SALIVARY HORMONAL RESPONSES TO DIFFERENT WATER-BASED EXERCISE PROTOCOLS IN YOUNG AND ELDERLY MEN

EDUARDO L. CADORE, FRANCISCO L.R. LHULLIER, CRISTINE L. ALBERTON, ANA PAULA V. ALMEIDA, KATIUCE B. SAPATA, ANDRÉ L. KORZENOWSKI, AND LUÍS FERNANDO M. KRUEL

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ABSTRACT

Cadore, EL, LHullier, FLR, Alberton, CL, Almeida, APV, Sapata, KB, Korzenowski, AL, and Kruel, LFM. Salivary hormonal responses to different water-based exercise protocols in young and elderly men. J Strength Cond Res 23(9): 2695–2701, 2009—Although adaptations to water-based resistance exercise and conventional water-based exercise have been investigated, little is known regarding acute anabolic and catabolic hormonal responses to these 2 types of exercise. The purpose of this study was to investigate the acute responses of salivary testosterone and cortisol to 2 water-based exercise protocols in which the different intensities were determined using Borg’s perceived exertion scale. Ten young (24 ± 2.7 yr) and 7 elderly men (65 ± 5.5 yr) who were familiar with exercise in water were subjects of the study. Salivary samples were collected at rest and 5 minutes after the 2 water-based exercise protocols. One session involved intermittent water resistance training at a Borg-scale intensity of 19 (W19), whereas the other involved continuous water aerobic training at an intensity of 13 (W13). The samples were used to determine salivary levels of free testosterone and cortisol. There was a significant increase on salivary testosterone in both groups after the W19 protocol (p < 0.05), but no such alteration was observed after W13. The testosterone response to the W19 protocol was significantly higher in young than in elderly men (p < 0.05). Although no modification on salivary cortisol was observed after either protocol, in young men, the cortisol response to W19 was higher than in elderly men (p < 0.05). Water-based exercise with emphasis on strength development was found to stimulate a more acute increase in salivary testosterone than water-based aerobic exercise, probably as a result of the higher intensity used in that training protocol. Given the known relationship between acute hormonal responses and chronic neuromuscular adaptations, the testosterone response after W19 should be considered when prescribing water-based exercise, especially to older populations.

KEY WORDS water exercise, testosterone, cortisol, resistance training

INTRODUCTION

In the literature, the performance of dynamic exercises in which water resistance is used as overload is reported to be an effective means of increasing muscular strength (7,10,31,35,39,41), muscular activation (35), aerobic power (Vo2max) (6,12,30,39,40), and functional capacity (5). Some studies have investigated water-based exercise training programs designed specifically to improve strength development (i.e., water resistance training) and have found significant increases in muscular strength in response to such training (7,35,39), with improvements greater than those found in studies involving the conventional aerobic-type water-based exercise model (31).

The intensity of water-based exercise sessions can be modulated by using devices to increase the frontal area and turbulent flow, as well as varying the velocity of motion (34). Because velocity is squared and proportional to drag force, increased velocity creates greater water resistance, which produces stronger muscular force and consequently higher strength adaptations (35).

Although studies have demonstrated adaptations induced by conventional aerobic exercise training in water and by water-based resistance training (i.e., increases in muscular strength and aerobic capacity), little is known regarding the anabolic and catabolic hormonal response to these types of exercise. On other hand, conventional resistance exercise (i.e., strength training) is known to stimulate acute increases in anabolic hormones, such as testosterone and growth hormone, and catabolic hormones, such as cortisol (2,3,9,22,23). This physiologic behavior is related to several factors, among which are intensity and volume: the higher the physiologic intensity and volume, the higher the hormonal responses (25,38). Age also influences response patterns, with
higher responses in young when compared with older subjects because of the reduction in the secretory capacity of Leydig cells during aging (23). In addition to the well-known importance of testosterone to neuromuscular metabolism, some authors have demonstrated that resistance exercise-induced acute increases in testosterone are strongly related to training-induced strength development and muscle mass (2).

