Salivary Testosterone and Cortisol Responses in Professional Rugby Players After Four Resistance Exercise Protocols

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Abstract

The acute response of free salivary testosterone (T) and cortisol (C) concentrations to four resistance exercise (RE) protocols in 23 elite men rugby players was investigated. We hypothesized that hormonal responses would differ among individuals after four distinct RE protocols: four sets of 10 repetitions (reps) at 70% of 1 repetition maximum (1RM) with 2 minutes' rest between sets (4 × 10–70%); three sets of five reps at 85% 1RM with 3 minutes' rest (3 × 5–85%); five sets of 15 reps at 55% 1RM with 1 minute's rest (5 × 15–55%); and three sets of five reps at 40% 1RM with 3 minutes' rest (3 × 5–40%). Each athlete completed each of the four RE protocols in a random order on separate days. T and C concentrations were measured before exercise (PRE), immediately after exercise (POST), and 30 minutes post exercise (30 POST). Each protocol consisted of four exercises: bench press, leg press, seated row, and squats. Pooled T data did not change as a result of RE, whereas C declined significantly. Individual athletes differed in their T response to each of the protocols, a difference that was masked when examining the pooled group data. When individual data were retrospectively tabulated according to the protocol in which each athlete showed the highest T response, a significant protocol-dependent T increase for all individuals was revealed. Therefore, RE induced significant individual, protocol-dependent hormonal changes lasting up to 30 minutes after exercise. These individual responses may have important ramifications for modulating adaptation to RE and could explain the variability often observed in studies of hormonal response to RE.

Key Words hypertrophy, maximal strength, strength endurance, power, endocrine response

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Introduction

Strength, lean mass, and power are critical components of a wide range of athletic pursuits (17), including professional rugby. As such, resistance exercise (RE) is regarded as an important part of an athlete's training schedule. Various RE protocols are reported to differentially improve components of the overall neuromuscular system, including maximal strength, muscular hypertrophy, strength endurance, and power (7).

RE has been shown to elicit acute hormonal responses in athletes (1,10,20), and at a fundamental level, it is thought that hormonal changes are necessary for muscular adaptation. Acute increases in insulin, growth hormone, and testosterone (T) concentration, for example, increase nutrient partitioning into tissue and protein synthesis (13,30), whereas increases in cortisol (C) concentration promote energy mobilization through tissue catabolism (9). The balance and timing of anabolic versus catabolic factors and the availability of dietary protein are considered essential to at least one aspect of muscle adaptation, namely muscle growth (25). It is accepted that specific loading parameters of RE influence the observed pattern of endocrine response (22). For example, Kraemer and colleagues (21) reported that protocol-specific, acute serum T increases were observed after hypertrophy and strength protocols. However, the magnitude and direction of anabolic responses have been reported to vary even in response to similar RE protocols among trained men (11,20,21,32). Furthermore, researchers have previously suggested that the T response to training may affect individual adaptation (2,16) and the ability to elicit a hypertrophic response (19).

T has been shown to increase the size and maximal voluntary strength of muscle, and it may improve the explosive power of muscle (3,4). Indeed, compelling data have demonstrated that pharmacologic blockade of T-specific receptors suppresses exercise-induced hypertrophy of skeletal muscle (14). Singh et al. (31) have also shown that T promotes the differentiation of pluripotent stem cells toward the myogenic lineage through an androgen receptor-dependent pathway. Furthermore, recent data showed that T could produce rapid intracellular calcium (Ca^{2+}) increases

and oscillations in skeletal muscle cells that could influence muscle cell hypertrophy and fiber-type transformations via a nongenomic pathway that is independent of muscle protein turnover (15).

Salivary sampling for analysis of hormones has advantages compared with blood sampling as it is a low stress, non-invasive method. Salivary hormone levels also reflect the free plasma concentration and bioactive component of steroid hormones (21,34), which is of primary importance as it is the biologically active fraction of T that is available to interact with androgen receptors (22). Despite these advantages, salivary measurement of biologically available levels of T and C has been largely neglected in the sports science field.

