Chronic Exercise Promotes Alterations in the Neuroendocrine Profile of Elderly People

Abstract

Aging and physical inactivity are 2 factors that favour the development of cardiovascular disease, metabolic syndrome, obesity, and diabetes. In contrast, adopting a habitual moderate exercise routine may be a nonpharmacological treatment alternative for neuroendocrine aging disorders. We aimed to assess the effects of moderate exercise training on the metabolic profiles of elderly people with sedentary lifestyles. Fourteen sedentary, healthy, elderly male volunteers participated in a moderate training regimen for 60 min/day, 3 days/week for 24 weeks at a work rate equivalent to their ventilatory aerobic threshold. The environment was maintained at a temperature of 23 ± 2°C, with a humidity of 60 ± 5%. Blood samples for analysis were collected at 3 intervals: at baseline (1 week before training began), and 3 and 6 months after training. The training promoted increased aerobic capacity (relative VO$_2$, and time and velocity to VO$_2$max; (p < 0.05)) and reduced serum α-MSH (p < 0.05) after 3 months of training when compared with the baseline data. In addition, serum thyroid hormone (T3 and T4) was reduced after 6 months of training compared with baseline levels. Our results demonstrate that a moderate exercise training protocol improves the metabolic profile of older people, and metabolic adaptation is dependent on time.

Introduction

The aging process is commonly defined as the accumulation of diverse deleterious changes that occur in cells and tissues; such changes are responsible for the increased risk of developing disease and eventual death [1]. These deleterious changes include loss of muscle mass, decreased maximal oxygen consumption (VO$_2$max) and an increased incidence of conditions, such as metabolic syndrome, obesity, diabetes, and sleep disorders [1, 2]. Changes in body weight are related to increased and/or decreased caloric intake and increased and/or decreased energy expenditure [3]. Feeding behaviour is regulated by several peripheral signals, such as leptin and insulin, thyroid hormones (T3 and T4), the hypothalamic-pituitary-adrenal axis, and IGF-1. This axis interacts with the hypothalamus in the central nervous system (CNS), activating or inhibiting neuropeptides and regulating appetite and energy homeostasis [4–7]. Metabolic disorders are closely related to a sedentary lifestyle and to lower levels of physical activity [8]. On the basis of these relationships, our group hypothesised that exercise training would be an effective nonpharmacological therapy to promote improvement in various metabolic disorders, including obesity, diabetes, and obstructive sleep apnoea syndrome; and exercise could improve the quality of sleep, thus preventing or reducing the comorbidities of aging [9]. Recently, our group demonstrated that elderly individuals undergoing a 6-month aerobic training program increased their physical capacity and showed greater improvement in their metabolic profile, such as improved insulin sensitivity, decreased plasma triacylglycerol, and increased adiponectin [10]. These effects were dependent on exercise intensity and duration [11].

Due to lack of data in the literature related to physical training, aging, and the interaction between central and peripheral hormones associated with feeding behaviour, the objective of this study was to evaluate a 6-month training program on the neuroendocrine status of elderly individuals.
**Subjects and Methods**

**Subjects**

The experimental protocol was approved by the Ethics Committee (No. 1592/07) of the Universidade Federal de São Paulo in accordance with the Declaration of Helsinki. All subjects were informed of the aims and risks of the study, and their written informed consent was obtained. Fourteen sedentary, healthy, elderly male volunteers living independently in São Paulo, Brazil, were recruited. The physical characteristics of the volunteers are presented in Table 1. All volunteers received a complete medical examination, and their training activities were approved by a sports medicine doctor prior to study inclusion. The exclusion criteria were the presence of cardiovascular pathologies or other diseases, either pre-existing or diagnosed during the clinical evaluation that could interfere with the response to training or the study results.

**Experimental design**

The volunteers arrived at the laboratory at 08:00 h for blood collection and then immediately underwent assessments of body composition to characterise their pretraining condition (total body mass, height, and body mass index calculation). After this procedure, the same individuals performed an incremental evaluation of cardiopulmonary exercise capacity to assess the level of physical fitness (maximum oxygen consumption) and to detect their ventilatory threshold in order to define the parameters to be used for the training program. All volunteers performed moderate exercise training sessions between 07:00 AM and 09:00 AM.

**Body composition**

Total body mass and fat percentages were measured by whole-body plethysmography (air displacement plethysmography, BOD POD body composition system; Life Measurement Instruments, Concord, CA, USA). Height was determined using a stadiometer. Additionally, the body mass index (BMI) was calculated as total body mass divided by height squared [12,13].

