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Influence of upper-body exercise order on hormonal responses in trained men

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ABSTRACT

The aim of this study was to examine acute hormonal responses after different sequences of an upper-body resistance-exercise session. Twenty men completed 2 sessions (3 sets; 70% 1-repetition maximum; 2 min passive rest between sets) of the same exercises in opposite sequences (larger to smaller vs. smaller to larger muscle-group exercises). Total testosterone (TT), free testosterone (FT), testosterone/cortisol (T/C) ratio, sex-hormone-binding globulin (SHBG), growth hormone (GH), and cortisol (C) concentrations were measured before and immediately after each sequence. The results indicate that the GH concentration increased after both sessions, but the increase was significantly greater ($p < 0.05$) after the sequence in which larger muscle-group exercises were performed prior to the smaller muscle-group exercises. No differences were observed between sessions for TT, FT, SHBG, C, or the T/C ratio at baseline or immediately after resistance exercise. These results indicate that performing larger muscle-group exercises first in an upper-body resistance-exercise session leads to a significantly greater GH response. This may have been due to the significantly greater exercise volume accomplished. In summary, the findings of this investigation support the common prescriptive recommendation to perform larger-muscle group exercises first during a resistance-exercise session.

Keywords: resistance training, endocrine responses, testosterone, cortisol, growth hormone, upper body

Introduction

Current guidelines for resistance-exercise program design recommend that large muscle-group exercises generally be performed first in a training session (ACSM 2009; Fleck and Kraemer 2004). This exercise-order recommendation has been supported by studies that found greater strength gains (Dias et al. 2010; Simão et al. 2010; Spineti et al. 2010) and hypertrophy (Simão et al. 2010; Spineti et al. 2010) in muscles that were trained at the beginning, rather than at the end, of a session during a long-term training program. Furthermore, studies examining the effect of exercise order (i.e., either large or small muscle-group exercises performed first in a training session) on repetition performance demonstrated significantly greater total repetitions (across all sets) for the same exercise when large muscle-group exercises were performed first than when they were performed last in a sequence (Bellezza et al. 2009; Farinatti et al. 2009; Gentil et al. 2007; Miranda et al. 2010; Sforzo and Touey 1996; Simão et al. 2005, 2007; Spreuwenberg et al. 2006). Thus, performing large muscle-group exercises first in a session results in a larger total

volume (load × repetitions) completed; however, whether the exercise-order effect on volume translates into acute differences in physiological responses, including the hormonal response after a session, has not yet been elucidated.

The importance of circulating hormones for muscle adaptations to resistance exercise is highlighted by the findings that suppression of circulating testosterone concentrations prevents resistance-exercise-induced hypertrophy in young healthy men (Kvorning et al. 2006), and that strength increased more when exercise sessions included an acute elevation in anabolic hormones (Hansen et al. 2001). Thus, an exercise-order effect on the acute hormonal response to a resistance-exercise session could help explain the differences in strength and hypertrophy found when large-muscle exercises are placed first or last in sessions. Resistance-exercise protocols are often structured to concentrate on upper- and lower-body muscle groups in separate sessions, and often on separate days; this is particularly true for hypertrophy-oriented protocols practiced by bodybuilders, rehabilitation sessions during physical therapy, and for individuals who have only upper-body capabilities. The hormonal response to an upper-body resistance session in men is generally much less than that from a lower-body exercise session, but the response is affected by the volume of the session (Migiano et al. 2010). Because the potential for acute hormonal responses is correspondingly less, the order of exercises for an upper-body session might be especially important for creating the most effective anabolic stimulus for that session.

Several studies have examined the effect of exercise order (Bellezza et al. 2009; Farinatti et al. 2009; Gentil et al. 2007; Miranda et al. 2010; Sforzo and Touey, 1996; Simão et al. 2005, 2007; Spreuwenberg et al. 2006), but none of these investigated the effect of exercise order on hormonal responses to an exercise session. Because the volume completed during a resistance-exercise session has been shown to vary with exercise order (Spreuwenberg et al. 2006), the magnitude of the acute hormonal responses could vary in a similar fashion. Therefore, the purpose of this study was to examine acute hormonal responses to an upper-body resistance-exercise session performed in opposite sequence (larger to smaller vs. smaller to larger muscle-group exercises).

