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Preliminary evidence that age and sex affect exercise-induced hTERT expression

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ABSTRACT

The ability to repair cellular damage is reduced with aging, resulting in cellular senescence. Telomeres shorten as cells divide but the rate of telomere attrition is modulated by telomerase, an enzyme that adds nucleotides to the chromosome. Shelterin is a protein complex that acts as a negative regulator of telomerase. The aim of the present study was to investigate age-related differences in telomerase and shelterin responses to acute exercise. We hypothesized that acute exercise would stimulate an increased activity of telomerase (measured by telomerase reverse transcriptase, hTERT) without an increase in activity of shelterin (measured by telomeric repeat binding factor 2, TRF2) in both young and older individuals and that hTERT response would be attenuated in older individuals. Young (22 ± 2 y, n = 11) and older (60 ± 2 y, n = 8) men and women performed 30 min of cycling. Blood was collected pre-exercise and 30, 60, and 90-min post-exercise. The trial induced a significant hTERT response in the cohort as a whole ($p < 0.05$) with greater increases in the young as compared to the older group (time-by-group interaction $p < 0.05$). As expected, TRF2 did not change in response to the trial, however older individuals had a higher TRF2 response at 60 min ($p < 0.05$). There was an unexpected sex difference, regardless of age, where men had significantly greater hTERT and TRF2 responses to the acute exercise as compared to women ($p < 0.05$). These data support the hypothesis that aging is associated with attenuated telomerase activation in response to high-intensity exercise; however, this was only evident in men.

1. Introduction

Telomeres, the protective cap at the terminal end of linear chromosomes, shorten with every cellular replication and, as a result, telomere length shortens with age (Aviv, 2006; Cherkas et al., 2008; Damjanovic et al., 2007; Daniali et al., 2013; de Lange, 2009; Epel et al., 2008; Gilson and Geli, 2007; Ludlow et al., 2008; Osthus et al., 2012; Sfeir et al., 2005). However, physical activity may protect against age-related telomere shortening as endurance-trained older individuals have similar telomere length as young adults (LaRocca et al., 2010) and young elite athletes have longer telomeres than their age-matched inactive peers (Muniesa et al., 2016). It has not been well elucidated how exercise can modify telomere biology but it may be, through increasing the activity and/or abundance of telomerase. Telomerase, a reverse transcriptase enzyme, reduces telomere attrition by adding nucleotides to the end of the chromosome shortly after DNA replication (Axelrad et al., 2013; Blackburn and Collins, 2011; Chilton et al., 2014; Collins, 2006; Cristofari et al., 2007; Gilson and Geli, 2007; Herrera et al., 1999; Hwang et al., 2014; Ludlow et al., 2008; Schmidt et al., 2014; Venteicher et al., 2009). The enzyme is composed of a functional protein, hTERT, and an ncRNA template, TERC. Because hTERT is the limiting factor in functional telomerase activity, it can be used as an index of telomerase function (Chilton et al., 2014). In addition, the molecular complex at the telomeric cap also contains shelterin. Shelterin is a complex of six proteins, including TRF2, which acts as a negative regulator of telomerase activity by controlling telomerase binding to the telomere (de Lange, 2009; Diotti and Loayza, 2011).

Lack of telomerase has been shown to accelerate aging in a cell model (Bernardes de Jesus et al., 2012) while acute exercise has been shown to stimulate telomerase activity, as measured by an increased gene expression of hTERT in young men (Chilton et al., 2014). What is not known is whether the same response would be observed in older individuals. We have previously demonstrated that acute exercise induces a protective effect to a subsequent oxidative stress challenge in young but not older adults (Nordin et al., 2014). Additionally, acute exercise increased antioxidant cell signaling in young men but this response was impaired in older men (Done et al., 2016). These data suggest that aging is associated with reduced ability to stimulate protective pathways regulated by cell signaling. We therefore hypothesized that exercise-induced telomerase activation would be attenuated with age. Telomerase activity was measured by changes in hTERT mRNA and shelterin was measured by TRF2 mRNA in response to a 30-minute

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bout of acute exercise, measured in peripheral blood mononuclear cells (PBMCs) at baseline and 30, 60, and 90-minute post-exercise. We predicted that hTERT would increase in response to acute exercise while TRF2 would not change. In addition, IL-6 mRNA was measured as a positive control to verify the success of the exercise stimulus. While earlier studies only included men, the present study included both men and women for sex parity. We used a high-intensity interval cycling protocol as the acute exercise stimulus because data from our laboratory comparing constant workload to a high-intensity interval protocol demonstrated a greater antioxidant enzyme response from the latter (Done et al., 2016). Evidence of sex effects on telomerase activity are lacking; therefore, our secondary aim was to explore potential sex differences in the exercise-induced telomerase response.

2. Methods

2.1. Subjects

Twelve young (18–25 y) and 8 older (55–75 y) recreationally active men and women were recruited from the community. Recreationally active was defined as having maximal oxygen consumption (VO_2 max) of less than 70th percentile based on age and sex. Study participants were generally healthy, non-smokers, with BMI's between 20 and 33 kg/m^2, and were not taking antioxidant supplements in excess of a multivitamin, or any non-steroidal anti-inflammatory (NSAIDS) drugs for two weeks prior to their study visit. Participants were free from cardiovascular, pulmonary, and metabolic diseases. In addition, regular meditation was an exclusion criterion because existing studies have identified meditation as a telomerase activity modifier (Jacobs et al., 2011; Lavretsky et al., 2013). Any individual who had experienced a myocardial infarction within the last six months or had a history of angina was also excluded. In addition, participants who exhibited clinically relevant arrhythmia at rest or during the maximal stress test were excluded. All participants signed a written informed consent approved by the Northern Arizona University Institutional Review Board.

