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Hormonal Responses after Various Resistance Exercise Protocols

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ABSTRACT

SMILIOS, I., T. PILIANIDIS, M. KARAMOUZIS, and S. P. TOKMAKIDIS. Hormonal Responses after Various Resistance Exercise Protocols. *Med. Sci. Sports Exerc.*, Vol. 35, No. 4, pp. 644–654, 2003. **Purpose:** This study examined the effects of the number of sets on testosterone, cortisol, and growth hormone (hGH) responses after maximum strength (MS), muscular hypertrophy (MH), and strength endurance (SE) protocols. **Methods:** Eleven young men performed multi-joint dynamic exercises using MS (5 reps at 88% of one-repetition maximum (1-RM), 3-min rest) and MH (10 reps at 75% of 1-RM, 2-min rest) protocols with 2, 4, and 6 sets at each exercise; and an SE (15 reps at 60% of 1-RM, 1-min rest) with 2 and 4 sets. Hormonal concentrations were measured before exercise, immediately after, and at 15 and 30 min of recovery. **Results:** The number of sets did not affect the hormonal responses after the MS protocol. Cortisol and hGH were higher ($P < 0.05$) after the four-set compared with the two-set sessions in the MH and SE protocols. No differences were observed between the six-set and the four-set sessions in the MH protocol. Cortisol and hGH were higher ($P < 0.05$) than the MS after the SE and MH protocols, and only when four and six sets were performed in the latter. hGH was higher than the MH after the SE protocol, whether two or four sets were executed, whereas cortisol ($P < 0.05$) was higher after the SE protocol only when two sets were performed. Testosterone did not change with any workout. **Conclusion:** The number of sets functions up to a point as a stimulus for increased hormonal concentrations in order to optimize adaptations with MH and SE protocols, and has no effect on a MS protocol. Furthermore, the number of sets may differentiate long-term adaptations with MS, MH, and SE protocols causing distinct hormonal responses. **Key Words:** STRENGTH, HYPERTROPHY, STRENGTH ENDURANCE, NUMBER OF SETS, TOTAL WORK

Resistance exercise is a potent stimulus for acute increases in the concentrations of circulating hormones such as testosterone, growth hormone (hGH), and cortisol (3,25,27). These responses may expand the possibility of hormone-receptor interactions within the muscle cells and along with the increased number of receptors after training (18) may enhance muscle protein turnover observed after resistance exercise (33). Testosterone and hGH administration increase muscle protein synthesis and promote muscle mass growth in humans (6,10), whereas data from animal studies reveal that androgen receptor antagonist (19) or hypophysectomy (1) suppresses hypertrophy induced by exercise. On the other hand, cortisol admin-

istration has a catabolic effect on myofibrillar proteins and suppresses protein synthesis (4,20). Moreover, hormonal responses play a significant role in tissue growth as well as in the regulation of energy substrate metabolism (22) during the recovery period after a training session. In addition, training studies have also shown that acute responses of hGH or changes in resting concentrations of testosterone and cortisol or testosterone to cortisol ratio, correlate well with changes in muscle size and strength (9,13,27).

Several resistance-training protocols have been developed to improve different aspects of the neuromuscular system such as maximum strength, muscular hypertrophy, and strength endurance (8). These protocols, which differ in the configuration of the acute program variables such as intensity, total work, and rest interval, cause different hormonal responses. Cortisol and hGH concentrations were found to be higher after a muscular hypertrophy protocol when compared with a maximum strength protocol, but testosterone concentrations did not seem to differ (15,23,24,25). Data on the hormonal responses after a strength endurance protocol are not existent, however, despite the fact that among other protocols, such a protocol is also recommended for training the muscular fitness of the population (2,7). Furthermore, it is not known whether the

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hormonal responses elicited by a strength endurance protocol are different from a maximum strength or a muscular hypertrophy protocol.

An important factor in designing a daily or a long-term resistance-training program is the volume of exercise defined as the total amount of work performed during each session. The appropriate amount of total work would be the one that would induce the highest anabolic hormonal responses, or an optimum combination of anabolic and catabolic hormonal responses, creating the most favorable environment for neuromuscular adaptations. A simple way to modify total work is to alter the number of sets performed at each exercise. Previous research has shown that the number of sets affects hormonal concentrations. More specifically, the performance of three sets at each exercise resulted in higher testosterone, hGH, and cortisol responses as compared with the performance of one set (11,30). However, it is unknown whether a higher number of sets would have caused higher hormonal concentrations as well or if there is a point above which an increase in the number of sets does not induce higher hormonal responses. Furthermore, the effects of the number of sets on hormonal responses have been studied only in a hypertrophy protocol and not in a maximum strength or a strength endurance protocol. A study on the above questions may provide important information for the selection of the appropriate number of sets when designing various resistance-training programs.

Total work may also contribute to the differences observed in the hormonal responses among the various resistance-exercise protocols. When an equal number of sets is performed at each exercise, total work is higher after a strength endurance protocol than after a hypertrophy protocol, whereas a maximum strength protocol yields the lowest total work. Changes in the number of sets modify the differences in the amount of total work among the training protocols, and the question arises whether this could affect the differences observed in the hormonal responses, as well.

The purpose of the present study was a) to examine the effects of the number of sets (i.e., 2, 4, and 6 sets) performed at each exercise on testosterone, cortisol, and hGH responses after a maximum strength, muscular hypertrophy, and strength endurance resistance-training protocol and b) to investigate whether the number of sets affects the variation in the hormonal responses among the three protocols where intensity, repetitions, and rest intervals within each protocol were kept constant.

METHODS

Subjects

Eleven men volunteered to participate in this study. Before the initiation of the study, a written informed consent was obtained from each subject, and the experimental protocol was approved by the Institutional Review Board Committee. The physical characteristics of the subjects were the following: age 23 ± 4 yr, height 181 ± 6 cm, body mass 80 ± 6 kg, and body fat $11 \pm 4\%$. Subjects had 2–8 yr of

resistance-training experience, but no one was a competitive lifter or trained systematically for any sport for the last 3 yr. They were training two to three times per week with a nonperiodized program for volume and intensity with loads 70–90% of one-repetition maximum (1-RM) and rest periods of 2–5 min between sets.

Experimental Design

All subjects performed eight workouts in order to compare the hormonal responses among a maximum strength, muscular hypertrophy, and strength endurance resistance exercise protocol as well as the effect of the number of sets within each protocol. The maximum strength and the muscular hypertrophy protocols were performed on three separate occasions with 2, 4, and 6 sets at each exercise, whereas the strength endurance protocol was executed on two separate occasions with 2 and 4 sets (even 4 sets were very stressful in the strength endurance protocol, and all subjects refused to perform 6 sets). In addition, the subjects participated in a control session in order to account for the effects of circadian rhythm on the hormonal concentrations. All exercise sessions, and the control session, were performed in random order with 1-wk intervals.

Exercise Protocols

Selection of exercises and strength measurement. The training protocols consisted of four exercises, which activated large muscle masses, performed in the following order: bench press, lateral pulldowns, squat, and overhead press. All exercises were executed using free weights except the lat pulldowns, which were performed using a Universal weight machine. Maximum strength at each exercise was measured with the 1-RM method.

