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Original Research

Influence of Resistance Training Exercise Order on Acute Thyroid Hormone Responses

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ABSTRACT

International Journal of Exercise Science 15(2): 760-770, 2022. The present study aimed to compare the exercise order of an acute bout of resistance exercise (RT) on acute thyroid hormonal responses. Eight (n = 8) healthy men were randomly separated into two experimental groups: A) the order from multi- to single-joint exercises (MJ-SJ) and B) the order from single- to multijoint exercises (SJ-MJ). For all exercises in both orders, the subjects were submitted to 3 sets of 10 repetitions, with rest intervals of 2 minutes between sets and 3 minutes between exercises. Blood samples were collected at rest and 0, 15, 30, 60 and 120 min after the end of the exercise session. In thyroid-stimulating hormone (TSH), differences between groups (MJ-SJ < SJ-MJ) were observed within 15 minutes after the session. In 3,5,3'-triiodothyronine (T3), differences between groups were observed between 30 (MJ-SJ > SJ-MJ) and 120 minutes (MJ-SJ < SJ-MJ) after the session. In 3,5,3',5'-tetraiodothyronine (T4), differences between groups (MJ-SJ > SJ-MJ) were observed within 15 minutes after the RT session. The order of RT exercises significantly changes the hormonal responses of TSH, T3 and T4. In addition, the exercise order should be chosen according to the individual's objectives.

KEY WORDS: Strength training, hypothalamus, physiology, endocrinology, thyroid axis.

INTRODUCTION

Resistance training (RT) is a physical exercise popularly used to improve muscle health, change body composition, improve quality of life and well-being, promote motor and functional capacities and prevent and/or treat several diseases (1). According to the American College of Sports Medicine (ACSM)(1), an appropriate RT program will manage the variables volume, intensity, exercise selection and order, number of sets and repetitions, frequency, and rest period. Therefore, variation and periodization can limit the overtraining likelihood and increase the ability to realize a developed level of muscular fitness (2). Among the variables, exercise order has been gaining increasing attention since the last ACSM (1) guidelines, which recommended an exercise order that includes large muscles and multijoint exercise at the beginning of the session (24).

An integrative review by Simão et al. (25) showed that the exercise order acutely affected the repetition performance in several sets, indicating that the total number of repetitions (volume) was higher when an exercise was placed at the beginning of a RT session, regardless of the number of joints or the relative amount of muscle mass involved, and the exercises performed at the end of the session were associated with fewer repetitions, regardless of whether the movement involved a small or large muscle group or was a single- (SJ) or multijoint (MJ) exercise. A recent systematic review, Nunes et al. (23) concluded that increases in muscular strength are the largest in the exercises performed at the beginning of an exercise session. For the goal of muscle hypertrophy, their meta-analysis also indicated that both the MJ-SJ and SJ-MJ exercise orders may produce similar results.

Regarding hormone outcomes, the training volume can change with the exercise order and can be affected by the magnitude of acute hormonal responses. Simão et al. (28) demonstrated significant differences in the growth hormone (GH) acute hormonal responses to a RT session performed in different exercise orders. The main conclusion of their study was that the RT increased the GH response after both orders. The relevance of some circulating hormones to RT muscle adaptations is highlighted by findings that suggest that suppression of circulating testosterone concentrations may impair RT-induced hypertrophy in healthy men (17). Additionally, Hansen et al. (16) showed that strength increased more when the RT exercise sessions included acute elevation of anabolic hormones.

Therefore, the effect of the exercise order on the acute hormonal response can help explain differences in strength and hypertrophy when certain exercises are placed at the beginning vs. the end of RT sessions. Due to the lack of studies on exercise order and hormonal responses to RT sessions, the topic has grown in appeal, and many doubts still permeate order-based exercise prescriptions.

Rodrigues et al. (9) observed that the prescription of RT based on SJ or MJ exercises is not supported given the hormonal responses, corroborating previous studies (25, 28). Such studies were carried out only through the analysis of hormones such as GH, total and free testosterone, and cortisol. However, thyroid axis hormones (THs) are fundamental for the interpretation of all metabolic responses, playing a central role in metabolic homeostasis (14). THs are the only hormones that require iodine for their synthesis, and they must be produced at adequate concentrations so that a normal number of other hormones can be produced. THs are formed inside thyroid gland follicles starting from iodine molecules, which must be present at four per molecule of 3,5,3′,5′-tetraiodothyronine (T4) and three per 3,5,3′-triiodothyronine (T3). Both hormones (T3 and T4) are set into the systemic circulation through the action of thyroid-stimulating hormone or thyrotropin (TSH)(3, 5, 10) and are fundamental to the growth and development of several organs and tissues.

