Experimental Gerontology 65 (2015) 16–22

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

# The effect of six months of elastic band resistance training, nutritional supplementation or cognitive training on chromosomal damage in institutionalized elderly

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ARTICLE INFO

*Article history:*
Received 7 November 2014
Received in revised form 16 January 2015
Accepted 3 March 2015
Available online 5 March 2015

Section Editor: Holly M Brown-Borg

*Keywords:*
Chromosomal damage
DNA damage
Exercise
Training
Aging
VAAS

ABSTRACT

Increased DNA and chromosomal damage are linked to aging and age-related diseases like cardiovascular diseases, diabetes or cancer. Physical activity and an optimal status of micro- and macronutrients are known to reduce the incidence of MN, a marker for chromosomal instability and mutagenicity. Once older people reach a certain age they change from a home-living situation to an institutionalized situation, which is often accompanied by malnutrition, depression and inactivity. We conducted the current study to investigate the effect of a six month progressive resistance training (RT), with or without protein and vitamin supplementation (RTS) or cognitive training (CT) only, on chromosomal damage measured by the cytokinesis block micronucleus cytome assay in 97 Austrian institutionalized women and men (65–98 years). All three intervention groups demonstrated a tendency of a reduced frequency of cells with MN (−15%) as well as for the total number of MN (−20%), however no significant time-effect was observed. Besides a significant increase in plasma B12 and red blood cell folate status, the six month change of B12 was negatively correlated with the six month change of the MN frequency in the RTS group ($r = -0.584$, $p = 0.009$). Our results suggest that in this age group either physical or cognitive training may result in similar biochemical changes and therefore enhance resistance against genomic instability. Supplementation with the vitamins B12 and folic acid could contribute to reduced chromosomal damage in institutionalized elderly.



## 1. Introduction

The loss of muscle mass, strength, function and the associated reduction of life quality is linked to the process of aging (Johnston et al., 2008; Seene et al., 2012). Physical activity, regular exercise, and especially resistance training are important in preventing severe muscle atrophy, enhancing function and maintaining physical independence in the elderly (Demontis et al., 2013; Arnold and Bautmans, 2014). The process of aging and the loss of muscle mass are both associated with higher levels of DNA damage and deteriorated antioxidant defense (Fulle et al., 2004; Gianni et al., 2004). It is well known that there are increased levels of chromosomal and DNA damage in people with chronic diseases such as cardiovascular diseases, type 2 diabetes and cancer (Battershill et al., 2008; Müllner et al., 2014). Resistance training, together with protein supplementation, seems to be most effective for increasing muscle mass and strength in the elderly (Shahar et al., 2013; Lustgarten et al., 2014). To lower the frequency of micronuclei (MN), which is a well-established marker for chromosomal damage and highly correlated with cellular mutation, an adequate status of the vitamins B12 and folate is recommended (Fenech and Bonassi, 2011; Ni et al., 2012).

Studies concerning the effect of exercise and training on MN frequency are rare. However, examinations on that topic show varying results. Regular physical activity seems to lower chromosomal damage (Goon et al., 2008; Huang et al., 2009), whereas acute exhausting exercise tends to increase the MN frequency (Schiffl et al., 1997). Training status also influences the body's ability to deal with exercise-induced oxidative stress. After an ironman triathlon, trained individuals showed no increase in chromosomal damage or even a reduced MN frequency

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http://dx.doi.org/10.1016/j.exger.2015.03.001
0531-5565/

(Reichhold et al., 2008), whereas only untrained subjects demonstrated an increased mutation rate even after 30 min of intense running (Umegaki et al., 1998).

As life expectancy is still growing, it is of great need to investigate health- and fitness-related markers in the oldest of the population (8th, 9th and 10th decade). Currently there is a general lack of data for this age group. This aspect is of particular interest since the living situation of many elderly individuals is changing in this period towards a transfer to new surroundings such as elderly homes, which is often accompanied by conditions of malnutrition, multiple medications or increased depression and loss of life quality (Smoliner et al., 2009).

We conducted the current study to investigate the effect of a six month progressive resistance training (using elastic bands), with or without protein and vitamin supplementation, on chromosomal damage in Austrian institutionalized elderly. The cytokinesis block micronucleus cytome assay (CBMN-assay) was performed to measure genome integrity. In the present study we hypothesized that increased physical fitness improves resistance against chromosomal instability in institutionalized elderly. Protein and vitamin supplementation should enhance this protective effect. With our study cohort subjects, being at or above life expectancy in Austria (study/Austria: women 82.86/83.25 years; men 84.85/77.95 years) (StatisticsAustria, 2014) and 75% of our study population being 80 years old or older, we were able to generate novel data on chromosomal damage and physical performance after a six month training period in this particular age group of institutionalized elderly.

