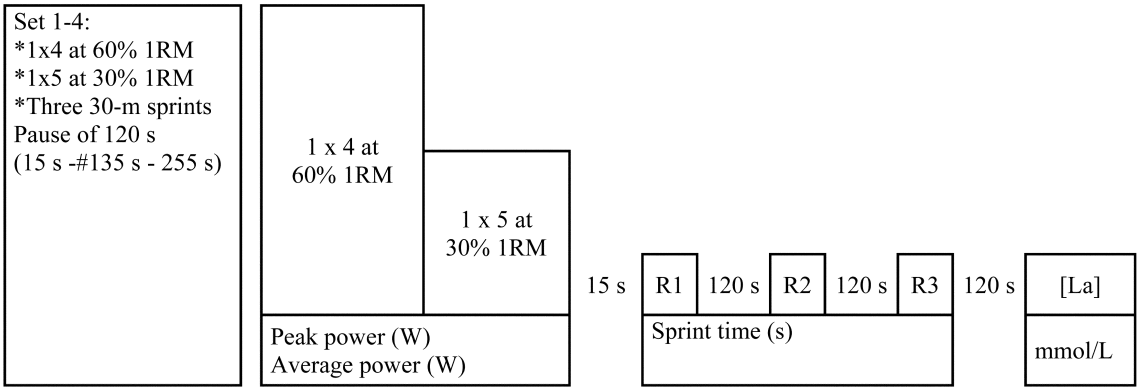

Fig. 3b. Design of experimental protocol 2 with complex training
1RM: 1 Repetition Maximum; 30-m: 30 meters Sprints; R1: first 30-m sprint; R2: second 30-m sprint; R3: third 30-m sprint; [La]: concentration of lactate; mmol/L: milimols per liter

RESULTS

Cortisol concentrations showed a significant decrease 24 hours after application of both protocols (P1, p=0.03; P2, p=0.02); progressions and changes are described in Table 2. The levels of CK-MB did not show significant in subjects who received changes P1 (p=0.12), but significant increases were observed 24 hours post effort in subjects who received P2 (p=0.002). Furthermore, both protocols resulted in significant increases in CK-Total concentrations (P1, p=0.01; P2, p=0.004). Progressions and changes are described in Table 2.

For both protocols, t min showed significant improvements between the control set and the four experimental sets (P1, p=0.0001; P2, p=0.0002); the t min results for the 30-m sprint during the control set and four experimental sets for P1 and P2 are displayed in Table 3.

DISCUSSION

Regarding the main goal of this study, the results of the Student's t-test revealed a significant decrease in the concentrations of cortisol 24 hours after application of both protocols of complex Training (P1, p=0.03; P2, p=0.02). Similarly, the blood levels of CK-MB and CK-Total showed significant increases with both protocols. In most of the literature consulted, the protocols used evidenced increases in the concentrations of CK[21–23] and cortisol post effort[6, 24], muscular fatigue as shown by electromyography[25], muscular damage post effort[1], and significant increases in the insulin-like growth factor I and IGF binding protein 3 ($p<0.05$)[4].

Regarding the second aim of the study, despite the fact that the levels of CK-MB and CK-Total increased considerably post effort, the results of ANOVA revealed a positive acute effect on t min for the 30-m sprint when applying complex train-

Table 2. Muscular damage indicators (mean ± SD) before and after the two protocols of complex training

Variables	Baseline	P1	P2
CK-MB (U/L)	20.7 ± 3.7	23.2 ± 6.4	24.1 ± 4.4^{++}
CK-Total (U/L)	145.7 ± 37.5	312.0 ± 137.2*	301.1 ± 96.3^{++}
Cortisol (µg/dl)	13.5 ± 2.8	11.0 ± 2.5*	11.6 ± 2.2^{+}

P1: experimental protocol 1; P2: experimental protocol 2; CK-MB: metabolic creatine kinase; CK-Total: total creatine kinase; U/L: units per liter; SD: standard deviation. *p<0.05 between baseline and P1; **p<0.01 between baseline and P1; $^{+}$p<0.05 between baseline and P2; $^{++}$p<0.01 between baseline and P2

Table 3. Post-activation potentiation (PAP) in the control set and four experimental sets (mean ± SD)

Variables	Control set	Set 1	Set 2	Set 3	Set 4
	Means ± SD	Means ± SD	Means ± SD	Means ± SD	Means ± SD
Time of 30-m sprint (s) p1**	4.57 ± 0.23	4.22 ± 0.20	4.27 ± 0.20	4.23 ± 0.23	4.23 ± 0.21
Time of 30-m sprint (s) p2**	4.57 ± 0.23	4.26 ± 0.17	4.28 ± 0.17	4.22 ± 0.16	4.22 ± 0.10

P1: experimental protocol 1; P2: experimental protocol 2; SD: standard deviation; ** p<0.001

ing, both for P1 (p<0.0001) and P2 (p<0.0002). Such effect was attributed to the PAP of the muscles involved in sprinting; this is supported by some studies that have shown significant differences in sprint times and/or velocities[16, 26, 27].

In conclusion, when applying either of the two complex training protocols, the blood levels of CK-MB and CK-Total increased considerably, though this did not affect the improvements in performance, as the results showed improvement in the 30-m test; the reductions in 30-m sprint time in the control set and the 4 experimental sets for P1 and P2 were attributed to PAP of the musculature involved in sprinting. In terms of cortisol levels, the levels dropped significantly 24 hours after application of both protocols; this decrease confirmed that the subjects were in an anabolic phase 24 hours post effort. Therefore, application of P1 and/or P2 seems to be a valid way of developing explosive strength in the lower limbs without producing negative alterations in athletes.

From a practical point of view, applying a protocol of complex training with four sets of back squats with 5 repetitions at 1RM 30% + 4 repetitions at 1RM 60% or four sets of 4 repetitions at 1RM 60% + 5 repetitions at 1RM 30% appears to be a good measure to achieve an increase in the levels of explosive strength, since training with this load generates PAP in the musculature involved in sprinting. Also, 24 hours post effort, subjects did not showed alterations of cortisol (major blood index for muscular damage), which indicates to coaches that complex training does not cause overtraining syndrome in athletes.

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