Title: Effect of long-term ultra-endurance training on telomere length and telomere regulatory protein expressions in vastus lateralis of healthy humans.

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Abstract

Introduction: Ultra-endurance races can last from several hours to several days and impose a high physical and psychological demand on the competitors. The question of whether this type of extremely strenuous activities induces adverse effects on body functions remains to be clarified. Telomere length is an important marker of the regenerative potential of tissues and can be affected both by physical and psychological stress. For instance, telomere length has been found severely shortened in skeletal muscle of athletes suffering from exercise-associated chronic fatigue and in leukocytes of mothers giving care to chronically ill children. The aim of this study was to assess whether the long-term practice of ultra-endurance activities affects skeletal muscle telomere length, telomerase expression and the expression of three other proteins with telomere regulatory properties.

Materials and method: Mean and minimum telomere length was measured by southern blot analysis in tissue samples from vastus lateralis muscle of 17 experienced ultra-endurance athletes (UE, mean age: 29.1±3.6; VO_{2max}: 59.9±5.2 mL·kg⁻¹·min⁻¹) and 13 healthy untrained controls (CON, mean age: 24.5±1.7). Expression of telomerase, tankyrase, TRF2 and POT1 was assessed with western blot analysis.

Results: Mean (UE: 10.1±1.5; CON: 10.5±0.5) and minimum (UE: 5.0±0.7; CON: 4.7±0.3) telomere lengths were not significantly different between UE and CON. Also, telomere length was not correlated to age, VO_{2max} or protein expressions in either of the groups. Concerning the expression of proteins, telomerase (UE: 4.4±2.9; CON: 0.8±1.0; p=0.008), TRF2 (UE: 2.2±1.0; CON: 0.5±0.4; P=0.005) and POT1 (UE: 4.0±0.9; CON: 1.1±1.6; p=0.005) protein expressions were significantly higher in UE compared to CON, while tankyrase protein expression was not significantly different between the two groups (UE: 0.9±0.66; CON: 0.5±0.6; p=0.181). In UE, POT1 was positively correlated to telomerase (r=0.83, p=0.012) and negatively correlated to tankyrase (r=-0.758, p=0.018). In CON, POT1 was positively correlated to telomerase (r=0.93, p=0.012).

Conclusion: This study shows for the first time that regular practice of ultra-endurance activities is not associated with abnormal shortening of skeletal muscle telomere length. More importantly, it has been found that telomere regulatory factors (telomerase, TRF2 and POT1) are highly expressed in skeletal muscle of ultra-endurance runners compared to healthy controls, indicating the possible involvement of telomere regulatory mechanisms in the maintenance of telomere length in skeletal muscle of athletes.
**Introduction**

**Multisport**

One of the toughest exercise challenges that are practiced today is multisport. Multisport, together with other adventure race sports such as triathlon, belongs to the ultra-endurance category of exercise. Ultra-endurance races can last from several hours to several days and typically consist of running/trekking, biking and paddling. These extreme events impose a high physical and psychological demand on the competitors, who seldom get more than a few hours of sleep (Lucas et al., 2008). Athletes competing in the 2003 Southern Traverse, a five day adventure race, completed the first 24h with a heart rate averaging 64% and a core temperature ranging between 36-39.2°C (Lucas, et al., 2008). The estimated energy expenditure is extremely high and can reach 80.000 kcal during a six-day event, which is an average of 500 kcal per hour (Enqvist et al., 2010). Research exploring this kind of strenuous exercise is still quite limited and mostly addresses frequency of illness (e.g. cardiac dysfunction and upper respiratory tract infection) and injuries (Gosling et al., 2010; Gosling et al., 2008). Only a few studies addressing issues such as skeletal muscle damage, inflammatory and immunological alterations (Neubauer et al., 2008), oxidative stress (Neubauer et al., 2009; Wagner et al., 2010), antioxidant response (Neubauer et al., 2010) and genome stability (Reichhold etal., 2009; Reichhold et al., 2009) have been performed. The question of whether this type of extremely strenuous activities might induce adverse effects on body functions still remains to be clarified. The following study intends to provide further information on the biological influences of ultra-endurance activities by investigating the effect of this type of activities on telomere length in skeletal muscle. Telomere length is an important marker of the regenerative potential of tissues and can be affected both by physical and psychological stress. For instance, telomere length has been found severely shortened in skeletal muscle of athletes suffering from exercise-associated chronic fatigue (Collins et al., 2003) and in leukocytes of mothers giving care to chronically ill children (Epel et al., 2004).