Few studies have examined and compared acute hormonal responses with commonly prescribed RE protocols in rugby players. Rugby players are a group of diverse athletes with very different skill set requirements, yet with quite similar training backgrounds. Therefore, the purpose of the current study was to examine the acute effects of four distinct RE protocols on salivary T and C concentrations in elite men rugby players.

Methods

Experimental Approach to the Problem

Four distinct RE protocols were superimposed on the normal training regimens of the athletes and completed in random order at least 2 days apart. Each athlete completed all four RE protocols. Saliva samples were obtained before (PRE), immediately after exercise (POST), and 30 minutes after exercise (30 POST). The hormonal response is defined as the difference between the PRE and POST samples; i.e., a decrease in T concentration would be represented by a negative response. The protocols were chosen as they reflect commonly prescribed RE protocols that span the spectrum of variables recognized as influencing hormonal response to exercise (24). Kraemer et al. (18) previously demonstrated significant hormonal differences immediately after and 30 minutes post exercise when load was manipulated. By replicating protocols from existing literature, it was also possible to compare and contrast existing serum data with salivary data.

Athletes performed the RE protocols in the morning at the stadium gymnasium where they were accustomed to training. Before the first protocol, individuals were instructed to replicate their presession behavior when returning to each of their subsequent protocols (e.g., sleep, exercise, and diet). Diet and sleep behaviors were recorded via questionnaire, and if the athlete did not adhere to these restrictions, the session was rescheduled. The four protocols were performed at the same time of day for each individual to avoid the effects of circadian rhythm on hormonal concentrations. Athletes were instructed to avoid hot drinks and hard foods (e.g., apples) for approximately 30 minutes before testing to minimize any risk of blood contamination in saliva.

Subjects

Twenty-three elite men rugby players ([mean ± *SD*] age: 25 ± 3 years; height 184.5 ± 9.0 cm; weight: 99.2 ± 10.1 kg; sum of eight skinfolds: 87.7 ± 22.4 mm; bench press one repetition maximum [1RM]: 131.5 ± 12.3 kg; 40-m sprint time: 5.23 ± 0.27 seconds) volunteered to participate in this study. Before the study, participants attended a presentation outlining the purpose and procedures involved. Written informed consent was provided, and ethical approval was obtained from the Waikato Institute of Technology Ethics Committee. Participants were well-trained professional athletes with at least 2 years of weight training experience (minimum of 15 hours' training per week). Of the 23 athletes, 15 completed all four protocols.

Procedures

Before the study, the body composition and maximal strength of the athletes were assessed. A level 3 International Society for the Advancement of Kinanthropometry anthropometrist performed the sum of eight skinfold assessments. Body weight was measured using free-standing electronic scales. The 1RM was determined for each exercise to allow calculation of loads using a previously reported method (36).

Sampling and Analysis

Saliva samples (approximately 5 mL) were collected by passive drool into a 10-mL graduated centrifuge tube (LBSCT1002; Labserve, Auckland, New Zealand). Samples were then stored at −20°C until assay. Athletes were seated for 10 minutes to allow equilibration before providing resting saliva samples (PRE). Saliva samples were also collected immediately after exercise (POST). The athletes then relaxed for 30 minutes before supplying a third saliva sample (30 POST). Athletes replicated their post-session behavior after each protocol. During the RE protocols and recovery period, the athletes were allowed water ad libitum.

Samples were analyzed in duplicate for T and C concentrations. Standard curves were constructed per the manufacturer's instructions, and additional internal standards were included. T results were obtained using an enzyme immunoassay kit (Salimetrics, State College, PA). The T test has a range of sensitivity from 3.70 to 360 $pg \cdot mL^{-1}$ and average intra- and interassay coefficients of variation (CVs) of 3.8% ± 2.6% and less than 10%, respectively. C results were also obtained via immunoassay (Salimetrics). The C test has a lower limit of sensitivity of 0.007 $ng \cdot mL^{-1}$ and average intra- and interassay CVs of 3.2% ± 2.4% and less than 10%, respectively. Assay plates were read using an Organon Teknika 230 S plate reader (Durham, NC).

