Salivary Hormone and Immune Responses to Three Resistance Exercise Schemes in Elite Female Athletes

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Abstract

Nunes, JA, Crewther, BT, Ugrinowitsch, C, Tricoli, V, Viveiros, L, de Rose Jr, D, and Aoki, MS. Salivary hormone and immune responses to three resistance exercise schemes in elite female athletes. *J Strength Cond Res* 25(8): 2322–2327, 2011—This study examined the salivary hormone and immune responses of elite female athletes to 3 different resistance exercise schemes. Fourteen female basketball players each performed an endurance scheme (ES—4 sets of 12 reps, 60% of 1 repetition maximum (1RM) load, 1-minute rest periods), a strength-hypertrophy scheme (SHS—1 set of 5RM, 1 set of 4RM, 1 set of 3RM, 1 set of 2RM, and 1set of 1RM with 3-minute rest periods, followed by 3 sets of 10RM with 2-minute rest periods) and a power scheme (PS—3 sets of 10 reps, 50% 1RM load, 3-minute rest periods) using the same exercises (bench press, squat, and biceps curl). Saliva samples were collected at 07:30 hours, pre-exercise (Pre) at 09:30 hours, postexercise (Post), and at 17:30 hours. Matching samples were also taken on a nonexercising control day. The samples were analyzed for testosterone, cortisol (C), and immunoglobulin A concentrations. The total volume of load lifted differed among the 3 schemes (SHS > ES > PS, $p < 0.05$). Postexercise C concentrations increased after all schemes, compared to control values ($p < 0.05$). In the SHS, the postexercise C response was also greater than pre-exercise data ($p < 0.05$). The current findings confirm that high-volume resistance exercise schemes can stimulate greater C secretion because of higher metabolic demand. In terms of practical applications, acute changes in C may be used to evaluate the metabolic demands of different resistance exercise schemes, or as a tool for monitoring training strain.

Key Words testosterone, cortisol, stress, IgA, saliva, load

Introduction

Resistance training is widely recommended to improve a range of motor abilities (24,31). These improvements can be achieved through both morphological (e.g., muscle size) and neural (e.g., motor unit synchronization) adaptations (15,17), and mediated, in part, by workout design. For instance, workout schemes using very heavy loads, low repetitions, and a moderate number of sets per exercise are often prescribed to maximize strength. Workouts employing lighter loads, a higher repetition range and explosive movements are often recommended for power development. To improve endurance, schemes that use lighter loads, high repetitions, and high volume are common.

Steroid hormones play a key role in modulating the training response of the neuromuscular system. For example, the anabolic effects of testosterone (T) and the catabolic effects of cortisol (C) help to control muscle growth and performance (6). Numerous studies have examined the T and C responses of men (athletic and nonathletic) to different workout schemes (1,5,12,16,20,22,29,32,35). However, little is known about the T and C responses of elite female athletes and the influence of different workouts (16,20). Such an analysis is important because resistance training is now widely employed by female athletes to improve neuromuscular performance.

Training strain is recognized as a potent stimulator for stress hormone (e.g., glucocorticoids and catecholamines) secretion (13,18). These hormones may themselves have deleterious effects on immune function including reduced natural killer cell activity, lymphocyte populations, lymphocyte proliferation, and antibody production (34). Immunoglobulin A (IgA) has been used to monitor immune function in women after resistance exercise (26,27) and other exercise forms (2,9,19,28). We are unaware of any studies that have examined the IgA responses of elite female athletes across different workout schemes. These data could provide additional information on the acute stressors imposed on the neuromuscular system by different training methods.
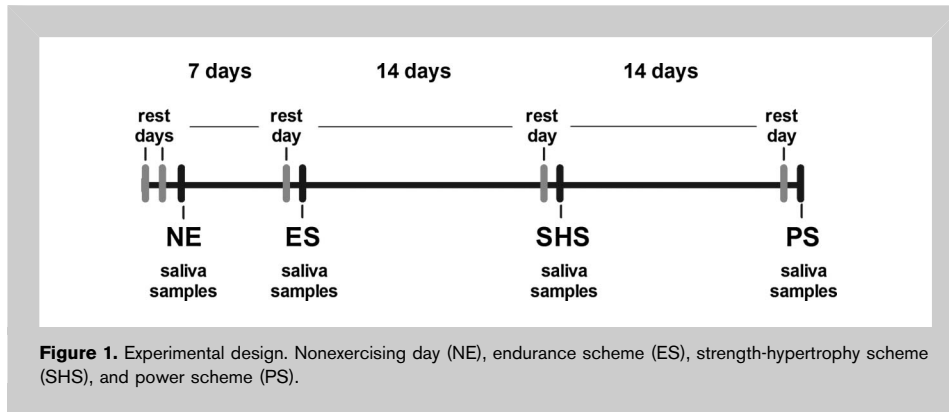
Figure 1. Experimental design. Nonexercising day (NE), endurance scheme (ES), strength-hypertrophy scheme (SHS), and power scheme (PS).

