

# Morphoquantitative analysis revealed Triceps Brachialis muscle hypertrophy by specific Resistance training equipment in rats

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## Abstract

**Aim:** Test the efficiency of a device and a specific protocol to stimulate muscular hypertrophy of an upper limb body muscle in rats. **Method:** We used 16 male Wistar rats divided into 3 groups: Initial Control (SC), Aged Control (SE), and Resistance training (T). We applied morphoquantitative measurements such as cross sectional area, myocytes (Vv[myo]) and interstitial (Vv[inters]) volume density in the Triceps Brachialis muscle (TB). The Ladder climbing equipment was utilized and each rat performed a total of 6 climbs with a 45 seconds of rest between each climb, in a frequency of five times per week during 16 weeks. The imposed overload was established according to a table for humans proposed and adapted from Heyward. **Results:** The animals reached 200% of the initial training load at the end of the training period. Morphometric analysis showed a 45% increase in the cross sectional area of the T group compared to SE ( $p < 0.05$ ). The stereological analysis confirmed also an increase in the Vv[myo] of the group T in comparison to SE. The Vv[inters] of the groups SC and T presented a meaningful difference in comparison to SE ( $p < 0.05$ ). **Conclusion:** the resistance training device and protocol used was efficient to demonstrate significant morphoquantitative alteration in the TB muscle size. The present results affirm that Ladder climbing equipment can also be used to induce upper limb muscle hypertrophy.

**Keywords:** morphometry, stereology, skeletal muscle, strength training.

## 1 Introduction

Muscle overload is an important factor for offsetting muscle loss with advancing age. Melton, Khosla, Crowson et al. (2000) mentions that an individual loses approximately 3 to 8% of muscle mass per decade after the age of thirty, and may also present a higher loss after the age of sixty. Partial restoration of muscle mass and function can be achieved through chronic exercise programs (HURLEY and ROTH, 2000; ROTH, FERRELL and HURLEY, 2000). Several studies have shown that resistance training increases protein synthesis and hypertrophy of muscle fibers while minimizing the loss of muscle mass and function changes (PORTER, 2001; NARICI, REEVES, MORSE et al., 2004; TAAFFE, 2006).

Although the effect of resistance exercise be amply demonstrated, little is known about the effects of this intervention on the different types of skeletal muscle. The muscular hypertrophy induced by some resistance training models (RTM) is mostly shown in the lower limb body muscles (DESCHENES, JUDELSON, KRAEMER et al., 2000; KRAUSE NETO, MAIFRINO and GAMA, 2011; NICASTRO, ZANCHI, LUZ et al., 2012).

Researches including humans have many features that can make it difficult to choose the best methodology to study muscular hypertrophy. However, many animal models have been developed, allowing most precise control of training parameters (ALWAY, DEGENS, KRISHNAMURTHY et al., 2005). The advantages and disadvantages of using animal training models to research

muscular hypertrophy is an actual discussion in the literature (ALWAY, DEGENS, KRISHNAMURTHY et al., 2005). The large differences found about the model, protocol and other parameters makes difficult to choose the best intervention for experimental research (NICASTRO, ZANCHI, LUZ et al., 2012). Therefore, the purpose of the present study was to test the efficiency of a specific RTM and protocol to stimulate the muscular hypertrophy of *Triceps Brachialis* muscle of rats.

## 2 Methods

This study was approved (protocol 015/2006) and performed at the Laboratory of Morphoquantitative Studies and Immunohistochemistry of the São Judas Tadeu University. We used 16 sedentary male Wistar rats (*Rattus norvegicus*), with 13 months of age ( $526.0 \pm 105.3$  g), housed in boxes of polypropylene (three each) and kept under controlled environmental conditions of temperature (22 °C) and lighting (12-hour cycle of light and 12 hours dark). For all groups were provided: reference commercial diet for rats (2.0 g protein / kg body weight) and water ad libitum.

The animals were divided into 3 groups: Group SC - sedentary and control of the procedures and euthanized at 13 months of age (n = 5), Group SE - aged and sedentary animals that were euthanized at 16 months of age (n = 6), Group T - animals which performed resistance training protocol and euthanized at 16 months of age (n = 5).

### 2.1 Resistance training protocol

The protocol was made respecting the animal's active period so done at dark. The group's division was made according to the performance of each animal during training adaptation. Slower rats were classified as SE and the agiler were named T.

The equipment used was a vertical ladder made of wood with iron steps (Figure 1). The height of the equipment (ladder) is 110 cm with a slope of 80° (DUNCAN, DAVID and GORDON, 1998). This model was chosen because to climb the ladder the animal uses both upper and lower limb body muscles. The training program was based in the principle of overload and it was established from the proposal by Heyward (1998). According to this author the classification of muscle strength is based on the relationship between the overload used to perform certain exercise and body weight of the individual (Table 1). Weekly, all animals were weighted and their loads adjusted (Gehaka BG 110 semi-analytical balance brand). The animals performed a five days adaptation to protocol prior to training. The procedure consisted of six consecutive climbs with 45 seconds of rest. The animals in the SC and SE group climbed the ladder without any load once a day, five times a week during the adaptation protocol until it was euthanized. The animals in group T performed six climbs separated by 45 second rest, with a frequency of five times a week during 16 weeks. The loads were attached by a musket tape added to the animal tail (Figure 1). We used the referential "Good" which corresponds to 75% of the animal's body weight. The increases were done every week by adjusting the overload to the animal's body weight.

