ORIGINAL ARTICLE

Running head: resistance training and body water

Resistance training promotes increase in intracellular hydration in men and women

Authors: Alex Silva Ribeiro[1], Ademar Avelar[1], Brad J. Schoenfeld[2], Raphael Mendes Ritti-Dias[3], Leandro Ricardo Altimari[1], Edilson Serpeloni Cyrino[1]

[1]*Group of Study and Research in Metabolism, Nutrition, and Exercise. Londrina State University. Londrina, Brazil;* [2]*Exercise Science Department, CUNY Lehman College, Bronx, New York;* [3]*School of Physical Education, Pernambuco University, Recife, Pernambuco, Brazil.*

Abstract

The main purpose of the present study was to investigate the effect of 16 weeks of resistance training (RT) on body water in men and women. Thirty men (68.4 ± 9.0 kg, 174.5 ± 6.6 cm, and 22.7 ± 4.4 years) and 34 women (58.8 ± 11.9 kg, 162.6 ± 6.2 cm, and 22.7 ± 4.1 years) underwent progressive RT for 16 weeks (2 phases, 8 weeks each), 3 times per week, that consisted of 10 to 12 whole body exercises with 3 sets of 8-12 maximum repetitions. Total body water (intracellular and extracellular content) and skeletal muscle mass were assessed using a spectral bioelectrical impedance device (Xitron 4200 Bioimpedance Spectrum Analyzer). Total body water, intracellular water, and skeletal muscle mass increased significantly ($P < 0.05$) over time in men (+7.5%, +8.2%, and +4.2%, respectively) and women (+7.6%, +11.0%, +3.9%, respectively), with no sex by time interaction ($P > 0.05$). We conclude that progressive RT promotes an increase in body water, principally by intracellular content, however the hydration status is not influence by gender.

Keywords: *strength training, skeletal muscle, gender, cellular hydration*

Introduction

Resistance training (RT) is a modality of physical exercise used for performance enhancement and health promotion purposes. Thus, RT has been recommended for a variety of different populations due to its numerous morphological, neuromuscular, physiological, and metabolic benefits (ACSM, 2009).

Increasing skeletal muscle mass (SMM) is a primary goal of many recreational individuals of both sexes who are engaged in RT programs. Mechanisms by which RT promotes increases in SMM have been attributed to various factors including mechanical, metabolic, and hormonal processes (ACSM, 2009; Schoenfeld, 2010, 2012, 2013). Among the metabolic factors, one of the potential mechanisms that may contribute to muscle hypertrophy is via an increase in intracellular water content (Schoenfeld, 2012, 2013). This phenomenon, termed cell swelling, serves as a physiological regulator of cell function stimulating anabolic processes both by increasing protein syntheses and decreasing protein breakdown (Grant, Gow, Zammit, & Shennan, 2000; Haussinger, 1996; Haussinger, Roth, Lang, & Gerok, 1993; Millar, Barber, Lomax, Travers, & Shennan, 1997).

Fast-twitch fibers play an important role in cellular hydration as they are particularly sensitive to osmotic changes (Frigeri, Nicchia, Verbavatz, Valenti, & Svelto, 1998; Sjogaard, Adams, & Saltin, 1985). In addition, cellular swelling is maximized by exercise that relies heavily on glycolysis (Schoenfeld, 2012, 2013). Considering that percentage of fast-twitch fibers are proportionally higher in men than in women (Sale, MacDougall, Alway, & Sutton, 1987; Schantz, Randall-Fox, Hutchison, Tyden, & Astrand, 1983), and that men have higher efficiency in glycogen degradation during exercise compared with women (Tarnopolsky, 2008), we cannot rule out the possibility that men and women may present different chronic adaptations in cellular hydration state pursuant to resistance exercise.

Since men and women engaged in RT often seek to increase muscle mass, and these subjects are frequently recruited for studies to verify the impact of RT programs on body composition, it may be useful to investigate whether gender affects the myocellular hydration status. Moreover, it is important to determine whether resistance training designed to promote hypertrophy results in long-term changes in cell water content. Therefore, the purpose of this study was to analyze the impact of 16 weeks of hypertrophy-type resistance training on body water in men and women.