26.2 ± 3.9 years, 183.1 ± 9.8 cm, and 74.5 ± 10.1 kg, respectively. Each player was involved in a training squad for the Brazilian National Team but played in major national leagues in different countries. Each participant had at least 5 years of high level-training experience and were currently performing 10–12 training sessions per week, consisting of strength and power conditioning, skill and team work, speed, and anaerobic fitness. Each subject was screened for musculoskeletal, neurological, and shoulder and elbow joint problems. Subjects were informed of the experimental risks and signed an informed consent form before the investigation. Because of methodological limitations, we did not control for the menstrual cycle phase or oral contraceptive usage (11). The investigation was approved by an Institutional Review Board for use of human subjects.

This study examined the salivary T, C, and IgA responses of elite female athletes to 3 different workout schemes. Based on previous research (5,29,35), it was hypothesized that the total volume of load lifted across each scheme would be an important regulator of the acute hormonal and immune responses.

METHODS

Experimental Approach to the Problem

An experimental study with a crossover design was used to examine the salivary hormone and immune responses of elite female athletes across 3 workout schemes. Participants attended 4 sessions over a period of 40 days. In the first session, saliva samples were taken across a nonexercising control day (NE). In the 3 remaining sessions, saliva sampling was repeated, while participants performed an endurance scheme (ES), a strength-hypertrophy scheme (SHS), and a power scheme (PS). The saliva samples were analyzed for T, C, and IgA concentrations. The experimental design is shown in Figure 1.

Subjects

Fourteen elite female basketball players volunteered for this study. The mean (±SD) age, height and body mass of participants were
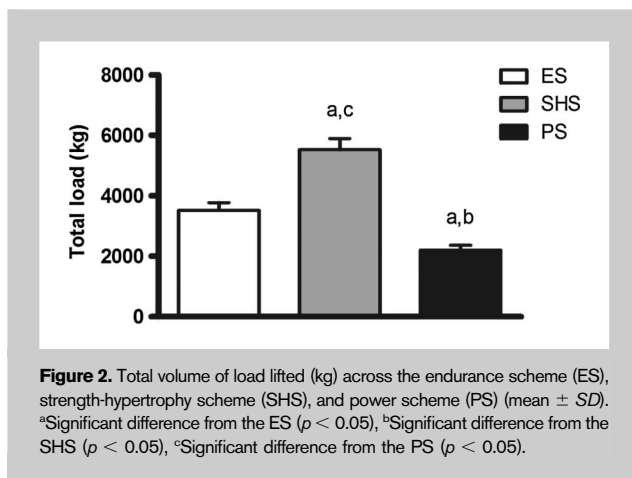

