**ABSTRACT**

Introduction: Fast population aging is a global reality. Today’s major challenge is to promote the healthy aging of more and more people by acting on factors that can be modified, such as physical exercise. Regular exercise could contribute to the prevention of chronic diseases associated with aging. Research has been conducted on the physical training response of elderly individuals, but there is not yet any consensus on the influence of strength training or IL-6 polymorphism on levels of inflammatory markers such as IL-6 and muscle damage marker CK, particularly in healthy elderly male individuals. Objectives: The aim of this study was to evaluate the relationship of IL-6 promoter -174 C/G gene polymorphism on systemic IL-6 responses and muscle damage after eccentric strength training in elderly men. Methods: This is a prospective, high-quality study. Gene frequency of polymorphism of promoter gene IL6 G-174C was identified using the Hardy-Weinberg test in 28 older male volunteers. The relationships of each genotype with IL-6 and CK serum levels were analyzed. CK and IL-6 levels were determined at pre-training and 0h, 3h, 24h, and 48h post-training periods. Results: Differences in baseline and post-training IL-6 levels of genotypic groups were observed for all time periods analyzed (p = 0.029). Eccentric exercise efficiently reduced post-intervention muscle damage, thus showing a statistical difference between the pre- and post-intervention time points (p< 0.0005). Conclusion: Eccentric training influenced CK and IL-6 modulation independently of the polymorphism of the IL-6 promoter gene -174 C/G.

**Keywords:** Elderly; Strength Training; Inflammation.

**RESUMEN**

Introducción: El rápido envejecimiento poblacional es una realidad global. El principal desafío actual es promover el envejecimiento saludable de cada vez más personas, actuando en factores que pueden ser modificados, como por ejemplo, el ejercicio físico. El ejercicio regular puede contribuir a la prevención de enfermedades crónicas asociadas al envejecimiento. Investigaciones han sido realizadas sobre las respuestas del ejercicio físico al entrenamiento físico, pero aún no hay consenso sobre la influencia del entrenamiento de fuerza o del polimorfismo del IL-6 sobre los niveles de ICT y de marcadores musculares, principalmente, en hombres ancianos. Objetivos: El objetivo del presente estudio consistía en evaluar la influencia del polimorfismo del gen promotor IL-6 -174 C/G sobre las respuestas sistémicas del IL-6 y daños musculares después del entrenamiento de fuerza excéntrica en hombres ancianos. Métodos: Se realizó un estudio prospectivo de alta calidad. Se identificó la frecuencia genotípica del polimorfismo del gen promotor IL6 G-174C mediante el test de Hardy-Weinberg en 28 hombres mayores voluntarios. Se analizaron las relaciones de cada genotipo con los niveles séricos de IL-6 y CK. Se observaron diferencias en los niveles de IL-6 y CK para los grupos genotípicos en todos los periodos analizados (p<0,029). El ejercicio excéntrico redujo eficientemente los daños musculares después del entrenamiento, mostrando una diferencia estadísticamente significativa entre los puntos de tiempo pre e intervención (p<0,0005). Conclusion: El entrenamiento excéntrico influenció a la modulación de CK e IL-6 independientemente del polimorfismo del gen promotor IL-6 -174 C/G.

**Descritores:** Idoso; Treinamento de resistência; Inflamação.
INTRODUCTION

The level of autonomy and the maintenance of elderly’s independence are dependent on functional capacity preservation, which is influenced by aging process typical alterations, like skeletal muscle functions and inflammatory response.1

The regular practice of physical exercises throughout the life cycle has been increasingly stimulated as a strategy for achieving healthy aging,2,3 generating questions about which protocols and modalities of physical exercises should be prescribed for elderly individuals. In this matter, the different inflammatory responses arising accordingly to the type, intensity, volume and frequency of training and the specificities of each gender4 should be considered.

Eccentric exercise is an important resource in the recovery and prevention of musculoskeletal system injury5,6 due to the strengthening of muscle and connective tissue,7 as well as neural adaptations.8

Adaptations generated by eccentric training should be appropriate to different people. The decrease in number and size of muscle fibers, especially of type II, seems to be the major reason for age reduced muscle mass. Therefore, physiological changes such as dehydration, mitochondrial dysfunction, inflammation9 of muscle tissue, hormonal alterations, metabolic disorders, decreased oxygen consumption, loss of mobility and muscle function, increased muscle fatigue and skeletal fractures, as well as higher risk of falls can be observed.10