**Ventilatory threshold and maximal oxygen consumption**

The maximal oxygen consumption (VO2 max) and ventilatory threshold for each volunteer were determined using an incremental exercise test on a treadmill. The initial velocity was 2.5 km/h, and the speed was increased by 1.0 km/h each minute until voluntary exhaustion. Expired gas was collected at the end of each stage to determine the aerobic threshold. Respiratory and metabolic variables were obtained breath by breath by measuring gaseous respiratory exchanges with a metabolic system (COSMED PFT4, Rome, Italy). After testing, the invalid data were discarded and analysis was performed after 20 s. The test yielded the following variables: VO2 max, ventilator anaerobic threshold (VATI), heart rate at VATI, and threshold load (W). Criteria to determine oxygen consumption at VATI followed those proposed by Wasserman et al. [14]: 1) exponential increase in ventilation; 2) abrupt increase in respiratory quotient (R); 3) systematic increase in oxygen ventilator equivalent without a change in PEI or CO2 equivalent and 4) increase in exhaled fraction of O2 (FeO2%). The VATI equivalent was defined as that state in which oxygen consumption was between 60% and 70% of VO2 max, the patient was at 70–80% of heart rate reserve, and between the values 12 and 14 on the Borg scale of perceived exertion (moderate aerobic exercise).

**Training protocol**

The training consisted of running for 60 min/day, 3 days/week for 24 weeks at a work rate equivalent to the subject’s VATI. In the first 2 weeks, the volunteers performed 30 min of exercise. In the third week, they performed 45 min of exercise. Then, from the fourth week onward, the volunteers performed 60 min of exercise. The environment was kept at a temperature of 23 ± 2 °C with the humidity maintained at 60 ± 5%. Data were collected at 3 time points: at baseline (1 week before training began), and after 3 and 6 months of training. All data were collected after 24 h of rest to exclude any acute effects of exercise.

**Blood collection and biochemical analyses**

Fasting (12 h) blood samples (20 ml) were collected from an antecubital vein in sterile tubes containing heparin before training and 24 h after the last exercise session. Blood samples were centrifuged at 650 g for 15 min. The plasma samples were stored at −80 °C and analysed within 1 week. Plasma leptin, NPY, α-MSH and MCH concentrations were measured using ELISA kits from Phoenix Pharmaceuticals, Inc. (Belmont, California, USA). Serum pituitary hormones (TSH, GH, testosterone, and IGF-1), cortisol and thyroid hormone [triiodothyronine (T3) and thyroxin (T4)] levels were measured using RIA and commercial kits (Orion Diagnostic).

**Statistical analysis**

The data distribution was previously checked by the Bartlett’s test for equal variances, and the data are reported as the mean and standard deviation. The differences in the plasma parameters among situations (before and after 3 and 6 months of exercise training) were assessed by one-way ANOVA with repeated measures. When applicable, the Tukey’s post hoc test was used.
Discussion

The results of this study showed that moderate aerobic training improved the parameters related to physical capacity, led to an increase in the concentration of the neuropeptide α-MSH after 3 months of training, and promoted changes in the T3 and T4 concentrations after 6 months of intervention. Thus, improvement in physical fitness led to changes in the neuroendocrine status of elderly individuals, and these adjustments were time-dependent.

Aging and physical inactivity are 2 physiological conditions that drive various neuroendocrine changes, such as decreased energy expenditure, increased food intake decreased insulin sensitivity, and increased proinflammatory cytokines [1,3,8]. All these physiological processes increase the likelihood that an individual will develop diseases, such as obesity, diabetes, and sleep disorders [8,15–19]. Aging affects energy homeostasis and regulation of food intake. These processes are controlled through the hypothalamus by the integration of signals from the peripheral circulation and central nervous systems [7,20]. Changes in the concentrations of hypothalamic neuropeptides related to feeding behaviour are closely linked to the control of energy homeostasis and food intake [21]. In our study, we found increased levels of the anorexigenic neuropeptide α-MSH after 3 months of training. However, the levels of this neuropeptide were similar to baseline values after 6 months, showing that exercise training modulates the action of this neuropeptide. Leptin and insulin are the main peripheral signals that simultaneously inhibit NPY neurons and stimulate POMC/α-MSH-producing neurons in the hypothalamic arcuate nucleus [22–24]. One of the possible causes for the increase of this neuropeptide after 3 months of training could be an improved sensitivity to insulin action in the hypothalamus [24]. Administration of exogenous insulin decreases hypothalamic activity in a dose dependent manner. Conversely, central administration of insulin antibodies results in increased food intake and body weight gain [22]. Thus, insulin provides a negative feedback signal to the CNS, which is proportional to the energy stored in peripheral tissues. Benoît et al. showed the importance of the effects of insulin in the regulation of food intake and body weight control via activation of melanocortin receptors in the hypothalamus [23]. In our study, we found no training-related differences in the concentrations of NPY and leptin. Lira et al. [10] found a