Saliva provides a convenient, noninvasive means of determining steroid hormonal concentrations (11,20,32). Many studies have demonstrated significant correlations between circulating and salivary levels of both free testosterone (27,42) and cortisol (21,27,37). Given these correlations, studies have been carried out using salivary measurements to evaluate the influence of several physical exercise modalities on steroid hormonal concentrations (16,17,19,24,26,29).

Based on the relationship between acute hormonal responses and strength increases induced by resistance training, as well as the influence of exercise intensity on the hormonal response pattern, it is reasonable to suggest that a high-intensity water-based exercise protocol designed to increase muscular strength could stimulate a greater increase in salivary testosterone than a similar aerobic-type water-based exercise performed at a lower intensity. Thus, to obtain information on the possible relevance of the endocrine system in the adaptations resulting from 2 different models of water-based exercise protocols performed at different intensities, the purpose of the present study was to investigate the acute salivary testosterone and cortisol responses induced by water-based resistance exercise and conventional aerobic water-based exercise. In addition, because of the influence of aging on hormonal responses, it was also decided to investigate the hormonal responses to water-based exercise in young and elderly subjects. Our hypothesis is that the most intense water-based protocol will provide a more powerful stimulus for increases in the hormone concentrations in saliva. Furthermore, on the basis of the data available in the literature (25), we can speculate that the young group will be more responsive to the exercise protocols.

**METHODS**

*Experimental Approach to the Problem*

To investigate the hormonal responses to water-based exercise, we chose 2 variables that have a known influence on hormonal responses to resistance exercise: intensity and age (22,23,25). Thus, in the present study, 2 age-defined groups (young and elderly men) performed 2 different common water-based exercise protocols. Subjects attended the test center on 3 different days. First, the individuals were interviewed regarding their medical history and physical activity, then their anthropomorphic characteristics were measured, and they had a training session during which they became familiar with the Borg scale (8), the training models, and the exercises to be performed. After that, subjects randomly attended the test center on 2 other days with a 1-week interval to perform the water-based aerobic exercise protocol and the water-based resistance exercise protocol. Before and 5 minutes after each training session, saliva samples were collected from the subjects to determine hormonal concentrations.

**Subjects**

Ten young (24.5 ± 3 yr) and 7 elderly (65 ± 5.5 yr) healthy men volunteered to participate in this study. All participants had some recreational experience of water-based exercises such as swimming, aquatic polo, or aquatic jogging, but none of them were engaged in any type of regular or systematic type of physical training. An anamnesis a priori demonstrated no difference between groups in the status of training (i.e., trained, untrained). The exclusion criteria were a history of renal, hepatic, cardiovascular, pituitary, or metabolic disease; the use of anabolic steroids or any other medication that could affect the musculoskeletal or endocrine metabolism; smoking; and adherence to a reduced-calorie or low-fat diet, ketogenic diet, or over-the-counter ergogenic aids that could affect hormonal levels within the past year. All subjects were carefully informed about the potential risks and discomforts of the project and signed a written consent form before their participation in the study. This investigation was approved by the Ethics Committee of the Federal University of Rio Grande do Sul (UFRGS) and is in accordance with the Declaration of Helsinki. Table 1 shows the physical characteristics. There were significant differences between the groups in terms of age and body mass (p < 0.01).

**Procedures**

*Water-Based Exercise Protocols.* To assess the acute hormonal response to the water-based exercises, 2 different sessions were organized: 1 session involved intermittent water resistance training and the other involved continuous water aerobic training. The training intensity was determined by means of the rate of perceived exertion (RPE) (8). A separate water-based class was held to familiarize the subjects with the exercises and Borg's RPE. First, Borg's recommendations were followed during the introduction to the RPE scale (8). Subsequently, in the water-based class, each exercise was performed progressively and randomly at all effort levels, ranging from 6 until 20. In both exercise sessions, a low-intensity warm-up was performed before either the

<table>
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<th>Table 1. Physical characteristics.</th>
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<td><strong>Young (n = 10)</strong></td>
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<td>Age (yr)</td>
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<td>Body mass (kg)</td>
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Significant differences between groups, *p < 0.05.
water-based aerobic or resistance training. During the protocols, an expert trainee gave verbal encouragement to the subjects to perform the exercises at in the proposed intensity.

