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Chronic and acute effects of endurance training on telomere length

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Abstract

Telomere shortening is considered a cellular marker of health status and biological ageing. Exercise may influence the health and lifespan of an individual by affecting telomere length (TL). However, it is unclear whether different endurance exercise levels may have beneficial or detrimental effects on biological aging. The aims of the study were to assess both chronic and acute effects of endurance training on TL after an exceptional and extreme trail race. TL was assessed in 20 endurance athletes (17 males; age = 45.4±9.2 years) and 42 age- and gender-matched sedentary controls (32 males; age = 45.9±9.5 years) with quantitative real-time PCR at baseline conditions. Of the 20 runners enrolled in the 'Tor des Géants ®' ultra-distance trail race, 15 athletes (12 males; age = 47.2±8.5 years) were re-evaluated at the intermediate point and 14 athletes (11 males; age = 47.1±8.8 years) completed the competition and were analysed at the final point. Comparison between the two groups (endurance athletes vs. sedentary controls) revealed a significant difference in TL (1.28±0.4 vs. 1.02±0.3, $P = 0.005$). TL was better preserved in elder endurance runners compared with the same age control group (1.3±0.27 vs. 0.91±0.21, $P = 0.003$). TL was significantly reduced at the intermediate (0.88±0.36 vs. 1.11±0.34, $P = 0.002$) and final point compared with baseline measurements (0.86±0.4 vs. 1.11±0.34, $P = 0.0006$) for athletes engaged in the ultra-marathon race. Our data suggest that chronic endurance training may provide protective effects on TL attenuating biological aging. Conversely, acute exposure to an ultra-distance endurance trail race implies telomere shortening probably caused by oxidative DNA damage.

Introduction

Telomeres are DNA tandem repeats of six bases, (TTAGGG)*n*, located at the end of chromosomes. While acting as buffers to prevent loss of genetic information, telomeric DNA inevitably shortens in human somatic cells with each cell division at a rate of 50–200 bp until reaching a critical length (Hayflick limit) (1). This shortening is due to oxidative stress and the end replication problem where DNA polymerase cannot replicate the very ends of the lagging strand. Oxidative stress preferentially damages telomeric regions over other genomic DNA regions and inhibits telomerase activity *in vitro* in various cell types (2). Short or dysfunctional telomeres are often recognized as DNA double strand breaks (DSBs), triggering cell-cycle arrest and result in cellular senescence or apoptosis. Thus, telomeres play an important role in cellular aging and may be considered as an 'internal clock' which terminates a cell's lifespan (3). Telomere

shortening is associated with aging and shorter leukocyte telomere length (TL) has been demonstrated to predict cancer, cardiovascular disease and mortality (4,5). Several health-related factors, including nutrition and exercise, may have an impact on the health and lifespan of an individual by affecting TL (6–8). Moderate amounts of physical activity are typically associated with good health and improved survival compared with a sedentary lifestyle (9,10). Endurance athletes provide a unique model for understanding the effects of physical activity induced by repeatedly engaging in very long periods of aerobic exercise. There is growing evidence about the relationship between habitual physical activity and longer TL (11–15), but research studies of the protective effects of chronic endurance training on TL have shown conflicting results (16–21). Exhaustive acute exposure to endurance exercise is also associated with accelerated oxygen radical generation [reactive oxygen species (ROS)] and oxidative stress, which may threaten the athlete's health and well-being (22,23). However, to date, there is a lack of data on the possible acute effects of extreme endurance running on TL. A 'unique and exceptional race' such as the 'Tor des Géants ®' ultra distance trail running (one of the most difficult mountain marathon races in the world where the ultra-endurance activity is associated to high altitude exposure and sleep deprivation) has the potential to provide evidence of the possible effects on TL induced by acute extreme exposures. Therefore, the present study was designed to investigate how different levels of endurance exercise may influence the telomere biology. Our aims were to assess the chronic and acute effects of endurance training on TL after an exceptional and extreme race.