**Telomeres**

The chromosomes, which are made of twin strands of deoxyribonucleic acids wrapped around histone proteins, are located within the nucleus of the eukaryotic cells and contain the genetic information. During cell divisions, chromosomes are not able to fully
replicate their ends, which could lead to an important loss of genetic information. To prevent the loss of important genetic information, the ends of chromosomes are capped by DNA sequences called telomeres (Blackburn, 1990). A telomere is a sequence of six repeated nucleotides, in the order TTAGGG, and their complementary bases. The human telomere is approximately 10-15 Kb long (Martinez and Blasco, 2010) and due to the “end replication problem” (i.e. an incomplete replication of the 5’ end at each cell division) the telomere gradually becomes shorter with every round of cell division. Based on in vitro studies, it has been suggested that the telomere becomes critically short after ~50 population doublings, a stage commonly termed as the “Hayflick limit” (Hayflick, 1961). When this happens the cell loses its ability to divide and enters replicative senescence to avoid genome instability. As telomeres are shortened at each cell division telomere length is commonly regarded as a marker of the replicative history and regenerative potential of tissues.

**Telomeres and the shelterin complex**

The telomere ends in a single stranded “overhang” constituted of the 3´ G-rich telomeric strand. This overhang is approximately 200 nucleotides long and is characterized by its T-loop formation, i.e. the 3´ end is folded back against the double stranded region creating a “loop”. The T-loop is thought to have a protective function and to restrict telomere end accessibility (de Lange, 2005). The T-loop is most likely shaped by action of the “shelterin complex”. The shelterin complex is composed of six specialized proteins: telomeric repeat binding factor 1 and 2 (TRF1 and 2), TRF-interacting protein 2 (TIN2), protection of telomeres 1 (POT1), repressor/activator protein 1 (RAP1) and POT1-TIN2 organizing protein (TPP1, also known as TINT1, PTO or PIP1) (de Lange, 2005). These factors are suggested to have a fundamental role in telomere protection. It has been hypothesized that the shelterin complex regulates telomeres through length-dependent feedback systems and through remodeling of the telomere end structure (de Lange, 2005). For example TRF2 and POT1 have been suggested to restrict telomerase accessibility and thereby prevent telomere elongation (Martinez and Blasco, 2010). TRF1-interacting ankyrin-related ADPribose polymerase (tankyrase1), on the other hand, has been suggested to make the shelterin complex become unstable and thus increase telomerase accessibility (Smith and de Lange, 2000). A current model suggest that critically short telomeres are not able to adequately bound sufficient amounts of the
shelterin complex to suppress the DNA damage response (Martinez and Blasco, 2010), which eventually trigger senescence or apoptosis. However, the biological actions of these telomere regulatory proteins are still very poorly understood and most of the available information originates from in vitro studies.