Resistance Exercise Protocols

The four RE protocols were supervised, and each involved four exercises (bench press, leg press, seated row, and squats) that activated large muscle masses. Bench presses and squats were performed using free weights, whereas the leg press and seated row were performed using machines.

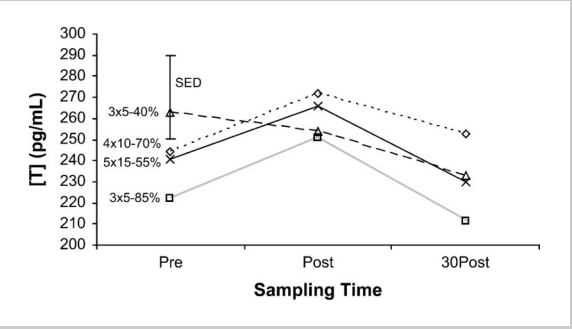

Figure 1. Pooled free salivary testosterone [T] for each protocol in professional athletes ($n = 23$) before (PRE), immediately after (POST), and 30 minutes after (30 POST) four resistance-training protocols (4 × 10–70%; 3 × 5–85%; 5 × 15–55%; 3 × 5–40%). Standard error of the differences (*SED*) is shown as an error bar. No significant differences were observed throughout the experimental period.

The four protocols were based on those used in previous studies (32,37). The 4 × 10–70% protocol consisted of four sets of 10 repetitions at 70% of each individual's 1RM. There was 2 minutes' rest between sets. The 3 × 5–85% protocol consisted of three sets of five repetitions at 85% 1RM with 3 minutes' rest between sets. The 5 × 15–55% protocol consisted of five sets of 15 repetitions at 55% 1RM with 1 minute's rest between sets. The 3 × 5–40% protocol consisted of three sets of five repetitions at 40% 1RM with 3 minutes' rest between sets. Athletes were instructed to perform the exercises in the 3 × 5–40% protocol with the intention of producing the greatest rate of force development possible. Each protocol lasted approximately 60 minutes.

Statistical Analyses

Log-transformed hormone data were analyzed using restricted maximal likelihood in GenStat Release 7 (Laws Agricultural Trust, Rothamsted Experimental Station). Particular comparisons were made using the pooled variation on the log scale to account for the high variance associated with hormonal data. Data are presented back-transformed to the original scale. Pooled data for each of the protocols included all athletes, even the eight that did not undertake all four protocols because of injuries or other commitments. The α level for significance was set at $P \leq 0.05$.

Results

Free T Concentration [T]

The mean pooled T concentration ([T]) measured before each RE protocol was not significantly different among protocols and ranged between 0.77 and 0.92 nmol·L^{-1} (222–263 pg·ml^{-1}). This is at the high end of the typical range for basal free T expected in men of this age (26). There were no statistically significant differences ($P > 0.05$) in pooled [T] PRE to POST RE protocols, and no differences were observed among the protocols (Figure 1). Pooled 30 POST [T] was within 5% of PRE values after 4 × 10–70%, 3 × 5–85%, and

Table 1. Individual absolute change in free salivary testosterone concentration [T] in professional athletes ($n = 15$).

	Absolute PRE to POST change in [T] (pg·mL^{-1})			
Subject	4 × 10–70%	3 × 5–85%	5 × 15–55%	3 × 5–40%
1	173	21	1	−220
7	214	26	−129	−129
10	246	−86	−299	−258
12	56	−69	−59	−33
Average	172.3 ± 83.1	−27.0 ± 58.8	−121.5 ± 129.7	−160.0 ± 100.5
2	−37	226	21	148
4	24	114	−170	42
5	63	144	−29	54
11	−5	108	35	36
15	32	144	137	−116
Average	15.4 ± 38.0	147.2 ± 47.1	−1.2 ± 112.0	32.8 ± 94.8
3	−93	33	106	−5
8	−19	173	206	71
9	10	53	104	30
13	259	41	853	160
Average	39.3 ± 152.8	75.0 ± 65.8	317.3 ± 360.3	64.0 ± 71.1
6	−219	−97	−49	314
14	−107	100	16	154
Average	−163.0 ± 79.2	1.5 ± 139.3	−16.5 ± 46.0	234.0 ± 113.1

Individuals were grouped according to the protocol in which the largest change in [T] was observed. PRE = before exercise; POST = immediately after exercise.