The regular practice of exercises by elderly could contribute to prevention of systemic inflammation markers increases and non-transmissible chronic processes associated with aging progression.2,11 However, the studies are controversial and results about inflammatory markers vary according to training protocol used.12,13

There is no consensus on the influence of strength training on inflammatory markers concentrations such as IL-6, nor on the influence of IL-6 polymorphism on these markers and muscle damage in healthy elderly individuals, especially for the male population.5,12,13

Among the main mediators of senile inflammation, IL-6 plays a central role in the inflammatory process and in the negative prognosis of some aging associated diseases, especially in sedentary individuals.1,4,10

However, serum levels of IL-6 also change during and after physical exercise of different modalities and intensities. When IL-6 is produced by muscles following physical exercise stimulus, it seems to play a beneficial role to the body and also helps muscle recovery through satellite cells activation.6,11-13

The literature is controversial regarding the relation of the IL-6 polymorphism with the serum increases of this protein after training, of resistance or not. Some studies indicate association between the polymorphism -174C / G and the increase of the serum IL-6 after training session.14 On the other hand, there are studies that did not identify this association.

The aim of the present work was to evaluate the relations of the IL-6 promoter -174 C / G gene polymorphism on systemic IL-6 responses and muscle damage after eccentric strength training in elderly men.

METHODS

The present research, of experimental and quantitative nature, was carried out with older human subjects accordingly to fundamental ethical requirements of Resolution CNS 510/16, including Catholic University of Brasilia Research Ethics Committee approval (UCB/CEP 272-10 report). Independent variables were the high damage eccentric training model and the IL-6 promoter -174 C / G gene polymorphism, and dependent variables were IL-6 and creatine kinase (CK) plasma concentrations, body composition, isometric and eccentric strength and plasmatic lipid profile.

Elderly subjects, aged from 66 to 75 years old, were invited to participate in the study through an explanatory speech, after what they read and signed the informed consent form. To determine the level of physical activity, the International Physical Activity Questionnaire (IPAQ), full version, was applied. The initial sample consisted of 46 male subjects, 60 years of age or older and physically active.

Individuals with changes in pressure levels (n=3), cardiovascular or pulmonary disease manifestations (n=2) and orthopedic alterations, under beta blockers treatment (n=6), and those who did not fulfill the health status examination (N = 3) were excluded from the sample, totaling 14 exclusions before the trials. Those who did not accomplished all blood sample collections during the trials (n=4) were also excluded. The final sample was constituted by 28 individuals.

Electrical bioimpedance tests for evaluating participants’ body composition were carried out on the BIA Biodynamics model 310®, software version 6and manufacturer’s recommendations were followed.

Muscle strength was assessed by Biodex System ® 4 Pro isotonic dynamometer (Biodex Medical Systems Inc., USA). The muscle group evaluated was that of knee extensors of the dominant limb. The participants were oriented and trained for the specific knee extension movement, when the dynamometer support arm moved down (speed toward = 45º / s) and when it moved up (speed Away = 120º / s). The arm only exerted resistance in the downward displacement, what generated an active eccentric force. The range of motion of the knee joint was previously collected by a CARCI manual goniometer, limiting extension by 135º and flexion by 90º.

Twenty-four hours before the training, the participants were familiarized with the equipment by performing two sets of seven repetitions...
with a 120-second interval between sets, and 24 hours after the familiarization, the training was performed with 10 sets of seven repetitions with an interval of 120 seconds between sets, in which the initial phase was knee extension and the final was knee flexion. The participants were positioned on the equipment according to the manufacturer’s instructions and protocol was adapted from Willoughby, VanEnk & Taylor.15

Participant blood samples were collected at five different time periods: before the eccentric training sessions and at 0h, 3h, 24h and 48 hours after their end. Five mL of blood samples were collected by a qualified professional under hygienic conditions and transferred to sealable tubes, immediately refrigerated and transported to the analyzing laboratory, where the tubes were centrifuged and then aliquoted to the different analyses.

Serum concentrations of IL-6 inflammatory mediator before and after the intervention were determined by the enzyme-linked immunosorbent assays method.16 The samples were processed in duplicate and calibration curve points in triplicate. Creatine kinase (CK) serum concentrations were determined by kinetic-spectrophotometric methodology. The threshold value for CK, indicating muscle damage, was 155 U/L.6,7 Serum lipid profiles, including total cholesterol - COLt and fractions were measured in the AutoHumalyzer apparatus manufactured by Human - GMBH (Germany).