for multiple comparisons. The analysis was carried out using STATISTICA software version 6.0, and the significance level was set at p < 0.05.

Results

The individual physiological and anthropometric characteristics of the volunteers before the exercise training and after 3 and 6 months of training are described in Table 1. Additionally, Table 1 shows that moderate aerobic training for 3 and 6 months effectively improved aerobic capacity as demonstrated by the increase in relative VO2max (p < 0.004), time of test (p < 0.0001) and speed (p < 0.0001) to VO2 compared with the pretraining values.

Table 2 shows that the 6-month period of aerobic training changed the concentrations of T3 and T4 levels compared with baseline levels and 3-month post-training levels (p < 0.05). However, no significant difference between the pretraining and 6-month sampling times was observed for TSH or free T4.

With respect to hormones related to eating behaviour, we found that α-MSH levels increased relative to baseline after 3 months of aerobic training. No differences as a function of training were detected between the groups studied.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>After 3 months</th>
<th>After 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3 (ng/ml)</td>
<td>118.289 ± 19.799</td>
<td>114.733 ± 21.541</td>
<td>102.167 ± 14.940*</td>
</tr>
<tr>
<td>T4 (ng/ml)</td>
<td>8.089 ± 1.332</td>
<td>8.667 ± 1.100</td>
<td>7.308 ± 1.141**</td>
</tr>
<tr>
<td>T4F (ngml)</td>
<td>1.069 ± 0.210</td>
<td>1.048 ± 0.244</td>
<td>0.915 ± 0.158</td>
</tr>
<tr>
<td>TSH (μlU/ml)</td>
<td>3.522 ± 2.884</td>
<td>3.058 ± 1.540</td>
<td>2.804 ± 2.277</td>
</tr>
</tbody>
</table>

Table 2. Thyroid hormones of elderly subjects who underwent a 6-month exercise training.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>After 3 months</th>
<th>After 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (nM/l)</td>
<td>15.043 ± 5.156</td>
<td>12.792 ± 3.867</td>
<td>14.669 ± 3.960</td>
</tr>
<tr>
<td>GH (μlU/ml)</td>
<td>1.300 ± 2.097</td>
<td>1.024 ± 1.412</td>
<td>0.971 ± 1.822</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>123.243 ± 42.164</td>
<td>103.031 ± 26.103</td>
<td>101.962 ± 36.596</td>
</tr>
<tr>
<td>Testosterone (ng/dl)</td>
<td>484.879 ± 139.442</td>
<td>431.750 ± 149.426</td>
<td>468.815 ± 175.918</td>
</tr>
</tbody>
</table>

Table 3. Hormones related to eating behaviour of elderly subjects who underwent a 6-month aerobic training.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>After 3 months</th>
<th>After 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (μg/ml)</td>
<td>21.655 ± 0.713</td>
<td>22.555 ± 0.291</td>
<td>24.645 ± 0.415</td>
</tr>
<tr>
<td>NPY (ng/ml)</td>
<td>0.234 ± 0.063</td>
<td>0.239 ± 0.104</td>
<td>0.213 ± 0.065</td>
</tr>
<tr>
<td>MCH (ng/ml)</td>
<td>1.804 ± 0.506</td>
<td>1.662 ± 0.360</td>
<td>1.819 ± 0.416</td>
</tr>
<tr>
<td>α-MSH (ng/ml)</td>
<td>0.330 ± 0.113</td>
<td>0.454 ± 0.181*</td>
<td>0.370 ± 0.098</td>
</tr>
</tbody>
</table>

Table 4. Anabolic and catabolic hormones of elderly subjects who underwent a 6-month aerobic training.

