The influence of physical exercise and sports on telomere length

A model for telomere length and telomerase activity regulation based on a comparative assessment of literature

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ABSTRACT

Based upon a comprehensive analysis of current literature and by combining a molecular biology and a sports science perspective, this review examines (1) if a correlation between physical activity load and telomere length (TL) exists, and (2) comprehensively analyses and integrates molecular pathways regulating exercise dependent TL dynamics. The focus is on TL in leukocytes and muscle tissue in middle to advanced aged subjects. Regarding item (1), a strong tendency for an increase in mean leukocyte TL was found for exercise energy expenditures up to about $2 \cdot 10^3$ kcal/week, while for higher activity levels no conclusive statement can be made. Conversely, research on skeletal muscle TL so far is quite limited but suggests that physical exercise with prolonged eccentric muscle contractions rather acts to shorten telomeres, while sports with little eccentric contractions might rather act to lengthen telomeres. As to item (2), a model for hypothetical pathways for exercise dependent telomerase activity regulation is proposed by consolidating findings of different studies in different cells. Consistent with this pathway model, various studies report increased telomerase transcription or activation by exercise.

Moreover, a qualitative overall model for endurance exercise related TL dynamics is presented. It considers telomeres as dynamic structures in equilibrium between telomere shortening (e.g., cellular turnover, oxidative stress, inflammation) and telomere lengthening (e.g., telomerase activity, telomerase recruitment) effects. A negative feedback-loop mediated by enhanced telomerase recruitment to short telomeres is assumed to counteract too excessive TL alterations. Finally, a proposal is put forth for future research on exercise dependent telomere dynamics by adopting a systems biology approach to develop mathematical models that properly integrate the complexity of the interacting variables.

1 Introduction and Scope

Although the term telomere is widely used in text books of molecular biology as well as in the scientific literature, and despite the recognition of telomeres as a distinct and essential structural feature of chromosomes involved in controlling cellular divisions and senescence (Alberts et al., 2007c; Carulli and Annicchiarico, 2014), a clear-cut and unequivocal definition of the term does not seem to exist. One interpretation (i.e. DNA level) refers to telomeres as the specific, tandemly repeated nucleotide sequences at the end of chromosomal eukaryotic DNA (Cherkas et al., 2008; Werner et al., 2009). The other (i.e. chromosome level) applies the term to the nucleoprotein structures or complexes at the end of chromosomes (i.e. the end caps of chromosomes), which are composed of both the repetitive nucleotide chain ends of the DNA and the associated molecules (Alberts et al., 2007c; Blasco, 2007a; Maicher et al., 2014). Notably, the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) does not list the term. Hence, depending on the specific context, both interpretations will be used in this paper.

As to their main functions, telomeres enable the ends of chromosomes to be efficiently replicated and they protect the end of chromosomes from deterioration or from fusion with neighboring chromosomes, as well as from being mistaken by the cell for a broken DNA molecule in need of repair (Alberts et al., 2007c). On the DNA level, the sequence of repeat units in humans and other vertebrates is GGGTTA (Alberts et al., 2007a). As to the telomere length (TL), human telomeres typically measure between 5 and 15 kbp (i.e. 800 to 2,500 repeat units). However, as in other eukaryotic organisms, chromosomes in humans usually consist of a variable number of such repeats and hence of variable TL values, respectively (Samassekou et al., 2010).

While telomeres play a crucial role in cell divisions particularly in the mechanism of replication, the enzymes that duplicate DNA cannot continue the duplication on the lagging strand all the way to the end of a chromosome. This is known as the *end replication problem* and results in telomere shortening in each duplication step (Levy et al., 1992). Furthermore, telomeres may be shortened by reactive oxygen species (ROS) and inflammation (Carulli and Annicchiarico, 2014), culminating in loss rates of about 50 to 200 bp per cell division (Ohki et al., 2001). Once TL becomes too short, telomeres trigger the onset of cellular senescence (Carulli and Annicchiarico, 2014). Therefore, TL is critically associated with the remaining proliferative capacity of a cell (Samassekou et al., 2010). On the other hand, numerous cell types such as stem cells and activated lymphocytes, have the ability to resynthesize telomeric repeats primarily via the complexly regulated enzyme telomerase (Weng, 2001). These telomere lengthening mechanisms counteract the telomere shortening mechanisms, although little is known about the magnitude and balance of such competitive effects.

A topic of special interest is related to the influence of physical exercise and sports on TL. Despite physical activity (PA) in general is seen as some sort of a “magic bullet” for the prevention and/or cure of most civilization diseases, at least strenuous physical exercise is known to trigger all three of the known telomere shortening mechanisms: (a) increased damage in skeletal muscle cells and thus increased stem cell proliferation for repair (Karalaki et al., 2009; Natale et al., 2003; Rae et al., 2010), (b) increased ROS levels due to accelerated metabo-

lism (Radak et al., 2013), and (c) release of inflammatory mediators (Natale et al., 2003). On the other hand, studies also exist that physical exercise may act to enhance (a) telomerase activity (Chilton et al., 2014; Werner et al., 2009), (b) anti-oxidative-enzyme activity (Urso and Clarkson, 2003), and (c) anti-inflammatory functions (Peake et al., 2005). The overall effect of physical activity on TL has also been reviewed by (Kadi and Ponsot, 2010; Ludlow et al., 2013). And yet, due to at least partially conflicting results, the competitive interaction and balance between telomere lengthening and shortening as affected by sports remains unclear.

Hence, this paper aims at addressing and elucidating the following two questions:

(1) *Does physical exercise exert any verifiable effect on telomere length? If yes, is there a correlation between PA load (i.e., average exercise energy expenditure per week or accumulated total exercise energy expenditure) and TL alterations?*

(2) *What is known and can be stated about the balance between telomere lengthening and shortening effects in relation to physical exercise along with details on underlying regulatory pathways?*

In terms of overall methodological approach, the above questions are elaborated based on a comprehensive literature review. The focus of the literature review is on investigations of middle-to-advanced age subjects (age of ≥40 years). This restriction is based on the assumption that any TL alterations due to physical activity will accumulate over time and thus should be more significant after decades of PA. Also, TL values are known to decrease with age in most cell types and tissues (Takubo et al., 2002). Hence, the probability of developing critically short telomeres (below a threshold value for cellular senescence) also substantially increases with age.

A second restriction was made as to the emphasis on specific cell types. TL and telomerase activity in multicellular organisms is primarily of relevance in life-long proliferating cells, that is in adult stem cells. Hence, strong attention is given in this paper to investigations on adult muscle stem cells (i.e. satellite cells). TL in satellite cells is of special interest, since exercise acts to particularly damage muscle tissue (Rae et al., 2010), thus requiring enhanced muscle stem cell proliferation. The cell proliferation and therefore the DNA replication is accompanied by telomere shortening due to the end replication problem, on the one hand, and by telomere lengthening via telomerase, on the other. The second main cell types selected were leucocytes. Due to easy sample taking of blood, a wide body of literature exists. While leucocytes are not stem cells, they may nonetheless reflect the TL of their haematopoietic ancestor stem cells to a certain degree. Moreover, lymphocytes - as a subgroup of leucocytes - can also be reactivated to proliferate and express telomerase (Weng, 2001).

2 Background and Methodology

In view of two recent reviews on the influence of physical activity on TL (Kadi and Ponsot, 2010; Ludlow et al., 2013), and while it is recognized that numerous other individual and biobehavioral factors affecting TL exist (see Table 1 (Starkweather et al., 2014)), in the following primarily those aspects will be summarized that relate to the aims of the present work stated in the introduction (Chapter 1). This includes a brief treatment of the structure of telomeres, followed by a discussion of telomere length regulatory mechanisms. Then, the biology of

satellite cells and leukocytes will be covered. A final sub-chapter on methodological aspects with a special focus on methodological limitations concludes this chapter.

Table 1: Factors other than physical exercise with increasing (+) or decreasing (-) effect on telomere length acc. to (Starkweather et al., 2014).

Individual factors	Biobehavioral factors (+)	Biobehavioral factors (-)
age	Resiliency	perceived stress
sex	high educational attainment	childhood adversities
race/ethnicity	longer sleep duration	duration of major depressive disorder
paternal age at birth		low educational attainment
genetic mutations		sedentary lifestyle
		short sleep duration
		smoking
		obesity

2.1 Telomere Structure

As emphasized before, the term telomere is used to refer to the specific repeats of nucleotide sequences at the end of the DNA (DNA level), on the one hand, and to the entire nucleoprotein structures or complexes at the end of chromosomes (chromosome level), on the other.