Materials and methods

Subjects

Twenty men (age, 22.4 ± 2.7 years; weight, 80.3 ± 5.7 kg; height, 180 ± 8 cm; body mass index, 21.5 ± 0.3 kg·m−2) with at least 2 years (≥3 times per week) of consistent recreational resistance training participated in this study. All subjects had experience in all of the selected exercises, had no medical conditions that might be aggravated by participation in or affect the outcome of the study, and did not use nutritional supplements or other ergogenic aids. All subjects provided written informed consent to participate, and were asked to not perform any resistance exercises other than those prescribed as part of the study. The experimental

procedures were in accordance with the Declaration of Helsinki, and the study protocol was approved by the Research Ethics Committee of the Institution.

Maximal strength assessment

Four testing sessions were conducted (prior to completing the 2 experimental sessions) to assess 1-repetition maximum (1-RM) strength for the following exercises: free-weight bench press (BP) with a straight bar; seated machine front-lat pull down (LPD); seated machine shoulder press (SP) with dumbbells; standing free-weight biceps curl (BC) with a straight bar; and seated machine triceps extension (TE). The 1-RM testing sessions were separated by 48 h. The 1-RM was determined in fewer than 5 attempts, with 5 min between attempts; 10 min was allowed prior to beginning the test for the next exercise. No exercise was allowed in the 48 h between tests, so as not to confound the test–retest reliability. To standardize the test protocol, the following strategies were adopted (Simão et al. 2007): standardized instructions concerning the testing procedure were given to subjects; verbal encouragement was provided during the testing procedure; and the mass of all weights and bars was determined using a precision scale.

Experimental sessions

Seven days after the fourth strength testing session, subjects performed 1 of the 2 exercise sequences in a randomized crossover design. The 2 sessions consisted of the same exercises, but performed in opposite sequences. Sequence A began with compound (larger muscle group) exercises and progressed toward assistance (smaller muscle group) exercises for BP, LPD, SP, BC, and TE. Conversely, sequence B was performed in the reverse order (i.e., TE, BC, SP, LPD, and BP). Warm-up prior to each exercise sequence consisted of 2 sets of 20 repetitions for the first exercise of the session (BP for sequence A and TE for sequence B) at 40% of the predetermined 1-RM load. All exercises in both sequences were performed for 3 sets at 70% of the predetermined 1-RM for each exercise. Sets and exercises were separated by 2 min of passive rest. The second sequence was performed 7 days after performance of the first assigned exercise sequence.

During the experimental sessions, subjects were instructed to perform repetitions to the point of voluntary exhaustion. Repetition velocity was not controlled, and no pause was permitted between the eccentric and concentric phases of each repetition. Total volume was calculated (load × repetitions) for each experimental session. All subjects performed each of their respective sessions at the same time of day, and all testing was performed between 0600 and 0900 to control for diurnal hormonal variations (McCaulley et al. 2009). Subjects were instructed to record their diet for 2 days prior to performing the first assigned exercise sequence and replicated the diet prior to performing the second trial. During the study, all subjects were asked to continue with their normal activities of daily living.

Hormone testing and analysis

The subjects fasted and slept for approximately 8 h prior to each morning blood sample and experimental session. Ambient temperature and humidity were fixed at 20–25 °C and 40%–65%, respectively. The baseline resting blood sample (i.e., 10 mL of blood drawn from the antecubital vein) was collected with subjects sitting in a slightly reclined position 15 min before each experimental session. This procedure was repeated immediately after each exercise session. Serum total testosterone (TT), free testosterone (FT), cortisol, sex-hormone-binding globulin (SHBG), and GH concentrations were measured in duplicate and determined using a commercially available radioimmunoassay kit (Coat-A-Count, DPC, Los Angeles, Calif., USA), in accordance with the manufacturer's instructions. The testosterone/cortisol (T/C) ratio was calculated also. To minimize the effects of interassay variation, all samples from one subject were analyzed in the same assay. Intra-assay coefficients of variation were all less than 5.0%.

Statistical analyses

Data are presented as the means ± standard deviation. A priori, it was determined that 10 subjects per group was adequate to substantiate the 0.05 alpha level, with a Cohen probability level of at least 0.80 for each dependent variable (nQuery Advisor software, Statistical Solutions, Saugus, Mass., USA). Intraclass correlation coefficients and paired t tests were used to assess the reliability of the maximal strength assessments. The data were initially analyzed using the Shapiro–Wilk normality test and the homoscedasticity test (Bartlett criterion). All variables presented normal distribution and homoscedasticity.