Materials and methods

Study population

The study population comprised 20 endurance athletes (17 males; age = 45.4 ± 9.2 years) enrolled during the 2014 edition of the 'Tor des Géants ®' which takes place in the Aosta Valley, Italy, in September. Athletes have to run 330 km with an overall positive slope of 24 000 m with a maximum time to complete the race of 150 h. Endurance athletes may have refreshments in the 'Punti Vita' assistance points situated along the route. The athletes are expected to manage their own health status during the race, together with the sleep cycles, in order to complete the race safely and properly. The endurance athletes in the study were experienced runners with an average training distance of 59.4 km per week and an average of 13.15 years of ultra trail running. A group of 42 sedentary subjects (32 males; age = 45.9 ± 9.5 years) was selected and matched to the endurance athletes as a control group. The healthy control subjects were selected if they were currently physically inactive and had no competitive sports history. A complete assessment of the health status of each participant was performed with a structured questionnaire including questions on hypertension, hypercholesterolemia, diabetes and smoking habits. Individuals who smoked at least three cigarettes per day at the time of the analysis were considered as smokers; ex-smokers had stopped smoking at least 6 months before study inclusion and non-smokers had never smoked. Saliva samples were drawn from all subjects for subsequent TL analysis. After receiving a description of the procedures, each subject gave his written informed consent prior to testing. The local Ethics Committee (Pisa, Italy) approved the experimental protocol.

Experimental overview

Three test sessions were planned for the study. The first session, 'Baseline', took place 1 or 2 days before the race depending on the availability of the athletes. After the first half of the race, a second test session was held at the intermediate 'punto vita' in Donnas (km 148.7). At the end of the race, in Courmayeur, athletes were evaluated for the third time, within an hour of their arrival and concluding any physical effort. Of the 20 athletes initially enrolled, 15 subjects (12 males; age = 47.2 ± 8.5 years) who reached Donnas were re-evaluated, while only 14 (11 males; age = 47.1 ± 8.8 years) completed the competition and were assessed within an hour of concluding the endurance trail race.

Sample collection and DNA extraction

Saliva samples were collected with an Oragene DNA Sample Collection Kit. Subjects had to spit ~2 ml of saliva into a plastic container. Once saliva collection was complete the containers were sealed, releasing the Oragene-DNA stabilizing agent that limits DNA degradation and bacterial growth. Saliva samples were subsequently stored in liquid nitrogen biobank (–196°C) until further analysis. Saliva DNA was extracted with a QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All DNAs were extracted using the same procedure and in the same assay in order to reduce variability in the recovery of DNA from different samples. The ratio of the absorbance at 260 and 280 nm (A260/280) was used to assess the purity of DNA. An absorbance ratio 1.7 < A260/280 < 1.9 was considered acceptable.

TL analysis

TL was measured in genomic DNA from whole saliva by Monochrome Multiplex Quantitative PCR (MMQPCR) method (24). MMQPCR measures the relative TL by determining the ratio of telomere repeat copy number (T) to single copy gene (SG) copy number (T/S ratio) in experimental samples relative to a reference sample. Forward and reverse primer sequences for telomere and β-globin gene (SG), PCR mix and the thermal cycling profile were previously described (24). All real-time PCR reactions were performed in triplicate in three different runs on a 384-well CFX RT-PCR System (Bio-Rad), with a negative and positive control included. Samples used to construct a standard curve for telomere and SG DNAs in combination were included in each assay to assess amplification efficiency and linearity. We analyzed within- and between-run coefficients of variation (CVs,%) in different samples in order to evaluate the reproducibility of TL analysis. Within-run imprecision was assessed by evaluating results of three replicates of three different samples analysed on the same day. Between run imprecision was evaluated over 10 days analysing three replicates of three different samples. The intra-assay mean value range of the coefficient of variation from the results of the three different DNA sample assays was 2.2–5.3%. The inter-assay mean value range between values obtained from independent replicates for the T/S ratio was 4.6–7%.

Statistical analysis

Statistical analyses of the data were performed with the StatView statistical package, version 5.0.1 (Abacus Concepts, Berkeley, CA). Data are expressed as the mean ± standard deviation. Differences between the means of two continuous variables were evaluated by the Student's *t*-test. Differences in non-continuous variables and genotype distribution were tested by χ^2 analysis. Data for three or more independent groups were analysed by analysis of variance and significant differences among pairs of means were tested with the Fisher's exact test. Regression analysis with the Pearson's test was used to evaluate the relationship between the two continuous variables. The association of selected variables with the TL was assessed

by logistic regression analysis using univariate and stepwise multivariate procedures. The following variables were included into the analysis: age, gender and endurance exposure. Odd ratios (OR) with the corresponding 95% confidence interval (CI) were estimated. A two-tailed *P* value ≤0.05 was chosen as the level of significance.