Starting with the DNA level, there is a 3'-overhang of the G-rich strand that measures between 35 and 600 nucleotides at the very end of the 5-15 kbp long telomeres. An initial G-overhang results from the end-replication problem on one chromosome end (the template being the lagging strand) and from resection of the C-rich strand on the opposite end (the template being the leading strand). In telomerase positive cells the G-overhang can be elongated by telomerase (Chai et al., 2006). This G-overhang bends on itself and invades telomeric repeat containing DNA, forming a telomeric loop (T-loop). The resulting triple stranded DNA structure is called displacement loop or D-loop (Greider, 1999). In this manner the 3'-overhang is sequestered, protecting telomeres from being recognized as double-stranded breaks. The T-loop is maintained and protected by telomeric and non-telomeric proteins (Palm and de Lange, 2008).

On the chromosome level, there are six telomeric proteins that form a protein complex called shelterin or telosome. These telomeric proteins are TRF1, TRF2 (telomere repeat factor-1, -2), POT1 (protection of telomere-1), TIN2 (TRF1-interacting protein 2), Rap1 (repressor/activator protein 1), and TPP1 (tripeptidyl peptidase 1). They protect telomeres by maintaining telomere structure, repress the DNA repair machinery at telomeres, and regulate telomere length (Palm and de Lange, 2008). The G-strand overhang is bound by the POT1/TPP1 heterodimer. This G-rich strand serves as an anchor for telomerase. The telomerase core components are an RNA subunit (TERC) and a reverse transcriptase subunit (TERT). These core components are associated with dyskerin (Dkc1) and other cofactors. The G-strand overhang pairs with TERC, which also serves as template for telomeric repeat addition by TERT (Samassekou et al., 2010). Adjacent to the telomeres there are gene poor regions called subtelomeres (Blasco, 2007b). Mammalian telomeres and subtelomeres are enriched in trimethylated histones (H3K9 and H4K20) and heterochromatin protein 1 (HP1). In addition, subtelomeric but

not telomeric DNA is methylated (Blasco, 2007a), and telomeric DNA can associate with telomeric repeat-containing RNAs (TERRAs) forming a structure called G-quadruplex (Xu et al., 2008). The structural complexity of telomeres poses quite a challenge on the replication machinery (Sampathi and Chai, 2011). How these challenges are overcome is reviewed in (Sampathi and Chai, 2011).

2.2 General Telomere Length Regulatory Mechanisms

As alluded to before, telomere length in stem cells is determined by the interplay of telomere shortening, for example due to the end of replication problem, oxidative stress and inflammation (Levy et al., 1992; Natale et al., 2003; Radak et al., 2013), and telomere lengthening mainly due to telomerase activity (Weng, 2001). Additionally, there are other ways for telomere lengthening termed alternative lengthening of telomeres (ALT). An important model for ALT is homologous recombination-dependent DNA replication. Here, the leading strand's 3'-overhang of a telomere recombinates with any other telomeric repeat containing DNA (i.e. telomere of another chromosome or sister chromatid) which serves as template for elongation of the 3'-overhang (Biessmann and Mason, 2003; Cesare and Reddel, 2010). Subsequently, polymerase complements the lagging strand (Cesare and Reddel, 2010). Despite being categorized as ALT, recombination-mediated telomere synthesis is thought to be the archetypal telomere lengthening mechanism when eukaryotes first evolved linear chromosomes before telomerase has evolved (de Lange, 2004; Neumann et al., 2013). Present studies indicate that ALT activity still occurs at least in some human cancers and normal mouse somatic tissues (Neumann et al., 2013). Still, telomerase plays the most prominent role in telomere elongation but is very complexly regulated.

Before discussing further details on general TL regulation mechanisms, it needs to be mentioned there is also a single nucleotide polymorphism in the human telomerase reverse transcriptase subunit (hTERT) promoter region (C-1327T; rs2735940) influencing telomerase activity (Ludlow et al., 2008; Matsubara et al., 2006a; Matsubara et al., 2006b). The TT genotype exhibits a significantly greater telomerase enzyme activity than both, the CT and CC genotype, at least in peripheral blood mononuclear cells (PBMCs). It is unclear, however, whether these changes in telomerase activity result in longer mean telomere length (TL_{mean}) (Ludlow et al., 2008; Matsubara et al., 2006b). In most organisms, TERT exists as single copy gene. Hemizygosity leads to stem cell diseases and amplification of TERT to increased tumor risk (Cifuentes-Rojas and Shippen, 2012).

TERT gene transcription is enhanced by binding of various transcription factors to the promoter and decreased by negative regulators or epigenetic silencing. TERT pre-mRNA can then be spliced into 10 different variants, which are correlated with changes in telomerase activity (Cifuentes-Rojas and Shippen, 2012). Most prominently, the splice variant TERTα is proposed to act as dominant negative inhibitor (Cifuentes-Rojas and Shippen, 2012; Colgin et al., 2000; Yi et al., 2000). Furthermore, a possible role of miRNAs in post-transcriptional TERT regulation was discussed recently in (Chilton et al., 2014).

The biogenesis and assembly of the telomerase ribonucleoprotein represent other avenues of regulation. Telomerase enzyme stability may be decreased by the MKRN1 ubiquitin ligase (E3). CHIP (C terminus of Hsc70-interacting protein), a co-chaperone with E3 ubiquitin ligase, interacts with hTERT especially around the G2/M

transition of the cell cycle. It polyubiquitinates hTERT in the cytoplasm and thereby blocks entry into the nucleus and reduces hTERT stability. In addition, phosphorylation of hTERT by the proteinkinase c-Abl negatively regulates telomerase activity, while phosphorylation by AKT correlates with increased telomerase activity presumably resulting from hTERT translocation from the cytoplasm to the nucleus. Another means of telomerase negative regulation is subnuclear localisation from the nucleoplasm to the nucleolus (Cifuentes-Rojas and Shippen, 2012).

Beside hTERT regulation, also hTERC regulation is thought to contribute to telomerase regulation. Various proteins can activate or repress hTERC transcription, which also appears to be controlled epigenetically. Moreover, hTERC is subject to post-transcriptional modification by pseudouridylation. However, it is still an open issue whether this pseudouridylation affects telomerase activity *in vivo* (Cifuentes-Rojas and Shippen, 2012).

Finally, telomerase must be recruited to accessible telomeres. This adds further mechanisms of regulation, which depend on the components and structural state of telomeric chromatin. The shelterin component TPP1, is implicated in telomerase recruitment. TPP1 forms a subcomplex with POT1, another shelterin component, thereby stimulating the interaction of POT1 with the single-strand 3'-overhang on the chromosome end. Repeat addition processivity of telomerase is influenced by TPP1 *in vitro* (Cifuentes-Rojas and Shippen, 2012). POT1-TPP1 binding seems critical for POT1-mediated TL control and telomere-end protection (Xin et al., 2007).

Mechanisms depending on the components or structural state of telomeric chromatin elegantly sense telomere length. In this regard a long non-coding nuclear RNA named TERRA (for telomeric repeat-containing RNA) and histone modifications at telomeres are of special interest. TERRA forms an integral part of human telomeric heterochromatin. Its transcription starts in the subtelomeric region and ends within the telomeric region (Azzalin et al., 2007), implicating that it is transcribed from the C-rich strand and therefore complementary to TERC (Maicher et al., 2014; Redon et al., 2010). Interestingly, transcription is increased at very short telomeres (Arnoult et al., 2012; Maicher et al., 2014) and TERRA levels peak at the G1/S transition and continuously decrease during the S phase (Maicher et al., 2014; Porro et al., 2010). TERRA not only has a potential function in mediating heterochromatin formation, it also blocks TERC by direct base pairing *in vitro*. *In vivo*, however, it leads to telomerase dependent telomere elongation especially at very short telomeres that exhibit a high TERRA concentration. It is thought that this due to TERRA acting as a scaffold for the formation of telomerase clusters which are then delivered especially to the telomere TERRA was transcribed from. Furthermore TERRA sequesters hnRNPA1, an RNA binding protein that *in vitro* not only binds single stranded telomeric repeats but also inhibits telomerase. Telomere bound TERRA might also facilitate ALT via homologous recombination (Maicher et al., 2014).

Considering the above findings, the apparent contradictory effects on telomere elongation of TERRA (mediating heterochromatin formation vs. recruitment of telomerase) might be resolved by the differential TERRA expression at the beginning and the ending of the S phase. At the beginning high levels of TERRA might recruit telomerase to telomeres and the continuously decreasing levels of TERRA throughout the S phase could lead to chromatin decondensation, allowing telomerase to access and elongate telomeres.