**Telomerase can elongate telomeres**

Since the regenerative potential of cells is expected to decrease with the progressive shortening of telomeres, chromosomal un-capping and cell senescence, are proposed to be one of the main mechanisms contributing to organismal ageing (Monaghan, 2010; Oeseburg et al., 2010). The exhaustion of the regenerative capacity of specific cell types such as stem cells, has been suggested to accelerate ageing due to impaired renewal of damaged tissues (Donate and Blasco, 2011). However, there is one factor that appears indispensable to cells that are required to undergo many divisions to maintain tissue integrity and cell viability, telomerase. Two years ago Elizabeth Blackburn, Carol Greider and Jack Szostak were awarded the Nobel Prize in Physiology and Medicine for their pioneer research on telomeres and the discovery of telomerase. Telomerase is a ribonucleoprotein able to elongate telomeres in vitro (Blackburn et al., 1989). An RNA template that can be used to add new TTAGGG repeats to the telomere end is incorporated in the enzyme, thus enabling telomerase to elongate the telomere. Telomerase is highly expressed in cells that display stable telomere length and unlimited proliferative capacity, e.g. germ cells and a majority of spontaneously immortalized tumor cell lines (Engelhardt and Martens, 1998) and also in stem cells and immune cells (Flores et al., 2006). Somatic cells, however, are thought to lack this important ribonucleoprotein.

Since telomerase is considered absent in somatic cells, post-mitotic tissues such as skeletal muscles are thought to lack telomerase. Yet it should be recognized that telomerase activity has been reported in many normal tissues, including skeletal muscle (Radak et al., 2001). The disparity between views might be due to differences in the study context, i.e. in vitro and in vivo. Currently, knowledge in this area is mainly based on in vitro studies. While in vitro studies provide important knowledge, a limitation to such systems is that they cannot fully mimic the environmental influences of an in vivo context where the telomeres and telomere regulatory factors are subject to the influence
of various local and circulatory factors. In fact, a strong stress-dependence of cell culture lifespan has been proposed, thus implicating that telomere-driven senescence highly depends on culture conditions and external factors, such as oxidative stress (von Zglinicki, 2002).

**Telomeres and oxidative stress**

The loss of telomeric sequences is typically attributed to the end replication problem. However, a loss of only about 10 out of the in total 50-200 lost base pairs is attributed to the incomplete synthesis of the lagging 5´strand (Monaghan, 2010). The loss of the remaining base pairs is due to yet unknown mechanisms. In this respect, it is suggested that the remaining base pair loss occurs because of the action of oxidative stress on telomeres (Zglinicki 2002), especially since the guanine rich telomere sequence is highly vulnerable to oxidative damage (Monaghan, 2010). In this respect it has also been proposed that inflammation could be a factor aggravating telomere erosion in vivo (Kadi and Ponsot, 2010).

**Skeletal muscle telomere length and physical training**

Although being a post-mitotic tissue skeletal muscle should not be considered a stable tissue (Kadi and Ponsot, 2010), due to the presence of satellite cells. Satellite cells, considered as muscle stem cells, are undifferentiated myogenic precursor cells located between the sarcolemma and the basal lamina (Mauro, 1961). They are normally quiescent in resting skeletal muscle and progress through the cell cycle first when they become activated by growth factors and mitogens. Proliferating satellite cells can either return to quiescence, replenishing the satellite cell pool, or proceed through differentiation to provide new myonuclei or generate new muscle fibers (Kadi et al., 2008) whereby restoring damaged muscle fibers and supporting muscle hypertrophy. The need for muscle regeneration increases in physically active individuals; for example a 141% increase in satellite cell number was observed as early as 24 hours after an eccentric exercise intervention in young men (Dreyer et al., 2006) and increases in satellite cell pool were observed in elderly subjects both after resistance and endurance training (Kadi and Ponsot, 2010). Even in already well-trained subjects endurance training has been proven to have a significant effect on satellite cell proliferation (Mackey et al., 2007). Yet, the largest increase in satellite cells has been observed after
exercise interventions causing severe myofiber damage, such as maximal eccentric or electrically induced contractions (Kadi and Ponsot, 2010). In theory, frequent satellite cell proliferation should be accompanied by shorter telomeres with every round of replication. As a result, regular physical exercise, especially in extremely demanding activities such as practicing ultra-endurance, should induce an accelerated shortening of the skeletal muscle telomere. The number of studies on the effect of regular training on telomeres is limited. Severely shortened telomeres have been discovered in a group of athletes suffering from overtraining symptoms (Collins et al., 2003). Kadi et al. (2008) evaluated telomere length in experienced power-lifters and reported no shortening of telomeres in this population. Interestingly, there was even a tendency towards longer telomeres in the group of experienced power-lifters (Kadi et al., 2008). More recently, Rae et al (2010) also did not observe a shortening of telomeres in skeletal muscle of experienced endurance runners. The possibility that extremely strenuous exercise activities can negatively alter telomere length in skeletal muscle cannot however be excluded.