Results

Demographic and clinical characteristics of the study population are reported in Table 1. In the whole sample, TL did not inversely correlate with age for the entire sample ($r = -0.16$, $P = 0.2$). However, in separate analyses, TL had a clear tendency to be inversely related to age for the control group ($r = -0.29$, $P = 0.058$). No significant association was found between TL and gender ($P = 0.6$), hypertension ($P = 0.1$), hypercholesterolemia ($P = 0.1$) or smoking habits ($P = 0.4$).

Chronic effects of endurance exercise on TL

A first analysis aimed to evaluate the chronic effects of endurance exercise on TL through the measurement of TL in 20 athletes who regularly engaged in ultra-endurance aerobic exercise and 42 sedentary matched-controls. The comparison revealed a significant difference in TL in the two groups (1.28 ± 0.4 vs. 1.02 ± 0.3, $P = 0.005$). Differences in TL between athletes and sedentary control subjects are graphically displayed in Figure 1. The difference remained statistically significant in a multivariate-adjusted regression model (OR = 0.1, 95% CI: 0.03–0.4, $P = 0.001$). Based on tertiles of sample age, the subjects were divided into the three groups, with the cutoff points for each tertile being ≤39, >39 to <53, and ≥ 53 years, respectively. Older endurance athletes had significantly increased TL compared with sedentary peers (1.3 ± 0.27 vs. 0.91 ± 0.21, $P = 0.003$) whereas there was no significant difference among younger age groups (Figure 2).

Table 1. Clinical characteristics of the study population

Clinical variable	Endurance athletes, *n* = 20	Sedentary controls, *n* = 42	*P* value
Mean SD, age (years)	45.4 (9.2)	45.9 (9.5)	ns
Gender, male *n* (%)	17 (85)	32 (76)	ns
Hypertension, *n* (%)	1(5)	6 (14.3)	ns
Hypercholesterolemia, *n* (%)	1(5)	3 (7.1)	ns
Diabetes mellitus, *n* (%)	0 (0)	0 (0)	ns
Smoking habit			
Smokers, *n* (%)	0 (0)	1 (2.4)	ns
Ex-smokers, *n* (%)	2(10)	15 (35.7)	
Non-smokers, *n* (%)	18 (90)	26 (61.9)	
Other pathology, *n* (%)	0 (0)	0 (0)	ns

Acute effects of ultra-endurance exercise on TL

In order to assess the acute effects of an ultra-endurance trail race on TL, TL of 14 endurance athletes who completed the competition were compared in the three test sessions. TL was significantly reduced in endurance athletes evaluated at the intermediate (0.88 ± 0.36 vs. 1.11 ± 0.34, $P = 0.002$) and final points compared with the baseline analysis (0.86 ± 0.4 vs. 1.11 ± 0.34, $P = 0.0006$), as reported in Figure 3.

Discussion

Our results provide evidence that repeated engagement in endurance exercise may have a protective effect on cellular aging as TL was better preserved in older ultra-distance trail runners compared with the same age control group. In younger people, we did not find any effect of exposure to endurance aerobic exercise on TL. Conversely, we demonstrated a significant telomere shortening in athletes acutely exposed to an exceptional and extreme race such as the 'Tor des Géants ®'.

Telomere shortening is widely considered to be a marker of health status and biological ageing and has been recognized as an important cause of chromosomal instability (5). TL may be considered a dynamic feature which can undergo considerable change over several months (25). Several studies have reported that the rate of telomere shortening or lengthening may be accelerated significantly by exhaustive exercise (26,27) or exposure to other stressors (28–30) over a shorter period of time (days or hours). TL regulation is a complex process influenced by a multitude of factors. It is well known that various lifestyle factors affect TL such as nutrition, exercise, psychological stress and other health-related factors (31). Moderate amounts of physical activity are typically related to high quality health and reduced biological aging (6–15) compared to a sedentary lifestyle. Ultra-distance trail runners represent an excellent model of fitness involved in repeated exposure to ultra-endurance aerobic exercise. They have 17% greater longevity in contrast with the general population (32) and numerous studies have reported decreased mortality with more frequent exercise (33,34). However, the impact of repeated, endurance aerobic exercise on TL and biological aging remains unclear. Previous marathon runners were found to exhibit unchanged TLs in leukocytes and muscle cells compared to sedentary controls (16,17). In contrast, other studies have showed that endurance-trained athletes exhibit longer leukocyte telomeres