The accessibility of telomeres for telomerase is regulated by telomeric chromatin (de)condensation as reviewed by (Blasco, 2007a). In brief, normal length telomeres contain several marks for constitutive heterochromatin, such as DNA hypermethylation, histone hypermethylation (H3K9 and H4K20), hypoacetylation of histones H3 and H4, and heterochromatin protein HP1 binding. Therefore, they seem to be hardly accessible for telomerase. However, as telomeres get shorter, these markers for heterochromatin are reduced and histones become acetylated. Thus, the access for telomerase and proteins involved in ALT is facilitated, allowing for telomere lengthening. Once an adequate telomere length is attained, the heterochromatic state is reestablished, e.g. by the SUV39H and SUV4-20H histone methyltransferases, proteins of the retinoblastoma (RB) family, HP1 and DNA methyltransferases (Blasco, 2007a).

2.3 Satellite Cells and muscle regeneration

Muscle injury induces strong changes in muscle cells and the extracellular matrix. Especially eccentric contractions, where the activated muscle is forcibly lengthened, induce disruptions of myofilament structures in sarcomeres, damage to sarcolemma and loss of fiber integrity. Muscle regeneration after such an injury has similarities to muscle development during embryogenesis. The initial phase of muscle repair is characterized by production of pro-inflammatory cytokines in the muscle and by degeneration of the damaged tissue. Almost simultaneously, previous quiescent myogenic cells, i.e. satellite cells, are first activated, then proliferate and differentiate, and finally fuse with a damaged but viable muscle fiber (Adams, 2006; Karalaki et al., 2009; Peake et al., 2005). The self-renewal of the satellite cell pool may occur via symmetric or asymmetric cell division of stem cells, or dedifferentiation of partially committed myoblasts (Pell, 2014). The growth in muscle girth (hypertrophy) also depends on satellite cell proliferation which adds more nuclei to the growing muscle fiber, while the muscle fiber count does not change. However, two main changes occur: the number of myonuclei per fiber increases, and also the size and number of myofibrils supported each myonucleus increases (Alberts et al., 2007c). The satellite cell nuclei represent less than 10% of the total myonuclei population. Unfortunately, satellite cell turnover is not known, but the average cell age in intercostal skeletal muscle is 15.1 years (Spalding et al., 2005). Considering subjects with continuously decreasing satellite cell TL over time, the following correlation is expected. The TL in a muscle fiber nucleus reflects the TL of its ancestral satellite cell at the time a particular muscle fiber nucleus originated. Any minimum TL (TL_{min}) in the muscle tissue sample therefore reflects the TL value of the most recently incorporated muscle fiber nuclei and thus the momentary TL of the satellite cells (Ponsot et al., 2008).

Regarding the importance of telomerase in muscle stem cells, it is particularly noteworthy, that during a period of proliferative expansion, a segregation of older and younger template DNA strands has been observed. This template strand co-segregation was strongly associated with asymmetric cell divisions, where the more immature daughter inherited the older template strand (Conboy et al., 2007). Considering the end replication problem, one might argue that this mechanism preserves TL in satellite cells in a telomerase independent way. However, replication leads to a 3'-overhang at only one end of the dsDNA. At the other (blunt) end, the C-rich strand of the template needs to be resected to create a 3'-overhang (Chai et al., 2006). Therefore, template strand co-

segregation would lead to telomere shortening at one and the same end in each replication, while TL at the other end would be preserved. Since the survival of a cell is limited by the shortest telomere (Hemann et al.), possible template strand co-segregation would therefore reinforce, not supersede the importance of telomerase in satellite cells.

2.4 Biology of Leukocytes

Leukocytes or white blood cells (WBCs) can be grouped into the three major categories (Alberts et al., 2007c) (relative cell number among WBCs in % from (Dugdale, 2013)): (a) granulocytes (40-60% neutrophils, 1-4% eosinophils, 0.5-1% basophils), (b) monocytes (2-8%; become macrophages once they leave the blood stream), and (c) lymphocytes (20-40%; B cells, T cells, natural killer cells). TL of hematopoietic stem cells and hence also of mature leukocytes decrease with number of cell division and hence age. During leukocyte maturation in the bone marrow, telomerase activity is gradually downregulated (Weng, 2001).

Many of the studies reviewed for this paper were done on PBMCs or other mononuclear cells (MNCs) that comprise mainly lymphocytes but also monocytes, dendritic cells and some of the basophils (Miyahira, 2012). As explained above, lymphocytes can also be reactivated to proliferate and express telomerase, so that at least some of the PBMCs in a sample also contain quantifiable telomerase activity.

2.5 Methodology, Methods and Limitations

A key methodological problem of any investigations on TL alterations with time is that TL changes occur rather slowly and pronounced differences become apparent only after decades. This implies that biomedical studies also need to cover more than a decade of physical activity. However, since one cannot prescribe groups of humans to systematically and significantly increase or decrease their exercise habits over such a long period just for the purpose of conducting a study, there are apparently no long-term intervention studies available (i.e., studies in which subjects of a control group and intervention groups are assigned a specifically defined PA program and are followed prospectively over time). From a study design point of view this limits investigations on TL or telomerase activity to longitudinal studies (i.e., studies in which subjects are followed prospectively over time without intervention) and cross-sectional studies (i.e., comparison of TL values of representative population subsets differing in PA load at a given point in time). Even longitudinal studies are rare, so that most studies are cross-sectional. Using rodents as models solves this problem but comes with other caveats. While humans are short telomere mammals and use TL as a tumor-suppressive mechanism, rodents are long telomere mammals which do not use this mechanism. Furthermore, rodents are telomerase positive in the majority of somatic tissues and their TERT gene is regulated differently to humans. Nonetheless, age related TL shortening also occurs in rodents and the long telomere bias is reduced by using strains with relatively short telomeres (e.g. CAST/Ei mice as reviewed in (Ludlow et al., 2013)).

Concerning investigations on muscle tissue, basic methodological problems are that samples can be taken from various muscles and that samples might also be contaminated with fibroblasts (O'Connor et al., 2009). Furthermore, momentary satellite cell length is usually not measured directly. Instead, in most studies TL was meas-

ured from DNA extracted from muscle tissue samples not from isolated satellite cells. As pointed out before, under specific assumptions TL_{min} may be assumed to reflect the momentary TL of the satellite cells (see Section 2.3). However, in cases where certain stimuli act to lengthen satellite cell TL, TL_{min} in skeletal muscle no longer reflects momentary satellite cell TL. In order to gain information on satellite cell TL dynamics on a shorter time scale than decades, satellite cell TL must be measured directly in isolated satellite cells.

As to investigations of leukocytes, which include lymphocytes, it must be taken into account that lymphocytes comprise various subsets, and that relative and absolute cell counts in a blood sample might change as response to exercise (Chilton et al., 2014). Additionally, changes of telomerase activity due to exercise may be confounded by lymphocyte activation and telomerase expression due to antigen contact (Weng, 2001).

Turning to experimental methods, TL is most frequently measured either by terminal restriction fragment (TRF) southern blot analysis or by quantitative real-time polymerase chain reaction (qPCR) assay. For TRF analysis DNA is extracted from the sample, inspected for integrity and digested by frequently cutting restriction enzymes which, however, do not cut telomeric repeats. Then the digested DNA is resolved by gel electrophoresis, transferred to a membrane and hybridized with labeled probes. Hence, the TRF length equals TL plus a segment of the adjacent uncut subtelomeric DNA. Nevertheless, TRF length provides a measure of the corresponding TL values. An advantage of the TRF method is that it allows for the determination of the TRF length distribution of the sample (Kimura et al., 2010) so that both mean and minimum TRF length (TRF_{mean} and TRF_{min}) can be analyzed. The determination of TL_{min} or the percentage of telomeres below a defined length (both reflected in the TRF length distribution) is of interest for at least two reasons. First, the survival of a cell is limited by the shortest telomere irrespective of the average TL (Hemann et al.). Second, at least for subjects with continuously decreasing satellite cell TL, TL_{min} reflects the momentary TL of the satellite cells (see Section 2.3). Finally, when utilizing TRF analysis it must be kept in mind that very short TRFs are not detected on the gel (Aubert et al., 2012; Ludlow et al., 2013). Also, the segment of the included subtelomeric DNA may vary in length between individuals due to restriction site polymorphisms (Cawthon, 2002).

The qPCR method is more sophisticated and described in detail elsewhere (Cawthon, 2002). In a given DNA sample undergoing a polymerase chain reaction, it compares the ratio of telomere DNA content to chromosomal (single copy gene) DNA content (Ludlow et al., 2013). It therefore provides no information on TL_{min}. While the qPCR method is highly correlated with the TRF method, there is a significant variability between labs and in sample preparation techniques, thus making comparisons of data from different studies difficult.

Finally, telomerase activity is usually measured by the telomeric repeat amplification protocol (TRAP). For that, telomerase is extracted from a sample and incubated in a solution containing an artificial substrate, similar to a telomere, which is then elongated by telomerase. The elongated substrate is subsequently amplified via PCR and resolved via a polyacrylamide gel electrophoresis. The electropherogram shows several peaks with 6 bp distance. Telomerase activity is than calculated from the area under the peaks (Sachsinger, 2003).