Figure 2. Total volume of load lifted (kg) across the endurance scheme (ES), strength-hypertrophy scheme (SHS), and power scheme (PS) (mean ± SD). [a]Significant difference from the ES ($p < 0.05$), [b]Significant difference from the SHS ($p < 0.05$), [c]Significant difference from the PS ($p < 0.05$).
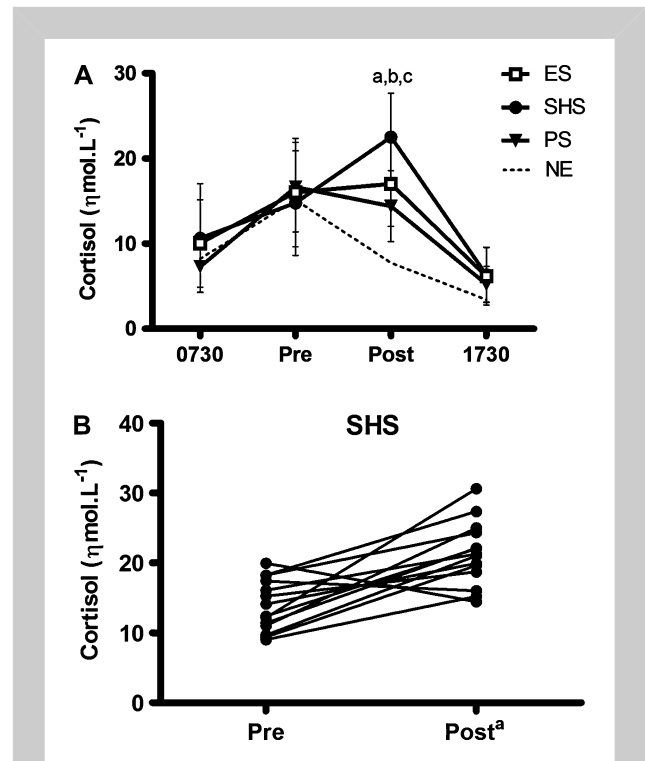

Figure 3. A, Salivary cortisol (ηmol·L^{-1}) concentrations across the endurance scheme (ES), strength–hypertrophy scheme (SHS), power scheme (PS), and nonexercising day (NE) (mean ± SD). [a]SHS different from pre-exercise $p < 0.05$. [b]All schemes different from nonexercising day (NE) ($p < 0.05$). B, Individual salivary cortisol (ηmol·L^{-1}) concentrations in the strength-hypertrophy scheme (SHS) (mean ± SD). [a]Different from pre-exercise ($p < 0.05$).

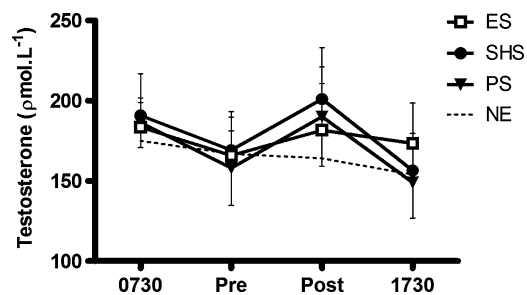**Figure 4.** Salivary testosterone ($pmol·L^{-1}$) concentrations across the endurance scheme (ES), strength–hypertrophy scheme (SHS), power scheme (PS), and nonexercising day (NE) (mean ± *SD*).

Workout Design

The design of each workout was based on previous recommendations (15) and adapted to meet the specific training requirements of the study population. The ES consisted of 4 sets of 12 reps using a 60% 1 repetition maximum (1RM) load and 1-minute rest periods between sets and exercises. The SHS comprised 5 sets using 1–5RM loads performed in a pyramid scheme (1 set of 5RM, 1 set of 4RM, 1 set of 3RM, 1 set of 2RM, and 1set of 1RM) with 3-minute rest periods, followed by 3 sets using a 10RM load with 2-minute rest periods. The PS consisted of 3 sets of 10 reps (maximal velocity) using a 50% 1RM load and 3-minute rest periods between sets and exercises. For consistency, the same exercises (i.e., bench press, squat and biceps curl) were performed for each workout. The total volume of load lifted across each workout scheme was calculated as follows: total repetitions × total sets × load intensity (21). Before each session, a standard warm-up was performed comprising light aerobic exercise, stretching, and submaximal lifts with each exercise.

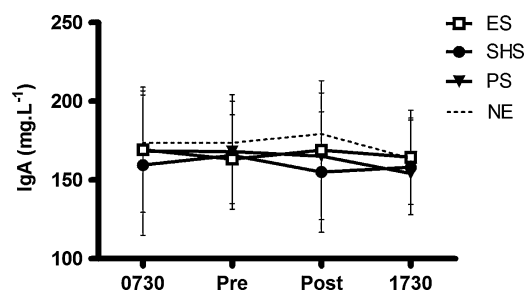The 3 workouts were performed in a consecutive order, each separated by 14 days, which was unavoidable because of the prior scheduling of the training camp. The likelihood of order effects, along with any training-induced changes, was mitigated by the advanced training background of subjects and their familiarity with the testing exercises and procedures. Each workout began at the same time of the day (09:30 hours) to account for diurnal variation and was preceded by 1 day of complete rest. The lifting techniques employed in this study have been described in more detail elsewhere (7,20,22). Briefly, the exercises in the ES and the SHS were performed using controlled eccentric and concentric movements, whereas the PS performed the exercises using controlled eccentric movements followed by explosive concentric movements.