Given the above discussion, the aim of this study was to assess whether the long-term practice of ultra-endurance activities affects skeletal muscle telomere length, telomerase expression and the expression of three other proteins with telomere regulatory properties. To investigate this, a group of experienced ultra-endurance (UE) was compared to a group of healthy untrained controls (CON).

**Materials and methods**

**Subjects**
Seventeen male ultra-endurance athletes (UE) \( \mathrm{VO}_{2\max} = 59.9 \pm 5.2 \ \mathrm{mL} \cdot \mathrm{kg}^{-1} \cdot \mathrm{min}^{-1} \) and thirteen healthy untrained controls (CON) volunteered to participate in this study. Subject characteristics are presented in table 1. All UE subjects were experienced ultra-endurance athletes and had competed in several very long duration races (> 48 h). Many of them had finished top ten in at least one world major race (e.g. World Championship, “Primal Quest”). The subjects were informed of the experimental procedures and possible discomforts associated with the study and gave their written informed consent to participate. The study was conducted according to the policy statement set forth in the Declaration of Helsinki and approved by the Regional Ethical Review Board in
Stockholm (Dnr 2005/1019-31/2). The study is a collaborative project between Örebro University and the Swedish School of sport and Health Sciences in Stockholm.

**Table 1.** Sample size and age of subjects.

<table>
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<th>N</th>
<th>Age</th>
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<tr>
<td>Ultra-endurance (UE)</td>
<td>17</td>
<td>29.1±3.6</td>
</tr>
<tr>
<td>Control (CON)</td>
<td>13</td>
<td>24.5±1.7</td>
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**VO$_{2\text{max}}$ test**

UE subjects performed an incremental all-out test on a cycle ergometer for determination of VO$_{2\text{max}}$ (Monark ergomedic 893E, Monark Exercise, Varberg, Sweden). Heart rate was continuously recorded using a Polar 610s heart rate monitor (Polar Electro Oy, Kempele, Finland) and VO2 was measured using an online ergospirometry system (AMIS 2001, Inovision A/S Denmark).

**Muscle biopsies**

All muscle biopsies were obtained under local anesthesia (1-2 ml Carbocain; 20 mg/ml, AstraZeneca, Södertälje, Sweden) from the belly of the vastus lateralis muscle using a Weil Blakesley conchotome (Wisex, Mölndal, Sweden). An incision was made in the skin and a muscle sample was taken. The muscle samples were mounted on Tissue Tek OCT Compound (Tissue Tek®, Miles laboratories, Elkhart, USA), quickly frozen in liquid nitrogen and stored at −80°C until analyzed.

