Does Resistance Training Modulate Food Intake and Hypothalamic Neuropeptides mRNA Expression in Rats?

Fábio Medici Lorenzeti, Daniela Fojo Seixas Chaves, Guilherme Giannini Artioli, Humberto Nicastro and Antonio Herbert Lancha Jr

Laboratory of Applied Nutrition and Metabolism, School of Physical Education and Sport University of São Paulo

Abstract

The aim of the present study was to evaluate if the food consumption patterns and the expression of hypothalamic neuropeptides of young rats (~4 months) is modulated in response to a 12-week resistance training protocol at 80% of the previously established maximum force. For this purpose young male Wistar rats were randomly divided in two groups: sedentary control group (CON) and a trained group (TR). To compare the different treatments at each time point, we used analysis of variance (ANOVA). For the analysis of mRNA levels between trained and control groups we used the paired t test. Our results showed no
significant differences between groups for body weight, food consumption and the expression of hypothalamic neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) (p > 0.05). Therefore, our data suggests that a 12-week strength training protocol does not affect the voluntary food intake, weight and NPY and POMC mRNA expression in an animal model for resistance exercise training.

**Keywords:** physical exercise, energy intake, NPY, POMC, rats

**INTRODUCTION**

Resistance exercise is characterized by an acute state of negative energy balance. It is intuitive then that acute exercise would stimulate a compensatory post-exercise hunger leading to subsequent food intake and restoration of energy homeostasis [1].

Less is known about the effects of resistance exercise on post-exercise energy homeostasis, but there is evidence that a single bout of high-intensity exercise can produce a prolonged metabolic perturbation in both men and women [2,3].

The reasons for the failure to immediately compensate for the increased energy expenditure from exercise are not clear, but might be at least partially explained by exercise-induced changes in neural and hormonal stimuli that influence sensations of hunger and satiety.

The hypothalamic neuropeptide Y (NPY) and the pro-opiomelanocortin (POMC) are the most studied networks that regulate energy intake. Both systems receive information about the nutritional status and the level of energy storage through signals from insulin and leptin, which are mediated by specific receptors located on POMC and NPY neurons present predominantly in the arcuate nucleus (ARC) [4-7].

Neuropeptide Y (NPY) is one the most potent orexigenic peptides found in the brain. It stimulates food intake with a preferential effect on carbohydrate intake and its effects are mediated through at least two receptors, the Y1 and Y5 receptors [6]. The NPY system for feeding regulation is mostly located in the hypothalamus. It is formed of the arcuate nucleus (ARC), where the peptide is synthesized, and the paraventricular (PVN), dorsomedial (DMN) and ventromedial (VMN) nuclei and perifornical area where it is active [6,7].

In POMC neurons, the neuropeptide precursor POMC is cleaved to α-melanocyte-stimulating hormone (α-MSH), which after secretion activates melanocortin 4 receptors expressed on secondary neuron populations, located in the paraventricular nucleus (PVN) of the hypothalamus, among other nuclei. Thus, membrane depolarization of POMC neurons leads to α-MSH release, MC4R activation and ultimately decreases food intake and increases energy expenditure. However, both the downstream pathways and the nature of the MC4R expressing neurons are only incompletely understood [8-11].
Physical exercise has been shown to play an important role in regulating the expression of hypothalamic neurotransmitter and MCR4 receptor, which may ultimately lead to decreased food intake [12].

Thus, the present study sought to evaluate the effect of strength training on food intake and mRNA expression of hypothalamic neuropeptides related to food intake in young Wistar rats.

**METHODOLOGY**

**Animals**

This research was approved by the Ethical Committee for Animal Research of the local committee and the experiments were conducted in accordance with the National Research Council's Guidelines for the Care and Use of Laboratory Animals.

Sixteen male Wistar rats (400-430g) were kept in individual cages in inverted light/dark cycle and had free access to food and water. They were randomly divided in two groups: a sedentary control group (CON) and a trained group (TR). The device used for strength training has been previously described and validated [13,14].

During the intervention, data regarding body weight and food intake of control and trained groups was collected.

After the training period the rats were killed by decapitation and hypothalamus was carefully extracted, flash frozen and stored at -80°C for later RT-PCR analysis.

**Training protocol**

The training protocol lasted 12 weeks, consisting of 35 sessions of resistance training (one session a day, three times a week). In each session, rats performed a total of 30 repetitions, which lasted about 30 minutes. The load used for each repetition was 80% according to the maximum force, and already used in other experiments previously conducted in our laboratory with rats [13].

**Food consumption**

Rats were fed of a standard diet *ad libitum* and food consumption was measured daily.

