Experimental Gerontology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Experimental Gerontology

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

A standardized randomized 6-month aerobic exercise-training down-regulated pro-inflammatory genes, but up-regulated anti-inflammatory, neuron survival and axon growth-related genes

Osigbemhe Iyalomhe [a], Yuanxiu Chen [b], Joanne Allard [c], Oyonumo Ntekim [d], Sheree Johnson [c], Vernon Bond [a], David Goerlitz [e], James Li [e], Thomas O. Obisesan [a,b,*]

[a] Division of Geriatrics, Department of Medicine, Howard University Hospital, 2041 Georgia Ave NW, Washington, DC 20060, USA
[b] Clinical Translational Science Center, Howard University Hospital, 2041 Georgia Ave NW, Washington, DC 20060, USA
[c] Department of Physiology and Biophysics, Howard University Hospital, 2041 Georgia Ave NW, Washington, DC 20060, USA
[d] Department of Health, Human Performance, and Leisure Studies, College of Arts and Science, Howard University College of Medicine, 520 W St NW, Washington, DC 20059, USA
[e] Department of Molecular Biology and Informatics, Georgetown University Medical Center, 400 Reservoir Rd NW, Washington, DC 20057, USA

ARTICLE INFO

Article history:
Received 9 February 2015
Received in revised form 12 May 2015
Accepted 13 May 2015
Available online xxxx

Section Editor: Christian Humpel

Keywords:
Mild cognitive impairment
Microarray
Gene expression
Aerobic exercise

ABSTRACT

There is considerable support for the view that aerobic exercise may confer cognitive benefits to mild cognitively impaired elderly persons. However, the biological mechanisms mediating these effects are not entirely clear. As a preliminary step towards informing this gap in knowledge, we enrolled older adults confirmed to have mild cognitive impairment (MCI) in a 6-month exercise program. Male and female subjects were randomized into a 6-month program of either aerobic or stretch (control) exercise. Data collected from the first 10 completers, aerobic exercise (n = 5) or stretch (control) exercise (n = 5), were used to determine intervention-induced changes in the global gene expression profiles of the aerobic and stretch groups. Using microarray, we identified genes with altered expression (relative to baseline values) in response to the 6-month exercise intervention. Genes whose expression were altered by at least two-fold, and met the *p*-value cutoff of 0.01 were inputted into the Ingenuity Pathway Knowledge Base Library to generate gene-interaction networks. After a 6-month aerobic exercise-training, genes promoting inflammation became down-regulated, whereas genes having anti-inflammatory properties and those modulating immune function or promoting neuron survival and axon growth, became up-regulated (all fold change $\geq \pm 2.0$, $p < 0.01$). These changes were not observed in the stretch group. Importantly, the differences in the expression profiles correlated with significant improvement in maximal oxygen uptake (VO_2max) in the aerobic program as opposed to the stretch group. We conclude that three distinct cellular pathways may collectively influence the training effects of aerobic exercise in MCI subjects. We plan to confirm these effects using rt-PCR and correlate such changes with the cognitive phenotype.

1. Introduction

Neuroinflammation, a prevalent feature of Alzheimer's disease (AD), is heralded by insoluble protein deposition, reactive astrocytes, activated microglia, dystrophic neurites, and intracellular neurofibrillary tangles (NFTs) (Selkoe, 2001; Lue et al., 1996; Hoozemans et al., 2006). Abundant evidence suggests that inflammatory mechanisms within the central nervous system (CNS) likely promote cognitive deterioration (Lee et al., 2009). AD progression is marked by uncontrolled glial cell activation, neuroinflammation and consequent neuronal/synaptic dysfunction, inciting a vicious cycle of increased glial activation and neuronal damage (Mrak and Griffin, 2005). Therefore, strategies to reduce neuroinflammation may attenuate the risk of sporadic AD (Vlad et al., 2008).