Total genomic DNA was obtained from whole blood samples as described in laboratory manuals using the QIAGEN KIT. The frequency of the C/G alleles was determined by the polymerase chain reaction technique (PCR-RFLP). Finally, amplification of the promoter region 174 and genomic sequencing with Big Dye was performed.10

Statistical analysis comprised Hardy-Weinberg equilibrium determined by χ² test to compare observed and expected genotypic frequencies. Continuous data were expressed as means ± SD or simple means (with 95% confidence interval), if applicable. Shapiro-Wilk tests were applied to verify the normal distribution of study variables. Repeated measures of ANOVA were used to compare the action of CK and IL-6 over the 48 hours post-training period. The sphericity component was checked by the Mauchley test. When the sphericity assumption was not found, the importance of the F-ratios was adjusted according to the Greenhouse-Geisser procedure. The Tukey Post-hoc test with the Bonferroni Arrangement was applied in the occurrence of importance. Comparisons of genotypic subtypes were made using the independent t-test. The areas under the curve (AUC) of CK and IL-6 were calculated by the trapezoidal method (Table 1).

RESULTS

Allelic and genotypic frequencies for the IL-6 GG and CC/CG polymorphism groups did not significantly differ according to Hardy-Weinberg test (χ² = 3.11, p = 0.078). The major part of participants (75%) were homozygous for the G allele, followed by the heterozygotes (21%) and the rare combination of the homozygosity of the C allele (4%).

There was no significant difference between age, anthropometric measurements or lipid profile for IL-6 genotypes. (Table 1)

To determine genotype-phenotype correlations, serum CK and IL-6 levels were compared between groups. (Table 2) There was no significant difference between the baseline, peak and area under the curve (AUC) of IL-6 concentrations in relation to genotype groups.

The training intervention caused statistically significant changes in the serum concentration of CK over time (p<0.0005). (Figure 1) Eccentric training increased the serum CK levels from 149.1 ± 98, 0 U/l on pre-workout to 234.0 ± 106.2 U/ in 24 h post-training period.

significant differences between the groups were found for IL-6 serum concentrations (table 2), once the GG baseline was higher than the GC/CC group (p = 0.016). In response to exercise, the group had significantly higher IL-6 serum peak (p = 0.041) and AUC (p = 0.029) than CC/CG group.

Due to these differences, IL-6 genotype kinetics were analyzed. (Figure 2) The eccentric exercise intervention did not lead to any statistically significant changes in IL-6 concentration over time for the GG group (p = 0.632), and the GC and CC (p = 0.305). IL-6 concentrations were significantly higher (p <0.05) for the GG group during all time period measures.

There was no significant difference between the mean baseline strength of the 10-series training related to the genotype groups. From the fifth set’s, the participants strength was significantly smaller than the first set’s (ps0.05).

Table 1. Physical characteristics for subjects and genotypes (mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>GG</th>
<th>CC/CG</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>70.7 ± 4.0</td>
<td>71.6 ± 4.0</td>
<td>0.627</td>
</tr>
<tr>
<td>Stature (cm)</td>
<td>166 ± 7.2</td>
<td>166 ± 5.2</td>
<td>0.960</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.9 ± 13.2</td>
<td>78.7 ± 8.5</td>
<td>0.284</td>
</tr>
<tr>
<td>%FAT</td>
<td>27.0 ± 5.4</td>
<td>30.8 ± 2.8</td>
<td>0.088</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>52.7 ± 7.6</td>
<td>54.3 ± 5.6</td>
<td>0.619</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.3 ± 4.0</td>
<td>28.6 ± 3.6</td>
<td>0.201</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>206 ± 56</td>
<td>202 ± 42</td>
<td>0.887</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>42 ± 10</td>
<td>37 ± 8</td>
<td>0.298</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>136 ± 45</td>
<td>122 ± 32</td>
<td>0.497</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>31 ± 12</td>
<td>43 ± 22</td>
<td>0.229</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>151 ± 58</td>
<td>212 ± 111</td>
<td>0.207</td>
</tr>
</tbody>
</table>

Table 2. Activity of Creatine kinase (CK) and content of interleukin 6 (IL-6) for IL6 genotypes groups in baseline, peak and area under the curve (AUC) during 48 h.