Participants were instructed to maintain their normal dietary intake 1 day before, and on, each day of testing. Dietary intake was prescribed and monitored by a qualified nutritionist to address the specific individual needs of each player during the training camp. Thus, we were able to standardize nutritional intake on an individual level. During each workout, participants were allowed to drink water ad libitum, but no additional food or supplements were taken. Player hydration levels were also monitored by the nutritionist during the training camp. Participants kept training logs to monitor nutritional intake, hydration, and training loads across the experimental period.

Sample Collection

On the nonexercise day (NE), saliva samples were collected from participants at 07:30, 09:30, 11:00, and 17:30 hours. The NE samples were performed after 2 days of complete rest. On the testing days, saliva samples were collected from participants at 07:30 hours, pre-exercise (Pre) at 09:30 hours, postexercise (Post, 15 minutes), with a final sample taken at 17:30 hours. The first sample on each day was collected before a standardized breakfast. The saliva samples were collected in sterile containers and stored at $-80°$ C before assay. Salivary T, C, and IgA were assayed using commercial diagnostic kits (Salivary Testosterone enzyme immunoassay (EIA) kit, Salimetrics©; Salivary Cortisol EIA kit, DSL©, Salivary Secretory IgA EIA, Salimetrics©) and the kit instructions. The interassay coefficients of variation for the T, C, and IgA assays were 3.7 and 6.9%, 2.5 and 7.8%, 4.2 and 9.1%, respectively, based on high and low control samples in each kit. The samples for each participant were analyzed in the same assay to avoid interassay variance. The steroid hormones measured in saliva represent the blood-free portion (6).

Statistical Analyses

Standard statistical methods were used for calculating means (±*SD*) for the hormonal and immune variables. Before analysis, each data set was assessed for normality using the Kruskal–Wallis test and visual checks for kurtosis and skewness. Within-group changes and between-group differences in the hormonal and immune variables were assessed using a 2-way (scheme × time) analysis of variance with repeated measurements and Tukey's post hoc analysis. The criterion level for significance was set at $p \leq 0.05$.

Figure 5. Salivary immunoglobulin A (IgA) ($mg·L^{-1}$) concentrations across the endurance scheme (ES), strength-hypertrophy scheme (SHS), power scheme (PS), and nonexercising day (NE) (mean ± *SD*).

RESULTS

Significant differences in total load volume were noted between the 3 workout schemes (Figure 2). Total volume of load lifted across the SHS was greater than in the ES and PS ($p < 0.05$), with the load lifted in the ES also found to be greater than in the PS ($p < 0.05$).

The salivary C responses to each scheme and control data are plotted in Figure 3. Postexercise C concentrations increased in the SHS, ES, and PS, compared with control values (NE) ($p < 0.05$; Figure 3A). In the SHS, postexercise C concentrations were also elevated from pre-exercise data ($p < 0.05$; Figure 3B). There was also a trend for a greater postexercise C response in the SHS, from that seen in the ES and PS ($p < 0.08$).

Figure 4 shows the salivary T responses to each workout scheme and control data. There were no significant changes in salivary T concentrations across the ES, SHS, or PS, when compared to pre-exercise values and corresponding non-exercising data (NE) ($p > 0.05$). Likewise, there were no scheme differences in T concentrations at any time point ($p > 0.05$).

Figure 5 shows the salivary IgA responses to each scheme and nonexercising data (NE). In response to the ES, SHS, and PS, we found no significant changes in salivary IgA concentrations from pre-exercise values or matching control data (NE) ($p > 0.05$). Likewise, no differences in IgA concentrations were observed across the 3 schemes at any time ($p > 0.05$).

DISCUSSION

The main findings of this study were as follows: (a) postexercise salivary C concentrations increased in all *3 workout schemes* compared to nonexercising data (NE), (b) postexercise salivary C concentrations were elevated in the SHS compared with pre-exercise values, and (c) there were no changes in the T and IgA responses to each scheme.