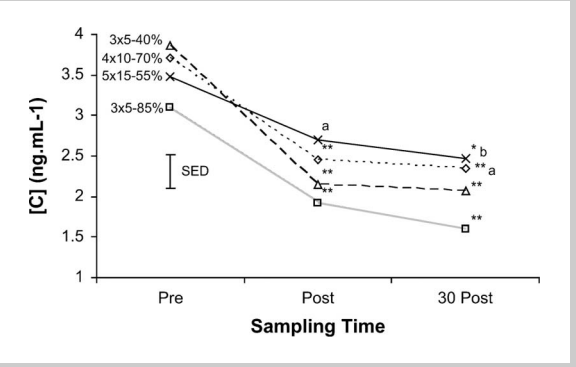

Figure 2. Pooled free salivary cortisol concentration [C] for each protocol in professional athletes ($n = 23$) before (PRE), immediately after (POST), and 30 minutes after (30 POST) four resistance-training protocols (4 × 10–70%; 3 × 5–85%; 5 × 15–55%; 3 × 5–40%). Standard error of the differences (*SED*) is shown as an error bar. $^{**}P < 0.01$ versus corresponding PRE value. $^{*}P < 0.05$ versus corresponding PRE value. $^{a}P < 0.05$ versus corresponding 3 × 5–85% value. $^{b}P < 0.01$ versus corresponding 3 × 5–85% value.

5 × 15–55% protocols but continued to decrease to less than baseline after 3 × 5–40% ($P > 0.05$) (Figure 1).

Nevertheless, data indicated individual-specific T responses to the protocols. Of the 23 athletes, 15 completed all four RE protocols. These subjects' data were retrospectively tabulated into subgroups according to the protocol in which they demonstrated the greatest absolute PRE to POST [T] increase (Table 1). Individuals were selected into these subgroups based on the premise that a maximal anabolic response was one of the aims of RE and provided an optimal environment for adaptation.

C Concentration [C]

The pooled mean C concentration ([C]) measured before each RE protocol was not significantly different among protocol groups and ranged from 8.53 to 10.65 nmol·L^{-1} (3.09–3.86 ng·ml^{-1}). These levels are typical of the age group studied in terms of non-stressed basal C levels (26). Significant PRE to POST [C] decreases ($P < 0.01$) were observed as a result of the 4 × 10–70%, 3 × 5–85%, and 3 × 5–40% protocols but not the 5 × 15–55% protocol (Figure 2). The pooled POST [C] was significantly different ($P < 0.05$) between the 5 × 15–55% and 3 × 5–85% protocols. Pooled 30POST [C] continued to decrease below POST [C] values in all protocols, and a significant difference was observed between the 5 × 15–55% and 3 × 5–85% ($P < 0.01$) and the 4 × 10–70% and 3 × 5–85% ($P < 0.05$) protocols (Figure 2).

Discussion

The present study identified large individual differences in T response to four distinct RE protocols. On four occasions, separated by at least 2 days, participants performed a different RE protocol (4 × 10–70%, 3 × 5–85%, 5 × 15–55%, or 3 × 5–40%). Hormone concentration was determined PRE, POST, and 30 POST exercise. The results of the present study indicate a trend toward an increase in [T] when individual data were pooled, although this increase was small and did not reach significance.

The 4 × 10–70% protocol used in the present study produced a nonsignificant acute T increase of 11.3% ± 19.7%, which is of a magnitude similar to that in previous studies that utilized similar RE protocols (20,32). Others have reported serum total T increases of up to 72% in trained men (10,18). Häkkinen and Pakarinen (11) reported increases of 23.8% and 22.4% in total and free serum T, respectively, in response to a hypertrophy protocol. By contrast, Bosco and colleagues (5) reported a decrease in total serum T in response to a similar hypertrophy-type protocol. A decrease in salivary T concentration has also been reported by Kraemer et al. (21) at the midpoint of a hypertrophy-type protocol that was significantly different from a control condition.