Although THs may be altered during exercise and are fundamental to the interpretation of metabolic changes, no studies have analyzed the effect of different exercise orders on TSH, T3 and T4, pointing to a gap in the literature. The present study intends to compare the exercise order of an acute bout of resistance exercise (from MJ to SJ vs. from SJ to MJ) on acute thyroid hormonal responses. Therefore, we hypothesized that both exercise orders can change acutely the THs concentrations.

METHODS

Participants
Eight (n = 8) healthy, trained men were selected (Table 1). Every participant in this study was familiar with the suggested exercises. They have only practiced resistance training and it was recommended the maintenance of their daily habits. The exclusion criteria were history of injury or limited strength, smoking, sedentary lifestyle, and nutritional or pharmacological ergogenic aids usage history. Based on a post-hoc analysis, an "*N*" of 8 individuals was calculated, after having adopted a power of 0.85, α = 0.05, correlation coefficient of 0.5, the Nonsphericity correction of 1 and an effect size of 0.45. It was found, using the *G * Power* 3.1 software, that the sample was sufficient to provide 89.4% of statistical power. This research project was approved by the Research Ethics Committee of the Rio de Janeiro Federal University, based on Resolution Nº 466, of December 12th, 2012 (protocol Nº 3.900.001) in accordance to the ethical standards of the International Journal of Exercise Science (22) and the Declaration of Helsinki (except for registration in a database).

Table 1. Sample descriptive data.

	Age (years)	High (cm)	Mass (kg)	BMI (kg/cm^2)	RTE
Mean	28.75	185.12	87.37	25.5	3
SD	1.56	3.95	3.77	0.25	0.86

BMI = Body Mass Index; RTE = Resistance Training Experience; SD = Standard Deviation.

Protocol
Exercises were performed following two different exercise orders. RTMJ-SJ order started with multijoint exercises and progressed to single-joint exercises (bench press, lat pulldown, shoulder press and triceps extension). The SJ-MJ order started off with single-joint exercise and progressed to multijoint exercises (triceps extension, shoulder press, lat pulldown and bench press). For every exercise in both orders, the individuals were subjected to three sets of 10

repetitions (80% 10RM), with 2-minute rest intervals between sets and 3 minutes between exercises (1). Vein blood samples (5 ml) from the superficial arm veins (vein puncture) were collected on a separate day before any exercise testing began and 0, 15, 30, 60 and 120 minutes after the end of each MJ-SJ or SJ-MJ session. In addition to visual control, the range of motion was delimited by elastic cords.

The 10-RM testing sessions were performed either in the MJ-SJ order or in the SJ-MJ order. The 10-RM testing began with a warm-up set at 50% of the perceived 10-RM load for each exercise. The load was then progressively increased until 10-RM was achieved. The 10-RM was determined in fewer than three attempts, with a rest interval of 5 min between each attempt and 10 min before starting the 10-RM assessment for the next exercise in either sequence. The participants came for a second visit, at which the 10-RM testing was repeated (retest); the highest successful lift was recorded as the 10-RM load (29). All 10-RM testing sessions were supervised by a professional to ensure the correct execution of the RT exercises. To verify the real effect of the order of exercises on hormonal responses and ensure equity of intensity, participants performed the 10-RM test and retest in the order MJ-SJ and then performed the RT session in the MJ-SJ order. Then, they performed the test and retest as well as the RT session in the reverse order (SJ-MJ)(11), according to the flowchart below:

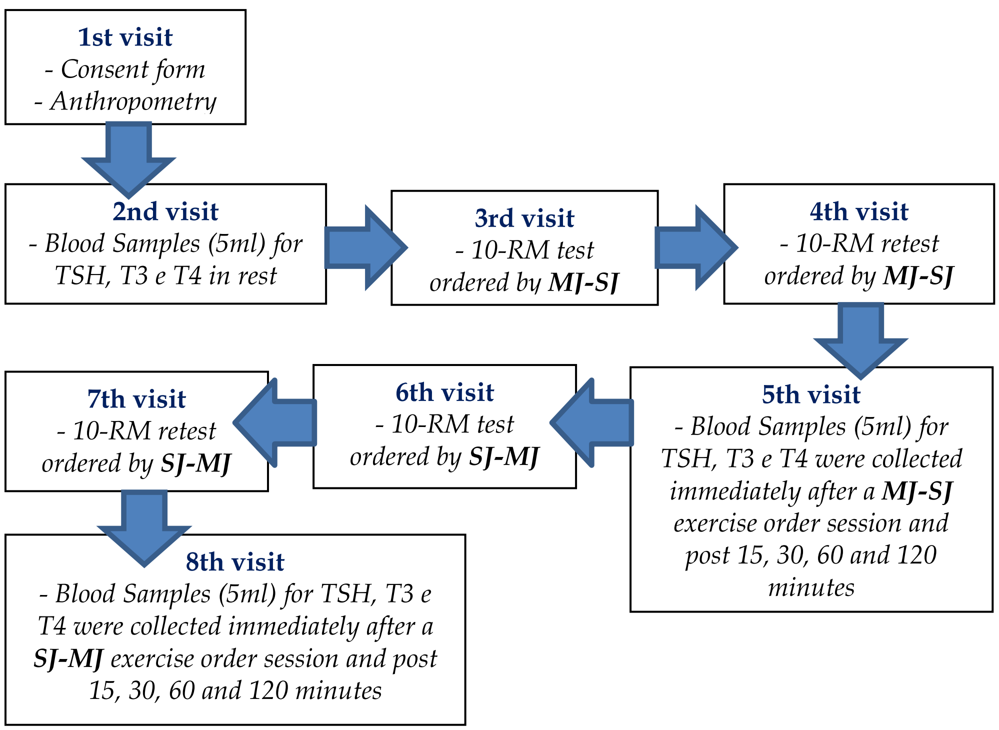

Figure 1. Description flowchart of the visits.

The present study was performed in a total of 8 visits on nonconsecutive days (48h intervals) that always occurred at the same time. The participants reported that they were not using any nutritional or pharmacological ergogenic aids during the study period.

1st visit: Every participant signed the consent form, according to the Helsinki Declaration, after a complete explanation of the study methodology and organization. Next, their body composition was measured by the skinfold test, and their body mass index (BMI) was calculated. Body mass was measured in a platform (*Filizola*) with 0.1 kg precision. Height was measured with a stadiometer (*Cardiomed*) with 0.1 cm precision. All individuals were measured barefooted and wearing swimming trunks.

2nd visit: Subjects returned to the laboratory to supply blood samples (5 ml) for TSH, T3 and T4 analysis after supervision of a 30 min rest period.

3rd visit: Exercise intensity was determined by the trial-and-error method for each participant (ordered by MJ-SJ), considering 10 as the highest repetition charge (10-RM). To determine the load of each exercise, all participants had a maximum of 5 tries of each exercise, with intervals that alternated from 3 to 5 minutes. After each load evaluation, a 10-minute interval was observed between the exercises.

4th visit: After 48 hours, a new 10-RM test was taken (retest) following the pattern adopted during the last visit, in the following order (MJ-SJ): bench press, behind-the-neck lat pulldown, shoulder press and triceps extension. The values presented excellent reproducibility between test and retest (intraclass correlation coefficient (ICC) 0.90-0.99).

5th visit: The subjects were submitted to the MJ-SJ exercise order, and right after the last repetition of the last exercise, blood samples were collected to measure their TSH, T3 and T4 levels. Samples were also collected 15, 30, 60 and 120 minutes after the end of the session.

6th visit: Exercise loads were again determined by the 10-RM test, but this time with the reverse order (SJ-MJ).

7th visit: After 48 hours, subjects took the 10-RM retest following the SJ-MJ exercise order. The values presented excellent reproducibility between test and retest (ICC 0.90-0.99).

8th visit: Subjects were submitted to the SJ-MJ exercise order. Immediately and 15, 30, 60 and 120 minutes after the last repetition of the last exercise, blood was collected to measure TSH, T3 and T4.

All visits occurred in the morning between 8:00 and 10:00, with 48 h between them. Participants were instructed to keep their meals and activities the same. To minimize errors during the exercises, verbal encouraging was adopted, which is a strategy suggested by Simão et al. (29). Equipment mass was confirmed on a platform scale.