A 2-way repeated-measures ANOVA (sequence × time point) was conducted to compare hormone concentrations. Significant effects were followed by Fisher's least significant difference post hoc analysis to examine pair-wise differences. Student's t test was conducted to compare the total repetitions completed for each exercise with the total volume of each sequence. The scale proposed by Rhea et al. (2004) was used to compare effects sizes in hormonal responses (the difference between pretest and post-test scores divided by the pretest standard derivation). An alpha level of $p < 0.05$ was used to determine significance for all comparisons. Statistica 6.0 software (Statsoft; Tulsa, Okla., USA) was used for all analyses.

Results

Excellent test–retest reliability was demonstrated in determining maximal strength for each exercise. The intraclass correlation coefficients were as follows: BP, r = 0.94; LPD, r = 0.96; SP, r = 0.94; BC, r = 0.94; and TE, r = 0.96. A paired Student's t test did not indicate significant differences between the 1-RM tests for any of the exercises. The total number of repetitions for each exercise was significantly greater for sequence A than for sequence B, with the exception of the SP; the total volume was also significantly greater for sequence A (see Table 1).

Table 1. Total exercise repetitions and total volume for sequence A and sequence B (mean ± standard deviation).

	Bench press	Lat pull down	Shoulder press	Biceps curl	Triceps extension	Total volume (kg)
Sequence A	26.7±2.8*	28.1±5.1*	21.3±5.2	18.6±6.2*	18±6.3*	22683±8.583*
Sequence B	14.1±5.8	19.1±4.0	26.6±8.1	27.4±3.2	26.8±4.2	13684±5.632

*p ≤ 0.05 vs. sequence B.

Table 2. Total testosterone, free testosterone, testosterone/cortisol ratio (T/C), and cortisol levels at rest (baseline) and immediately after resistance exercise.

	Baseline		After exercise	
	Sequence A	Sequence B	Sequence A	Sequence B
Testosterone (ng·dL^{-1})	498.63±139.62	490.32±104.31	581.52±146.21	556.20±137.52
Free testosterone (pg·dL^{-1})	117.82±11.78	119±23.21	132.10±36.70	140.42±34.31
Cortisol (ng·dL^{-1})	15.78±3.78	17.98±2.45	22±3.83*	24.23±2.36*
T/C ratio	34.61±5.11	28.21±2.11	27.32±2.91	23.93±2.23

Note: Data are expressed as the mean ± standard deviation. T/C, testosterone/cortisol.
*p ≤ 0.05 for baseline vs. immediately after resistance exercise.

Table 3. Sex-hormone-binding globulin (SHBG) and growth hormone (GH) levels at baseline and immediately after resistance exercise.

	Baseline		After exercise	
	Sequence A	Sequence B	Sequence A	Sequence B
SHBG (nmol·L^{-1})	24.02±5.71	26.27±3.41	28.04±8.42*	27.47±8.87
GH (ng·dL^{-1})	0.45±0.09	0.66±0.14	6.82±2.32*†	2.90±2.42

Note: Data are expressed as the mean ± standard deviation. SHBG, sex-hormone-binding globulin; GH, growth hormone.
*p ≤ 0.05 for baseline vs. immediately after resistance exercise.
†p ≤ 0.05 for difference between sequences immediately after resistance exercise.

TT, FT, and the T/C ratio were not significantly different at baseline and immediately after exercise (see Table 2). Conversely, cortisol concentration significantly (p < 0.05) increased immediately after sequence A (26.7%) and sequence B (26.9%) (see Table 2). There were no significant differences between sequences for TT, FT, the T/C ratio, or cortisol at baseline or immediately after resistance exercise.

Table 4. Effect-size comparison between sequences.

	Testosterone (magnitude)	Free testosterone (magnitude)	T/C ratio (magnitude)	Cortisol (magnitude)	SHBG (magnitude)	GH (magnitude)
Sequence A	0.59 (small)	1.20 (moderate)	−1.43 (moderate)	1.65 (large)	0.72 (small)	70.78 (large)
Sequence B	0.63 (small)	0.90 (moderate)	−2.06 (large)	2.55 (large)	0.65 (small)	16.07 (large)

Note: T/C, testosterone/cortisol; SHBG, sex-hormone-binding globulin; GH, growth hormone.