Maximum strength protocol (MS). In the MS protocol, the initial intensity was 88% of the 1-RM for the bench press, lat pulldowns, and squat and 80% for the overhead press; five repetitions were performed at each set, and the rest interval between sets was 3 min. The intensity was reduced after the second set in order to allow the subjects to complete five repetitions at all sets (i.e., bench press, lat pulldowns and squat combined: third set 83.85 ± 3.33 , fourth set 82.38 ± 3.64 , fifth set 80.83 ± 4.37 , and sixth set $79.51 \pm 4.48\%$ of the 1-RM; overhead press: third set 75.23 ± 5.78 , fourth set 73.24 ± 7.13 , fifth set 71.97 ± 8.14 , and sixth set $70.92 \pm 7.58\%$ of the 1-RM).

Muscular hypertrophy protocol (MH). In the MH protocol, the initial intensity was 75% of the 1-RM for the bench press, lat pulldowns, and squat and 68% for the overhead press; 10 repetitions were performed at each set, and the rest interval between sets was 2 min. The intensity was reduced after the second set in order to allow the subjects to complete ten repetitions at all sets (i.e., bench press, lat pulldowns, and squat combined: third set 69.16 ± 4.43 , fourth set 65.11 ± 5.32 , fifth set 61.03 ± 6.28 , and sixth set $58.94 \pm 6.68\%$ of the 1-RM; overhead press: third set 57.4 ± 7.67 , fourth set 52.34 ± 8.58 , fifth set 45.15 ± 9.98 , and sixth set $43.42 \pm 10.31\%$ of the 1-RM).

TABLE 1. Total work (J) produced at each resistance exercise protocol.

Sets	Resistance Exercise Protocol		
	Maximum Strength	Muscular Hypertrophy	Strength Endurance
2	17,327.8	30,166.3	38,362.4
4	33,280.4	58,013.1	69,757.6
6	49,536.5	82,234	

Strength endurance protocol (SE). In the SE protocol, the initial intensity was 60% of the 1-RM for the bench press, lat pulldowns, and squat and 52% for the overhead press; 15 repetitions were performed at each set, and the rest interval between sets was 1 min. The intensity was reduced after the second set in order to allow the subjects to complete 15 repetitions at all sets (i.e., bench press, lat pulldowns, and squat combined: third set 51.66 ± 5.52 and fourth set $45.3 \pm 6.47\%$ of the 1-RM; overhead press: third set 40.09 ± 5.3 and fourth set $30.68 \pm 7.65\%$ of the 1-RM).

All subjects completed the first two sets with the initial load, and thereafter the load was adjusted only when the subjects were unable to complete without assistance the required number of repetitions. At all training protocols, the rest interval between exercises was 6 min and the rest interval between sets was similar within each protocol regardless of the number of sets performed at each exercise.

Calculation of total work. All exercises were structured according to the anatomical characteristics of each subject with grip widths and positions marked and kept constant for each exercise throughout the study. Lifting work was calculated as the weight load \times the vertical distance moved per repetition \times the number of repetitions. The weights of the body segments of the subjects \times the vertical distance of the center of gravity of the body segments, which were moved, were also included in the calculations (Table 1). The location of body segments centers of gravity and the estimation of body segment weights from the total body weight were assessed with the use of anthropometrics tables (34). The distances were obtained from measurements with the subjects and the equipment in the starting and ending exercise positions.

Experimental Protocol

The subjects reported in the laboratory at 9:00 a.m. or at 11:30 a.m. after an overnight fast. Subjects avoided caffeine and alcohol consumption for 24 h and did not perform physical exercise for 48 h before the experimental sessions. To avoid the effects of the circadian rhythm on hormonal concentrations, each subject performed the experimental sessions at the same time of day. The time of day that each exercise session began was adjusted, so that the 30-min recovery period was approximately similar for all experimental sessions when blood samples were obtained. Before each session, the subjects rested for 15 min in the supine position, and then a preexercise blood sample was drawn via an indwelling venous catheter placed into an antecubital vein. Blood samples were also drawn immediately after exercise, as well as at 15 min and at 30 min after exercise for the determination of lactate, testosterone, cortisol, and

hGH concentrations. All blood samples were drawn with the subjects in the supine position. In the control session, the same procedure was followed. The subjects did not perform any exercise protocol but sat passively for 60 min while blood samples were obtained at the same time points as in the experimental exercise sessions.

Before the start of the exercise protocols, stretching exercises for the muscle groups activated with selected lifts were performed for 10 min. Before each lift, subjects performed a warm-up set with 80% of the repetitions and the resistance used in the respective exercise protocol. The duration of the exercise protocols varied depending on the number of sets performed at each exercise. When two sets were performed, the duration was 30–35 min; when four sets were performed, 50–65 min; and when six sets were performed, 80–90 min. To complete the required number of repetitions, some assistance was provided during the last repetition of the strength protocol, the last two repetitions of the hypertrophy protocol and the last three repetitions of the strength endurance protocol without, however, removing the weight so that the subjects would exert maximum effort. During the training workouts and the recovery periods, subjects were allowed to drink water *ad libitum*.

Blood Analyses

Ten milliliters were drawn at each sampling time; 200 μL of this whole blood were immediately added to 400- μL trichloroacetic acid and centrifuged at 2500 rpm for 15 min. The supernatant was removed and frozen in -80°C until later analyzed for lactate concentrations using an enzymatic method (procedure no. 826-UV, Sigma Chemical Co., St Louis, MO). Blood was analyzed for hemoglobin using the cyanmethemoglobin method (procedure No. 525-A, Sigma Chemical Co.) and for hematocrit by the microcapillary technique. Percent changes in plasma volume were calculated using the hematocrit and hemoglobin values according to Dill and Costill (5). The remaining blood was centrifuged at 2500 rpm for 15 min. Serum was removed, separated into aliquots, and frozen at -80°C until analyzed. Serum was analyzed by luminescence immunoassay (LIA) for testosterone with assay sensitivity $< 0.03 \text{ nmol}\cdot\text{L}^{-1}$ and intra- and inter-assay coefficient of variability (CV) of 2.5% and 4.8%, respectively (Ortho-Clinical Diagnostics, Johnson & Johnson Inc., Rochester NY), and for cortisol with assay sensitivity $< 3 \text{ nmol}\cdot\text{L}^{-1}$ and intra- and inter-assay CV of 3.75% and 5.9%, respectively (Ortho-Clinical Diagnostics). Serum was analyzed by immunoradiometric assay for hGH with assay sensitivity $0.09 \mu\text{g}\cdot\text{L}^{-1}$ and intra- and inter-assay CV of 3.9% and 5.2%, respectively (Medicorp Inc., Montreal, Canada).

Statistical Analyses

A two-way ANOVA (exercise protocol \times time) with repeated measures in both factors was used to examine a) the effects of the number of sets in the MS, MH, and SE protocols; and b) the differences among the three protocols in the hormonal concentrations at the various time points.