3 Overview and Assessment of Literature

The available literature on the influence of physical exercise on telomere length and on exercise related regulatory mechanisms will now be analyzed in terms of the two questions raised in the introduction. Following an overview and analysis of the impacts of physical exercise on telomere length, evidence for the existence of exercise related TL regulatory mechanisms will be discussed.

3.1 Influence of Physical Exercise on Telomere Length

As to the literature on exercise related effects on TL, unfortunately but not unexpectedly, many of the studies differ in terms of their research approach and make use of different physical quantities (units) for defining and measuring the PA load and the exercise energy expenditure (EEE) of the subjects, respectively. This, in addition to the already mentioned inherent methodological limitations, complicates the comparison of data from different investigations. Nevertheless, for most cases it was possible to obtain good estimates of exercise energy expenditure (EEE) per week in units of kcal/week by making the following simplifying but feasible assumptions for inter-conversion of various PA quantities:

- 1h of physical exercise corresponds to an EEE of 600 kcal.
- 1 km of running corresponds to 1.0 kcal per kg body weight (Margaria et al., 1963); in cases of lacking body weight data, a body weight of 71 kg was assumed.
- For PA loads given in metabolic equivalent hours (METh), the following inter-conversion was used: (METh) x (body weight) equals EEE in kcal; in cases of lacking body weight data, again a body weight of 71 kg was assumed.

As a key prerequisite for comparing and contrasting literature data and findings below, use will be made of literature results in *harmonized (standardized) units* (i.e. EEE in kcal/week) by applying the above inter-conversions. Results in the units used in the original literature along with additional information of relevance will be provided in footnotes. Moreover and as already mentioned, the vast majority of studies relating long-term physical exercise to TL is of cross-sectional study design and focuses on leukocytes as an easy accessible sample of cells. Therefore, and for reasons of better comprehension, the following discussion on the impacts of physical exercise on TL will start with leucocytes then followed by treatment of skeletal muscles.

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In this section, first the relevant literature will be briefly reviewed and recapitulated in terms of the results obtained in their *harmonized* form as explained above. A common scheme is used that allows for the integration of most of the literature results in a comparable graphical form.

The core of the data for the comparative data analysis in search of a correlation between leukocyte TL and PA load is provided by 7 cross-sectional studies which are listed in Table 2 along with specifics of the various investigations performed. Three of these studies are *multi-group studies* involving at least three up to five groups of subjects differing in terms the weekly PA load (i.e. EEE class). Four studies are *two-group studies* involving only two groups of subjects, one exercising group and a control group (the study by Werner et al. (Werner et al., 2009) is listed twice in the table to account for the two subject groups of different age).

Table 2: Comparison of 7 cross-sectional studies investigating associations between leukocyte TL_{mean} and PA load listing specific details of the studies.

	Multi-point studies			Two-point studies				
Study	**Cherkas et al. 2008 (Cherkas et al., 2008)**	**Ludlow et al. 2008 (Ludlow et al., 2008)**	**Du et al. 2012 (Du et al., 2012)**	**Werner et al. 2009 (Werner et al., 2009)**	**Werner et al. 2009 (Werner et al., 2009)**	**LaRocca et al. 2010 (LaRocca et al., 2010)**	**Denham et al. 2013 (Denham et al., 2013)**	**Mathur et al 2013 (Mathur et al., 2013)**
Method	TRF_{mean}	qPCR	qPCR	qPCR Flow-FISH	qPCR Flow-FISH	TRF_{mean}*	qPCR	qPCR**
Cells	leukocytes	PBMCs	leuko-cytes	leukocytes, lymphocytes, granulocytes	leukocytes, lymphocytes, granulocytes	leukocytes	leukocytes	lymphocytes, granulocytes
Time of blood test	morning (fasted)	-	-	morning (fasted)	morning (fasted)	24 h exercise and 12 h food abstinence	-	-
Subject age (years)	48.8±12.9	50-70	59	20.4±3.3	51.1 (7.8)	62±2	43.6±9.2	54±4
Time span of exercise inquiry	past year	from age 30	past year	-	-	-	-	-
Training history	-	-	-	-	35±2.7 years	≥5 years	≥2 years	14±11 years but all ≥5 years
Subjects investigated	not specified	healthy subjects	women	track and field national team	marathon runners, triathletes	habitually exercising	male ultra-marathon runners	marathon runners
Total no. of subjects	2,401	69	7,813	58	46	32	123	32

*Information provided in review by (Ludlow et al., 2013)

Before illustrating and comparing the results of these 7 cross-sectional studies in graphical form, they will first be discussed briefly below outlining the essential features and key results in terms of the context of the present paper. Also included in this discussion are literature studies that did not allow for deducing comparable harmonized data, nevertheless add some further interesting insight.

Multi-group studies on the effect of exercise on leukocyte telomere length

To begin with, Cherkas et al. (Cherkas et al., 2008) studied mean leukocyte TL in 2401 twin subjects aged 48.8±12.9 years and assessed their exercising habits of the past year in a questionnaire. The subjects' PA level ranged from "inactive" (av. 160 kcal/week) to "heavy activity" (av. $1.99 \cdot 10^3$ kcal/week)[1]. Mean leukocyte *TL "... was positively associated with increasing PA level in leisure time. This association remained significant after adjustment for age, sex, body mass index, smoking, socioeconomic status, and PA at work. ... [This] finding was confirmed in a small sub-group of twin pairs discordant for PA level."* (Cherkas et al., 2008). Moreover, subjects that reported more than $4 \cdot 10^3$ kcal/week[2] exercise in their 20s had significantly longer telomeres than inactive subjects, when data were again adjusted for age, sex and blood extraction time.

Ludlow et al. (Ludlow et al., 2008) measured TL_{mean} in PBMCs of 50 to 70 year old healthy subjects. PA levels ranged from sedentary subjects to master athletes (incl. senior Olympians), with EEE gathered via interviews and quantified in kcal/week. Subjects reported their PA levels starting from their 30s, and only subjects performing regular and uniform exercise since their 30s were included in the study. The investigators assigned the subjects into quartiles of incremental EEE. They found an inverted U relationship between EEE and TL with a peak between 991 and 2340 kcal/week. Subjects burning more than 3541 kcal/week through exercise had equally short telomeres as those burning less than 990 kcal/week.

Similar results were obtained from Du et al. (Du et al., 2012) for 7813 women aged 59±7 years whose activity levels over the past year were assessed by questionnaire, and blood samples for TL measurement were taken 1-2 years later. Even though the authors stated that *"greater moderate- or vigorous-intensity activity was ... associated with increased [mean leukocyte] TL"*, a closer look on the data, however, again reveals peak leukocyte TL_{mean} values at $1.2 \cdot 10^3$ - $2.4 \cdot 10^3$ kcal/week[3] for moderate/vigorous intensity activity. Above the peak value at about $1.8 \cdot 10^3$ kcal/week, leukocyte TL_{mean} values initially again slightly decreased. In subjects doing more than $4.2 \cdot 10^3$ kcal/week[4], TL_{mean} values almost declined to values obtained in subjects doing less than 600 kcal/week[5]. Nonetheless, by conducting an F test on the data to assess a potential nonlinear (2^{nd} order) correlation, the authors came to the conclusion that there is no statistical evidence for a nonlinear relation between moderate/vigorous activity ($P = 0.42$) and leukocyte TL_{mean}. Interestingly, however, when performing an Microsoft® excel analysis on their data, the coefficient of correlation for the data is better for a polynomial than for a linear regression (i.e. for EEE in kcal/week on the x-axis, $R^2 = 0.9$ (polynomial) vs. 0.78 (linear)).

[1] 16 min/week ("inactive") and 199 min/week ("heavy activity")
[2] 6 h/week
[3] 2-4 h/week
[4] 7 h/week
[5] 1 h/week

The above findings are at least partially contradicted by another study (Werner et al., 2009) which compares TL_{mean} values in leukocytes, lymphocytes and granulocytes of marathon athletes and triathletes, aged 51.1±7.8 years, to TL_{mean} values in inactive healthy non-smokers. The athletes reported 35.5±2.7 years of training history, with $5.7\pm1.1\cdot10^3$ kcal/week[6] running or $5.8\pm2.0\cdot10^3$ kcal/week[7] overall exercise (incl. other exercise), respectively. Athletes were associated with longer telomeres in leukocytes with an even greater effect in lymphocytes and especially granulocytes. Additionally, this study also compared TL_{mean} in young track and field athletes with TL_{mean} in inactive controls. TL_{mean} in both groups was similar but longer than in aged inactive controls.