Vein blood samples (5 ml) from the superficial arm veins (vein puncture) were collected by a trained nurse wearing sterile surgical gloves with needles and syringes. The collection occurred at the second visit (above) and 0, 15, 30, 60 and 120 minutes after the end of the evaluated exercise session. The environmental temperature was set between 20-25 °C, and the relative humidity was set between 40-65%. The serum was separated by centrifugation at 1500 *g* for 10 min and then stored at −20°C prior to analysis. To evaluate changes in plasma hormone levels, TSH, T3 and T4 serum levels were determined by electrochemiluminescence immunoassay (Elecsys, Roche Diagnostics, GmbH, Manheim, Germany), which was performed following the recommendations of the manufacturer. To quantify hormone concentrations, all samples were ran on one plate.

Statistical Analysis

Initially, a test to evaluate the data normality gathered was done (Shapiro-Wilk test) and a Levene's test for homogeneity of variances. The variables were normally distributed and homogeneous ($p > 0.05$). Additionally, the reliability between 10-RM test and retest sessions was analyzed using a two-factor random effect model intraclass correlation coefficient, and the paired *t* test was run to check the reproducibility of the values between the 10RM test and retest. Next, to compare the mean and standard deviation (SD), we used Two-way ANOVA with repeated measures [condition (MJ-SJ order vs. SJ-MJ order) versus timeline (Baseline vs. after vs. 15min-after vs. 30min-after vs. 60min-after vs. 120min-after)] using Bonferroni's post hoc test. The critical value of significance adopted for every one of the analyses was $p < 0.05$. Statistical analyses were conducted using the statistical software package GraphPad Prism 8.0.

RESULTS

The ANOVA results demonstrated significant interaction (F (5.84) = 4.022; $p = 0.0025$) for TSH results. The intragroup analysis showed significant TSH increases ($p < 0.001$) when compared to the rest levels of TSH after RT in both exercise orders (MJ-SJ and SJ-MJ). Those values remained significantly high ($p < 0.001$) until 15 minutes after the end of the session, as shown in Figure 2. In the intergroup (MJ-SJ vs. SJ-MJ) analysis, significant differences ($p < 0.001$) in the TSH levels 15 minutes after the end of the session were also observed, as shown in Figure 2.

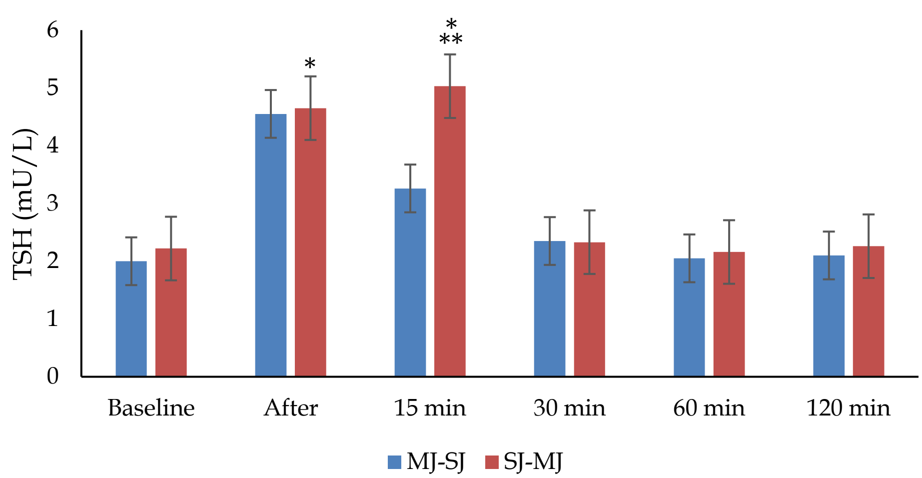

Figure 2. TSH levels after different resistance exercise orders. * Represents significant difference from the respective rest. **Represents significant difference between the orders.

The ANOVA results demonstrated significant interaction (*F* (5.84) = 8.787; $p = 0.0001$) for T4 results. The intragroup analysis showed significant increases ($p < 0.001$) between the rest value of T4 and the T4 level 15 minutes after RT in the MJ-SJ order (Figure 3). However, the intragroup analysis did not show significant differences ($p > 0.05$) in the T4 levels in the SJ-MJ order. In the intergroup (MJ-SJ vs. SJ-MJ) analysis, significant differences ($p < 0.001$) in the T4 levels 15 minutes after the end of the session were also observed, as shown in Figure 3.