Both exercise protocols used the same muscle group and lasted 18 minutes. However, in the intermittent water resistance exercise session, a sequence of local exercises was performed, alternating exercise between upper and lower limbs, at an anaerobic intensity. On other hand, in the continuous water aerobic exercise session, exercises with both upper and lower limbs were performed at the same time, using an aerobic intensity.

In the water-based resistance exercises (W19), 2 bouts were performed with 4 sets of 15 seconds of effort for each exercise (3 exercises performed sequentially with 10-s breaks) at RPE 19, with a rest of 70 seconds between sets and 2 minutes of active rest, RPE 9, between bouts. The exercises performed in the first bout were a) simultaneous bilateral horizontal shoulder flexion and extension; b) unilateral right frontal kick; and c) unilateral left frontal kick. In the second bout, the following exercises for the same muscle group were performed: a) unilateral right horizontal shoulder flexion and extension with lateral support on the bar; b) unilateral left horizontal shoulder flexion and extension with lateral support on the bar; and c) alternated bilateral kick with dorsal support on the bar (1).

In the water-based aerobic exercise protocol (W13), the session consisted of 3 bouts of 6 minutes, with exercises performed continuously at RPE 13. The exercises performed in each bout were a) frontal kick to 90° with horizontal shoulder flexion and extension; b) cross-country ski with horizontal shoulder flexion and extension; and c) stationary running with horizontal shoulder flexion and extension (1).

Saliva Collection and Analysis. Saliva samples were obtained between 8:00 and 8:40 AM, after an overnight fast and 2 days with no training session. The time of blood collection was chosen because of its use in studies conducted with these procedures for the control of the circadian hormonal range (2,3,9,22). First, subjects were asked to rinse their mouths with water to remove any residual food and asked to refrain from drinking caffeine before the collection. They sat in a slightly reclined position during 10 minutes and, after that, 1 mL of unstimulated saliva was collected by passive dribble into a receptacle tube. After collection at rest, subjects performed the exercise protocol and, after 5 minutes, another 1 mL of saliva was collected using similar techniques. The saliva samples were transferred to Eppendorf tubes and subsequently stored at –20°C until analyzed. Resting and exercise-induced salivary concentrations of free testosterone and cortisol were determined in duplicate, using radioimmunoassay kits (ICN Biomedicals, Irvine, CA, USA) with sensitivity adapted to saliva (33,36). Care was taken with the procedure to eliminate any matrix effects caused by the analysis of the saliva biocompartment. To make the physiologic solution similar to salivary composition, the standard and control samples used for the salivary assays were diluted 1:20 (33,36) with an incubation period of 12 hours. To eliminate interassay variance, all samples were analyzed within the same assay batch, and all intra-assay variances were 6.3%
or less. Antibody sensitivities were 20 pg/mL for salivary testosterone and 0.05 μg/dL for salivary cortisol. We achieved this saliva sensitivity using both a lower range of standard and a twice sample volume, calculated using the formula of minimum detectable dose: $\text{MDD} = x \left( \frac{m}{\sqrt{n}} \right)$ sensitivity, in which $x$ is the number of 0 values observed on the dose-response curve, $m$ is the number of SDs used, and $n$ is the number of replicates (13). We found a test-retest reliability coefficient ranging from 0.9 to 0.92.

**Nutritional Assessment.** Table 2 shows the nutritional intake of the subjects in the days before the collection days. A questionnaire was used to screen the meal intake by subjects and to calculate the total energy and percentage of macronutrients in the diet to control the nutritional status of subjects before the 2 water-based exercise protocols. Before the questionnaire, subjects were instructed by verbal and written form to report each meal intake in controlled days. There were no differences between the days before the exercise protocol days in the total intake of energy, carbohydrate, lipids, and proteins.