A study by (LaRocca et al., 2010) led to similar results on a group of habitually exercising 55 to 72 year olds. The exercising group did $5.3\pm0.8\cdot10^3$ kcal/week[8] exercise for at least five years. They had significant longer leukocyte TL_{mean} values than sedentary controls. This study also investigated leukocyte TL_{mean} in young endurance trained and young sedentary subjects, but did not find significant differences. Also, leukocyte TL_{mean} was found to be positively correlated to the maximum oxygen consumption VO_{2max} measured in ml/(kg·min).

Analogously, Denham et al. (Denham et al., 2013) observed that 43.6±9.2 year old ultra-endurance runners who had trained $2.8\cdot10^3$ - $7.1\cdot10^3$ kcal/week[9] for a minimum of two years had 11 % longer leukocyte TL_{mean} values than age and gender matched, and apparently healthy, untrained controls. *"The difference remained statistically significant after adjustment for cardiovascular risk factors."* (Denham et al., 2013).

In sharp contrast to the findings by (Werner et al., 2009), (LaRocca et al., 2010) and (Denham et al., 2013), there is yet another two-group study conducted by (Mathur et al., 2013) which contradicts the results of the former. 54±4 year old marathon runners, training $3.6\pm1.0\cdot10^3$ kcal/week[10] and having 14 years of training history on average, were reported to exhibit similar leukocyte and granulocyte TL_{mean} compared to age- and sex-matched healthy, sedentary non-smokers. In fact, this is the only study, where average leukocyte TL_{mean} of athletes (marathon runners) turned out to be slightly shorter than of sedentary controls.

Non-harmonization capable studies on the effect of exercise on leukocyte telomere length

There are numerous other multi-point studies on effects of exercise on leukocyte TL, unfortunately lacking easily quantifiable measures for activity level.Woo et al. (Woo et al., 2008) divided 2006 Chinese 72.4±5.1 year old subjects into quartiles of leukocyte TL_{mean}. Both, before nor after adjusting for age, body mass index, smoking, and self-perceived socioeconomic ranking could a significant difference in TL_{mean} values across quartiles of PA be found. Nevertheless, there was a slight increasing trend for TL_{mean} from PA quartiles 1 to 3.

[6] 80.5 ± 15.6 km running for the av. subject weight of 71 kg
[7] 9.6±3.3 h/week
[8] 75±11 METh/week
[9] 40-100 km/week
[10] 51±14 km/week

Likewise, (Cassidy et al., 2010) divided 2284 women, ca. 50-70 years old, with broadly distributed PA loads of about $1.35 \cdot 10^3$ kcal/week[11] on average into quintiles of leukocyte TL_{mean} and found no association with PA.

On the other hand, (Savela et al., 2013) provide support for an inverted U relationship between leukocyte TL_{mean} and PA level. Their study is interesting in that activity levels and other variables were obtained in 1974, and not only the leukocyte TRF_{mean} length but also the proportion of short telomeres (<5 kbp) was measured 29 years later. In 1974 the subjects comprised 204 actively working, healthy men with high socioeconomic status and without diabetes, clinical cardiovascular disease or regular medications. The subjects were assigned to three activity levels, with level 2 described as "walking, cycling, skiing, gardening, bowling, fishing or other light exercise weekly". After adjusting for age, BMI, cholesterol and smoking in 1974, in 2003 the ranking for the meanwhile on average 76 year old subjects was as follows: the moderate PA group had a statistically significant longer average leukocyte TRF_{mean} value (8.27 kbp) than the low PA group (8.10 kbp) or the high PA group (8.10 kbp). Moreover, the proportion of short telomeres was found to be the lowest in the moderate PA group.

Beside all these purely cross-sectional studies, (Weischer et al., 2014), in a cross-sectional and longitudinal study, measured leukocyte TL_{mean} and assessed activity levels in two sets of examinations about 10 years apart. In total, 4576 subjects reflecting the adult Danish population (age 20 and above at the outset) were investigated. Subjects were classified inactive, if they reported less than $2.4 \cdot 10^3$ kcal/week[12] exercise, a value which roughly corresponds to exercise levels associated with peak leukocyte TL_{mean} in other studies (see above and (Du et al., 2012; Ludlow et al., 2008)). After assigning the participants into quartiles of leukocyte TL_{mean} and upon performing a cross-sectional analysis on each of the two time separated data sets, a clear inverse correlation was found between the leukocyte TL_{mean} values and the percentage of inactive subjects in a given quartile (i.e. the shorter the leukocyte TL_{mean} range of a quartile, the higher the percentage of inactive subjects in that quartile).

Turning to the longitudinal analysis by (Weischer et al., 2014), inter-observational changes in TL_{mean} (ΔTL_{mean}) and inter-observational cumulative PA (in h) were recorded for each of the study participants individually, with four main results. First, the average ΔTL_{mean} amounted to -193 bp in the inter-observation period (-19,3 bp/year). Second, despite this overall average decline, leukocyte TL_{mean} was found to incline in 44% of the subjects (i.e. on an individual level). Third, a significant negative relationship between ΔTL_{mean} and leukocyte TL_{mean} in the first examination was found. Fourth, no correlation between inter-observational cumulative PA and ΔTL_{mean} could be established. The latter finding is surprising, considering the result of the cross-sectional analysis of small leukocyte TL_{mean} being more common in inactive subjects. Thus for the longitudinal analysis one may have expected a positive relation between inter-observational cumulative PA and ΔTL_{mean} which, however was not found. The authors speculated that the discrepancy between their cross-sectional and longitudinal findings may partly be due to a so-called 'collider bias'. For example, as inactivity is linked to shorter telomeres at the beginning of the observation period, there is a higher likelihood for mortality of such individuals, if they simultaneously have high TL loss rates. As individuals that have passed away cannot be reexamined after 10

[11] 19.7 METh/week for the av. subject weight of 68.6 kg
[12] 4 h/week

years, this creates a bias for the inactive group towards "apparently" smaller loss rates than expected. An alternative explanation for the results of this study will be provided in Chapter 4.

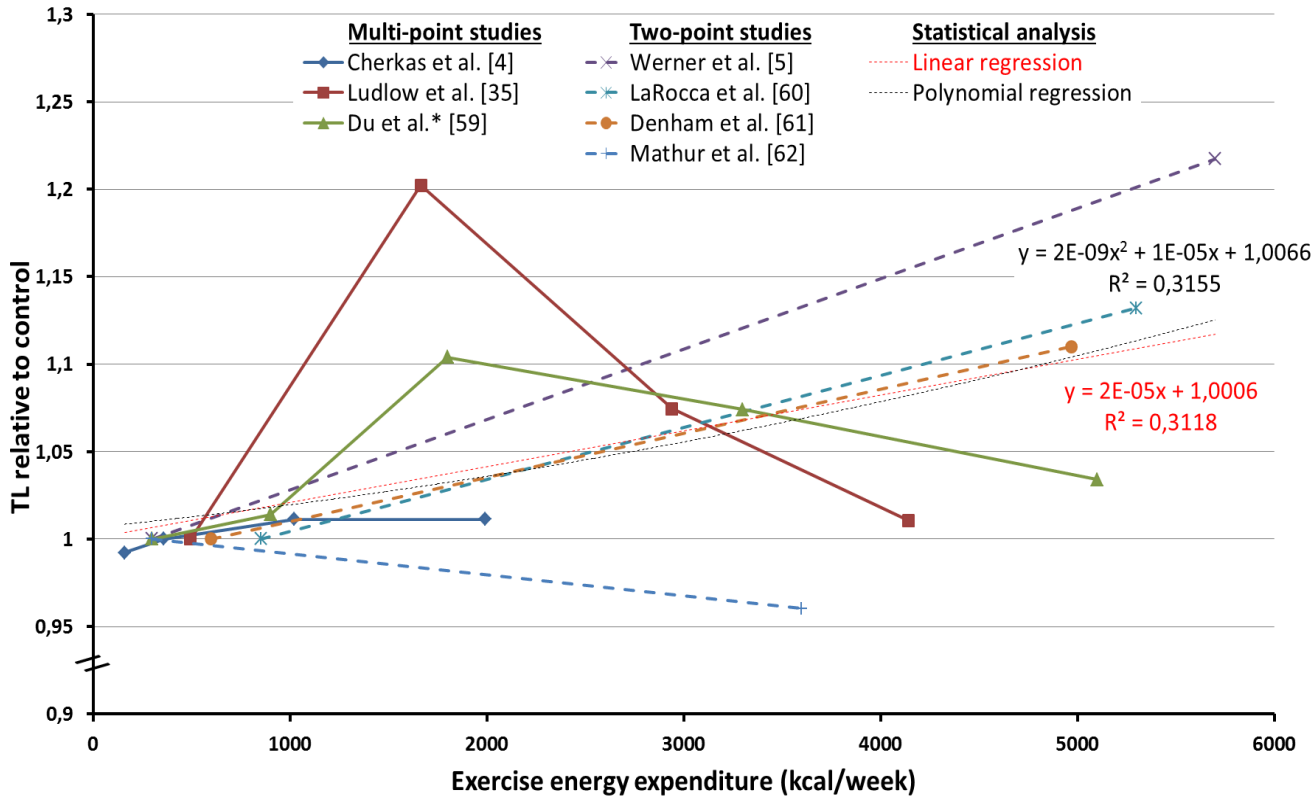

Fig. 1: Associations between exercise energy expenditure (EEE) and mean leukocyte TL from seven cross-sectional studies. Different specifications for amount of exercise between the studies were harmonized (unified) by best-estimate of weekly EEE values of the subjects. Solid lines are for multi-group studies, dashed lines for two-group studies, and dotted lines for regression analysis of the data.