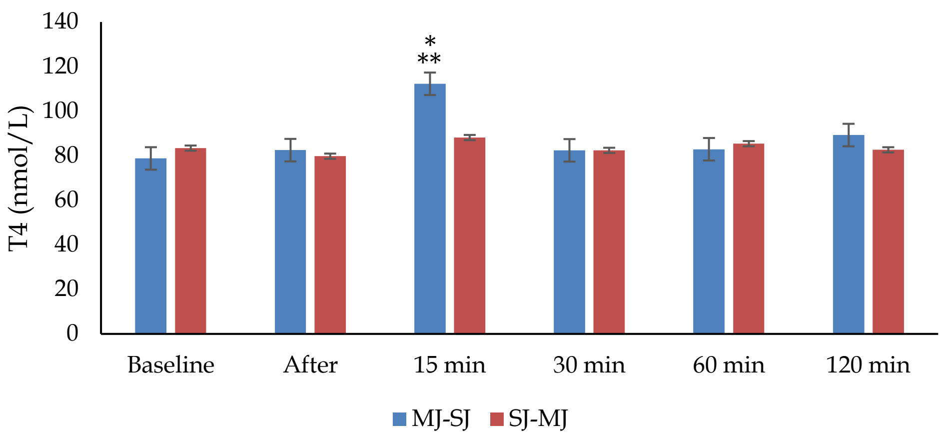

Figure 3. T4 levels after different resistance exercise orders. * Represents significant difference from the respective rest. **Represents significant difference between orders.

The ANOVA results also demonstrated significant interaction (*F* (5.84) = 18.33; $p < 0.0001$) for T3 results. The intragroup analysis showed that the rest value of T3 and the T3 level 30 minutes after RT in the MJ-SJ order ($p < 0.001$) (Figure 4). The results showed that T3 remained significantly high ($p < 0.01$) until 120 minutes after the end of the session, as shown in Figure 4. The SJ-MJ intragroup analysis showed a significant decrease ($p < 0.001$) in T3 at 30 minutes compared to the T3 rest value (Figure 4). The intergroup (MJ-SJ vs. SJ-MJ) analysis showed significant differences ($p < 0.05$) in the T3 levels both 30 and 120 minutes after the end of the session, as shown in Figure 4.

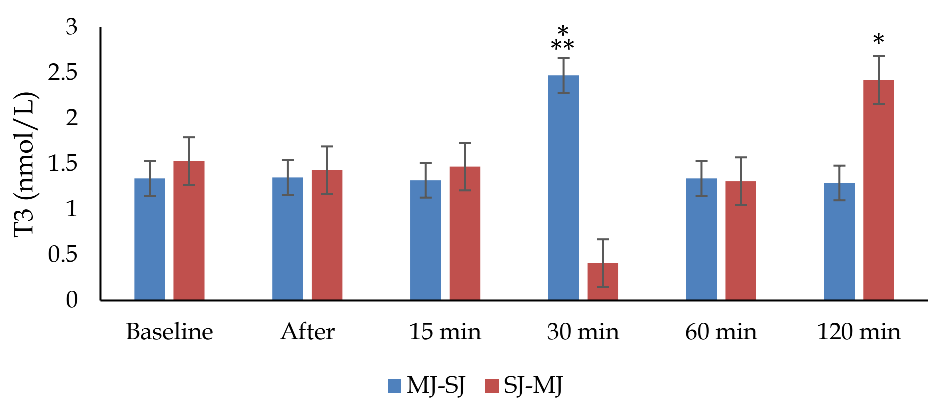

Figure 4. T3 levels after different resistance exercise orders. * Represents significant difference from the respective rest. **Represents significant difference between orders.

DISCUSSION

RT is a potent stimulus of serum hormone levels (13), and those responses may be influenced by the training volume (20), intensity (19), number of sets (15), rest intervals between sets (6) and exercise order (9, 28). This is the first study to verify the influence of different exercise orders on TH levels.

Confirming the initial hypotheses, the main results of the present study showed that exercise order can affect the hormonal responses of TSH, T3 and T4. There was a significant increase in the TSH levels in both groups right after exercise. Our study implemented different exercise protocols in which a TSH increase was found immediately after the end of the exercise. In addition, in this scenario, we observed that the TSH levels were significantly higher when the SJ-MJ order was followed than when the MJ-SJ order was followed 15 minutes after the end of the session. TSH levels are related to metabolic work (10), and as the participants usually trained in the MJ-SJ order, a higher metabolic stress occurred in the SJ-MJ order (9).