The effects of the number of sets were examined with a separate analysis for each protocol, and three separate analyses were also carried out, for the performance of 2, 4, and 6 sets at each exercise, in order to examine the differences among the training protocols. Significant differences between means were located with the Tukey HSD procedure. The significance level was set at $P < 0.05$.

RESULTS

Blood Lactate

Effects of the number of sets. Maximum strength protocol. There were no differences in lactate concentrations among the performance of 2, 4, or 6 sets ($P > 0.05$; Fig. 1A). Blood lactate concentrations were higher ($P < 0.05$) throughout the recovery period in comparison with control session regardless of the number of sets performed.

Muscular hypertrophy protocol. When four sets were executed, lactate concentrations were higher ($P < 0.05$) during the recovery period compared to the execution of two and six sets (Fig. 1B). No differences ($P > 0.05$) were found between the performance of two and six sets. After all exercise sessions, lactate concentrations were higher ($P < 0.05$) during the recovery compared with control session.

Strength endurance protocol. No differences were observed between the two- and four-set sessions in lactate concentrations ($P > 0.05$; Fig. 1C). Lactate was higher ($P < 0.05$) after both the two- and four-set exercise sessions compared with the control session.

Effects of resistance exercise protocol. Lactate concentrations, whether 2, 4, or 6 sets were performed, were higher ($P < 0.05$) after the MH and SE protocols than after the MS protocol, at all postexercise time points (Fig. 2). When two sets were performed, lactate was higher ($P < 0.05$) after the SE workout than the MH workout at all postexercise time points. However, after the execution of four sets, the highest lactate increase ($P < 0.05$) occurred after the SE workout only immediately after exercise (Fig. 2B).

Serum Testosterone

Effects of the number of sets. Maximum strength protocol. No differences ($P > 0.05$) were observed in testosterone concentrations whether 2, 4, or 6 sets were performed (Fig. 3A). Testosterone concentrations did not differ ($P > 0.05$) between the three exercise conditions and the control session at any time point.

Muscular hypertrophy protocol. Testosterone concentrations did not differ ($P > 0.05$) among the performance of 2, 4, or 6 sets (Fig. 3B). No differences were observed ($P > 0.05$) between the three exercise conditions and the control session at any time point. When four sets were performed, testosterone concentrations were higher immediately after exercise compared with before exercise values.

Strength endurance protocol. No differences were found between the performance of two and four sets in testosterone concentrations ($P > 0.05$). Although testosterone was

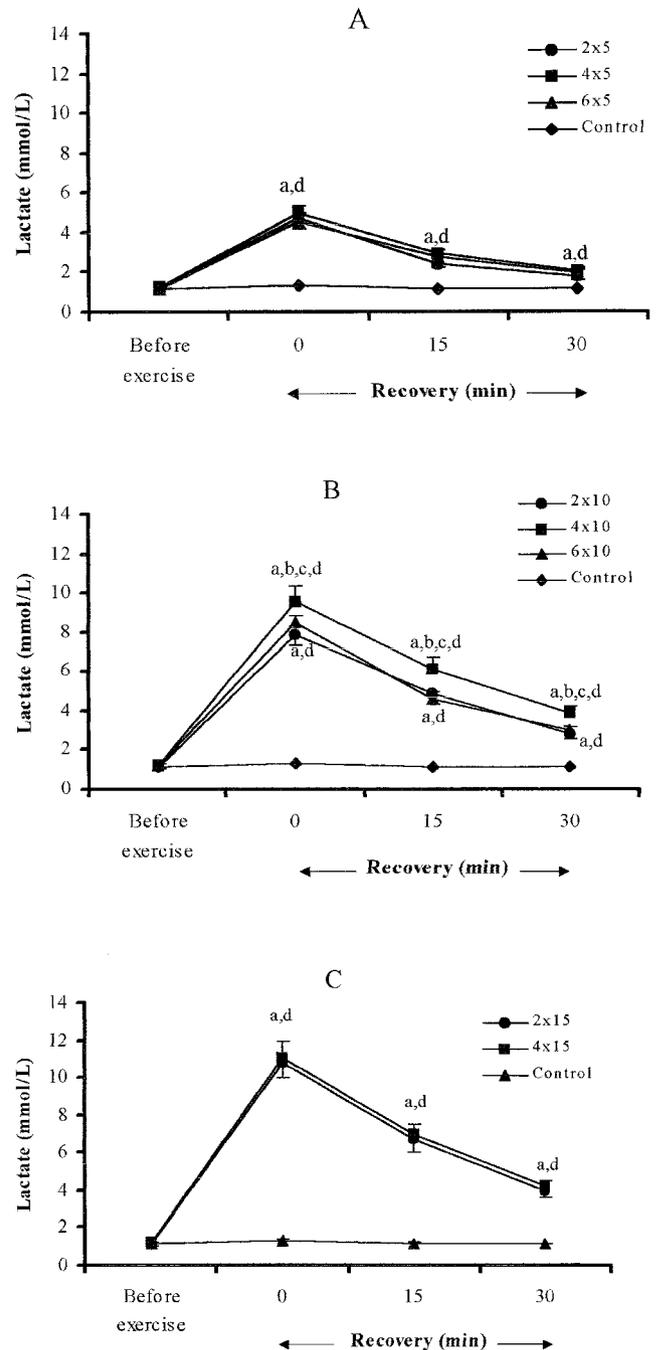


FIGURE 1—Lactate concentrations ($\bar{x} \pm SE$) when 2, 4, and 6 sets were performed at each exercise in a maximum strength (A), muscular hypertrophy (B), and strength endurance (C) resistance exercise protocol and a control session. Note: a, $P < 0.05$ from corresponding control session value; b, $P < 0.05$ from corresponding two-set values; c, $P < 0.05$ from corresponding six-set values; d, $P < 0.05$ from corresponding before exercise value.

higher after the exercise sessions compared with the control session, during the recovery period, no significant differences were observed. When four sets were performed, testosterone concentrations were higher ($P < 0.05$) immediately after exercise compared with the values before exercise (Fig. 3C).