Methods

Experimental design

The study was carried out over a period of 22 weeks, with 16 weeks dedicated to the RT program and 6 weeks used for evaluations. Anthropometric and body water measurements were performed at weeks 1-2, 11-12 and 21-22. The first body water measurement took place one week before the start of training (Monday and Tuesday). The second and third evaluations were done on the following Monday and Tuesday after the end of each training phase (Friday) so that there was 48-72 hours afforded between training and assessment of body water. Supervised progressive RT was performed between weeks 3-10, 13-20. All sessions were supervised by trained personnel. The subjects were instructed to maintain their normal level of physical activity and were specifically asked not to start a new exercise regimen during study period.

Participants

Subjects were recruited from a university as well as local advertisement and then volunteered to participate in this study. Initially, 51 women and 44 men completed a detailed health history questionnaire. The subjects were admitted to the study if they had no signs or symptoms of disease, no orthopedic injuries, were inactive or moderately active individuals (physical activity less than twice a week), and had not been regularly engaged in any RT program during the last six months before the beginning of the study. Thirty men (68.4 ± 9.0 kg, 174.5 ± 6.6 cm, and 22.7 ± 4.4 years) and 34 women (58.8 ± 11.9 kg, 162.6 ± 6.2 cm, and 22.7 ± 4.1 years) finished the study and therefore were included in the analysis. The reasons for the dropouts included insufficient attendance to training sessions (< 85% of the total sessions) and voluntary abandonment for different reasons. It is noteworthy that none of the participants abandoned the study due to injury. All women included in the analysis were in the same phase of their menstrual cycle at the three time-points designated for evaluation. Twenty-one women were at the follicular phase, and thirteen women were at the luteal phase when the body water was assessed. The follicular phase was assumed as the first day of menstruation until the fourteenth day, and the luteal phase was considered as half of the cycle (fifteenth day) until the day that precedes menstruation. All women who were menstruating at the time of evaluation were asked about large weight gain (2 to 4 kg) (Heyward, 1998), but none of these participants reported any such changes. Written informed consent was obtained from the subjects after a detailed description of all procedures was provided. This investigation was approved by the local University Ethics Committee (Process 028/2012).

Instructions for measurements

Subjects were instructed to urinate approximately 30 minutes before the measurements, refrain from ingesting food or drink in the last four hours, avoid strenuous physical exercise for at least 24 hours, refrain consumption of alcoholic and caffeinated beverages for at least 48 hours, and avoid the use of diuretics during 7 days prior each assessment.

Anthropometry

Body mass was measured to the nearest 0.1 kg using a calibrated electronic scale (Filizola, model ID 110, São Paulo, Brazil), with the subjects wearing light workout clothing and no shoes. Height was measured using a wooden stadiometer to the nearest 0.1 cm while subjects were standing without shoes. Body mass index was calculated as the body mass in kilograms divided by the square of the height in meters.

Body water and skeletal muscle mass

Total body water (TBW), intracellular water (ICW) and extracellular water (ECW) content were assessed using a spectral bioelectrical impedance device (Xitron 4200 Bioimpedance Spectrum Analyzer). Bioelectrical impedance spectroscopy has been shown to be a valid tool for assessment of TBW and its various compartments in both men and women across a wide array of populations (Armstrong et al., 1997; Matias et al., 2013; Matthie et al., 1998; Moon et al., 2008). Before measurement the participants were instructed to remove all objects containing metal. Measurements were performed on a table that was isolated from electrical conductors, with subjects lying supine along the table´s longitudinal centerline axis, legs abducted at an angle of 45^{o}, and hands pronated. After cleaning the skin with alcohol, 2 electrodes were placed on surface of the right hand and 2 on the right foot in accordance with procedures described elsewhere (Hoffer, Meador, & Simpson, 1969; Nyboer, 1959). Before each measurement day the equipment was calibrated as per the manufacturer´s recommendations. The values generated by equipment software for ICW and ECW were used for analysis, the TBW was estimated by the sum of ICW and ECW. Based on the test-retest, the technical error of measurement, coeficient of variation, and intraclass correlation coeficient for TBW were 0.5 L, 1.1%, and 0.99 respectively. The SMM was estimated by the preditive equation proposed by Janssen et al. (Janssen, Heymsfield, Baumgartner, & Ross, 2000):

$\text{SMM (kg)} = [(\text{Ht}^2/R \times 0.401) + (\text{gender} \times 3.825) + (\text{age} \times -0.071)] + 5.102$

where: Ht is height in cm; R is BIA resistance in ohms; for gender, men = 1 and women = 0; age is in years.