**Statistical Analyses.** Data are shown as mean ± SD. Normality and homogeneity was analyzed using the Shapiro-Wilk and Levene tests, respectively. Because of the nonparametric characteristics, Box-Cox transformations were performed on the salivary testosterone and cortisol data. However, the original values are shown in the tables to offer the reader a real notion of the concentration levels. Paired t-tests were used for comparison of the at-rest values from the 2 protocols and comparison of nutritional status on the days before the experimental days. Indeed, independent t-tests were used to compare the at-rest concentrations of the groups. Hierarchic factorial analysis was used for comparisons of the pre- and postexercise values, between groups and between protocols. Where significant differences were found, Tukey post hoc tests were used in their analysis. The level of significance used was $p \leq 0.05$, statistical power was 80%, and the analyses were made in the Sisvar software for Windows version 4.0 (15).

**RESULTS**

Figures 1 to 4 show the results. There were no intragroup differences in the salivary concentrations of free testosterone and cortisol found at rest between the different experimental days. Although, in both at-rest situations (before W19 and W13), the young subjects showed higher concentrations of salivary testosterone compared with elderly subjects ($p < 0.05$), this was not the case with cortisol. There was a significant increase in salivary testosterone after the W19 protocol in both groups ($p < 0.05$), although no significant
changes were observed in testosterone after the W13 protocol. During the W19 protocol, the salivary testosterone response was higher in young compared with elderly subjects ($p < 0.05$). There were no significant increases in salivary cortisol after both water-based exercise protocols. Likewise, there were no differences in the cortisol response between the 2 intensities investigated. In the most intense protocol (W19), the salivary cortisol response was higher in the young subjects than the elderly ($p < 0.05$).

**Discussion**

The main result of the present study is to demonstrate that the most intense protocol (i.e., W19) provoked greater responsiveness in the free salivary testosterone in both the investigated groups. Furthermore, in the W19 protocol, the free salivary testosterone and cortisol were higher in the young than in the elderly subjects, although no significant modifications were observed in the cortisol in either group after this protocol.

In regard to the testosterone, the fact that a significant increase was only seen in the most intense protocol, with emphasis on the development of muscular strength, corroborates results reported in the literature regarding hormonal response to strength training. Although we found no reports investigating the response of testosterone and cortisol to water-based protocols in our review, some authors have demonstrated that, during strength training, in sufficient volume, the greater the physiologic intensity, the greater the hormonal response observed (38). However, in the present study, the training models, as well as the intensity, were different. In W13, a continuous training model was used, in which the subjects performed the exercises at an aerobic intensity, without intervals between series, thus exercising for longer than in W19, in which the subjects performed the training with intervals at the maximum possible speed. Although the volume performed is an important variable in hormonal response (25,38), in the present study, the intensity seen had more influence on hormonal response in both groups, as observed in our results. Although the subjects performed exercise for longer during the W13 session, the physiologic intensity was probably not sufficient to stimulate the testosterone response.

The absence of differences in magnitude of the response seen in both types of training was possibly caused by the great variability of the samples. Nonetheless, in the present study, only the most intense protocol, performed at the maximum speed of each individual, and thus generating the greatest force possible in the water, produced significant increases in the testosterone levels of both groups. This may have occurred because of the higher anaerobic component of the W19 protocol (28). A high correlation has been shown between lactate and the testosterone response during anaerobic exercise. Moreover, during in vitro experiments, the infusion of lactate into rat testicles generated a dose-dependent increase in testosterone, indicating that the accumulation of lactate is a powerful action mechanism for the increase of testosterone during exercise (28). In addition, the higher level of sympathetic activity resulting from the higher-intensity may have played a part in stimulating the testosterone (14).