Effects of resistance exercise protocol. The MS, MH, and the SE protocols did not differ ($P > 0.05$) in

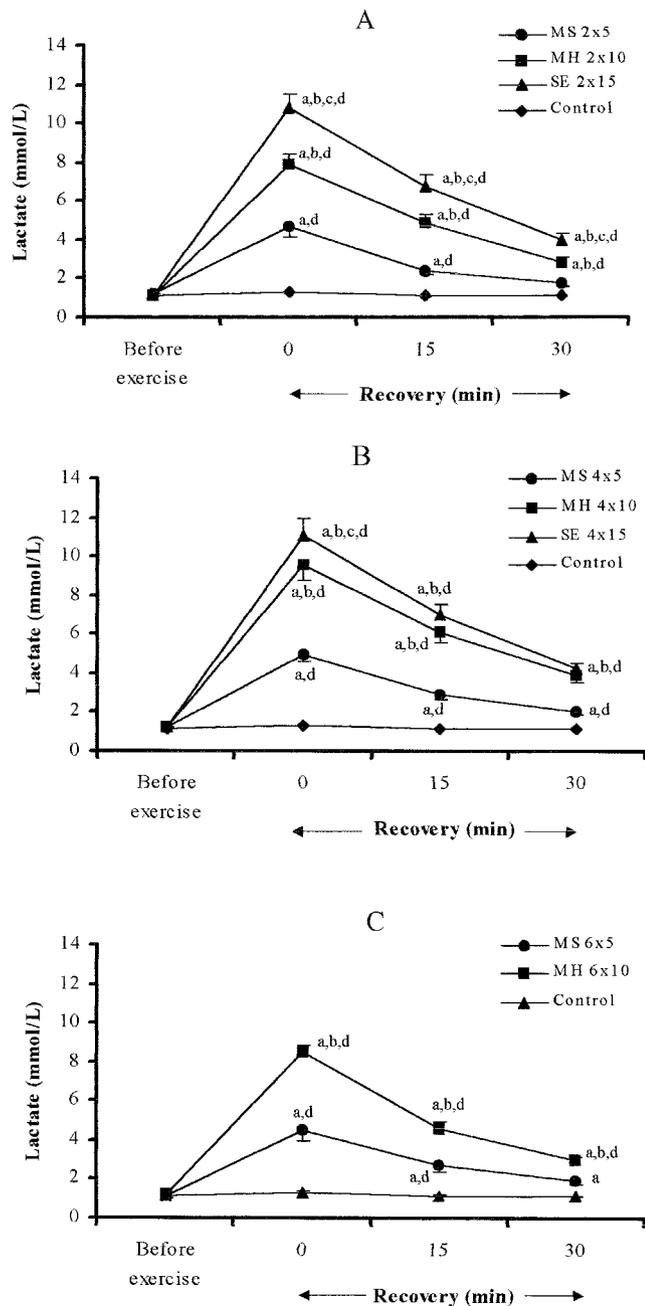


FIGURE 2—Lactate concentrations ($\bar{x} \pm SE$) after a maximum strength (MS), muscular hypertrophy (MH), and strength endurance (SE) resistance exercise protocol when 2 sets (A), 4 sets (B), and 6 sets (C) were performed at each exercise and a control session. Note: a, $P < 0.05$ from corresponding control session value; b, $P < 0.05$ from corresponding maximum strength protocol value; c, $P < 0.05$ from corresponding muscular hypertrophy protocol value; d, $P < 0.05$ from before exercise value.

testosterone response at any time point in the immediate recovery period whether 2, 4, or 6 sets were performed at each exercise (Fig. 4).

Serum hGH

Effects of the number of sets. *Maximum strength protocol.* When four sets were performed, hGH concentrations were higher ($P < 0.05$) immediately after exercise

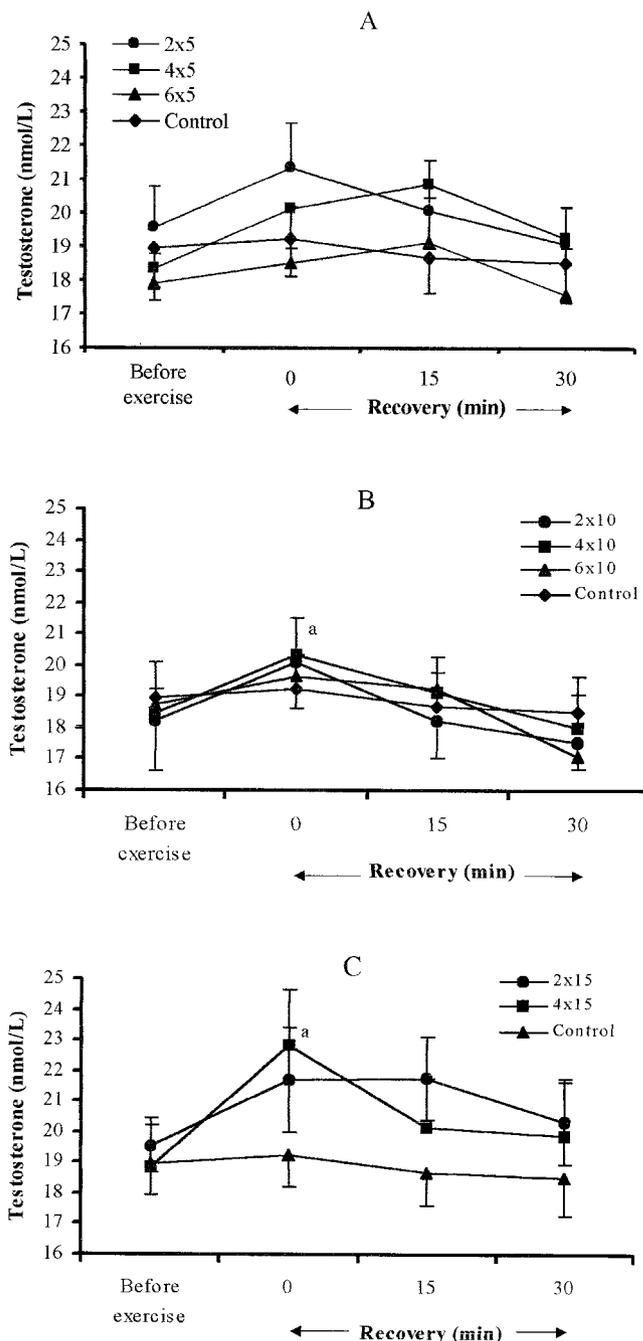


FIGURE 3—Testosterone concentrations ($\bar{x} \pm SE$) when 2, 4, and 6 sets were performed at each exercise in a maximum strength (A), muscular hypertrophy (B), and strength endurance (C) resistance exercise protocol and a control session. Note: a, $P < 0.05$ from before exercise value.

than when two sets were performed and at 15 min of recovery than when six sets were performed. hGH concentrations were higher ($P < 0.05$) during the whole recovery period after the performance of two and four sets and during the first 15 min of the recovery after six sets compared with the control session (Fig. 5A).

Muscular hypertrophy protocol. The concentrations of hGH after the four- and the six-set sessions were higher ($P < 0.05$) in the first 15 min of recovery compared with the two-set session (Fig. 5B). No differences ($P > 0.05$) were