Legend to EEE: for converting exercising hours and running kilometers into kilocalories, an energy consumption of 600 kcal/h and 1 kcal/km/kg bodyweight (Margaria et al., 1963) was assumed; in case of lacking data, a body weight of 71 kg was assumed for the conversion of running km or metabolic equivalent hours (METh) into kcal, , (see also text in Section 3.1)

Legend to TL: for each study, the TL of the subset of subjects whose EEE was closest to 500 kcal/week served as basis for assessment of relative TL (*values for Du et al. are given as multiples by an unknown factor of the true TL relative to the control group).

Legend to regression analysis: linear (red) and and polynomial (black) regression analysis was performed for all data points of all seven studies taken together.

As an interim summary, the results of the 7 cross-sectional studies allowing for "comparative data harmonization" are depicted in Fig. 1 as normalized TL_{mean} values (i.e. TL_{mean}(exercise group)/ TL_{mean}(control group); for each study, the group with a weekly EEE closest to 500 kcal/week was chosen as control) vs. weekly EEE. In terms of EEE allocation, the figure is based on mean values for the weekly exercise loads of a group as reported in the original papers after harmonizing these numbers in a manner described at the beginning of Section 3.1. Taken together, there is only a weak but positive correlation between EEE and leukocyte TL_{mean}. At least there is consensus that there is hardly any evidence that exercising for as much as 35 years at levels up to about $5 – 6 \cdot 10^3$ kcal/week shortens leukocyte TL_{mean}. Indeed, increased physical activity might even be positively associated with increased leukocyte TL_{mean} values for activity levels up to roughly $2 \cdot 10^3$ kcal/week. Results for activity levels beyond $2 \cdot 10^3$ kcal/week are inconclusive and range from declines to back to quasi the same TL_{mean} values

of inactive subjects (Ludlow et al., 2008); (Du et al., 2012) (Mathur et al., 2013)) to strong increased leukocyte TL_{mean} values, i.e. up to >20% compared to inactives (Werner et al., 2009) ; (LaRocca et al., 2010) (Denham et al., 2013).

3.1.2 Impacts of Exercise on Skeletal Muscle Telomere Length

Satellite cells of skeletal muscles are of special interest to this paper, because training can either cause muscle damage or be a stimulus for hypertrophy, both requiring increased satellite cell proliferation (Adams, 2006; Alberts et al., 2007c; Karalaki et al., 2009; Peake et al., 2005). To obtain muscle fiber TL, biopsies have to be performed, which explains why fewer studies exist than on leukocytes. Hence, to broaden the data base, the literature reviewed here includes studies on animals and humans.

To determine effects of endurance training in mice, (Ludlow et al., 2012) compared TL_{mean} values in skeletal muscle (gastrocnemius), cardiac muscle and liver of 8-week-old sedentary, one-year-old sedentary and one-year-old active CAST/Ei mice. Active mice were voluntarily wheel-running (6,475±1,396 meters/day) since they were 8-weeks-old. They had significantly shorter skeletal muscle TL_{mean} values than both, 8-week and one-year-old inactive mice, whose TL was similar. Interestingly, telomerase activity and TERT gene expression were significantly higher in the one-year-old active mice than in the one-year-old sedentary mice (see also Section 3.2.2). In contrast to skeletal muscle cells, TL_{mean} values in cardiac muscle cells as well as in liver cells were significantly shorter in one-year-old sedentary mice than in both other groups (Ludlow et al., 2012).

To determine effects of endurance training in humans, (Rae et al., 2010) obtained detailed training records and minimum TRF length of the vastus lateralis muscle in 17 well recovered 42.4±6.9 year old endurance runners. They reported mean weekly running distances corresponding to between $2.01 \cdot 10^3$ and $6.29 \cdot 10^3$ kcal/week[13] for 15.4±5.8 years, and they participated in competitions for the last 7 years at least. The 19 controls never did more than two days of "social exercise" per week nor did they participate in competitive sports. None of the athletes but 10 controls were smokers. Minimum TRF length was not significantly different between runners and control group as well as between the smokers and non-smokers in the control group. However, when the training records of the athletes were analyzed, minimum TRF length was negatively correlated with both the training years and the training hours spent in the entire career (Rae et al., 2010). This trend was also observed for the log running distance (log km) of the entire career. Moreover, personal best times for 21.1 and 42.2 km were queried for the entire career and the last year. Although not significant, there were trends for athletes with shorter minimum TRF lengths to show greater declines in performance over time for both distances. Taking the two results together (1: similar TRF_{min} in runners and sedentary; 2: declining TRF_{min} values for increasing total training volume), these data might as well suggest an inverted U relationship between exercise level and TRF_{min}, if TRF_{min} values were interpolated for the missing data between control group and the runners with the fewest total training hours or years. Alternatively, and although there were no significant differences between smokers and non-smokers of the control group in (Rae et al., 2010), smoking was negatively associated with TL in leukocytes of

[13] between 29.5 and 92.2 km for the av. subject weight of 68.2 kg

larger cohorts in (Morlá et al., 2006; Valdes et al., 2005). Hence the nexus between running and TRF_{min} might in fact be negative over all levels of training load if smoking had acted to shorten telomeres in the controls.

Another study was conducted by (Osthus et al., 2012) on a group of 5 male, competitive cross country skiers >65 years of age. The cross country skiers participated in races in the previous years and had a VO_{2max} of 45.4±6.7 ml/(kg·min), but further information on training history is lacking. Age and gender matched controls were also physically active, i.e. played senior soccer or danced at least twice a week, but had never competed at higher levels in any sports. Questioning (Rae et al., 2010), the cross country skiers had significantly larger TRF_{mean} values in vastus lateralis than the controls. In (Osthus et al., 2012) a study was also performed on 5 male competitive cross country skiers and track runners 20-30 years old. By trend, but not significantly, TRF_{mean} in vastus lateralis was larger in the athletes than in age and gender matched moderately active controls. A correlation between longer telomeres and higher VO_{2max} was also found and was more prominent in the groups of athletes. In addition to statements in (Osthus et al., 2012), it is worthwhile to note that both of the young groups had on average longer TRF_{mean} values in vastus lateralis than each of the older groups. This result, however, was not mirrored in a study on tibialis anterior muscle (Ponsot et al., 2008), where TRF_{min} and TRF_{mean} in young (25±4 years) and old (75±4 years) healthy subjects, who did moderate exercise for $6{\cdot}10^2$ – $1.2{\cdot}10^3$ kcal/week[14] were not significantly different.

The above two studies (Osthus et al., 2012; Rae et al., 2010) have in common, that they were done on supposedly healthy athletes. However, there is a condition known as the fatigued athlete myopathic syndrome (FAMS) (St Clair Gibson et al., 2000). Athletes suffering FAMS have history of high-volume training for many years and are chronically fatigued. Histological evidence indicates extensive muscle regeneration (Collins et al., 2003; Vihko et al., 1978, 1979), which results in an elevated demand for satellite cell proliferation (Collins et al., 2003). Consequently, if muscles are damaged over an extended period of time, telomeres can be expected to be shorter in skeletal muscle of FAMS athletes. To prove this hypothesis, (Collins et al., 2003) compared TRF_{min} and TRF_{mean} values in vastus lateralis muscle from 13 athletes aged 42.0±10.2 years suffering from FAMS with 13 healthy controls matched for age and, according to the authors, also for training volume. Type I fiber ("slow twitch") proportions were similar in the FAMS and control group. The mean TRF_{min} value of the FAMS group (4.0±1.8 kbp) was significantly shorter than that of the control group (5.4±0.6 kbp), but the mean TRF_{mean} value was only insignificantly shorter in FAMS than in controls (7.8±2.8 kbp vs. 9.6±1.1 kbp). However, a closer look on the available data to the training records along with own calculations, reveals a higher weekly training volume of the FAMS athletes before they got symptoms than of controls (mean±SD (number of subjects): 67.8±24.3 km/week (10) vs. 52.0±12.7 km/week (10); one-tailed p-value = 0.06 in an independent two-sample t-test). On the other hand, the training/racing career of FAMS athletes was shorter (11.8±5.6 years vs. 14.2±6.9 years). Nonetheless, one might argue that the shorter telomeres in FAMS athletes can be explained exclusively by the increased training load.