Our current study reports a nonsignificant acute T increase of 13.1% ± 19.9% after 3 × 5–85% exercise ($P > 0.05$), which is similar to the range reported by others (20,32). In terms of literature investigating serum total T responses to similar maximal strength-type exercise, increases of up to 28% have been observed in resistance-trained men (20). Others have shown no significant change when reporting pooled group data (32).

The current 5 × 15–55% protocol produced a nonsignificant acute T increase (10.5% ± 19.7%; $P > 0.05$). Using a similar protocol, Smilios et al. (32) reported larger acute serum T increases (approximately 21% ± 10%; $P < 0.05$). No significant acute T change (3.5% ± 19.7%; $P > 0.05$) was observed in the current study in response to the 3 × 5–40% protocol. This contrasts results of similar dynamic power protocols that reported small but significant serum T increases of approximately 15–30% in trained men (29,35).

Large variability in hormonal responses to RE, as indicated by large standard errors, was evident in our study. The sampling procedure or variability within the population studied may have contributed to this variation. Alternatively, the variability could be explained if individuals responded in a distinct manner to protocol variables. An important observation in this study was that, when an individual's hormonal response was compared among protocols, a pronounced T response to one or occasionally two protocols was evident. This contrasts the results observed in the pooled data.

The protocol considered optimal in terms of anabolic response (as defined by an absolute increase in bioavailable T concentration) differed among individuals. This is a unique observation as it offers one potential reason why studies have varied in terms of observed hormonal responses to RE. Indeed, Kraemer et al. (19) noted that differences in exercise-induced hormonal patterns seemed to affect the ability of training men to elicit a hypertrophic response. Alen et al. (2) observed a positive correlation between individual changes in free T and maximal isometric force and concluded that this emphasized the "importance of biologically active free

testosterone for trainability." Furthermore, Jensen et al. (16) suggested that interindividual differences in total serum response might affect individual adaptation to training despite pooled data revealing no significant increase in response to either strength or endurance training. These results suggest that pooling data can have an impact on both the validity of the results and the interpretation of study findings.

Our data identified four subgroups of specific protocol responders (4 × 10–70%: $n = 4$; 3 × 5–85%: $n = 5$; 5 × 15–55%: $n = 4$; 3 × 5–40%: $n = 2$). If this reflects differences at a population level, it would be remarkably easy to skew pooled results depending on subject homogeneity. It would seem that each individual needs to act as his or her own control among protocols and that pooled data require careful interpretation. Our protocols were not repeated sufficiently to validate the apparent individual nature of responses, but future studies should be designed to address this potential individuality.

In terms of unconjugated C concentrations, the results of the present study indicate significant decreases in pooled data as a result of the exercise protocols. In addition, the C response of the athletes to the four protocols showed significant differences. The present study reports a significant acute C decrease (44.3% ± 20.6%; $P < 0.01$) in response to the 3 × 5–40% protocol. In contrast, other authors have reported no change (35) in serum C levels when a similar exercise type was performed. The 3 × 5–85% protocol used in the current study produced a significant acute C decrease (38.2% ± 20.6%; $P < 0.01$). This is comparable to the results reported by Smilios et al. (32), who found serum C decreases of approximately 22%. Other authors have reported no significant serum C change immediately post exercise in trained men as a result of maximal strength-type protocols (12,28). We report a significant acute C decrease (33.6% ± 20.6 %; $P = 0.01$) as a result of the 4 × 10–70% protocol. This result contrasts most of reported serum C responses to similar hypertrophy-type protocols (e.g., Gothshalk et al. [10] approximately 17%; Kraemer et al. [23] approximately 50%; and Häkkinen and Pakarinen [11] approximately 149%). Finally, we report an acute decrease in C (22.2% ± 20.6%; $P = 0.096$) as a result of the 5 × 15–55% protocol. This contrasts the results of the four exercise, four sets, 15 repetitions, 60% 1RM, strength endurance protocol described by Smilios et al. (32), who reported a significant approximately 27% increase in serum C.