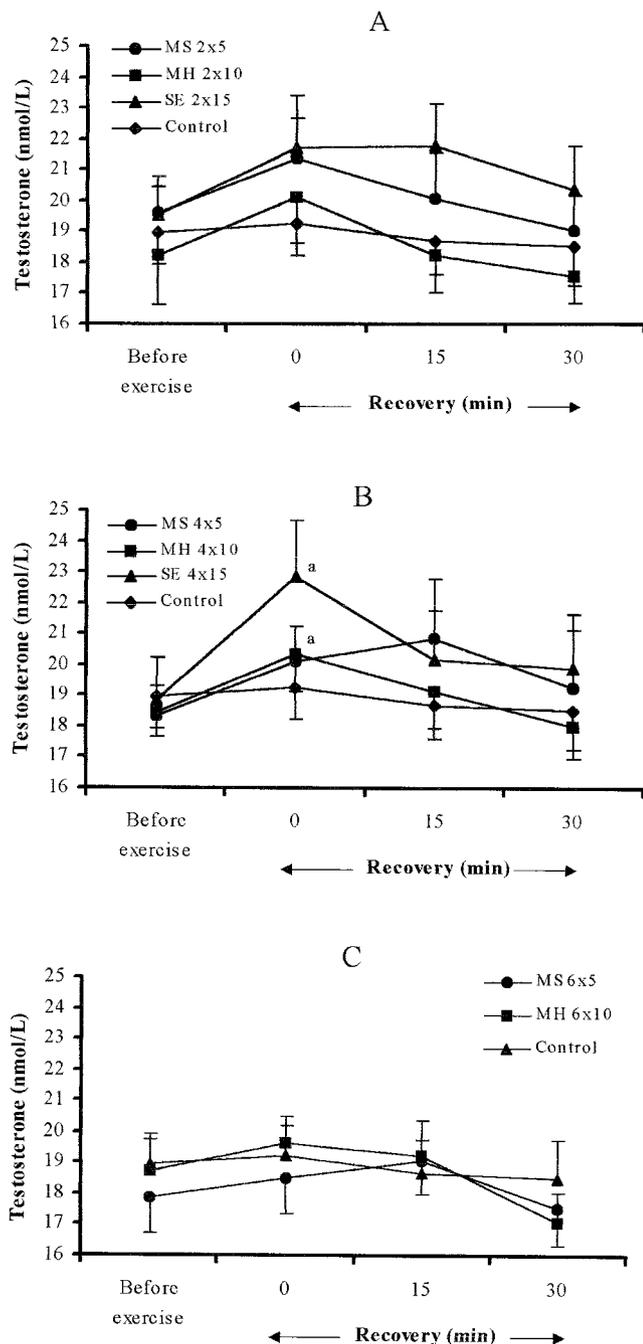


FIGURE 4—Testosterone concentrations ($\bar{x} \pm SE$) after a maximum strength (MS), muscular hypertrophy (MH), and strength endurance (SE) resistance exercise protocol when 2 sets (A), 4 sets (B), and 6 sets (C) were performed at each exercise and a control session. Note: a, $P < 0.05$ from corresponding control session value.

observed between the four- and six-set sessions. Compared to the control session, hGH concentrations were higher ($P < 0.05$) immediately after exercise with the performance of two sets, during the whole recovery period with four sets and during the first 15 min of recovery with six sets.

Strength endurance protocol. In the SE protocol, hGH concentrations were higher ($P < 0.05$) immediately after exercise after performing four sets than two sets, whereas at 15 min, the difference between the two sessions was not quite significant ($P = 0.06$). Both exercise sessions yielded

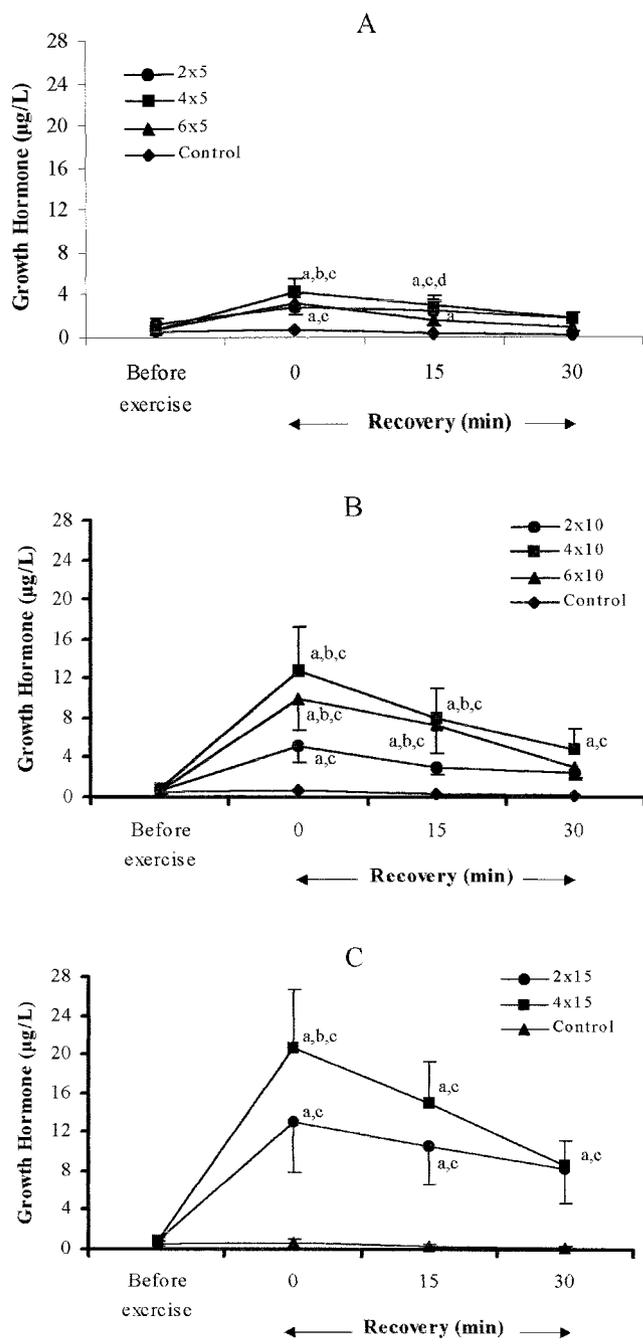


FIGURE 5—hGH concentrations ($\bar{x} \pm SE$) when 2, 4, and 6 sets were performed at each exercise in a maximum strength (A), muscular hypertrophy (B), and strength endurance (C) resistance exercise protocol and a control session. Note: a, $P < 0.05$ from corresponding control session value; b, $P < 0.05$ from corresponding two-set values; c, $P < 0.05$ from corresponding before exercise value; d, $P < 0.05$ from corresponding six-set values.

higher hGH concentrations compared with the control session at all postexercise time points (Fig. 5C).

Effects of resistance exercise protocol. Two-set sessions. hGH concentrations were higher ($P < 0.05$) after the SE compared with the maximum strength and muscular hypertrophy protocols (Fig. 4A). The MS and the MH workouts did not yield any significant differences in hGH throughout the measurements (Fig. 6A).