Physical activity can reduce inflammation (Braskie et al., 2014), and has been demonstrated to improve memory in MCI and possibly AD subjects (Buchman et al., 2012). In support of this view, but through a yet undiscerned mechanism, moderate exercise reduced amyloid plaque burden in transgenic mouse models of AD, and improved cognitive function (Adlard et al., 2005). Independently, aerobic exercise can modulate immune response, increase the population of regulatory T cells, decrease the population of inflammatory monocytes, and down-regulate inflammation (Boas et al., 1996; Gleeson et al., 2011; McTiernan, 2008, 2004; Ouchi et al., 2011; Spence et al., 2010). However, whether pro-inflammatory proteins facilitating cell death or anti-inflammatory genes promoting neuronal survival and axonal growth are involved in this regulatory mechanism has not been systematically examined in MCI subjects. Importantly, data are lacking on the mechanisms underlying the

* Corresponding author at: Division of Geriatrics, Howard University Hospital, 2041 Georgia Ave NW, Washington, DC 20060, USA.
E-mail address: Tobisesan@howard.edu (T.O. Obisesan).

http://dx.doi.org/10.1016/j.exger.2015.05.005
0531-5565/

beneficial effects of exercise in African American (AA) MCI subjects. As a preliminary step to this investigative exercise, we examined the training-induced changes in the overall gene expression profile from blood cells of MCI volunteers randomized into a 6-month aerobic exercise-training program versus a stretch-exercise control group. We hypothesized that aerobic training adaptation would promote favorable changes in the expression of pro-inflammatory or anti-inflammatory genes, and of genes modulating immune response or promoting neuronal survival. Since MCI is prodromal for AD, insight into these mechanisms may inform the development of interventions and treatment regimens for AD patients.

2. Material and methods

The protocols used in this investigation were approved by the Howard University Institutional Review Board (IRB). As required for studies involving human subjects, all participants completed a signed informed consent form prior to enrollment in the study.

2.1. Level I screening

Main eligibility criteria used for inclusion consisted of the following: age > 55 years; ability to exercise vigorously without causing harm to self; diagnostic designation as MCI according to Petersen criteria (Petersen, 2004) using education adjusted scores; have a committed study partner; be in good general health; and willing to exercise for the entire study duration; and undergo required medical and study-related assessments. After obtaining informed consent from volunteers, we collected data on demographics and general medical history. Based on this initial evaluation, we excluded volunteers with the following history: head trauma, uncontrolled diabetes mellitus and hypertension; current chronic renal, liver, respiratory or neurologic disorders; recent myocardial infarction (within the previous 6 months), and unstable angina. Also excluded were persons using hormone replacement therapy (HRT) or medications that may affect memory (e.g., anticholinergics, sedative hypnotics, narcotics, and antiparkinsonian agents). Volunteers having unstable medical conditions, evidenced by starting new medications within 6 weeks of enrollment, and those having history of chronic alcohol and drug abuse, were also excluded from the study. Subjects were allowed to continue using medications to treat AD (Reminyl, Aricept, Exelon, Namenda, and Ginkgo Biloba).

Diagnosis of MCI was made using the following criteria: memory complaints, education adjusted Mini-Mental State Examination (MMSE) scores, where the adjusted MMSE = raw MMSE − (0.471 × [education − 12]) + (0.131 × [age − 70]) with a score of 24–30 being inclusive (Mungas et al., 1996), objective memory loss ascertained by performance on education adjusted scores on the Wechsler Memory Scale Logical Memory II, Clinical Dementia Rating Scale (CDR) of ≤0.5, modified Hachinski score <4, and Geriatrics Depression Scale (GDS) <6. We also excluded persons with probable dementia according to National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (MINCDS/ADRDA) criteria, and those having memory loss from medical, neurological, or psychiatric conditions, medication effects or delirium.