A frequency of 50kHz was used to calculate SMM.

Dietary intake

Participants were instructed by a nutritionist to complete a food record on 3 nonconsecutive days (2 week days and 1 weekend day) at weeks 1-2, and 21-22. Subjects were given specific instructions regarding the recording of portion sizes and quantities to identify all food and fluid intake. Total dietary energy, protein, carbohydrate, and fat content were calculated using nutrition analysis software (Avanutri Processor Nutrition Software, Rio de Janeiro, Brazil; Version 3.1.4). All subjects were asked to maintain their normal diet throughout the study period.

Resistance training

A supervised progressive RT program designed to induce muscular hypertrophy was performed into 2 phases of 8 weeks each. Training was carried out 3 times per week on nonconsecutive days (Monday, Wednesday, and Friday) (ACSM, 2009). All subjects were individually supervised by experienced instructors during each training session in order to reduce deviations from the study protocol and to ensure subject safety. Subjects performed RT using a combination of free weights and machines, and the exercises included total and segmental movements of the upper limbs, trunk and lower limbs. The progressive RT program in the first phase consisted of 9 exercises selected to stress the major muscle groups in the following order: bench press, leg press 45^o, wide-grip behind-the-neck pulldown, leg extension, side lateral raise, lying leg curl, triceps pushdown, calf press on the leg press machine, and arm curl.

In the second phase, the RT program was altered, and 11 exercises were performed in the following order: bench press, incline dumbbell fly, wide-grip behind-the-neck pulldown, seated cable rows, seated barbell military press, lying triceps press, arm curl, leg extension, leg press 45^o, lying leg curl, and seated calf raise. After the resistance exercises, the abdominal crunch exercise was performed on the floor using subject’s bodyweight (3 sets of 50-100 repetitions in both phases). After the resistance exercises, 3 sets of the abdominal crunch exercise were performed on the floor in both training phases with subjects encouraged to total between 50-100 repetitions over the course of the 3 sets.

For both the phases, all subjects performed 3 sets of 8-12 maximum repetitions for each of the exercises except for calf exercises (3 sets of 15-20 maximum repetitions), and were instructed to perform repetitions with a concentric-to-eccentric phase ratio of 1:2. The rest period between sets lasted 60-90 s, with a 2-3 min rest interval between each exercise. Subjects were encouraged to perform all sets to voluntary concentric muscular failure. The training load was consistent with the prescribed number of repetitions for the 3 sets of each exercise. The load was adjusted weekly using the weight test by maximum repetitions (Rodrigues & Rocha, 2003). The subjects were instructed not to perform any other type of exercise during the study period.

Statistical analysis

Normality was checked by Shapiro-Wilk´s test. The data were expressed as mean ± standard deviation. Levene’s test was used to analyze the homogeneity of variances. Two-way analysis of variance (ANOVA) for repeated measures was used for intra- and inter-group comparisons, when the baseline values were different a two-way analysis of covariance (ANCOVA) for repeated measures was applied. In variables where sphericity was violated as indicated by

Mauchly´s test, the analyses were adjusted using a Greenhouse-Geisser correction. When F-ratio was significant, Bonferroni's post hoc test was applied to identify the mean differences. Baseline differences, and relative changes between men and women, were explored with an independent t-test. The effect size (ES) was calculated to verify the magnitude of the differences by Cohen's d (Cohen, 1988) where an ES of 0.20-0.49 was considered as small, 0.50-0.79 as moderate and ≥ 0.80 as large (9).For all statistical analyses, significance was accepted at $P < 0.05$. The data were stored and analyzed using STATISTICA software version 7.0 (STATSOFT INC., TULSA, OK, USA).

Results

Total energy and macronutrients daily intake at pre and post-training are shown in Table I. There were no significant main effects or sex by time interactions ($P > 0.05$) indicating that relative daily energy and macronutrients intake did not differ between men and women or over time.

***** INSERT TABLE I*****

Changes in SMM are presented in Table II. Both men and women showed significant increases ($P < 0.001$) in SMM from pre-training to post-training (men = +4.2%, women = +3.9%).