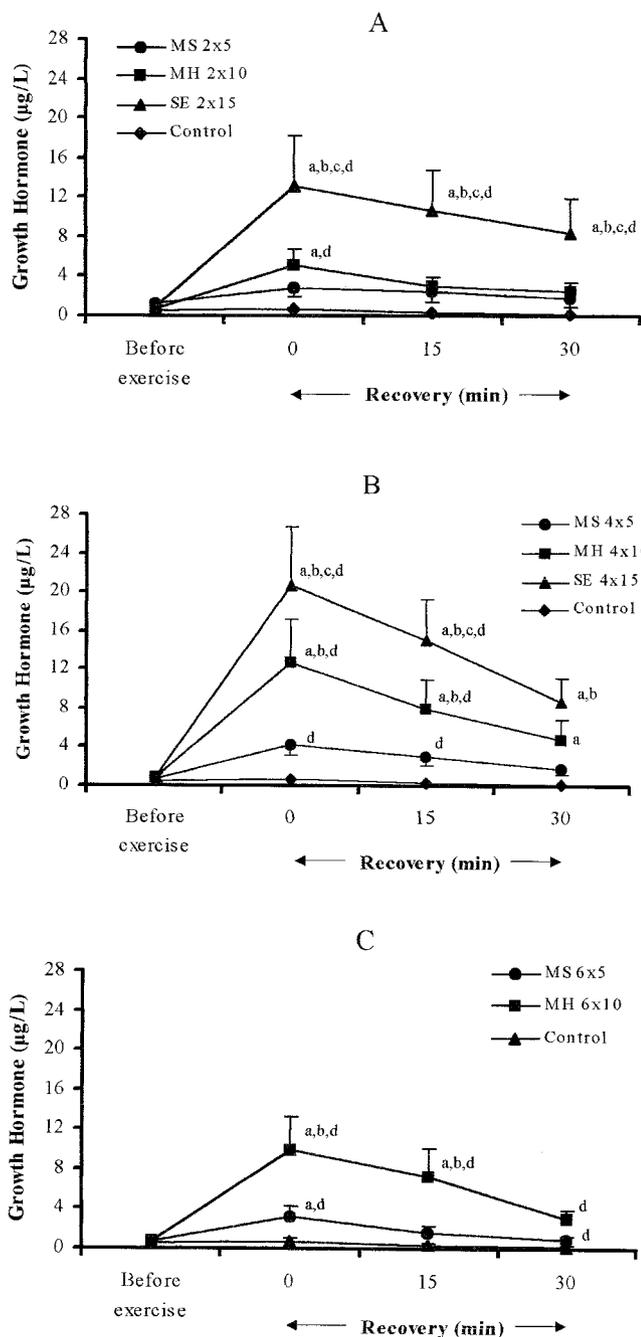


FIGURE 6—hGH concentrations ($\bar{x} \pm SE$) after a maximum strength (MS), muscular hypertrophy (MH), and strength endurance (SE) resistance exercise protocol when 2 sets (A), 4 sets (B), and 6 sets (C) were performed at each exercise and a control session. Note: a, $P < 0.05$ from corresponding control session value; b, $P < 0.05$ from corresponding maximum strength protocol value; c, $P < 0.05$ from corresponding muscular hypertrophy protocol value; d, $P < 0.05$ from before exercise value.

Four-set sessions. hGH concentrations were higher ($P < 0.05$) after the MH and SE workouts during the 30-min recovery period compared with the MS workout (Fig. 4B). Furthermore, hGH concentrations in the first 15 min of recovery were higher ($P < 0.05$) after the SE protocol than after the MH protocol (Fig. 6B).

Six-set sessions. hGH concentrations in the first 15 min of recovery were higher ($P < 0.05$) after the hypertrophy

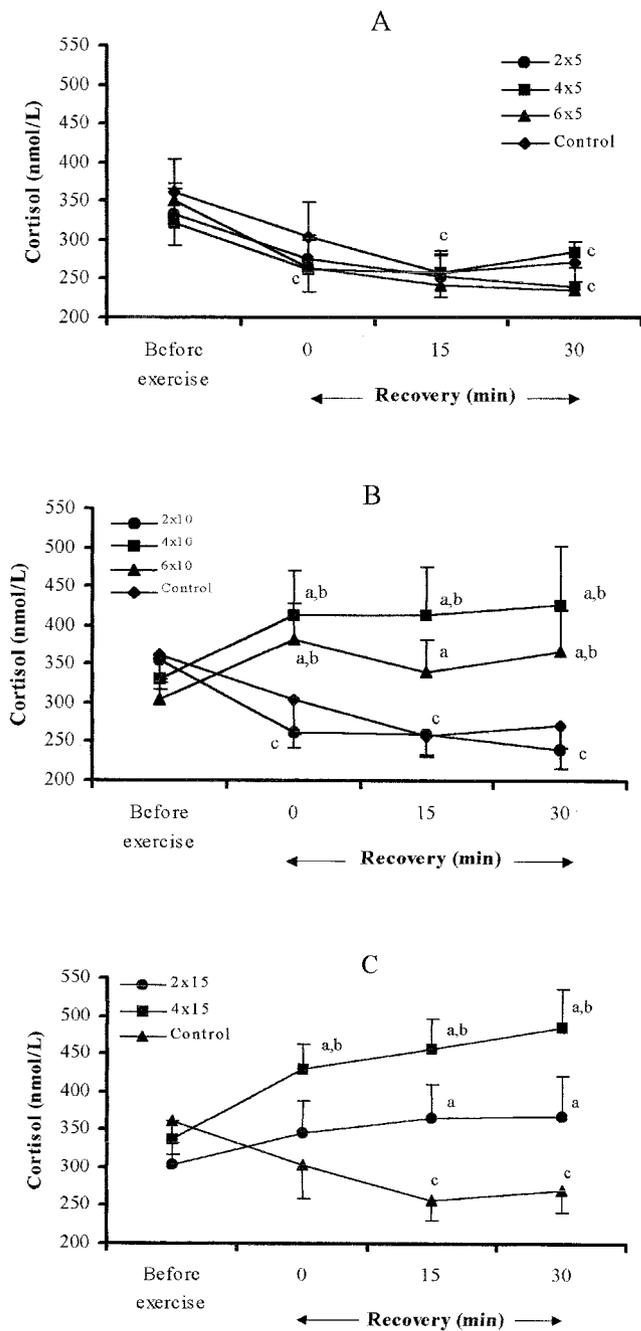


FIGURE 7—Cortisol concentrations ($\bar{x} \pm SE$) when 2, 4, and 6 sets were performed at each exercise in a maximum strength (A), muscular hypertrophy (B), and strength endurance (C) resistance exercise protocol and a control session. Note: a, $P < 0.05$ from corresponding control session value; b, $P < 0.05$ from corresponding two-set values; c $P < 0.05$ from corresponding before exercise value.

protocol compared with the maximum strength protocol and the control session (Fig. 6C).

Serum Cortisol

Effects of the number of sets. *Maximum strength protocol.* The number of sets did not affect cortisol concentrations. After all exercise sessions, cortisol decreased significantly in the recovery period compared with the concen-

trations before exercise. However, no exercise session differed from the control session ($P > 0.05$; Fig. 7A).

Muscular hypertrophy protocol. Cortisol concentrations were higher after the performance of four and six sets compared with the performance of two sets and the control session ($P < 0.05$; Fig. 7B). No differences ($P > 0.05$) were observed between the four- and six-set sessions as well as between the two-set and control sessions.

Strength endurance protocol. After the performance of four sets, cortisol concentrations were higher than those observed after the two sets and the control sessions ($P < 0.05$; Fig. 7C). Cortisol at the 15 min and at the 30 min of recovery was higher ($P < 0.05$) after the two-set session compared with the control session.