2.2. Level II screening

From the remaining eligible volunteers, we acquired vital signs and anthropometric measurements, and then performed detailed general physical and neurological examinations. We collected fasting blood samples to assess plasma lipoprotein/lipids, hemoglobin and hematocrit, basic metabolic panel (BMP), rapid plasma reagin (RPR) and plasma levels of thyroxine (T_4), and thyroid stimulating hormone from all eligible volunteers.

The remaining qualified and willing volunteers underwent a maximal treadmill exercise test using the Bruce protocol (Bruce and Hornsten, 1969) to screen for cardiovascular disease (CVD). The test was terminated when the subject could no longer continue, or CVD signs and symptoms occurred (American College of Sports Medicine Position Stand, 1998a). CVD indicators including blood pressure, heart rate, and ECG were recorded before the test, at the end of every exercise stage, and every 2 min for 6 min after discontinuing the test. The test was terminated if the subject had >2-mV ST-segment depression, extra systole, chest pain, arrhythmias, hypotension or dizziness, or significant ST segment elevation during the treadmill tests (American College of Sports Medicine Position Stand, 1998a; White and Evans, 2001). The maximal oxygen consumption (VO_2max) was determined using a validated customized on-line system (K4b^2 by Cosmed, Chicago Illinois). As part of the initial evaluation and prior to the treadmill screening tests, each participant had a brain MRI to exclude significant intracranial pathology (such as clinically significant cerebrovascular disease including cortical infarcts, strategically located subcortical gray matter or extensive white matter abnormalities). Prior to randomization, study partners and subjects were instructed to maintain regular caloric intake during the study period, and maintain an American Heart Association Step 1 diet: <30% of energy from fat, ~55% from carbohydrate, ~15% from protein, and cholesterol intake <300 mg/day.

2.3. Randomization and blinding procedures

Randomization of subjects occurred prior to baseline tests. All staff, except those directly monitoring exercise-training, were blinded to group assignments. The data were de-identified using assigned unique identifiers for labeling and tracking.

2.4. Baseline testing

At the completion of dietary stabilization and randomization, subjects were familiarized with treadmill screening tests, before undergoing the actual treadmill exercise test. We then determined baseline VO_2max and endurance capacity, using a modified Bruce protocol. Discontinuation criteria for this test were similar to those used for the level II screening test.

During the 24-hour period prior to blood drawing for baseline tests, participants were instructed not to have anything by mouth, consume no alcohol or smoke cigarettes, and to not use any anti-inflammatory medications. Additionally, subjects were told to refrain from exercise for 72 h prior to testing, and to confirm that they had no infection in the week prior to testing. For the gene expression analyses, and prior to the initiation of exercise-training, we collected overnight fasting blood samples using sterile techniques by personnel trained in phlebotomy, and stored samples in heparinized collection tubes. Subsequently, the samples were centrifuged at 500 *g* after which the buffy coat layer containing leukocyte population was removed and stored in aliquots at −80 °C.

2.5. Aerobic exercise-training protocol

We inferred each subject's maximum heart rate from baseline VO_2max tests. Both the intervention (aerobic exercise) and control (stretch exercise) groups underwent supervised training 3 times/week.

The aerobic exercise protocol complied with the American College of Sports Medicine Guidelines (ACSM) (American College of Sports Medicine position stand, 1990), and included treadmill walking or jogging, stair-stepping, and elliptical climbing. Subjects underwent a warm up period, followed by exercise-training and an appropriate cool-down period. Initial training sessions lasted 20 min at 50% VO_2max, while study staff used records of exercise heart rate and duration to monitor protocol compliance. During each session, training duration increased by 5 min/week until subjects completed 40 min of exercise at 50%

Please cite this article as: Iyalomhe, O., et al., A standardized randomized 6-month aerobic exercise-training down-regulated pro-inflammatory genes, but up-regulated anti-inflammat..., Exp. Gerontol. (2015), http://dx.doi.org/10.1016/j.exger.2015.05.005