***** INSERT TABLE II*****

Figure 1 shows the TBW, ICW and ECW at 3 moments of the study for men and women (Panels A, C, and E) as well as for the whole sample analysis (Panels B, D, and F). There was no significant sex by time interaction for TBW (F = 0.90, $P = 0.435$), ICW (F = 0.47, P = 0.625), and ECW (F = 0.31, *P* = 0.733). ANCOVA indicated that there was no main effect of sex for the 3 components analyzed (TBW: F = 4.76, *P* = 0.186; ICW: F = 0.03, *P* = 0.855; ECW: F = 0.35, *P* = 0.555). A significant main effect of time was observed for the TBW (F = 4.3, *P* < 0.05) and ICW (F = 20.25, *P* < 0.05), in which both men and women increase from pre-training to post-training in TBW (men = +7.5%, women = +7.6%) and ICW (men = +8.2%, women = +11.0%), however for ECW the main effect of time was not observed (F = 1.26, *P* = 0.284). Also a significant increase from pre-training to post-training was observed considering whole sample in TBW and ICW (+7.6% and + 9.5%, respectively). The ESs for TBW were considered moderate and small for men (0.56) and women (0.48), respectively. For ICW moderate ESs were observed for men (0.69) and women (0.64). For ECW the ESs observed were considered small for men (0.33) and women (0.21). When considering the whole sample, a moderate ESs were observed for TBW (0.35) and ICW (0.40), however with no effect for ECW (0.19).

***** INSERT FIGURE 1*****

Figure 1. Total body water, intracellular and extracellular water in men and women (Panels A, C, and E), and whole sample (Panels B, D, and F) at different moments of the study. **P* < 0.05 vs. Pre-training. There was no significant sex by time interaction (*P* > 0.05). Data are expressed as mean ± standard deviation.

Relative changes in SMM, TBW, ICW and ECW are presented in figure 2. There were no statistical differences (*P* > 0.05) between men and women for all the components analyzed.

***** INSERT FIGURE 2 *****

Figure 2. Relative changes between pre-training from post-training on the body composition components in men and women. SMM = skeletal muscle mass. TBW = total body water, ICW = intracellular water, ECW = extracellular water. Data are expressed as mean ± standard deviation.

Discussion

The main and novel findings of the present study were twofold: 1) that hypertrophy-oriented resistance training produced significant long-term increases in ICW, and; 2) that these changes were not influenced by gender. The present study examined the effect of RT on TBW and its fraction ICW and ECW, and we observed that the increase in TBW occurred predominantly due to an increment in ICW. The increase in ICW content may be related to several factors. Cellular hydration is maximized by exercises that rely heavily on glycolysis, with the resultant lactate accumulation acting as the primary contributor to osmotic changes in skeletal muscle (Frigeri et al., 1998; Sjogaard et al., 1985). Furthermore, RT is a model of exercise regimens that cause increase in glycogen storage (MacDougall, Ward, Sale, & Sutton, 1977), and given that glycogen has an osmotic effect whereby it draws three grams of water into the cell for every gram of glycogen (Chan, Johnson, Moore, Kapadia, & Dudley, 1982), an increase in glycogen stores may mediate a favorable muscle protein balance over time by cellular hydration. In addition, fast-twitch fibers are particularly sensitive to osmotic changes, presumably related to a high concentration of water transport channels called aquaporin-4 (Frigeri et al., 1998). Considering that the percentage of fast-twitch fibers are proportionally higher in men than in women (Sale et al., 1987; Schantz et al., 1983), and given that females have a deficient metabolism in glycogen degradation compared with males (Tarnopolsky, 2008), it was logical to expect that the additive responses in ICW content were related to the sex differences. However, the results of this study showed these responses occurred independently of gender. This finding highlights that other factors rather than the ICW increase may contribute to some chronic adaptive differences in hypertrophic responses observed in men and women (Hubal et al., 2005; Ivey et al., 2000; Peterson, Pistilli, Haff, Hoffman, & Gordon, 2011; Roth et al., 2001).