Our results showed that T4 levels presented inverted responses, being significantly higher in the MJ-SJ order than the SJ-MJ order 15 minutes after the end of the session. At this time, the TSH levels were higher in the SJ-MJ order. Regarding the T3 levels, although the MJ-SJ order showed higher values 30 minutes after the end of the session, the SJ-MJ order showed high values 120 minutes after the end of the session. It is known that the synthesis and secretion of THs are regulated by a negative feedback system that involves the hypothalamus, hypophysis, and thyroid. Thyrotropin-releasing hormone (TRH) is a tripeptide synthesized in the paraventricular nucleus of the hypothalamus and transported via the axon to the median eminence and then to the anterior pituitary via the portal capillary plexus, where it binds to the TRH receptors of the thyrotropes in the pituitary and then secretes thyrotropin (TSH)(31). TSH interacts with receptors on the thyroid follicular cell membrane, inducing the expression of proteins involved in HT biosynthesis, increasing thyroid cell activity, and stimulating hormone secretion.

Our results partially corroborate other studies in both humans and experimental models (7, 14). However, these results might be directly connected to the negative feedback system and the conversion of thyroid hormones by type I, II and III iodothyronine deiodinase enzymes, because it is well known that most of the circulating T3 comes from the deiodination of T4 by enzymes D1 and D2, which makes us believe that the increase in T3 levels in the MJ-SJ order 30 min after exercise may have occurred due to the conversion of T4 to T3, since at this time T4 levels were normal. According to Fortunato et al. (12), cortisol levels may decrease after exercise, inhibiting deiodinase enzymatic activity. On the other hand, since the SJ-MJ group had increased T3 levels at 120 min after exercise, we believe that in this experimental condition, the increase in T3 production occurred by a direct intrathyroid mechanism, since TSH remained high for longer periods.

To explain this metabolic stress post-RT involving the exercise order, we can highlight two important studies of the Simão group. In the first one, trained men and women were submitted to RT sessions in random orders, and the participants did fewer total repetitions of exercises at the end of the sequence than at the beginning, independent of whether the exercise involved multi- or SJ exercises (26). In the second study, in which women were submitted to two different RT sequences (from smallest to largest muscle groups and from largest to smallest muscle groups), the participants again managed significantly fewer total repetitions of exercises performed later in the sequence, regardless of whether the exercises involved relatively large or small muscle groups (27).

We also believe that the alterations observed in the levels of THs may be related to the activation of the muscle metaboreflex. The activation of this reflex is mediated by the accumulation of metabolites produced during strenuous physical exercise. As a result, there is an increase in sympathetic nervous activity, causing hyperventilation and peripheral vasoconstriction. In addition, the baroreflex deactivation induced by physical activity promotes sympathoexcitation (4, 8, 21). Based on these premises, we believe that an increase in sympathetic activity is capable of activating not only the presympathetic neurons of the paraventricular nucleus but also the TRH-producing neurons present in this hypothalamic region, which are responsible for the activation of the hypothalamic-hypophyseal-thyroid axis. This mechanism would explain the increase in TSH levels right after the end of the activity. This hypothesis is reinforced by the study published by Weltman and collaborators. According to them, exercise-induced growth hormone release is dependent on sympathetic activation (30). Nevertheless, the activation of the muscle metaboreflex and the attenuation of the baroreflex response induced by physical activity explain not only the activation of the hypothalamic-hypophyseal-thyroid axis but also the significant increase in T3 levels. As sympathetic activity is one of the main factors responsible for the increase in D2 activity (18), it is not surprising that T3 levels are increased after physical activity.

Our results emphasized that some variables as control of autonomic nervous system variables, others biochemical indicators, sleep, and diet can influence to some extent the results analysis, becoming for lack of control during the study, limiting factors for inferences.

In summary, we have demonstrated that the order of RT exercises significantly influences the hormonal responses of TSH, T3 and T4 and, despite our data come from a descriptive study; it seems that the hormonal responses to the different session types occur by different pathways. The present findings lead us to believe that although the exercise order is capable of altering hormonal responses, the exercises should be chosen according to the training objectives (not based on the number of joints involved in the exercise).

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