[14] 1-2 h/week

And yet, there are at least two arguments against that. The first makes reference to own calculations illustrated in Table 3 and is based on the findings by (Rae et al., 2010) of a change in TRF_{min} in vastus lateralis of about -0.14 bp per hour of training or $-1.6 \cdot 10^3$ bases bp per Δlog(total km). The weekly training loads of FAMS and control groups (in h and km, respectively) of the study by (Collins et al., 2003) were extrapolated for 11.8 years (average duration of the career in which FAMS athletes developed their medical condition). The extrapolated values were multiplied either by -0.14 bp per hour of training or by $-1.6 \cdot 10^3$ bp per Δlog(total km) to obtain the so-expected difference in TRF_{min} for the different training loads of FAMS and control. These expected TRF_{min} differences turned out to be roughly one order of magnitude below the actually measured TRF_{min} difference of $1.4 \cdot 10^3$ bp. This is the more remarkable, since additional telomere shortening in the control group due to their longer career (on average plus 2.4 year) was not accounted for in above calculations. Second, there are many runners not diagnosed with FAMS who have higher training volumes and a longer training history than the average FAMS athlete of the study by Collins et al. (e.g., studies by (Werner et al., 2009) (Rae et al., 2010) and most likely (Denham et al., 2013)). Hence, there must be other risk factors for developing FAMS than a high running/training volume.

Table 3: Theoretical calculation to assess the expected TRF_{min} differences between FAMS and control athletes as caused by the corresponding training load differences. The measured TRF_{min} value of FAMS was on average $1.4 \cdot 10^3$ bp shorter than TRF_{min} of asymptomatic controls (Collins et al., 2003). This measured difference is roughly one magnitude bigger than the theoretically expected difference from TRF_{min} shortening rates of $1.6 \cdot 10^3$ bp per Δlog(total km) and $1.4 \cdot 10^{-1}$ bp per running hour (derived from (Rae et al., 2010)).

	Raw data (Collins et al., 2003)		Total running distance		Total running hours
	Weekly running distance	Average running speed	Extrapolated total distance*	log(extrapolated total distance)	Extrapolated total time*
Control group	52,0	12,4	$3.19 \cdot 10^4$	4,504	$2.57 \cdot 10^3$
FAMS group	67,8**	12,8	$4.16 \cdot 10^4$	4,619	$3.25 \cdot 10^3$
Difference				0,115	$6.77 \cdot 10^2$
Expected TRF_{min} difference (bp)				**$1.84 \cdot 10^2$**	**$9.5 \cdot 10^1$**

*Extrapolation to 11.8 years (i.e. average career length of FAMS athletes for which data is available (Collins et al., 2003)). **Before first symptoms for FAMS were noticed.

In this context it is remarkable that the vast majority of FAMS patients described in the literature were active in sports involving eccentric muscle contractions (mostly endurance runners, one triathlete and one squash player; all sports where eccentric muscle contractions occur when the foot strikes the ground). Very few cyclists, only one rower, canoeists, or swimmer, and no power lifters (all sports without eccentric contractions) are reported to suffer FAMS (Collins et al., 2003; Derman et al., 1997; Grobler et al., 2004; St Clair Gibson et al., 2000). Hence, one may hypothesize that especially endurance sports that also involve abundant eccentric muscle contractions may lead to FAMS. Moreover, a causal relationship between short telomeres and FAMS has yet to be established and possible other mechanisms for its emergence need to be investigated.

While the demand for satellite cell proliferation after endurance training, is probably mainly due to muscle damage repair, in strength training satellite cell proliferation is also required to add new nuclei to the hypertrophying muscle fiber (Alberts et al., 2007c). In this regard (Kadi et al., 2008) compared TRF_{min} and TRF_{mean} values in vastus lateralis muscle of 7 power lifters (28.5±6.6 years) to 7 healthy control individuals (24.1±2.1 years) with no history of strength training. The power lifters had been practicing three to four times a week corresponding to 7 h/week for 8±3 years. Controls exercised less than once a week but did cycling and walking for transport. Both, TRF_{min} and TRF_{mean} tended to be longer in the power lifter group. Additionally, from the power lifters data for personal bests for the exercises 'squat' and 'deadlift' (both require action of the vastus lateralis muscle) and also for 'benchpress' were collected. Interestingly, TRF_{min} values were found to be negatively correlated with personal bests in 'squat' and 'deadlift', and TRF_{mean} values tended to be negatively correlated with personal bests in 'deadlift'. No correlation between skeletal muscle TRF_{min} and TRF_{mean} values and the number of weekly training sessions or the number of years of powerlifting practice was found. Also, no relationship between the age of the subjects and skeletal muscle TRF_{min} and TRF_{mean} values could be established. Assuming that higher personal bests imply higher weights lifted during training, the finding of a negative correlation between personal bests and skeletal muscle TL by Kadi et al. for power lifters may be considered equivalent to the finding of a negative correlations between TRF_{min} values and career length or total career running time, respectively, by (Rae et al., 2010). Moreover, when applying the same rational as for the results by Rae et al., there might in fact also be an inverted U relationship for weights lifted during training and skeletal muscle TL in the study by Kadi et al. However, the causality could also be the other way round. Thus, it is conceivable that individual athletes with satellite cells more prone to proliferation not only have shorter skeletal muscle telomeres but also develop bigger muscles, and as a consequence of the latter achieve better personal bests.

Consolidating and generalizing all these findings, it appears that the effects of PA on skeletal muscle TL vary between the types of sports performed. Endurance sports involving eccentric contractions (e.g. running) are seemingly associated with declining TL already at quite low PA levels. For strength training (e.g. powerlifting) higher personal bests were associated with lower TL, but the average powerlifter did not have shorter telomeres than non-power lifters. For these two PA types, there might also be a dose dependent inverted U-type relationship between TL vs. PA load. By contrast, endurance sports with little eccentric contractions (e.g. cross-country skiing) are associated with higher TL, but there is no detailed information on the training load of the subjects.

These patterns could perhaps be explained by recalling the established interdependencies that (a) eccentric contractions lead to muscle damage and thus satellite cell proliferation (Karalaki et al., 2009), (b) hypertrophy also leads to satellite cell proliferation (Alberts et al., 2007c), and (c) satellite cell proliferation may cause TL shortening due to the end replication problem (Levy et al., 1992). Eccentric endurance sports involving frequent eccentric contractions damage muscles the most. Strength training involving less frequent eccentric contractions and training schedules with sufficient regeneration phases for muscle damage repair and hypertrophy induces much less muscle damage. Moreover, it is plausible to assume that satellite cell proliferation due to strength training-induced hypertrophy may be limited since muscles cannot grow infinitely. Finally, endurance sports

with concentric contractions and little to no eccentric contractions damage muscles the least and like eccentric endurance sports only hardly induce hypertrophy.

And yet, current data on exercise induced TL alterations in satellite cells is still inadequate for deducing any final conclusions. Not only is the data base of existing studies insufficient and covers only a limited PA range up to about $6 \cdot 10^3$ kcal/week, there is also no study available that investigates training times exceeding $1.5 \cdot 10^4$ kcal/week[15] as they occur for instance in professional cycling (Lindner, 2005). These PA loads are by a factor of more than two higher than those in current studies published. During the very long training sessions and races in cycling, the glycogen stores of athletes can easily get depleted. Therefore, fatty acid metabolism for energy production and degradation of (muscle) protein for gluconeogenesis is enhanced (Konopka, 2006; Schmidt, 2007). It still has to be examined if this kind of muscle damage requires satellite cell proliferation for repair and thereby links glycogen store depletion to TL shortening.

3.2 Exercise Related Regulatory Mechanisms for Telomere Length

In order to explain the partly contradicting results of exercise on TL in the previous section, a closer look will now be taken on possible exercise induced TL regulatory mechanisms along with evidence for exercise dependent regulation of telomerase activity.

3.2.1 Evidence for Exercise Dependent Regulation of Telomerase Activity

As to evidence in support of exercise related telomerase activity, a recent study found significantly raised hTERT mRNA levels in WBCs 60' after 30' of exercise at 80% of VO_{2max} and also tendentially raised hTERT mRNA levels in distinct T lymphocyte subpopulations (Chilton et al., 2014). The same study also screened 1,300 miRNAs levels for changes. Four miRNAs showed changed expression after exercise as well as putative interactions with genes involved in telomere regulation *in silico*. Interestingly, the potentially TERT mRNA binding miRNA miR-181b concomitantly increased with its target, thereby effectively dismissing any significant miRNA/transcript interaction. Likewise, comparing the other three miRNA levels with mRNA levels of their putative corresponding target genes in the distinct T lymphocyte subtypes, no conclusive evidence could be found that any of these miRNAs really lead to putative target mRNA degradation in the subjects.