Effects of resistance exercise protocol. Two-set sessions. Cortisol concentrations were higher ($P < 0.05$) after the SE protocol than the MS and MH protocols (Fig. 8A). No differences ($P > 0.05$) were observed between the MS and MH workouts.

Four-set sessions. Serum cortisol concentrations were higher ($P < 0.05$) after the muscular hypertrophy and strength endurance workouts than after the maximum strength workout. No differences ($P > 0.05$) were observed in cortisol values between the MH and the SE protocols (Fig. 8B).

Six-set sessions. Cortisol concentrations after the muscular hypertrophy workout were higher ($P < 0.05$) than the maximum strength workout and the control session throughout the recovery period (Fig. 8C).

Plasma volume decreased during all resistance exercise protocols. The greatest changes were observed pre- to postexercise and were as follows (means \pm SD): maximum strength protocol: 8.49 ± 6.5 , 8.3 ± 2.69 , and $9.08 \pm 3.71\%$ when 2, 4, and 6 sets were performed, respectively; muscular hypertrophy protocol: 12.33 ± 5.17 , 12.41 ± 4.67 , and $9.87 \pm 2.85\%$, when 2, 4, and 6 sets were performed, respectively; strength endurance protocol: 15.7 ± 5.18 and $14.23 \pm 3.48\%$ when two and four sets were performed, respectively. Corrected and uncorrected data for plasma volume changes yielded similar results. Therefore, we have decided to present the uncorrected data because target tissues are exposed to absolute hormone concentrations.

DISCUSSION

The results of this study indicate that an increase in the number of sets performed at each exercise induces higher hGH and cortisol responses in a hypertrophy and a strength endurance protocol but does not affect acute hormonal responses after a strength protocol. In a hypertrophy workout, a limit exists in the number of sets that induce higher hormonal responses. The specific configuration of the program variables causes distinct hGH and cortisol responses among the three workouts. The variation in training volume by manipulating the number of sets affects the differences in hGH and cortisol among the training protocols.

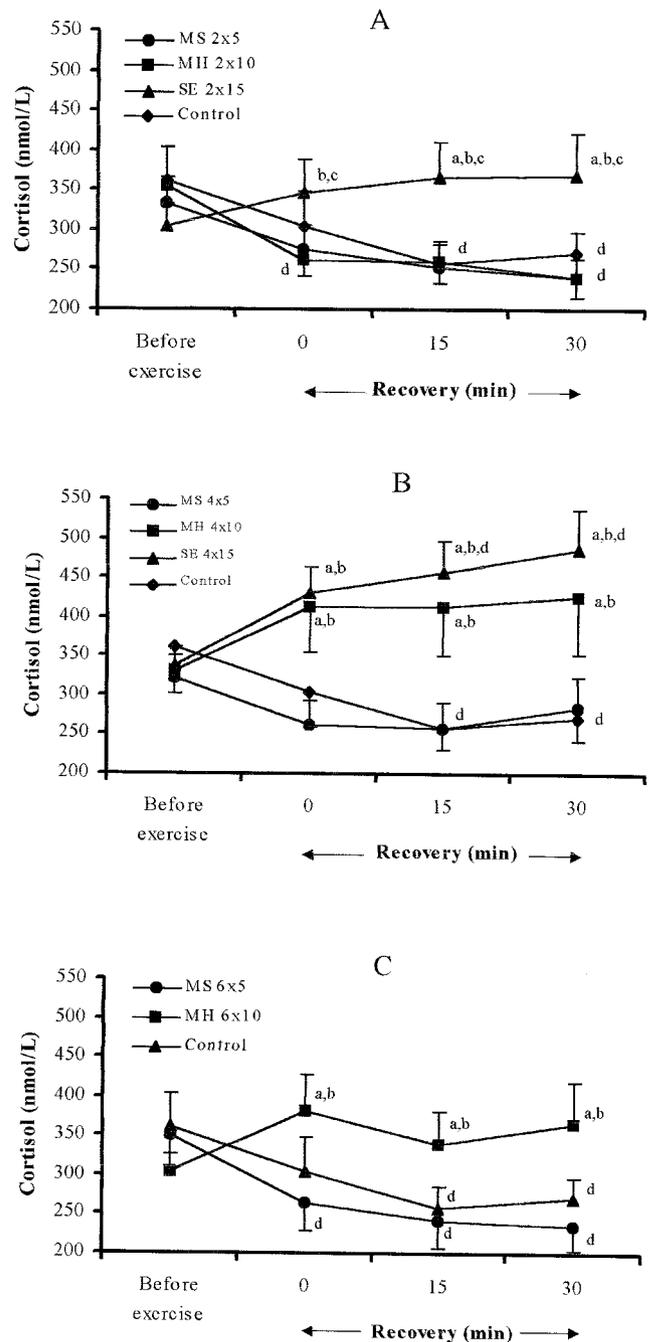


FIGURE 8—Cortisol concentrations ($\bar{x} \pm$ SE) after a maximum strength (MS), muscular hypertrophy (MH), and strength endurance (SE) resistance exercise protocol when 2 sets (A), 4 sets (B), and 6 sets (C) were performed at each exercise and a control session. Note: a, $P < 0.05$ from corresponding control session value; b, $P < 0.05$ from corresponding maximum strength protocol value; c, $P < 0.05$ from corresponding muscular hypertrophy protocol value; d, $P < 0.05$ from before exercise value.

Number of sets and maximum strength protocol.

A high number of sets may be used to optimize strength development after a heavy-load–low-repetition strength protocol (8). However, our results indicate that these adaptations may not be due to higher hormonal concentrations. Total work was doubled and tripled, but this had no effect on testosterone, hGH, and cortisol increases. In a strength protocol, an increase in total work does not seem to be a

stimulus for higher hormonal concentrations. Nevertheless, increased activation of intracellular mechanisms for tissue growth or an increase in maximal neural activation of the muscles (14), via the application of mechanical stress for a longer period of time, with a high number of sets in a strength protocol cannot be excluded.

Number of sets and muscular hypertrophy protocol. In agreement with other studies (11,30), a total work effect was observed with the hypertrophy protocol. The performance of four sets at each exercise caused higher hGH and cortisol responses than the performance of two sets, whereas no effect was observed on testosterone concentrations. These hormonal responses may contribute to the higher increases in strength and lean body mass and decreases in percent body fat observed after the long-term use of a multiple set as compared with a single-set program (26). Nevertheless, the increase in hGH concentrations and the absence of a cortisol response, when two sets were performed, reveal a hormonal environment that favors anabolic processes within the muscle. In the long term, these responses may contribute to the positive adaptations in body composition and strength capacities with low-set protocols during the early phase of resistance training (16,26). It was worth noting that when four sets were applied, both hGH and cortisol were increased. The magnitude, however, of hGH response was much larger than that of cortisol and this may compensate for the negative effect of cortisol on protein metabolism (29).