2.2. Study design

The study employed a cross-sectional design. Prior to the study trial, the participants went through a screening visit and a test of VO_2 max. The acute exercise trial was separated from the VO_2 max test by at least two days to control for any confounding effects of acute exercise. Regardless of age or sex, all participants performed a high-intensity interval protocol on a stationary cycle for 30 min at an intensity relative to their maximum capacity as described below. Blood samples were taken before the exercise and at 30, 60, and 90 min after the exercise bout.

2.3. Screening visit

Prior to any exercise testing the participants completed a health history questionnaire. Height, weight, waist circumference, and resting blood pressure were measured and a 12-lead resting EKG was obtained for screening any abnormalities that would exclude the participant.

2.4. Maximal oxygen consumption (VO_2 max) test

VO_2 max was measured with a graded exercise test performed on a cycle ergometer as previously described (Traustadóttir et al., 2012). The starting workload was selected based on the predicted maximal workload for each individual, and was increased every minute until volitional exhaustion. Participants were instructed to maintain a pedaling rate of 60–70 rpm throughout the test. Oxygen consumption was measured by indirect calorimetry using a metabolic measurement cart (Vmax29, CareFusion, Yorba Linda, CA). Heart function was monitored with continuous 12-lead EKG. VO_2 max was considered achieved if two of the following three criteria were met: a plateau in VO_2 with an increase in workload, a respiratory exchange ratio (RER) ≥ 1.10, and heart rate within 10 beats of age-predicted maximal heart rate (Kohrt et al., 1991). Standard contraindications to exercise testing, and termination criteria outlined by the American College of Sports Medicine were followed at all times.

2.5. High-intensity interval exercise trial

Subjects completed 30 min of cycling at varied workloads consistent with interval training. Breath-by-breath analysis of oxygen consumption was measured throughout the trial using indirect calorimetry to ensure target interval intensities were met. Participants began with a progressive six-minute warm-up broken into two minute steps corresponding to approximately 40-, 60-, and 80% VO_2 max. The instructions participants received to achieve target VO_2 utilized the Borg CR10 scale (Borg, 1998). Participants were asked to complete the first 2 min at an intensity corresponding with a three out of ten, the second 2 min at a five out of ten, and the last 2 min at a seven out of ten, while investigators monitored the oxygen consumption to ensure the appropriate intensities were achieved. The warm-up period was followed by a 3-minute recovery at an intensity corresponding to approximately 60% VO_2 max. Subjects then completed a total of seven high-intensity intervals, each comprised of 1 min of high-intensity followed by 2 min of recovery. For the high-intensity intervals, subjects were instructed to work at the highest absolute intensity they could maintain for one full minute. The target intensity of each interval was set at 90% VO_2 max. Subjects who did not achieve the target intensity within the first interval were given subsequent instructions to increase the workload for the following intervals. Recovery bouts were completed at a self-selected intensity.

2.6. Peripheral blood mononuclear cell isolation

Whole blood was collected with a 21-gauge butterfly needle (Becton Dickinson, Franklin Lakes, NJ) from a superficial antecubital vein. Samples were collected into EDTA vacutainers to prevent coagulation. Whole blood was diluted 1:1 with phosphate buffered saline (PBS) and layered onto Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden). Samples were spun at 900 $\times g$ for 30 min at room temperature, per manufacturer's directions. Following centrifugation, the lymphocyte rich layer was collected and washed twice in PBS with phosphatase inhibitors (Halt Phosphatase Cocktail, ThermoFisher, Waltham, Massachusetts) at 400 $\times g$ for 10 min at 4 °C. Cells were counted using Countess Automated Cell Counter (Life Technologies, Waltham, MA) and aliquots of 2×10^6 PBMCs were made and stored with 200 μL of RNAlater at −80 °C until analyses.

2.7. RT-qPCR – reverse transcriptase quantitative polymerase chain reaction

Real-time PCR analyses of human telomerase reverse transcriptase (*hTERT*), telomeric repeat binding factor 2 (*TRF2*), and interleukin 6 (IL-6) were performed in RNA extracted from PBMCs. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was selected as the housekeeping gene as previous studies have shown it to be a reliable, stably expressed gene in response to aerobic exercise in humans (Catoire et al., 2012; Mahoney et al., 2004). PBMC aliquots were lysed using buffers to manufacturer specifications (Qiagen, Hilden, Germany) and RNA was extracted. Sample RNA was quantified (Gen5 ver. 2.09, Winooski, VT) and tested for RNA purity prior to cDNA synthesis. Equal amounts of sample were reverse transcribed using the iScript RT kit (Bio-Rad, Hercules, CA). A 20 μL reaction of Ssofast SYBR green (Bio-Rad, Hercules, CA), target primers (ThermoFisher, Waltham, MA), nuclease free water and sample cDNA were mixed and aliquoted into 96-well optical plates. Each gene of interest was analyzed in triplicate using a Bio-Rad CFX96 TOUCH thermocycler with CFX Manager software (Bio-Rad,