The metabolic stimulus from RT leads to a series of intracellular events that ultimately regulates the ICW status, and this increase in ICW content causes non-contractile hypertrophy of the muscle as a whole (Schoenfeld, 2012, 2013). Although the exact physiological basis linking cell hydration with an anabolic drive is yet to be determined, evidence shows a correlation between cellular hydration with both an increase in protein synthesis and a decrease in proteolysis (Haussinger, 1996; Haussinger et al., 1993; Millar et al., 1997). In addition, it has been theorized that the stimulus associated with cell hydration status may trigger proliferation of satellite cells and facilitate their fusion to hypertrophying myofibers (Dangott, Schultz, & Mozdziak, 2000).

Accurate measurement of SMM can be determined with advanced technological equipment such as magnetic resonance imaging, ultrasound, computed tomography and dual energy x-ray absorptiometry. However, these procedures are expensive and have limited practical application. Therefore, other indirect methods with less financial cost and greater applicability in the field have been proposed. The present investigation estimated SMM using a spectral bioelectrical impedance equation, a mathematical model that shown to have good predictive validity for this purpose (Janssen et al., 2000). Our results indicate that the increase in SMM observed after the RT program was not influenced by sex. To our knowledge this is the first investigation that applied such methodology to compare men versus women, thereby limiting comparison with the literature. However, other studies that have investigated the effects of RT on fat-free mass in men and women also found that gender had no effect on the outcomes (Deruisseau et al., 2004; Hunter, 1985; Lemmer et al., 2001; Lemmer, Martel, Hurlbut, & Hurley, 2007). It is important to point out that similar relative changes in SMM between men and women suggests a higher absolute increase in men, since men generally have a higher absolute SMM than women (Miller, MacDougall, Tarnopolsky, & Sale, 1993).

The gains in SMM observed in this investigation occurred without changes in the food habits of the subjects. These results suggest that the macronutrients and energy intake observed throughout the progressive RT in this study were sufficient to support increased muscular hypertrophy. We believe that the monitoring of the eating habits of the participants is a strong point of the present study, since a majority of the previous investigations did not control for this variable.

The systematic variation in training volume and intensity can contribute significantly to optimize the development of RT outcomes (ACSM, 2009; Deschenes & Kraemer, 2002). In this regard, we used a strategy of variation and progression based on alterations in the program structure (number of exercises, exercise order, number of sets and repetitions). Indeed, we believe that the manipulation of training program variables in this study provided sufficient stimulus to maximize the muscular and morphological adaptations to training over time while limiting plateau. However, the time course of SMM and ICW adaptations were different, while the SMM adaptations predominated during the first 8 weeks of RT program, the ICW showed significant increase only after 16 weeks of RT.

Finally, it is worth noting that this investigation has some limitations. For one, the data found in our study cannot be extrapolated to other populations and are limited only to the time of training and moments applied in this investigation. Moreover, the method used for evaluating body water content is not considered a gold standard. However, spectral bioelectrical impedance has produced valid measurements of body water content when compared to a criterion method by deuterium or bromide dilution technique (Armstrong et al., 1997; Matias et al., 2013; Matthie et al., 1998; Moon et al., 2008), so it is considered a valid laboratory method to estimate TBW and its various components. On the other hand, to our knowledge, this is the first study to verify the adaptations in ICW and ECW content associated with RT in men and women. We believe that this article advances our understanding of the RT adaptations related to sexual dimorphism.

Conclusion

The results suggest that the progressive RT promotes an increase in TBW, particularly in ICW content, in both men and women. Furthermore, total water content does not seem to be influenced by gender at least during 16 weeks of intervention. Increasing SMM is a primary goal of many recreational individuals of both sexes who are engaged in RT programs. Elucidating our knowledge of the mechanisms by which the hypertrophy occurs, and how it is related with sexual dimorphism may be useful to exercise professionals and researchers, in order to allow the optimization of hypertrophy-oriented training programs in men and women, in particular for those individuals who seek to improve muscular hypertrophy.

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Figure captions

Figure 1. Total body water, intracellular and extracellular water in men and women (Panels A, C, and E), and whole sample (Panels B, D, and F) at different moments of the study. **P* < 0.05 vs. Pre-training. There was no significant sex by time interaction (*P* > 0.05). Data are expressed as mean ± standard deviation.

Figure 2. Relative changes between pre-training from post-training on the body composition components in men and women. SMM = skeletal muscle mass. TBW = total body water, ICW = intracellular water, ECW = extracellular water. Data are expressed as mean ± standard deviation.