## 2. Methods

### 2.1. Subjects

Ninety-seven institutionalized elderly women and men (aged 65–98 years), recruited from five different senior residencies in the area of Vienna (Curatorship of Viennese Retirement Homes), volunteered for the study. The subjects were mentally (Mini Mental State Examination ≥23) and physically (Short Physical Performance Battery >4) able to participate in the study. They were sedentary (less than 1 h of physical activity or exercise per week) and free of severe diseases that would contra-indicate medical training therapy or measurement of physical performance, including cardiovascular diseases, diabetic retinopathy and regular use of cortisone-containing drugs. The health condition of all study subjects was assessed by specialists in internal medicine and gerontology. Furthermore, regular strength training (more than once per week) in the last six months before inclusion was an exclusion criterion. Written informed consent was obtained from all participants before entry into the study in accordance with the Declaration of Helsinki. This study was approved by the ethics committee of the City of Vienna (EK-11-151-0811) and registered at ClinicalTrials.gov, NCT01775111.

### 2.2. Study design

The present study was conducted in a randomized, controlled, observer-blind design. The participants were randomly divided into three parallel groups – cognitive training (CT), resistance training (RT), RT + supplement (RTS) – and matched for gender. Blood samples were taken and physical performance tests were executed before (T1), after three months (T2) and after six months (T3) of intervention. The goal was to assess and compare the effects of either a resistance training intervention, a resistance training and nutritional supplementation intervention or a cognitive training intervention on institutionalized elderly.

### 2.3. Resistance training

The resistance training groups (RT and RTS) received two weekly sessions of resistance training, conducted on two non-consecutive days and supervised by a sport scientist. Training attendance was recorded every session. The only equipment used were elastic bands and a chair. The progressive resistance training protocol was designed based on the guidelines of the American College of Sports Medicine for resistance training with older subjects (Nelson et al., 2007). The main part consisted of ten exercises for the main muscle groups (legs, back, abdomen, chest, shoulder and arms). One training session started with 10 min of warm-up, continued with 30–40 min of strength training and ended with a 10 minute cool-down. To keep the training stimulus high enough, the exercise program was adjusted to the participants' individual needs, by either adapting the resistance of the elastic band (shorter or stronger band) or by modifying the exercise, by means of performing a more difficult version. In the initial phase (4 weeks) one set of 15 repetitions was performed in order to learn the correct form of each exercise. From the fifth week on the intensity and volume has progressively been increased from two sets of light exercises to two sets of heavy resistance. If the participants could easily perform two sets of 15 repetitions they were told to either take more resistance or to perform a more difficult version of the exercise.

### 2.4. Resistance training and supplementation

The RTS group followed the same training protocol as the RT group. Additionally they received a liquid supplement every morning, as well as directly after each training session. It consisted of 20.7 g protein (56 energy (En)%, 19.7 g whey protein, 3 g leucine, >10 g essential amino acids), 9.3 g carbohydrates (25 En%, 0.8 BE), 3.0 g fat (18 En%), 1.2 g roughage (2 En%), 800 IU (20 μg) of vitamin D, 250 mg calcium, vitamins C, E, B6 and B12, folic acid and magnesium (1 portion FortiFit, Nutricia). Total energy per drink was 150 kcal. The intake of the nutritional supplement was controlled at breakfast as well as after the training sessions.

### 2.5. Cognitive training

The CT groups performed coordinative or cognitive tasks two times per week, equally to the RT and RTS groups. In contrast to a classic control group, which would not get any treatment, the training frequency of this group was the same as in the other groups. Therefore the "bias" of regularly being part in group activities was minimized (socialization factor). The participants of the CT group mainly performed memory training and finger dexterity exercises in sitting position (Gatterer and Croy, 2004).

### 2.6. Cytokinesis block micronucleus cytome assay

Blood samples were taken early morning after an overnight fast using heparin, serum and EDTA tubes (Greiner Bio-One, Kremsmunster, Austria). Peripheral blood lymphocytes were isolated using Ficoll separation tubes (Greiner Bio- One). The cytokinesis block micronucleus cytome (CBMN) assay was conducted according to the protocol of Fenech (Fenech, 2007) and Wallner et al. (Wallner et al., 2012). Cells were stimulated to perform mitotic division with phytohemagglutinin (PAA, Pasching, Austria) using a concentration of $1 \times 10^6$ cells/ml in culture medium. Samples were incubated at 37 °C and 5% $CO_2$, and after 44 h, cytochalasin B (Sigma Aldrich, Vienna, Austria) was added to stop further cell division. Cells were spotted onto microscope slides, stained (Diff-Quick; Medion Diagnostics, Dudingen, Switzerland) and counted using a bright field microscope (1000-fold magnification; Olympus, Wien, Austria). For each sample, duplicates were performed and two slides of each duplicate were produced. From the four resulting slides, 500 cells per slide (2000 per subject) were counted equally to minimize experimental variation and eliminate scorer bias.

To assess chromosomal damage of blood lymphocytes, the frequency of MN, nucleoplasmic bridges and nuclear buds in 2000 binucleate (BN) cells was counted, as well as the number of apoptotic and necrotic cells. Furthermore, the nuclear division index (NDI) was calculated to