For the "index corresponding to treatment Good" was performed the following calculation:

Load / Body weight coefficient = Adaptation to Experimental Models

Body weight × coefficient = Load

Ex: 500g × 0.72 (Good) = 360g

### 2.2 Animals euthanasia

The animals were anesthetized with an intraperitoneal injection of thiopental (40 mg/kg body weight, Sigma®, USA). After euthanasia, we removed the Triceps brachialis muscle (TB) and prepared it for light microscopy techniques. Samples of muscle were removed and randomly cut evenly and isotropic (UIR). Muscle fragments were fixed in 10% buffered formaldehyde and dehydrated in increasing series of alcohols, diaphanized in xylol, embedded in paraffin, sectioned in slices of 7 µm thick and stained with hematoxylin and eosin (HE) for light microscopy analysis.

### 2.3 Morphometric study

We analyzed cross sections of slides containing TB muscle. Each slide contained four sections of which five fields were analyzed in each cut. The selection of fields was randomized so that all the contents of the slide were analyzed. A total of 15 fields on each slide were visualized, one slide for each animal in the study. Measurements were made on an image analysis program (version 4.8 AxioVision) attached to a light microscope (Zeiss) with 100-fold increase. The analysis was performed using the following steps: a field of tissue under the microscope was focused and the image was transmitted to the computer screen. The cells were circumvented and the program automatically provided the value of the area analyzed.

### 2.4 Stereological study

Stereological method seeks the interpretation of three-dimensional structural arrangement based in the analysis of the structure of cuts that provide only one-dimensional information. You can still perform quantitative studies (density per volume, and surface area) very efficiently using minimal cuts (MANDARIM-DE-LACERDA, 2003). To do this, the measures were used to estimate based in the volume density of myocytes (Vv[myo]) and interstitial space (Vv[inters]).



**Figure 1.** Demonstration of the load placed to animal's tail and training equipment.

To determine the estimated volume density ( $V_v$ ), the slides corresponding to each muscle of each group were photographed under the light microscope with final magnification of 50x. For this analysis were taken 15 photographs of each slide. A stereological analysis was performed using the Image J program. The program placed the image on a points system and all points that fell in myocytes and interstitial space were counted.

### 2.5 Statistical analysis

We used mean and standard deviation and ANOVA (post hoc Tukey) for data comparison between groups. For statistical significance was established  $p \leq 0.05$ . We used the software SPSS version 13.0.

## 3 Results

### 3.1 Analysis of the training load

The animals showed a progressive level of performance throughout the training protocol. At the end of the 16 weeks of training the animals carried 570-750 g of overload, which equates to approximately 161% to 201%, of the initial training load (Table 2). There was a linear evolution of load and total overload (corresponds to body weight over the load added to tail) of training.

### 3.2 Morphometric analysis

The cross sectional area of TB myocytes between groups SC and SE showed no significant difference (Table 3). However, T group showed a significant increase of 45% in comparison to SE ( $p \leq 0.000$ ).

### 3.3 Stereological analysis

Stereological data is show in Table 4 and 5. SE group decreased 13% of the  $V_v[\text{myo}]$  compared to SC ( $p \leq 0.012$ ). T presented a 18%  $V_v[\text{myo}]$  increase compared to SE ( $p \leq 0.001$ ). The  $V_v[\text{inters}]$  of the SE increased 17% compared to SC ( $p \leq 0.012$ ). However, T showed a 21% decrease in the  $V_v[\text{inters}]$  compared to SE ( $p \leq 0.001$ ).

## 4 Discussion

There is a great difficulty to find an ideal, or at least, suitable method to isolate and control the variables of training. Krisan, Collins, Crain et al. (2004) criticize the training unit described by Tamaki, Uchiyama and Nakano (1992) and suggests that this device is probably best suited for plyometric training, which encourages elastic components of the muscles in the eccentric phase, during transition to concentric phase. Therefore, the equipment suggested by Duncan, David and Gordon (1998), and Hornberger and Farrar (2004) seems to be more appropriate as there is no

**Table 1.** Classification proposed by Heyward (1998) to evaluate the muscular strength of humans.

Classification (age)	Men		Women	
	<60	>60	<60	>60
Superior	>0.90	>0.82	>0.56	>0.55
Excelent	0.80-0.89	0.72-0.81	0.49-0.55	0.48-0.54
Good	0.72-0.79	0.67-0.71	0.44-0.48	0.43-0.47
Regular	0.64-0.71	0.58-0.66	0.40-0.43	0.39-0.42
Weak	<0.63	<0.57	<0.39	<0.38

**Table 2.** Training load relationship between percentage of the initial load and animals body weight.

	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5
Percentage of the initial load	166%	161%	201%	172%	179%
Percentage of the initial body weight	120%	116%	144%	124%	128%

**Table 3.** Mean and standard deviation of the cross-sectional area of each group presented for the Triceps Brachialis (TB) muscle.

