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Research Paper

Resistance training and redox homeostasis: Correlation with age-associated genomic changes

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ABSTRACT

Regular physical activity is effective as prevention and treatment for different chronic conditions related to the ageing processes. In fact, a sedentary lifestyle has been linked to a worsening of cellular ageing biomarkers such as telomere length (TL) and/or specific epigenetic changes (e.g. DNA methylation), with increase of the propensity to aging-related diseases and premature death.

Extending our previous findings, we aimed to test the hypothesis that 12 weeks of low frequency, moderate intensity, explosive-type resistance training (EMRT) may attenuate age-associated genomic changes. To this aim, TL, global DNA methylation, TRF2, Ku80, SIRT1, SIRT2 and global protein acetylation, as well as other proteins involved in apoptotic pathway (Bcl-2, Bax and Caspase-3), antioxidant response (TrxR1 and MnSOD) and oxidative damage (myeloperoxidase) were evaluated before and after EMRT in whole blood or peripheral mononuclear cells (PBMCs) of elderly subjects.

Our findings confirm the potential of EMRT to induce an adaptive change in the antioxidant protein systems at systemic level and suggest a putative role of resistance training in the reduction of global DNA methylation. Moreover, we observed that EMRT counteracts the telomeres' shortening in a manner that proved to be directly correlated with the amelioration of redox homeostasis and efficacy of training regime, evaluated as improvement of both muscle's power/strength and functional parameters.

1. Introduction

With increasing age, genetic vulnerabilities, underlying diseases, and physiological impairments increase the risk for decline in physical functioning [50]. Despite it is known that physical activity (PA) prevents functional decline among older people, with a positive influence on health and survival compared with a sedentary lifestyle, inactivity continues to be a major public health problem [70,14,5]. Actually, a sedentary lifestyle has been linked to a worsening of cellular ageing biomarker such as telomere length (TL) in white-blood-cell [14], with increase of the propensity to aging-related diseases and premature death [50]. Telomeres are repetitive sequences of DNA (5'-TTAGGG$_n$-3') located at the ends of mammalian chromosomes. They decrease in length with age, reducing the stability and function of chromosomes [19]. Besides the end-replication problem, telomere-specific DNA damage induced by oxidative stress has been extensively studied as a factor that may contribute to telomere shortening [29]. TL has been indeed reported to predict cancer, mortality and cardiovascular events [36], where chronic oxidative stress seems to play a major role in the pathophysiology of chronic inflammation. Moreover, clinical data suggest that parameters of telomere biology in circulating cells can be used as indicators of the effect of therapeutic intervention for cardiovascular morbidity [21].

Circulating blood leukocytes represent a resource in telomere biology because of the high correlation between TL in these cells and those of other tissue types [25,72]. However, it must be considered that TL in different cell types may better reflect specific diseases, tissue-specific aging, or cell-specific adaptations [67]. To

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date, there are many evidences about the association between habitual PA and longer leukocyte telomere length (LTL) [14,17,37,40,71,18,57,39], but most of them derive from studies where aerobic endurance-exercise training was used as intervention [14,17,37,18,57]. Moreover, they have shown conflicting results, with telomeres that can take three different trajectories (expected shortening, maintenance, and lengthening). Nevertheless, little is known about the effect of resistance training on telomere length, so far. Resistance training has the potential to counteract the age-related decline of muscle strength, power output, and muscle mass in elderly populations. In fact, the magnitude of these adaptations seems to be similar to those observed in untrained young subjects [45,51]. Therefore, this type of training has clinical relevance due to its benefits on the neuromuscular system and on the daily living activities performance [5].

We recently showed that 12 weeks of low frequency, moderate intensity, explosive-type resistance training (EMRT) could be proposed as an effective exercise intervention for improving the overall health of older people. Indeed, EMRT not only enhanced the muscle strength, power, and functional performance without detrimental effects on cardiovascular and inflammatory parameters [6], but improved the general adaptive response to oxidative stress related to intense acute exercise [11].

Given the extensive evidence that aging can impact the genome modifying its structure-function relationship through epigenetic changing (i.e. DNA methylation) and alter telomere dynamics [30], the aim of the present study was to test the hypothesis that EMRT may attenuate the age-associated genomic changes. In particular, LTL, global DNA methylation and global protein acetylation, as well as the expression of TRF2, Ku80, SIRT1, SIRT2 and proteins involved in the apoptotic pathway (Bcl-2, Bax and Caspase-3) and the antioxidant response (TrxR1 and MnSOD) were evaluated before and after EMRT in peripheral blood mononuclear cells (PBMCs) of elderly subjects. Moreover, a correlation analysis was performed to verify whether LTL correlates with the markers of redox homeostasis or with the decline in functional parameters in ageing.