**Determination of mRNA by RT-PCR**

To determine the expression of mRNA for neuropeptide Y and POMC (pro-opiomelanocortin) we used the methodology previously described by Amaral et al. (2006) [15]. Total mRNA from hypothalamus was extracted using Trizol (Invitrogen, Carsbad, CA) according to manufacturer’s recommendations, purified through the use of a commercial kit (vero kit da qiagen usado) and quantified in a spectrophotometer for micro-samples (NanoDrop, ND 2000).
Seven micrograms of total RNA were reverse transcribed with MMLV (checar concentração200 U/ml) using oligo (dT) (50 mmol/L) in a reaction volume of 20 ml (5 x RT buffer, 10 mmol/L dNTP and 40 U/ml RNase inhibitor free). Reverse transcription involves 50 minutes of incubation at 42° C and 15 minutes of incubation at 70° C. The PCR products were subjected to electrophoresis on an agarose gel (1,5%) containing ethidium bromide and visualized by excitation with ultraviolet light. The photo documentation was performed using the System ImageQuant (GE Healthcare) and the band was quantified using the ImageQuantTM TL Software (GE Healthcare). In all samples the amplification of RPS-29 was performed and used as an internal control for the quantity and quality. The semi-quantitative expression (SE) of neuropeptide Y (NPY) was calculated by the formula SE = pixel product of the area / pixel area of the RPS-29 x 100. The primers used for PCR are: RPS-29 (NCBI: NM012876), sense 5´-AGGCAAGATGGGTCACCAGC-3´, antisense: 5´AGTCGAATCATCCATTCAGGTCG-3´ (fragment: 202 bp, Tm: 57° C, 27 amplification cycles), POMC (pro-opiomelanocortin) (NCBI: AF510391), sense: 5´-CTCCTGCTTCCAGACCTCAT-3´, antisense: 5´-TTGGGTACACCTTCACAGG-3´ (fragment: 398 bp, Tm: 63° C, amplification: 32 cycles), NPY (neuropeptide Y) (NCBI: NM012614), sense: 5´-AGAGATCCGACCTTGCAGATC-3´, antisense: 5´-AAGCACAACAGGGAAATGG-3´ (fragment: 236 bp, Tm: 62° C, amplification: 31 cycles).

Statistical analysis

To compare the different treatments at each time point, we used analysis of variance (Two-way ANOVA). For the analysis of mRNA levels between trained and control groups the paired t test was used. The results were expressed as mean ± standard error of mean (M ± SEM). And the level of significance was 0,05.

RESULTS

Figure 1 shows the relationship of average body weight before and after intervention for both groups. No significant differences between groups were found for body weight at both PRE (CON=401.38 ± 1.06g; TR=402.13 ± 1.46g) and POST intervention (CON=500.38 ± 48.64g; TR=507.63 ± 24.12g; p > 0.05).

Figure 2 shows that there was a reduction in food intake in the trained group at the midpoint of the intervention. However there was no significant difference between groups for food intake at the end of the study (PRE: CON=30.00 ± 0.00g; TR=29.00 ± 1.07g; POST: CON=29.00 ± 3.21g; TR=29.00 ± 3.21g).

In order to verify whether strength training could affect the expression of neuropeptides related to food intake, we examined the expression of NPY and
POMC. Our findings show that the expression of hypothalamic neuropeptides NPY and POMC were not different between the control and trained groups (Figure 3A and 3B; p > 0.05).

DISCUSSION

Several studies have investigated the effects of physical activity on energy intake in rodents [16] and humans [17,18]. However, these molecular mechanisms are not entirely clear. Evidence shows that exercise performed either acutely or chronically exert anorectic effects by modulating the expression of hypothalamic neurotransmitters [19]. The neurotransmitters NPY and POMC play a key role in the control of food intake by modulating the effects of hypothalamic orexigenic and anorexigenic [6,19,20]. In our study, 35 sessions of strength training with 80% of maximum load did not affect the voluntary food intake, weight or NPY and POMC mRNA expression in an animal model for resistance exercise training.

Studies showed the effect of aerobic exercise in lower postexercise hunger levels and lower relative food intake, these data support the notion that aerobic exercise induce a postexercise anorexia being related to decrease in appetite and to exercise-induced weight loss [21-25]. This was evident only with acute aerobic exercise, not strength training, suggesting a relationship between exercise intensity, which would explain our data.

Acknowledgments
This work (Grant No 2010/08329-3 and 2010/10852-6) was supported by the Brazilian Funding Agency (FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo)

References


Figure 1 – Relationship between weight before and after groups of control and trained. p = 0.07
Figure 2 – Relationship between food consumption at the beginning, middle and end of the intervention and control groups trained. Comparison of pre and post $p = 0.08$; * compared with the beginning of the intervention through $p = 0.0018$. 

![Food consumption graph](figure2.png)
Figure 3 – a) Relationship between NPY expression between the control and trained groups, \( p = 0.38 \); b) Relationship between POMC expression between the control and trained groups, \( p = 0.20 \)

Received: October, 2012