The observed [C] changes in our study consisted of uniform decreases among all protocols. [T] of the matching salivary sample either increased slightly or stayed constant. This suggests that saliva was not concentrated or diluted during the current experimental procedure. Although saliva is noninvasive and easy to collect, allowing less stressful compliance from subjects, various issues surround the extraction and analysis of saliva-obtained samples. The transference of the hormones and metabolites across biological membranes requires careful consideration. It has been reported that the lag time in partitioning between blood and saliva may not always be linear among stress states (6). Blood contamination and dilution of the saliva samples also pose difficulties. However, one study suggests that any significant blood contamination could be seen with the naked eye (24). Fluid intake in the oral cavity could temporarily lower sample content in a manner not easily controlled or correctable. Therefore, unusually low salivary hormone concentrations should be interpreted with care.

Our results are qualitatively similar to other studies utilizing direct blood parameters despite the use of salivary measures in our methodology. However, we observed some differences, in particular the magnitude and direction of C change. We contend that many parameters within hormonal studies, beyond the physical stimulus applied, need careful consideration with regard to interpretation. Indeed, humans express an anticipatory anxious response to stressful events, such as venopuncture, that has been shown to increase C levels (33). Therefore, the perception of the sampling technique imposed may confound stress responses to exercise when venopuncture is performed. By using the relatively stress-free and noninvasive salivary sampling method, the influence of any non–exercise-related C increase should be avoided.

Previous RE and other training experience must also be considered. Exercise-induced responses of untrained individuals, as indicated by Kraemer et al. (23), would be expected to vary from the responses of trained athletes. Elite strength athletes reportedly possess very limited abilities to increase their strength even during prolonged training periods (12). Because of the relatively high basal T reported in the current study, increases could seem more modest relative to a population in which basal levels were lower on average. Also, although the capacity to increase the free pool of T among varying populations may be similar, the stimuli needed to induce change may not. This may also play a role in the individuality of response that is suggested in this study.

Exercise as a stressor elicits typical hormonal profiles of response. These responses are known to be complex and modulated by psychological drive. In a competitive environment, these responses are not associated solely with a competition outcome, but also the perception of (or perceived contribution to) that outcome. Examples of this are seen in T and C responses in team and contact sports (8,33), as well as in chess matches (27).

Another factor to consider is that, psychologically, many rugby players may enjoy weight training as a "time out" from the rigors of contact training. This could affect hormonal stress responses relative to other athlete populations, which may perceive an equivalent workload of weight training differently. Aspects of novelty and stress of the situation are likely to be perceived in a manner based on experience, although the simple imposition of an experimental design may offer novelty to even seasoned trainers. In line with other stressors, adaptation and familiarity take place such that the

response is often linked to the perception of coping with the stimulus rather than to the actual physical nature of the stimulus. The psychological nature of hormonal response may mean that seasoned trainers respond differently to protocols with which they are unfamiliar or alternatively like or dislike. This response is in addition to the physiological stress caused by exertion.

To achieve consistency among hormonal studies, it will be necessary to explicitly control for all of these, and potentially many more, variables. Much of the equivocality in the literature may be explained if a more comprehensive framework for studying hormonal responses was available. Frequent measurement will be one of the factors needed to achieve this, and such measurements will need to be as low in invasiveness and perceived stress as possible.

Studies often report pooled data from subjects that are very similar in physique and sporting background (e.g., bodybuilders, rowers, and power lifters). Individual differences to protocols can be masked when pooled data are used. At any point in an athlete's training schedule, hormonal response is affected by numerous factors, making the process of functional adaptation highly complex. It is well known in the exercise prescription field that, as a result of these complexities, response-adaptation is highly individualized, with different individuals showing different responses to forms of training. To our knowledge, this is the first study to identify "individual" RE-induced hormonal responses in elite athletes.

Practical Applications

Essentially, it is highly likely that individual athletes respond differently to different RE protocols during their training cycle. Monitoring hormonal responses to exercise stimuli could prove the most accurate way to assess stress and manage maximal adaptation. The use of saliva as a noninvasive indicator of the levels of bioavailable hormones implicated in modulating adaptation provides a novel methodology for trainers and athletes. However, it needs to be shown that the hormonal changes as a result of exercise produce functional gain differences or these measurements have little relevance.

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