**Protein extraction**

Frozen muscle samples were homogenized using a polytron (i.e. a motorized homogenizer). Cytoplasmic and nuclear fractions were separated using the NE-PER™ Nuclear and Cytoplasmic Extraction Reagents Kit (Pierce Biotechnology). The NE-PER™ protocol involves stepwise lysis of cells and centrifugal isolation of nuclear and cytoplasmic protein fractions. Due the location of the chromosomes only the nuclear fraction was taken for further analysis. To calculate the protein concentration in each nuclear fraction a Bradford analysis was performed using the Bradford Reagent (Sigma-Aldrich) and Bovine Serum Albumin (BSA; Albumin solution for bovine serum, Sigma-Aldrich) standards.
Western blot analysis

Nuclear fractions was diluted in Laemmlly buffer and separated on 8% SDS-PAGE. The amount of protein loaded on each gel was 50 µg, 50 µg, 40 µg and 2.5 µg for telomerase, tankyrase, TRF2 and POT1, respectively. The proteins were migrated with a constant voltage of 200V and subsequently transferred to nitrocellulose membranes (Amersham Biosciences) at a constant voltage of 100V. The membranes were blocked over night at 4°C using BlottoA (Santa Cruz Biotechnology Inc) for telomerase, tankyrase-1 and POT-1, and BSA 4% (dissolved in TBS containing 0,1% Tween20) for TRF2. Membranes were incubated with primary antibodies: goat polyclonal anti-TRF2 (sc-32106, Santa Cruz Biotechnology Inc.), rabbit polyclonal anti-POT1 (sc-33789, Santa Cruz Biotechnology Inc.), rabbit polyclonal anti-tankyrase (sc-8337, Santa Cruz Biotechnology Inc.) and rabbit polyclonal anti-telomerase (600-401-252, Rockland Inc), for 2h at room temperature before repeated washing steps in TBS and TBS containing 0,1% Tween20. The antibodies for POT1, tankyrase-1 and telomerase were diluted in milk whereas the antibodies for TRF2 were diluted in BSA 4%, all dissolved in TBS containing 0,1% Tween20. Appropriate secondary antibodies were subsequently applied to the membranes: donkey anti-goat (sc-2020, Santa Cruz Biotechnology Inc.) and goat anti-rabbit (sc-2004, Santa Cruz Biotechnology Inc.). ECL detection reagent (Amersham Biosciences) was used for detection of signals using chemiluminescence. After developing the signals, the films were scanned and analyzed digitally using the UN-SCAN-IT software (version 6.1, Silk Scientific, USA).

All experiments were performed using the same reagents and antibody lots. Each gel contained a molecular weight ladder. Positive controls for all proteins were also loaded together with the samples on the gel. Mouse heart extract (sc-2254 Santa Cruz Biotechnology Inc, USA) served as positive control for POT-1 and for both tankyrase-1 and TRF2, Jurkat nuclear extract (sc-2132 Santa Cruz Biotechnology Inc, USA) was used. Positive control for telomerase was breast cancer nuclear extract.

Isolation of genomic DNA

Approximately 8-10 mg of muscle tissue was digested over-night in 500 µl digestion buffer containing 100 mM NaCl, 10 mM Tris-Cl (pH 8), 100 mM ethylene-diamine tetraacetic acid (EDTA, pH 8), 1% Triton X-100 and 40 units ml⁻¹ proteinase K. The digest was extracted with 1 volume of phenol/chloroform/isoamyl alcohol (25:24:1
respectively) and later precipitated with 1 volume of 7.5 M ammonium acetat and 100% ethanol (0.5:2). The DNA was stored 4°C after being washed in 70% ethanol and re-suspended in TE-buffer containing 10 mM Tris-Cl (pH 8), 1 mM EDTA (pH 7.5).

**Telomere length**

The restriction enzyme *Hinf I* (New England Biolabs, Ipswich, Massachusetts) was used to digest the genomic DNA, obtained as described above, at 37°C for 4h. The digested DNA was, together with two DNA ladders (1 kb plus and high molecular weight; Invitrogen, Carlsbad, California) migrated in 0.7% agarose gel for 20h in 4°C at a constant voltage of 90V. The gels were dried, denatured and neutralized. The length of the telomeric restriction fragments were visualized on X-ray films (BioMax MS, Estman Kodak, Rochester, New York) after hybridization to a 32P-labeled probe. The signal response was then analyzed using Scion Image v4.03 software (Sicon Corporation, Frederick, Maryland). With this software the intensity of the signal may be plotted as a function of the migration distance. Mean and minimum length of the telomeric restriction fragments are then obtained by converting the corresponding migration distance into kilo base pair (kbp) using the migration profile of the low (1-12 kb) and high (8-9 kb) DNA ladders loaded with the samples. Mean telomere length represents the telomere length from a majority of the post-mitotic myonuclei which has been incorporated in muscle fibers since birth and therefore have undergone few divisions. Minimum telomere length represents the telomere length from satellite cells and the most recently incorporated myonuclei (Ponsot and Kadi, 2008).