SHBG increased significantly after sequence A (14.2%), but not after sequence B, relative to baseline levels (Table 3). However, despite the significant increase in SHBG from pre- to postexercise for sequence A, no differences were noted between sequences for SHBG, either at baseline or after resistance exercise (see Table 3). There was a significant increase in GH concentration immediately after sequence A (98.3%), which was significantly greater (i.e., 63% greater) than after sequence B (see Table 3).

In the effect-size analysis, sequence A and sequence B demonstrated a small response for TT, a moderate response for FT, a large response for cortisol, a small response for SHBG, and a large response for GH. The effect size for the T/C ratio was different between the sequences, with sequence A eliciting a moderate response and the sequence B eliciting a large response (see Table 4).

Discussion

The main finding of this study is that exercise order affects the endocrine response to an upper-body resistance-exercise session. When the larger muscle-group exercises were placed first (i.e., sequence A), rather than last (i.e., sequence B), significantly greater circulating concentrations of GH were present immediately after exercise. These findings provide unique physiological support for the current recommendation for resistance-exercise prescription (i.e., the placement of compound exercises first in a session). To our knowledge, this is the first study to examine the effect of exercise order on the endocrine response to a resistance-exercise session performed in opposing sequences.

It should be noted that the GH and cortisol responses after both sequences (as well as the T/C ratio for sequence B) elicited large effect sizes, indicating that both sessions produced a high metabolic demand. It should also be noted that moderate effect sizes were elicited for the FT response after both sequences (as well as the T/C ratio after sequence A). These results indicate that from a practical standpoint, both sessions transiently altered the hormonal milieu in favor of muscle tissue building.

Consistent with previous studies (Bellezza et al. 2009; Farinatti et al. 2009; Gentil et al. 2007; Miranda et al. 2010; Sforzo and Touey, 1996; Simão et al. 2005, 2007; Spreuwenberg et al. 2006), our study indicates that performing an exercise toward the beginning of a sequence results in greater repetitions than performing the same exercise toward the end of a sequence. Specifically, the total repetitions for the BP and LPD were significantly greater when performed first (i.e., sequence A); conversely, the total repetitions for the TE and BC were significantly greater when performed first (i.e., sequence B). The repetitions completed for the SP, performed in the middle of both sequences, were not significantly different between sequences. Because the load was constant for all sets of a particular exercise, the volume completed (load × repetitions) was solely dependent on the total repetitions completed; thus, the total repetitions and volume were greater when the larger muscle-group exercises were performed first (sequence A).

It is well established that the total volume of work completed during a resistance exercise session influences the magnitude of the hormonal response, especially that of GH (Hansen et al. 2001; Migiano et al. 2010; Kraemer et al. 1990; Leite et al. 2011; Smilios et al. 2003). In accordance with these previous findings, in our study, sequence A (which involved a 40% greater volume of work) promoted a

significantly greater GH response than sequence B. The absolute GH responses, as well as the difference in responses between the 2 sequences, are similar to the GH responses found by Migiano et al. (2010) for unilateral and bilateral (approximately twice the work as unilateral) upper-body resistance exercise. The effect of total volume of work on GH secretion from the anterior pituitary is in large part due to the metabolic demand caused by the volume during the exercise session; this metabolic demand is generally reflected by the lactic acid response pattern (Kraemer et al. 1990; Smilios et al. 2003; Migiano et al. 2010). The drop in blood pH that is concurrent with a large increase in blood lactate concentration during exercise can increase GH secretion; conversely, infusion of the pH buffer sodium bicarbonate (NaHCO3) results in a reduction in GH both at rest and after exercise (Elias et al. 1997). The larger increase in GH found for sequence A (the sequence with a larger volume and, likely, a larger metabolic demand), compared with sequence B, could be explained by the larger metabolic demand and resultant larger reduction in pH for sequence A. Circulating growth hormones have important metabolic properties that are associated with muscular and skeletal growth (Kraemer 1988), and a correlation between the acute GH response to resistance exercise and muscle fiber hypertrophy has been demonstrated (McCall et al. 1999). In addition to the direct effects of GH (and the effect of GH on insulin-like growth factor), GH might also have an indirect effect on muscle. GH appears to facilitate the anabolic actions of testosterone because of the permissive and synergistic effects in promoting protein synthesis (Mauras et al. 2003). Thus, the larger acute response for GH found for sequence A might have important implications for adaptations to resistance exercise.