The 3 workout schemes all increased salivary C concentrations postexercise (vs. nonexercising data), with the SHS also producing an elevated response from pre-exercise and a trend toward a greater C response (vs. ES and PS). The latter finding may be explained by the total volume of load lifted on each protocol (SHS > ES > PS). This idea is supported by increases, or greater increases, in C across workouts characterized by high total volume of load lifted or work (5,12,22,23,29,35). For example, the salivary C concentrations of men were elevated after a hypertrophy scheme but not after a strength scheme and PS (5). These results reflected total volume of load lifted (as % of 1RM) across the hypertrophy (7,500% 1RM), strength (2,112% 1RM), and power (2,160% 1RM) schemes. Similarly, others have demonstrated *greater C* responses using 3 sets (vs. 1 set) per exercise (10,25). These findings confirm that high-volume workouts, most likely because of the greater metabolic demand, can enhance C secretion.

No changes in salivary T concentrations were observed after the 3 workout schemes. This finding is consistent with previous research (3,16,20) and suggests that female T is unresponsive to this type of exercise. Male T does respond to resistance exercise when using the same relative load as women do (16,20,33). One possible explanation lies in the greater T levels in men (10-fold > women) (6). Gender differences in muscle mass (men > women) (30) could also be important in stimulating a greater T response. A recent study reported an increase ($\sim$15%) in free T after 6 sets of 10 repetitions of squats in weight-trained women (33), but still less than that observed for men ($\sim$60%). Interpretation of these results is limited by the small number of men ($n = 8$) and women ($n = 7$) tested. Despite the general lack of T change, it appears that many other peptides and steroids (e.g., growth hormone, dehydroepiandrosterone, estradiol) do respond to resistance exercise in women (3,4,16,20). Thus, for women, these hormones might play a more prominent anabolic role during and after resistance exercise.

We found no changes in IgA concentrations after the 3 workout schemes. Recently, the immune responses of 2 schemes, 1 using a 50% 1RM load and another a 80% 1RM load, were compared in untrained older women (26). Both schemes produced similar increases in IgA, which could be explained by the fact that total volume of load lifted was equated between the training schemes. However, the applicability of these results to younger women is likely to be limited, especially to those whom are already highly adapted to physical training stimuli. Similar to our results, there were no changes in IgA concentrations after a strength workout in trained and untrained women (27). Furthermore, no changes or differences in immunoglobulin G and M were noted. It may be suggested that, for trained women, a greater training stimulus is needed to elicit a response in these salivary immune parameters compared with older untrained women.

Immunoglobulin A flow rate and secretion rate, circadian variations, and current immunity levels are all possible factors contributing to the IgA responses to exercise (2,9,19,28). To complicate matters, salivary IgA is only indicative of acute immunosuppression. Resistance exercise has also been shown to acutely modify other markers of the immune function (e.g., white blood cells, T-cells, leukocytes, and lymphocytes) (8,26,27), some of which occurred in the absence of any changes in IgA. It is also noteworthy that women possess higher glucocorticoid receptor protein content than do men (33), despite producing similar C responses to exercise and competition (14,23), which adds to the complex neuro–endocrine–immunological regulatory system responding to different stressors in the athletic environment.

We acknowledge that the menstrual cycle can influence the assessment and interpretation of female hormonal data during exercise studies. However, it is difficult to match or coordinate the same cycles, along with contraceptive use, in a group of elite female athletes. The fact that the workout schemes were performed in a consecutive, rather than a randomized, order presents another problem with interpretation. The confounding effects of other variables (e.g., concurrent training, periodization, psychological factors) are also noted, but these

issues do represent the actual training environment and the inherent limitations with performing research on elite athletes.

In conclusion, *the current* findings confirm that high-volume resistance exercise schemes can stimulate greater C secretion because of higher metabolic demand. *Conversely*, there were no changes in salivary T and IgA concentrations to any workout scheme.

PRACTICAL APPLICATIONS

The results of this study confirm the importance of the total volume of load lifted (repetitions × sets × load) as a regulator of the acute C responses to resistance exercise. These findings have implications for the use of C, for example, monitoring or testing the metabolic demands of different resistance-exercise schemes in elite female athletes. Our results also suggest that high-volume schemes might induce greater training strain and, if performed for a long period of time, could result in a state of nonfunctional overreaching. Therefore, sports coaches may use C as a tool for monitoring the amount of training strain imposed by different resistance exercise schemes.

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