**Statistics**

Differences in sample characteristics between the ultra-endurance group (UE) and controls (CON) were evaluated using un-paired t-test. One-way analysis of variance (ANOVA) on ranks was conducted to evaluate differences in mean and minimum telomere length and in protein expression between UE and CON, with exception of TRF2 protein expression that was evaluated using a parametric one-way ANOVA. Correlation analysis using Pearsons product-moment correlation coefficient was conducted to explore relationships between telomere length and expression of telomerase, TRF2, tankyrase-1 and POT-1. Statistical analysis was performed using SigmaPlot 11 software
(Systat Software Inc, San Jose, California). Statistical significance was set at $p < 0.05$. Data are presented as mean ± standard deviation.

Results

Mean (UE: 10.1±1.5; CON: 10.5±0.5) and minimum telomere (UE: 5.0±0.7; CON: 4.7±0.3) length of skeletal muscle DNA did not show any statistically significant differences between UE and CON (fig.1). However, a statistically significant difference in age occurred between the two groups (UE: 29.1±3.6; CON: 24.5±1.7; $p<0.001$). No correlation was found between telomere length and age, or between telomere length and VO$_2$max.

The expression of telomerase (UE: 4.4±2.9; CON: 0.8±1.0; $p=0.008$), TRF2 (UE: 2.2±1.0; CON: 0.5±0.4; $P=0.005$) and POT1 (UE: 4.0±0.9; CON: 1.1±1.6; $p=0.005$) were found to be significantly different between UE and CON, while tankyrase expression (UE: 0.9±0.66; CON: 0.5±0.6; $p=0.181$) was not significantly different between the two groups (fig.2). In UE, POT1 was positively correlated to telomerase ($r=0.826$, $p=0.012$) and negatively correlated to tankyrase ($r=-0.758$, $p=0.018$). In CON, POT1 was positively correlated to telomerase ($r=0.93$, $p=0.012$). However, there was no correlation between protein expression and telomere length.

Figure 1: Mean and minimum telomere length in the vastus lateralis muscle of UE and CON expressed in Kbp. No significant differences were found between the two groups.
Figure 2: Expression of telomere regulatory proteins (telomerase, tankyrase, TRF2 and POT1) in the vastus lateralis muscle of UE and CON. *p<0.01.

Discussion

This study shows for the first time that regular practice of ultra-endurance activities, one of the most physically and psychologically demanding sport activities, is not associated with abnormal shortening of skeletal muscle telomere length. More importantly, it has been found that telomere regulatory factors are highly expressed in skeletal muscle of ultra-endurance runners compared to healthy controls, indicating the possible involvement of telomere regulatory mechanisms in the maintenance of telomere length in skeletal muscle of athletes.

Mean telomere length represents the telomere length of a majority of the post-mitotic myonuclei which has been incorporated in muscle fibers since birth and therefore have undergone few divisions, while minimum telomere length represents the telomere length from satellite cells and the most recently incorporated myonuclei (Ponsot and Kadi, 2008). In this study mean and minimum telomere lengths are within the normal ranges previously reported in vastus lateralis muscle of untrained healthy individuals (Ponsot et al., 2008) and endurance runners (Collins, et al., 2003; Kadi, et al., 2008; Rae et al., 2010), and exceeds that of athletes suffering from overtraining symptoms (Collins
et al. 2003). A significant difference in age between the ultra-endurance athletes and controls was found. Given the fact that telomere length is thought to decrease with age, the significantly higher age in the ultra-endurance group would theoretically give reason for an already shorter telomere length in this group. However, the fact that there is no difference in either mean or minimum telomere length and that no correlations were found between age and telomere length, confirms that the difference in age between the two groups has had no impact on the telomere length data.