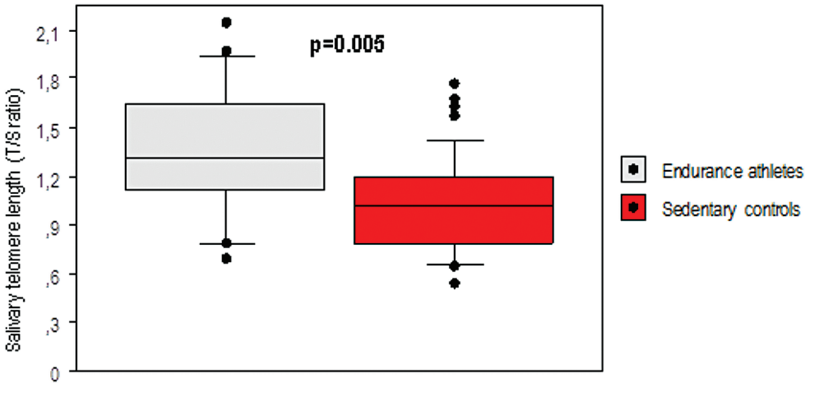

Figure 1. Chronic effects of regular engagement in endurance exercise on TL in the whole population.

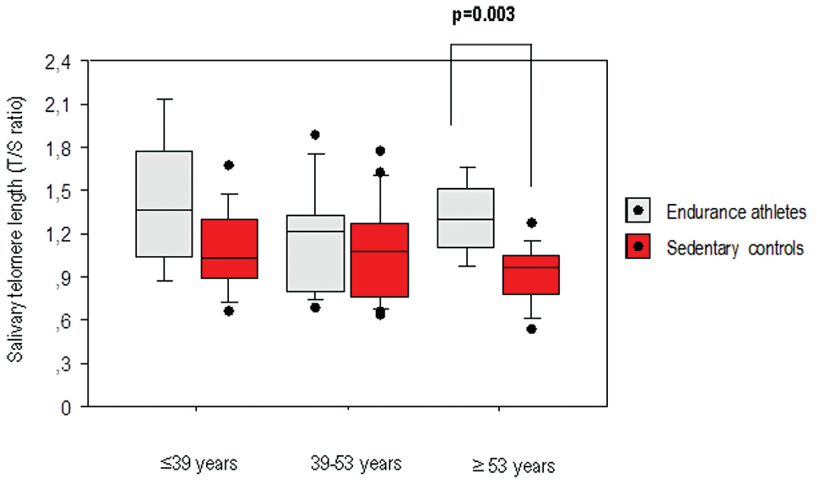

Figure 2. TL expressed as T/S ratio among athletes and sedentary controls stratified by age.

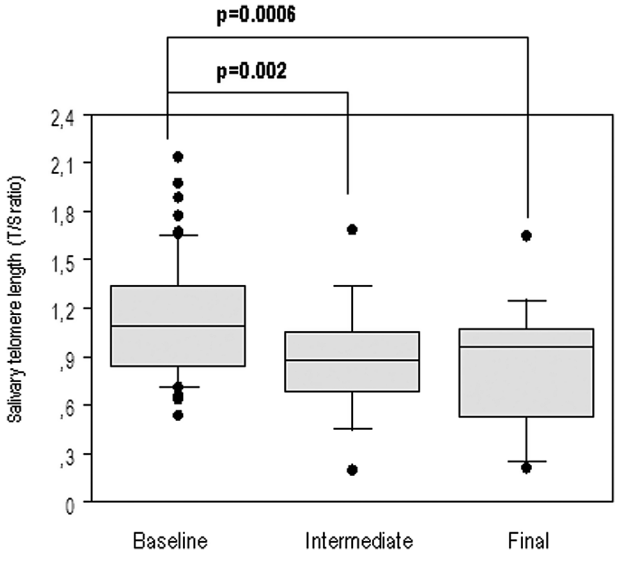

Figure 3. Acute effects of ultra-endurance trail race on TL.