*p < 0.05 vs. baseline. **p < 0.05 vs. 3 months of training. T3: triiodothyronine; T4: thyroxine; T4F: thyroxine free; TSH: thyroid-stimulating hormone, NPY: Neuropeptide Y. MCH: Melanin-concentrating hormone. α-MSH: melanocyte-stimulating hormones.
Several studies have shown that exercise can decrease the inflammatory status in diseases like obesity [25] and cancer [26,27]. Thus, exercise has anti-inflammatory properties. In addition to the increase in insulin sensitivity, Lira et al. found an increase in adiponectin, an anti-inflammatory cytokine, after 3 months of training. Furthermore, in addition to the effects of α-MSH within the central nervous system, this peptide may exhibit peripheral actions, including an anti-inflammatory effect [28], reduction of human neutrophil chemotaxis [29] and decreased production of TNF-α [28]. A review by Lipton et al. highlighted some anti-inflammatory functions of α-MSH: α-MSH inhibited the release of TNF-α, interleukin-1 beta, and interleukin-8 in vitro blood samples [29]. There is also evidence that α-MSH can alter the expression of proinflammatory cytokines by inhibiting NFκB, a transcription factor, while increasing the production of anti-inflammatory cytokines like IL-10 [29]. These observations are consistent with the data in the present study, which show that exercise produced anti-inflammatory effects. In addition to insulin and leptin, other peripheral hormones, including sex hormones, GH, cortisol and thyroid hormones, regulate energy homeostasis and are sensitive to changes in physical training [7]. Furthermore, several studies show that aging affects the signalling cascade of hormones in the endocrine system, leading to changes in hormone concentrations and neuroendocrine disorders [30–32]. In our study, the concentrations of anabolic and catabolic hormones were with the ranges of normal values for healthy subjects as reported in the literature [33], showing that our sedentary elderly individuals did not have any metabolic disorders initially.

After 6 months of a moderate aerobic training program, we noted a decreased concentration of T3 compared to baseline. In addition, the levels of T4 after 6 months of training were lower than those observed following 3 months of training. These results indicate that even without pathological metabolic changes, physical training induces changes in these hormones in elderly patients.

The improvement in maximal oxygen consumption found in our elderly subjects was mainly due to peripheral adaptations, specifically in skeletal muscle that led to enhanced oxidative metabolism and an associated increase in metabolic efficiency [34–36]. Lira et al. showed that after 3 months of aerobic training in elderly subjects, there was a decrease in the plasma concentration of fatty acids. These authors also suggested a possible increase in the uptake and oxidation of fatty acids, which promoted the decrease observed in the plasma [10]. In the same study, the values of nonesterified fatty acids (NEFAs) returned close to baseline after 6 months of training. This restoration towards baseline levels may have resulted from an increase in the supply of intramuscular triglycerides, which increased their oxidation [36,37]. Thyroid hormones have pleiotropic actions, that is, they may act differently as a function of the target tissue [38,39]. In skeletal muscle, thyroid hormones modulate the expression of mitochondrial enzymes linked to oxidative metabolism [40–42]. Moreover, O2 uptake at rest and basal the metabolic rate are usually regulated by the thyroid state. Thus, hypothyroidism and hyperthyroidism are opposite conditions associated with decreased and increased basal O2 uptake, respectively [43]. It is possible that after 6 months of training, our volunteers achieved an improvement in metabolic efficiency due to an increase in the number of mitochondria in their skeletal muscle [44] or increased stores of intramuscular triglycerides [37]. These effects would be reflected in the lower concentrations of T3 and T4 in the plasma of these individuals.

Martin et al., using a maximal incremental test protocol, found a decrease in the maximum oxygen consumption and a decreased arteriovenous oxygen difference 2 weeks after the administration of T3 in 18 healthy subjects [45]. These authors quantified enzymes related to oxidative metabolism in skeletal muscle biopsies and found a decline in muscle oxidative capacity after administration of T3. Specifically, a reduction in the enzymes necessary for oxidative metabolism of carbohydrates and fatty acids [45]. Rone et al. studied the relationship between fitness and T3 metabolism and found that the higher the fitness of the individual, the greater the rate of T3 metabolism [46].

In conclusion, our data show that moderate physical exercise improved the physical fitness of healthy older adult males, reduced serum α-MSH after 3 months of training and reduced serum thyroid hormones (T3 and T4) after 6 months of training. These results indicate time-dependent adaptations at the central and peripheral levels. A limitation of our study was that food intake was not controlled during the study protocol, despite the recommendation of maintaining the same eating habits. We also lacked a control group to compare changes not only within groups but also between groups. However, more studies are needed to better understand the relationship between exercise and improved metabolic profile in elderly individuals.

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