Previous studies (Kadi et al., 2008; Rae et al., 2010) have found that minimum telomere length of highly trained athletes is inversely related to performance (personal weight lifting records and years spent in running). In this study no such relationship was found, i.e. V\textsubscript{O2max} was not found inversely correlated to minimum telomere length. V\textsubscript{O2max} is frequently used to describe physical fitness level and is influenced both by genetic and environmental factors (Bouchard et al., 1998). Since V\textsubscript{O2max} is not the only parameter responsible for sport performance (i.e. the athlete with the highest V\textsubscript{O2max} is not automatically the strongest or fastest) the results from this study is not in conflict with the findings of a relationship between performance and minimum telomere length. As already proposed by Kadi et al., (2008) it is possible that when the exercise-induced satellite cell recruitment exceeds a given threshold the length regulatory mechanisms will fail to preserve telomere length. Consequently, there should be no adverse effects from regular training, even at very high intensities, if the training protocol is well-designed. This in turn would suggest that the abnormal shortening of skeletal muscle telomeres that is evident in some overtrained runners (Collins et al., 2003) reflects the athletes’ overtraining status rather than the fact that they are engaged in physical training. Collins et al. (2003) propose that poor antioxidant systems in overtrained athletes might make their DNA less protected against oxidative damage, particularly leaving the guanine rich telomere vulnerable. Given the above discussion, accelerated telomere shortening can be predicted to occur when the telomere length regulatory mechanism fails to compensate for particularly extensive exercise-induced inflammation, oxidative damage or muscle damage, such as the one occurring in overtrained athletes or in athletes performing above their physiological capacity.
This is the first study dealing with telomerase expression in human skeletal muscle. Two earlier studies have investigated telomerase activity in the blood of physically active subjects; Werner et al. (2009) showed an increased telomerase activity in middle-aged field-track athletes while Ludlow et al. (2008) observed no association between physical activity level and telomerase activity in over middle-aged subjects. It has been demonstrated that all cells with proliferative potential have ongoing or potential telomerase activity (Rubin, 2002). In this respect, telomerase is able to elongate telomeres (Blackburn, et al., 1989) and the shelterin complex is able to preserve them (de Lange, 2005). Therefore, since telomere length is maintained in ultra-endurance athletes, these factors could be expected to be up-regulated in their skeletal muscle. The finding that telomerase expression is more than five times higher in ultra-endurance athletes compared to controls suggest that up-regulation of this telomere elongation factor do occur in response to strenuous exercise. Since telomerase is able to elongate telomeres, an exercise-induced up-regulation could prevent accelerated telomere shortening even if satellite cell proliferation occurs at a high rate. This may well serve as an explanation to the fact that telomere length is within normal range in athletes engaging in ultra-endurance activities. Yet, much remains unknown regarding telomere elongation and telomerase action in the context of skeletal muscle. For example it is not known whether increased amounts of telomerase are always responsible for greater telomere elongation efficiency. It is also important to remember that high telomerase activity is not necessarily a positive event in normal cells since it is a characteristic of the majority of tumor cell lines.