The present study reveals a limit where the increase in the number of sets, in a hypertrophy protocol, induces higher hGH and cortisol responses. When six sets were executed at each exercise, hormonal responses were not larger from those observed after the execution of four sets. It appears that a very high number of sets may not create a more favorable hormonal environment for muscular adaptations. This finding is important for designing a resistance-training program because it could prevent increased training stress. It should be mentioned, however, that hormonal influences are only one of the factors for the development of muscle strength after resistance training. Other neural or muscular factors may be affected positively with a high number of sets. Another factor that may influence the interaction between the number of sets and hormonal responses is the training status of the individual. Multiple-set protocols are more effective for well-trained athletes (21), muscle protein turnover is reduced after resistance exercise in trained people (33), and hormonal responses are influenced by the training status of the individual (9). Therefore, it would be important to determine whether there is also a point where an increase in the number of sets fails to cause higher hormonal responses in well-trained athletes as it was observed in our recreational lifters who did not train for any sport.

Number of sets and strength endurance protocol. The strength endurance protocol, in the present study, was stressful to increase hGH and cortisol and failed to alter testosterone significantly although the latter was consistently higher compared with the control session. The rise of

hGH and cortisol concentrations may contribute to the regulation of glucose and glycogen metabolism, which was highly activated as it shown by the lactate data. These hormonal responses may also contribute to muscle tissue adaptations after long-term training. Previous studies showed that high-repetition–low-resistance training increased muscle mass (17). Furthermore, we also observed a significant volume effect on hormonal responses in the strength endurance protocol. The doubling of sets from two to four caused higher hGH and cortisol concentrations. Based on these hormonal responses, it could be hypothesized that the use of a moderate number of sets (3–4) would be more effective for neuromuscular adaptations than the use of a low (1–2) number of sets. Yet, in the present study, even the use of two sets induced a high activation of anaerobic metabolism and augmented hormonal responses indicating sufficient stress for adaptations after long-term training with a strength endurance protocol.

Hormonal responses in various protocols. The different configuration of the program variables of the resistance exercise protocols imposed a specific activation pattern in neuromuscular and metabolic processes. This in turn caused certain hormonal changes related to the total work of the acute exercise stress. Cortisol and hGH were higher after the hypertrophy workout than the strength workout when a moderate (four sets) and a high (six sets) number of sets were performed, whereas no differences were observed after the performance of two sets. It appears that with a low number of sets, similar long-term adaptations may be observed in the muscle tissue with the use of both protocols. A large amount of total work must be performed with a hypertrophy protocol in order to produce distinct hormonal responses and lead to different muscular adaptations from a strength protocol. Furthermore, cortisol and hGH were higher after the strength endurance workout as compared with the strength and hypertrophy workouts. However, when four sets were performed at each exercise, the hypertrophy and the strength endurance protocols did not differ with each other in cortisol concentrations despite the difference in total work (11,745 J) and the shorter rest interval (2 vs 1 min) in the latter protocol. The higher load used in the hypertrophy protocol may compensate for the higher total work and the shorter rest interval used in the strength endurance protocol. Thus, total exercise stress in the two protocols may be the reason for similar cortisol responses. It is likely that cortisol responses to resistance exercise depend on metabolic requirements and total exercise stress.

It is interesting to note that hGH concentrations were higher after the strength endurance protocol than after the hypertrophy protocol. The hGH is characterized by various metabolic actions (e.g., glycogenesis, synthesis of contractile or sarcoplasmic and mitochondrial proteins) that are probably dictated by the cellular needs depending on the exercise workout. Differences in the intensity of tension, as a function of the applied load, and the amount of tension, as a function of total work, imposed on the muscle may cause specific intracellular needs, which may differentiate hor-

monal actions within the cell. This may occur despite the similar or different hormonal concentrations in the circulation. For example, high tension applied for a short time, with low activation of anaerobic metabolism, in a strength protocol may activate intracellular mechanisms for muscle growth despite the low hormonal concentrations in the circulation. In the strength endurance protocol, the low tension applied for an extended period of time caused a high activation of anaerobic metabolism and so the hormonal responses may activate the process related more to the restoration of energy substrates. Furthermore, differences in muscle tissue activation and tissue damage with each workout may vary the acute and overnight hormonal responses and the functions within the muscle cells for energy provision, tissue regeneration, and overcompensation with new muscle tissue during the recovery period (22,28,31,32). Studies have shown that resting hormonal concentrations may change with resistance training using maximal and submaximal loads (13,26). However, no comparisons have been made among various resistance exercise protocols in the adaptations they induce in resting hormonal concentrations.

Testosterone concentrations did not change with any workout used in the present study compared with the control session. Similarly, other studies did not observe an effect of resistance exercise on testosterone concentrations (27,32), whereas others found significant increases (11,24). Testosterone concentrations have been even found to be suppressed (32) for 13 h after a stressful resistance exercise protocol. The different configuration of the program variables, the amount of muscle mass activated, the inclusion of a control session in the experimental design, and the individual variability in testosterone response may explain part of the differences observed in the above studies. Previous research has shown that a large difference in total work may differentiate testosterone responses between a strength and a hypertrophy protocol (15). However, when total work of both a strength and a hypertrophy workout was high (>30,000 J), no differences were observed in testosterone (24,25). Similarly, in most workouts of the present study total work was above 30,000 J, and no differences were observed in testosterone. Undoubtedly, differences could be observed above 30,000 J among protocols in testosterone, but it appears that when total work is high, other training variables (e.g., rest interval) may influence more testosterone responses. It seems that the exercise stimulus of the different protocols stimulates the hypophysis–Leyding cells axis, which controls testosterone secretion in the same mag-

nitude. However, differences in testosterone interaction with androgen receptors when using various workouts cannot be excluded.

Our purpose was to compare the hormonal responses among three different protocols, just as they were used in practice. No attempt was taken for research purposes to equate these protocols to total work or to have similar resting periods between sets throughout protocols. It should be mentioned, however, that a predetermined number of repetitions and resting periods had to be selected for each protocol although a range of repetitions or rest intervals could be used for each protocol (8). Both of these factors could cause different hormonal responses, and future research could examine these modifications.

Nevertheless, the intracellular processes activated by exercise determine the adaptations in the tissues. Resistance exercise increases muscle protein turnover (33) through the activation of molecular and cellular mechanisms (12). To our knowledge, it is unknown how different resistance exercise routines affect the above processes within the muscle cell. Moreover, despite the differences observed among the various resistance exercise protocols in the acute responses of circulating hormones, it is the interactions of the hormones with the receptors that contribute to these intracellular processes. Studies on the effects of the number of sets in various resistance exercise workouts on hormone-receptor interactions, the number of receptors in the muscle, and overnight hormonal concentrations will give further insight in the adaptations observed with resistance exercise.

In conclusion, the number of sets does not affect the hormonal responses observed after a maximum strength, muscular hypertrophy, and strength endurance resistance exercise protocol in a similar manner. There appears to be a limit above which an increase in the number of sets does not elicit higher hormonal concentrations. Furthermore, these different resistance exercise protocols produce distinct hormonal response patterns depending on the number of sets performed at each exercise. These results should be taken into account when trying to optimize the effectiveness of resistance training or to induce specific adaptations in the neuromuscular system with the use of different resistance training protocols.

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