Although there are few studies that compare the increase in strength resulting from water-based resistance training with the adaptations seen in conventional, water-based aerobic-intensity training, studies investigating water-based training specifically for strength found a higher percentage increase in strength than studies that investigated water-based exercise in which the movements were performed at lower execution speeds (35,39). The results of the present study suggest that, in addition to the higher specific muscle strength resulting from higher speeds, the response of the testosterone may be a physiologic factor mediating increments in muscular strength, because, in addition to the known role of testosterone in muscular metabolism as a stimulant of muscular protein synthesis and in the synthesis of neurotransmitters (23), some studies have shown a high correlation between the increase in muscular strength and the testosterone response in an acute strength training session (2,9).

A higher increase on salivary testosterone was seen in the W19 protocol in the young when compared with the elderly subjects. The diminished testosterone response to exercise seen in the elderly is associated with the andropause, characterized by the lower number of Leydig cells and their reduced secretory capacity, resulting from aging (23). Nevertheless, the results of the present study show that specific water-based exercise, when performed at high intensity, is capable of increasing salivary testosterone in elderly subjects. It was shown that 78% of the salivary testosterone is not bound because of the absence of sex hormone binding globulin in the saliva and is more closely correlated with the free circulating testosterone (20), with greater muscular bioactivity than total testosterone (23).

With regard to the cortisol, although some studies have demonstrated higher concentrations of cortisol at rest in elderly when compared with young subjects (23,25), the results of the present study show a higher increase in the young subjects, in response to the more intense session. The lower cortisol response in elderly subjects in the W19 protocol may have occurred because of the methodology of intensity control in the present study. Some studies have demonstrated that elderly subjects can overestimate the intensity when it is controlled by the subjective perception of effort (4,18). Therefore, although both groups carried out the protocol at an intensity of 19 on the Borg scale (8), it is possible that the young subjects performed the exercise at a higher physiologic load. Some authors have observed that the cortisol response to exercise occurs especially with higher metabolic overload (22,33,38).

Another possible explanation for the higher acute cortisol response seen in the young subjects could be the nature of the physical training session performed. With aging, there is
Hormonal Responses to Water-Based Exercise

a selective loss of type II fibers, leading to a reduced capacity to produce strength and power in the elderly (23). During water-based exercise, the tension generated in the muscles is dependent on the speed of execution of the movement, and possibly the younger individuals performed the movements at a higher speed and therefore with higher specific muscular tension. Although, in the present study, we did not measure parameters of muscular microlesion, we can speculate that higher tension may have generated greater tissue degradation and, thus, greater inflammatory response. Despite the known catabolic effect of cortisol, this hormone has an important tissue remodeling function (25). If the young subjects in the present study produced greater muscular damage, a greater cortisol response could have occurred in those subjects related to tissue remodelation (25). In fact, Kraemer et al. (22) observed a high correlation between the increase in cortisol levels and the increase in the plasma levels of creatine-kinase, a muscle damage indicator, after resistance exercise (22). Nonetheless, the absence of a significant increase in cortisol after exercise and the failure to measure inflammatory parameters makes this hypothesis speculative.

In conclusion, in the present study, it was seen that a high-intensity water-based resistance training session is a greater stimulus for an increase in salivary testosterone than conventional water-based aerobic training in young and elderly men. In addition to the greater specific tension suggested in the W19 protocol, the higher testosterone response could be a greater stimulus for the increase in muscle strength seen with this training model. However, care should be taken when interpreting these results because the investigation was limited to the pattern of hormonal response to water-based exercises in the oral cavity of the subjects included in the groups.

Practical Applications

The present results demonstrate that, if the main purpose of a training session is to stimulate testosterone, a water-based resistance exercise protocol is more suitable than a water-based aerobic exercise, including elderly subjects. On the basis of the relationship between the acute testosterone response to resistance exercise and the increase in the muscular strength caused by resistance training, it is important to prescribe training sessions that are sufficiently intense to optimize the stimulus to anabolic hormones, especially to increase strength and muscle mass in elderly subjects.

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