This study, as the first one addressing this issue, also found that the expression levels of tankyrase, TRF2 and POT1 were higher in ultra-endurance athletes compared to controls, although the difference between groups was only significant for TRF2 and POT1. The average TRF2 expression in the ultra-endurance group was more than four times higher than in controls. TRF2 binds directly to the telomeric repeats and has been found to be able to remodel artificial telomeric substrates into T-loops (Stansel et al., 2001), a structure that probably has a role in restricting telomere end accessibility (de Lange, 2005). Hereby it is likely that TFR2 restricts telomerase possibility to act on the telomere end, why it could be regarded as a negative regulator of telomere length. However, TRF2 has also been suggested to play a part in protecting the telomere from
DNA instability and recombination (Martinez and Blasco, 2010), which might be an important feature in protecting the telomere against the effects of oxidative stress. Thus, the up-regulation of TRF2 in ultra-endurance athletes might serve to protect telomeres against the deleterious effects of oxidative stress that occurs in response to strenuous exercise. Tankyrase1 has been suggested to inhibit TRF1 binding capacity to the telomeric repeats and, since TRF1 is in association with TRF2 (Martinez and Blasco, 2010), un-stabilizing the T-loop structure (De Boeck et al., 2009). An unstable T-loop is likely to increase telomere end accessibility and tankyrase1 may hereby counteract TRF2 i.e. promoting telomere elongation. Although not significantly higher in the ultra-endurance group compared to controls at the time of the biopsy sampling, it is possible that tankyrase fluctuates and thus increase or decrease the probability of telomerase acting on the telomere end. POT1, with almost four times higher expression in ultra-endurance athletes compared with controls, possesses a high affinity for binding to telomeric repeat tracts and hereby protects the telomere by forming a cap over the 5’end (de Lange, 2005). POT1 has been described both as a positive and a negative mediator of telomere length. Positive, in that way that POT1 when associated to TPP1 has been suggested to be involved in telomerase recruitment and processivity to the telomere end (Baumann and Price, 2010) and negative because long telomeres have been shown to be associated with a large amount of POT1 loaded on the telomeric overhang which is thought to decrease telomere end accessibility. Thus, longer telomeres have a greater probability of inhibiting telomerase action (de Lange, 2005). However, in this study we found no relationship between telomere length and POT1. On the other hand, there was a strong positive correlation between POT1 and telomerase in both groups. This might suggest the scenario where an up-regulation of POT1 recruits telomerase when needed and subsequently counterbalances its effect. POT1 was also found negatively correlated to tankyrase in the ultra-endurance group, a finding that can be regarded as logical since POT1 and tankyrase are suggested to have opposite effects on telomerase accessibility. Given the discussion above, the higher amounts of both TRF2 and POT1 are suggested to reduce telomere accessibility and hereby prevent the effects of excessive oxidative stress and uncontrolled telomere elongation in the presence of telomerase.
Conclusion

In conclusion, the main finding from this study was that the regular practice of strenuous endurance activities, such as ultra-endurance sport events, does not affect telomere length negatively. This suggests the existence of length regulatory mechanisms allowing for the control of telomere length, supported here by the up-regulation of telomerase, TRF2 and POT1 in ultra-endurance athletes in comparison to controls. Further, the up-regulation of TRF2 and POT1 may have an important role in protecting the telomere against the deleterious effects of excessive oxidative stress and uncontrolled telomere elongation in the presence of telomerase.

Future perspectives

To further expand on the issue of telomere length in overtrained athletes, the expression of telomere regulatory factors such as telomerase, tankyrase, TRF2 and POT1 should be investigated in athletes with overtraining symptoms. Supplementary analyses of telomerase expression using a method to measure telomerase activity (e.g. Telomerase PCR-ELISA) would bring further insights into the role of telomerase in preserving telomere length. Also it would be interesting to further explore the effects of oxidative stress on telomere attrition in athletes. This is particularly important since a study performed by (Gomez-Cabrera et al., 2008) has shown that supplementation with vitamin C decreases training efficiency and down-regulates enzymes with antioxidant properties. Further, this area of research would benefit from longitudinal studies. Studies based on the assessment of telomere length in the same subject over different time periods would bring further insights into the regulation of telomere length in humans.
References