<table>
<thead>
<tr>
<th></th>
<th>GG</th>
<th>CC/CG</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK (U/I)</td>
<td>164.5 (115.3 – 213.7)</td>
<td>102.9 (74.6 – 131.1)</td>
<td>0.249</td>
</tr>
<tr>
<td>BaseLine</td>
<td>262.7 (212.2 – 313.2)</td>
<td>2170 (142.8 – 291.2)</td>
<td>0.326</td>
</tr>
<tr>
<td>Peak</td>
<td>200.4 (173.1 – 239.1)</td>
<td>179.0 (123.8 – 235.6)</td>
<td>0.559</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.61 (2.97 – 6.25)</td>
<td>1.56 (0.01 – 3.28)</td>
<td>0.016</td>
</tr>
<tr>
<td>BaseLine</td>
<td>7.97 (4.20 – 11.74)</td>
<td>2.87 (1.41 – 4.32)</td>
<td>0.041</td>
</tr>
<tr>
<td>Peak</td>
<td>5.50 (3.22 – 7.78)</td>
<td>1.64 (0.52 – 2.77)</td>
<td>0.029</td>
</tr>
</tbody>
</table>

* Significant differences for pre-exercise, 0h, 3h and 48h post-exercise. # Significant differences for pre-exercise, 0h, 3h and 24h post-exercise (p<0.05).

Figure 1. Creatine quinase (CK) activity in response to eccentric exercise for the study population show.
DISCUSSION

Physical training has been stimulated to contrast sedentary lifestyle for all age groups and has generated a wide discussion about selected protocols, especially those involving resistance. In this study with older person, eccentric training with seven repetitions of ten series was investigated, and strength declining magnitude for the dominant limb in knee extension was observed. In fact, after five repetition series a significant decline in strength (p = 0.008) was already observed, indicating that exhaustion was reached. This result helps to define the number of series to be settled in a training trial of seven repetitions for elderly, a fact not described in some studies with similar methodology.

Participants were found to have body fat percentage above levels recommended by the American College of Sports Medicine. Ethnology of aging body fat increase content in not yet totally understood, but some studies found a strong relationship of fat accumulation with daily life activities (DLAs) decrease and nutritional transition, affecting all age groups. High prevalence of excess body fat is a relevant factor, since it is directly related to many chronic diseases, including diabetes and cardiovascular diseases, among others. It should be noted that, in this context, inflammatory markers play an important role and increased levels of these molecules, such as IL-6 have been widely related to chronic disease process. Distinct physical activity practices may modify the negative effects associated with chronic disease evolution, provided that they are performed in a safety and efficiency way.

Eccentric training intervention was used in this study because its therapeutic benefits are well described in available literature. However, its action on inflammatory response is still controversial, especially in the elderly. The intensity and duration of eccentric muscle activity may induce muscle damage, leading to muscular hypertrophy that can be monitored by the serum levels of CK.

In this study, CK serum levels reached maximum values (234.0 ± 106.2 U/l) after 24 hours of training activity, demonstrating that eccentric training may be related to the effects of this cytokine following the muscle damage for the studied population.

Upon analysis of the genotypes, it was observed that the polymorphism of the IL-6 promoter -174 G/C gene is related to IL-6 levels, but not to the pre-intervention and post-training CK serum concentration. The polymorphism of the IL-6 promoter -174 G/C gene showed great influence on the serum levels of IL-6 inflammatory marker (p = 0.016). A greater allelic frequency of G and a higher genotypic frequency of G homozygote were observed, corroborating literature findings. The presence of the C allele was determinant for lower serum levels of IL-6 basal moment (p = 0.016) as well as for IL-6 serum levels throughout the trials time periods (p = 0.029).

Result corroborates other research groups’ works, indicating that G homozygote population has higher IL-6 serum levels, being more propitious to diseases related to higher levels of this marker.

Both the modulatory effects of eccentric training and the influence of IL-6 cytokine level require further clarification. However, because IL-6 is a multifunctional cytokine, it plays an important role in metabolic signaling.

Indeed, independently of the genotype imposed by the IL-6 promoter region and the IL-6 serum concentration level, one can benefit from the positive effects of eccentric training in the muscle mass rehabilitation context. However, the G homozygote group, which has significantly higher basal serum levels (p = 0.016), requires more attention in order to elucidate the effects of serum concentrations of IL-6 on the human body, especially in the elderly.

CONCLUSIONS

IL-6 serum levels increased with muscle damage in elderly men submitted to eccentric physical training. However, this increase did not happen at the same intensity on different genotypic groups of promoter region of the gene -174 C / IL-6 polymorphism. It seems to be a relation of elderly IL-6 serum levels increases and the homozygote genotype G allele, even before the intervention proposed in this work.

ACKNOWLEDGMENT

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All authors declare no potential conflict of interest related to this article.
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