Muscle	Groups	Área ( $\mu\text{m}^2$ ) (mean $\pm$ sd)	P value
TB	SC	2,019.3 $\pm$ 1,080.3	-----
	SE	2,095.9 $\pm$ 1,031.5	0.335 (SC $\times$ SE)
	T	3,052.6 $\pm$ 1,681.7*	0.000 (SE $\times$ T)

Triceps Brachialis (TB) =\* $p \leq 0.01$  between SE vs T.

**Table 4.** Mean and standard deviation of the Myocytes volume density ( $V_v[\text{myo}]$ ) of each group presented for the Triceps Brachialis (TB) muscle.

Muscle	Groups	$V_v[\text{myo}]$ (%) (mean $\pm$ st)	P value
TB	SC	59.248 $\pm$ 7.429	0.564 (SC $\times$ T)
	SE	52.394 $\pm$ 5.978*	0.012 (SC $\times$ SE)
	T	61.594 $\pm$ 5.126*	0.001 (SE $\times$ T)

Triceps Brachialis (TB) =\* $p \leq 0.05$  between SC vs SE and SE vs T.

**Table 5.** Mean and standard deviation of the Interstitial space volume density (Vv[inters]) of each group presented for the Triceps Brachialis (TB) muscle.

Muscle	Groups	Vv[inters] (%) (mean ± st)	P value
TB	SC	40.280±7.428	0.516 (SC × T)
	SE	47.180±5.983*	0.012 (SC × SE)
	T	37.735±5.308*	0.001 (SE × T)

Triceps Brachialis (TB) = \*p≤0.05 between SC vs SE and SE vs T.

type of reward or foreign aid given to perform the task. However, many researches use questionable resources as external stimulation (KRISAN, COLLINS, CRAIN et al., 2004). This intervention is criticized because can alter the animals stress level and thereby modify the levels of force applied during exercise. These external factors do not resemble those experienced in human training. In our study, the T group performed the intervention without external stimulation. Thus, we supposed to be more close to the ideal training conditions.

The literature does not demonstrate a pattern between the load and the more appropriate place to fix it to animal's body. This is an important factor because it may increase the arm lever. Various studies does not even describe in which region the load was fixed (HARRIS, SLACK, PRESTOSA et al., 2010; MATHENY, MERRITT, ZANNIKOS et al., 2009). In the study of Hornberger and Farrar (2004), the arm lever was greater than that presented by Duncan, David and Gordon (1998). In this study we chose to fix the overload in the proximal part of the tail, based in the fact that the closest to the power resistance is, lower should be the load variation prescribed.

Many studies use a relatively short training duration such as twelve (KRISAN, COLLINS, CRAIN et al., 2004; CORAZZA, PAOLILLO, GROppo et al., 2013), eight (HORNBERGER and FARRAR, 2004), six (HARRIS, SLACK, PRESTOSA et al., 2010) and four weeks (BARAUNA, BATISTA JUNIOR, COSTA ROSA et al., 2005). Short training may be insufficient to produce an adaptation similar to those observed in humans. In this study we performed a period of 16 weeks hence were expected morphological adaptations to occur. After conducting extensive research in order to find in the literature a test load for rats, we were not successful because much of the calculation used to load progression found, were not standardized as noted in the research by Duncan, David and Gordon (1998). The authors increased training load about 5% of body weight. Heyward (1998) presents a table to test load in older men and women. In our work this table was adapted to the animal model.

We started the training using the coefficient "GOOD", which corresponds to 75% of the maximum load, assuming that the animals had apparently good health. From the 4th week of training, we use the coefficients: 0.80, 0.90, 1.00, 1.10 and 1.20 which correspond to 116% to 144% of the animal's body weight and respecting the overload principle. The animals showed an excellent level of performance so we increase the coefficients beyond levels proposed by Heyward (1998). Thus, we obtained expressive muscle gains similar to that observed by Duncan, David and Gordon (1998).

As far we know, this is the first study to test a RTM in order to promote muscular hypertrophy in an upper limb

body muscle of animals. Mostly of the studies analyzes lower limb body muscles such as Plantaris, Soleus, Extensor Digitorum longus, Gastrocnemius, Quadriceps Femoris and other (DESCHENES, JUDELSON, KRAEMER et al., 2000; McGEE, MUSTARD, HARDIE et al., 2008; BRUUSGAARD, JOHANSEN, EGNER et al., 2010). Our study showed that resistance training increased the cross sectional area of myocytes by 45% in comparison to SC and SE groups, confirming quantitatively the claims of Santarém (1999), who reports that older trained are able to preserve your muscle mass. So far, stereological analysis confirmed the morphometric results. We observed an increase in the mean Vv[myo] density of the group T compared to SE, and similarity between groups T and SC. These data show that resistance training is effective in increasing the volume density occupied by myocytes in order to compensate the loss of these numerical cells.

## 5 Conclusion

We concluded that the tested RTM and protocol was efficient to significant increase TB muscle size.

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