2. Material and methods

2.1. Study design

Recently we have conducted a controlled trial in which elderly subjects (70–75 years) were randomly divided into training and control group, to evaluate the effect of an EMRT protocol for 12 weeks on muscle strength, power and functional performance, as well as the adaptive response to oxidative stress induced by an acute exercise [11,6]. The study has been expanded utilizing subjects from both the trained group (TR: n=10) and from the control group (CTRL: n=10), successfully able to complete the blood samplings at baseline and after the EMRT protocol period. Our subset resulted to be fully representative of the original groups, being the anthropometric, physiological and functional characteristics overlapping the values of the previous study at both baseline (PRE) and POST-EMRT training (Table 1) [6]. Physical activity level was evaluated using the Modified Baecke Questionnaire for Older Adults [69]. Diet composition and nutrient intake, adjusted for total calories, were similar among the groups (data not shown). No significant difference between groups was reported comparing baseline level of all parameters analyzed and characteristics of participants. Unless differently stated, no gender differences were found concerning all parameters analyzed.

Participants were seated comfortably for 10–15 min prior to resting blood pressure and heart rate measurements. Further, height and weight were recorded during a clinical visit, from which BMI was calculated. Exclusion criteria included co-morbidities that could interfere with LTL analysis between active and inactive subjects (e.g., heart disease, heart attack, stroke, diabetes mellitus types 1 or 2, osteoarthritis of the hip or knee, Parkinson disease, cancer, psychological chronic stress or any other mood disorders). All participants were fully informed of the research design and associated benefits and risks of the investigation before signing an informed consent form approved by the relevant Ethics Committee.

Table 1
Baseline characteristics of participants.

Characteristic	TRAINED ($n=10$)	CONTROL ($n=10$)
Gender	5 males 5 females	5 males 5 females
Age (years)	72 ± 1	72 ± 1
Height (cm)	166 ± 9	167 ± 10
Body Mass (Kg)	70 ± 2	72 ± 3
BMI (Kg/m^2)	23 ± 2	25 ± 1
Systolic BP (mmHg)	123 ± 4	131 ± 4
Diastolic BP (mmHg)	78 ± 3	75 ± 3
Resting HR ($b \times min^{-1}$)	68 ± 9	69 ± 8
Physical activity level	20 ± 1	19 ± 2
Modified Baecke Questionnaire (score)		
Household	14 ± 1	13 ± 1
Sports	3 ± 1	4 ± 1
Leisure	3 ± 1	2 ± 1
Muscle power (Δ%)		
LegExt	35.9 ± 5.2[a,c]	-0.9 ± 1.9
CMJ	17.5 ± 4.3[a,c]	-2.0 ± 2.8
Muscle strength (Δ%)		
LegExt	16.4 ± 1.5[a,c]	-0.3 ± 1.5
Functional tests (Δ%)		
6-m walking	-9.0 ± 1.6[a,c]	1.4 ± 1.3
6-m walking loaded	-10.0 ± 2.1[a,c]	1.5 ± 2.2
Stair-climbing	-8.0 ± 3.1[a,b]	3.6 ± 2.0
Stair-climbing loaded	-12.0 ± 2.5[a,c]	-0.2 ± 2.7

All values represent means ± SEM. The delta percentage was calculated trough the standard formula: Δ%=[(POST-test score-PRE-test score)/PRE-test score] × 100.
BMI (body mass index); **BP** (blood pressure); **mmHg** (millimeters of mercury); **LegExt**, leg-extention; **CMJ**, countermovement jump.

[a] p values < 0.01.
[b] p < 0.05 for changes within-group after the intervention period.
[c] p values < 0.01 for changes between-group after the intervention period (TR vs. CTRL).

2.2. Blood sampling and PBMCs isolation

Before and after 12 weeks of intervention, fasted blood samples were drawn from the antecubital vein while subjects remained in reclined position. Samples in additive-free tubes (BD Biosciences, San Jose, CA, USA) were left at room temperature for coagulation for at least 1 h and then centrifuged (2,500 rpm × 10 min) for serum separation. Blood sampled in EDTA tubes (BD Biosciences) were used for plasma collection by centrifugation of whole blood (2,500 rpm × 10 min at 4 °C) and for PBMC isolation. Whole blood, serum, plasma, and PBMC samples were aliquoted and stored at −80 °C for further analyses. PBMCs were isolated by density gradient centrifugation from each sample (Ficoll-Paque plus; Amersham Pharmacia Biotech, Piscataway, NJ).

2.3. EMRT and functional parameters

Detailed information about the explosive-type resistance training (EMRT) protocol and the evaluation of functional parameters has been published elsewhere [6]. Briefly, low frequency EMRT (2 days/week) for 12 weeks was employed. For each machine, the resistance was set at 70% of the one-repetition maximum (1RM), and subjects performed 3–4 sets of 10–12