(18–21). LaRocca *et al.* (19) and, more recently, Østhus *et al.* (20) demonstrated a preserved effect of chronic exercise training on TL only in older people, not finding any association in younger participants. Accordingly, our results confirmed a better preserved TL in older ultra-distance trail runners compared with younger groups. Non-significant association in younger groups seems to be due to less exposure to physical activity in terms of activity years. In addition, differences in TL for older athletes and sedentary peers may also have clinical significance in relation to longevity. Several mechanisms might explain TL maintenance in endurance athletes. The major proximal pathway for maintenance is through telomerase activation which plays a critical molecular role in telomere maintenance (35). Werner *et al.* (18) reported a regulation of telomere-stabilizing proteins in mice and in humans and subsequent protection from stress-induced vascular apoptosis with chronic physical activity. Peripheral blood leukocytes isolated from endurance athletes showed increased telomerase activity, expression of telomere-stabilizing proteins and reduced leukocyte telomere erosion compared with untrained controls. A cell redistribution toward cells with longer telomeres or an influx of naïve cells into the circulation might represent an alternative mechanism. Thus, rather than elongation of TL on a per cell basis, it seemed to be in part due to replacement of cells in circulation. Naïve T cells naturally have longer TL than memory T cells but may also have longer TL than granulocytes (36). Any factors that stimulate more naïve T-cell influx may lead to lengthening and/or maintenance of TL. Improvements in health behaviour and exercise may be important factors involved in protection cells with longer telomeres, either more naïve cells or fewer senescent (12,37). On the contrary, research has shown that, in general, acute exposure to endurance exercise increases oxidative stress (38). Particularly, high-intensity acute exercise has been found to induce oxidative DNA damage after a few hours (22,39,40). Oxidative stress has been shown to accelerate telomere shortening and inhibits telomerase activity *in vitro* in various cell types (2). To date, divergent results are present on the oxidative effects of acute training on telomeres. Bruunsgaard *et al.* (26) highlighted a recruitment of lymphocytes with an activated phenotype and short telomeres in young and older humans; on the contrary, telomere lengthening was found in peripheral blood T-cells mobilized by acute exercise in humans (27). Chilton *et al.* (41) focused on the potential mechanisms underpinning the positive association between physical activity and leukocyte TL. Although TL was not analysed, the authors reported an upregulation of telomerase and a regulation of miRNAs with a potential role of controlling the downstream expression of genes involved in telomere homeostasis (41). To our knowledge, our study is the first to measure TL at multiple time points in people competing in an ultra-distance trail race. Our data provide evidence for telomere shortening at intermediate and final points probably due to the damage caused by acute oxidative stress in the telomeric DNA region. One explanation put forth for this is that antioxidant defenses are sufficient to meet an increase in ROS production during chronic endurance exercise, but as exercise-intensity increases, these defenses are surpassed resulting in significant oxidative stress. Telomere related effects of oxidative stress may be more pronounced in tissue compartments with high cellular turnover such as bone marrow. Since peripheral blood cells originate from bone marrow, telomere alterations in bone marrow progenitors seem to be reflected also in peripheral blood cells (42). Although TL is commonly assessed in peripheral white blood cells, Mitchell *et al.* (43) recently reported a significant positive correlation of 0.72 (P = 0.002) between leukocyte TL and TL measured in salivary DNA 74% of which comes from white blood cells, the same source of DNA in blood (44). In addition, a recent study found

significant positive correlations between TL measured in leukocytes, skeletal muscle, skin and subcutaneous fat, supporting the notion that TL measurements of any tissue may be approximately and equally useful as biomarkers of aging (45). In the present study, the analysis of TL from salivary samples appears to be a non-invasive, reliable and convenient approach in detecting chronic and acute TL changes over time at the individual level.

Study limitations

Our study findings should be interpreted bearing in mind some limitations. Firstly, with regard to TL and endurance exercise training, we cannot exclude the possibility of residual confounding because of unknown or unmeasured factors in the present study such as information on diet, oxidative stress and antioxidant intake. Secondly, a small sample size with a prevalence of male participants entails another important limitation. Finally, the design of our study being cross-sectional in nature does not allow for comment on the causality of these results. Randomized control clinical exercise trials would eliminate some of the bias associated with cross-sectional studies and help to clearly define the effects of endurance exercise on telomere biology.

Conclusion

In summary, our results corroborate the fact that chronic exposure to physical endurance activity seems to have beneficial 'anti-aging' effects, while acute extreme exposures are linked to detrimental effects with increased TL attrition. The investigation and delineation of molecular pathways modulated by exercise, which are responsible for modifications in TL, is doubtless of high priority.

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