None of the exercise orders induced changes in TT or FT from pre- to postexercise in our study. Crewther et al. (2008) and McCaulley et al. (2009) compared acute hormonal responses after 3 resistance-training schemes designed for different adaptational objectives: hypertrophy (4 sets of 10 repetitions at 70% of 1-RM, 3-min rest in squat), power (8 sets of 6 repetitions in jump squats at 0% of 1-RM, 3-min rest), and maximal strength (11 sets of 3 repetitions at 90% of 1-RM, 5-min in squat). They found that the hypertrophy scheme induced greater testosterone levels than the other schemes. In contrast, Migiano et al. (2010) found that neither unilateral nor bilateral (twice the volume) upper-body dumbbell exercises resulted in an exercise-induced increase in TT, despite a similar repetition and load range (4 sets of 10 repetitions at 80% of 1-RM for 5 exercises). Our data confirm the literature (Migiano et al. 2010) demonstrating that the involvement of only the upper-body musculature in trained men appears to be insufficient to induce significant increases in FT or TT. An exercise-order effect for testosterone is, therefore, not likely to exist for upper-body resistance-exercise sessions in trained men.

The SHBG concentration was significantly increased only after sequence A; this transport protein directly influences the biological availability (i.e., the amount of testosterone that is available to interact with nuclear steroid receptors) and the rate of degradation of testosterone. Testosterone bound to SHBG is not considered to be

bioavailable; thus, an increase in SHBG can result in a reduction in FT without a change in TT. In our study, however, FT was not affected by the increase in SHBG. Conversely, SHBG-bound testosterone is protected from degradation, so SHBG can serve as a reservoir of testosterone in the circulation. The results of this study indicate that when large muscle-group exercises are placed first within a session, a small increase in SHBG occurs. It has yet to be determined if such a small increase in SHBG affects adaptations to resistance exercise.

The cortisol concentration increased significantly for both sequence A and sequence B, but was not different between sequences, indicating that elevations in cortisol are independent of exercise order. The role of cortisol involves stimulation of catabolic or anti-anabolic processes, and elevations in cortisol are common after metabolically demanding resistance exercise (Crewther et al. 2008). Although Migiano et al. (2010) demonstrated that the cortisol response to upper-body-only resistance exercise in trained men is affected by volume, it appears that the smaller volume difference in our study was not sufficient to induce a differential cortisol response. On the basis of the findings for cortisol, placing larger muscle-group exercises first in a resistance-exercise session does not appear to produce a greater catabolic signal, despite the greater volume completed.

Circulating testosterone and cortisol have been proposed as physiological markers to evaluate the tissue-remodeling process after resistance exercise (Kraemer and Ratamess 2005). It has been suggested that the T/C ratio is an indirect parameter of the anabolic/catabolic milieu and an indicator of resistance-exercise-induced stress to the organism (Deschenes and Kraemer 2002; Fry and Kraemer 1997; Häkkinen and Pakarinen 1995). In our study, there was no change in the T/C ratio immediately after each sequence, despite the increase in cortisol. This lack of a change in the T/C ratio was likely due to the small but nonsignificant increase in testosterone, combined with the significant increase in cortisol. Our study suggests that for upper-body resistance-exercise sessions in trained men, the steroid hormone anabolic/catabolic milieu is not altered; thus, the added anabolic endocrine signal from this type of exercise session is mainly induced by GH.

In summary, the recommended practice of placing larger muscle-group exercises first in a sequence is supported by our findings. A significantly greater increase in GH was present immediately after sequence A, which placed the compound exercises toward the beginning (i.e., BP and LPD), rather than the end, of a sequence. Therefore, this study indicates that for an upper-body resistance exercise session, larger muscle-group exercises should be placed at the beginning to promote greater GH responses. Other hormonal parameters (TT, FT) were not affected by either exercise protocol, probably because of the relatively low muscle mass stimulated; increased secretion of these hormones probably requires additional involvement of the lower-body musculature. Future research should elucidate the influence of exercise order on hormonal responses to lower-body and whole-body resistance-exercise sessions.

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