Also, (Werner et al., 2009) see Section 3.1.1) found increased telomerase activity in PBMCs of young and middle-aged runners compared to age-matched sedentary controls. On the other hand, (Ludlow et al., 2008) see Section 3.1.1) found no statistically significant differences in PBMC telomerase activity between the exercise level quartiles, although there was a clear trend for increased telomerase activity across the four quartiles (in attomoles per 10,000 cells; 1^{st}: $1.35 \cdot 10^{-3}$; 2^{nd}: $1.68 \cdot 10^{-3}$; 3^{rd}: $2.26 \cdot 10^{-3}$; 4^{th}: $8.39 \cdot 10^{-3}$; P=0.84). The apparent discrepancy in the studies by Werner et al. and Ludlow et al. would probably vanish, if the same statistical analysis were applied to the subjects of the corresponding EEE classes in both studies (i.e. 1^{st} and 4^{th} quartile in study by Ludlow et al.).

[15] 25 h/week

In another study it was found that daily repeated marathon running on seven consecutive days did neither change telomerase activity or hTERT mRNA levels or hTERC mRNAs nor TL_{mean} values in PBMCs, when measured 22-24 h after the last marathon (Laye et al., 2012). Furthermore, studies on exercising mice (Ludlow et al., 2012; Werner et al., 2009) see also Section 3.1.2) revealed elevated telomerase activity in MNCs derived from spleen, blood and bone marrow. Elevated TERT mRNA levels and telomerase activity were found in aorta and even >48 h after the last exercise in gastrocnemius muscle, but no change in telomerase activity was found in cardiac muscle or liver compared to sedentary controls. The finding in gastrocnemius muscle is challenged, however, by a study that reported similar telomerase activity in skeletal muscle in exercising mice compared to sedentary controls (Radak et al., 2001).

Taken together, in leukocytes a single exercise session apparently increases hTERT mRNA at least transiently, and regular exercise might increase telomerase activity for longer periods. However, in highly active endurance athletes (e.g., ultra-endurance runners, cyclists), a multi-day stage race, for example, will probably not result in a further medium-term increment of hTERT mRNA or telomerase activity. These findings in leukocytes may yet be of limited relevance, since most leukocytes have a short life span, and the results may be confounded by leukocyte redistribution and lymphocyte activation. In any case, it remains an open question, whether stem cells exhibit a similar behavior.

3.2.2 Evidence for Exercise Dependent Regulatory Mechanisms Targeting Telomeric Chromatin Components

In order to efficiently lengthen telomeres by telomerase, not only its activity but also its spatial recruitment to telomeres is of prime importance. As discussed in Section 2.2, this recruitment is mediated and controlled by telomeric chromatin components and structure, both of which may be affected by PA. Regarding components of telomeric chromatin, mRNA levels of several shelterin components including POT1, which is involved in telomerase recruitment, were elevated in PBMCs after marathon running on seven consecutive days (Laye et al., 2012). Furthermore, the histone deacetylase SIRT6 mediates deacetylation of histone H3 lysine 9, 18, 19 and 56 residues, which leads to chromatin condensation (Kugel and Mostoslavsky, 2014). According to (Blasco, 2007a), this should impede telomerase access to telomeric chromatin. However, deacetylation of telomeric H3K9 and H3K56 residues during S phase is required for efficient recruitment of a helicase called WRN, which may be required for proper replication of lagging telomeric DNA and telomere end capping by shelterin. Overall, and despite these apparent contradictory effects of SIRT6 on TL regulation, a loss of SIRT6 leads to telomere sequence loss (Kugel and Mostoslavsky, 2014)). Having this in mind, it is noteworthy that SIRT6 mRNA levels were increased after exercise in unsorted human WBCs but not in sorted T lymphocyte subpopulations (Chilton et al., 2014). Likewise, SIRT6 mRNA and protein is significantly higher in skeletal muscle of elite athletes than in moderately active individuals (Lindholm et al., 2014). However, another study contradicts these enhancements, reporting that SIRT6 mRNA is decreased in human skeletal muscle cells 24 h after a single exercise session (Radak et al., 2011).

Recapitulating the current literature status, there is some evidence that exercise could counteract telomere attrition by recruiting telomerase to the telomeres via POT1. While a loss in SIRT6 is known to reduce TL, it still remains fairly unclear if and how exercise affects SIRT6.

3.2.3 Effects of Exercise on Telomere Length via Regulation of Apoptosis

An entirely different, plausible yet little noticed idea is that exercise has anti-apoptotic effects that reduce cellular turnover and therefore maintain telomere length (Werner et al., 2009; Werner et al., 2008). Interestingly, TERT not only actively lengthens telomeres, it also acts on the proteins Ku70, p16, p53 and checkpoint kinase 2 (Chk2). Together with Ku80, Ku70 is involved in DNA repair (Ouyang et al., 1997), p16 can induce (Minami et al., 2003) or protect from (Al-Mohanna et al., 2004) apoptosis, p53 can trigger apoptosis (Amaral et al., 2010), and Chk2 is central for inducing DNA damage triggered apoptosis (Ahn et al., 2004). In aged human PBMCs, athletes showed increased Ku70 and decreased p16, p53 and Chk2 protein levels compared to sedentary controls. The same changes were observed in aortas of $TERT^{+/+}$ but not in $TERT^{-/-}$ mice voluntarily running on a wheel for 21 days, indicating that TERT mediates these alterations. The physiological relevance of these changes is reflected in the finding, that exercising tendentially leads to better protection from chemically induced apoptosis in $TERT^{+/+}$ compared to $TERT^{-/-}$ mice (Werner et al., 2009). Additionally p53 may be involved in negative regulation of hTERT transcription (Cifuentes-Rojas and Shippen, 2012; Shats et al., 2004; Xu et al., 2000). The resulting double-negative feedback loop (TERT decreases p53 protein levels and p53 decreases TERT transcription) might lead to a switch-like response between the options “elongate telomeres” and proliferate or “undergo apoptosis” and thereby increase cellular turnover and telomere shortening. On the other hand, exercise leads to an increased production of reactive oxygen species and elevated levels of glucocorticoids and catecholamines that could induce apoptosis (Phaneuf and Leeuwenburgh, 2001). Hence, how exercise mediated influences on apoptosis affect telomere attrition rates and to which extent such effects are dose dependent is yet unclear.

4 Proposed Model for Exercise Dependent Telomere Length Dynamics and Telomerase Activity Regulation

In this chapter, first a qualitative overall model for endurance exercise related TL dynamics is presented. Subsequently a model of hypothetical pathways for exercise dependent telomerase activity regulation is proposed.

4.1 Qualitative Model for Exercise Dependent Telomere Length Dynamics

Building on the literature analysis and discussion in Chapter 3, a general and coarse qualitative model summarizing how various forms of endurance exercise (as input parameter) might influence TL in leukocytes and satellite cells (as output parameters) will now be proposed. In this context the study by (Weischer et al., 2014) see also Section 3.1.1) on TL in leukocytes is of special relevance, as it is the only combined longitudinal and cross-sectional investigation available. While the authors explained the discrepancies between their cross-sectional and longitudinal analysis partly in terms of a collider bias, an alternative interpretation may be as follows.

The negative relation between inter-observational cumulative PA and ΔTL_{mean} in the longitudinal analysis suggests that restoring forces act on TL. One such restoring force is likely exerted by telomerase, as it is preferentially recruited to short telomeres (see Section 2.2; (Maicher et al., 2014). These restoring forces may stabilize TL at a certain level or steady state condition. Nonetheless, cross-sectional analysis in studies by Weischer et al. and others (see Section 3.1) show that PA and other parameters may alter TL, i.e. can lead to perturbations from this TL level. Therefore, it appears that TL is in a dynamic equilibrium, whose position is shifted to smaller values by shortening effects/stimuli (e.g., number of proliferations in the past, oxidative stress, inflammation) and to higher values by lengthening effects/stimuli (mainly mediated by telomerase). The speed of the adjustment of a new steady state after the appearance or disappearance of such effects may be different between the various stimuli. According to this notion, TL does not change unless the shortening/lengthening stimuli change. Returning to the results of Weischer et al., and making the plausible assumption that people rarely change their PA habits, it is therefore no longer surprising that inter-observational cumulative PA was not correlated with changes in ΔTL_{mean}, while concomitantly (momentary) PA was very well linked to (momentary) leukocyte TL_{mean}. Since in this particular study, being physically active was linked to longer telomeres, the average physically active subject may have coincidentally dosed his/her exercise in a way that it increased e.g. anti-oxidative-enzyme activity (Urso and Clarkson, 2003) and/or anti-inflammatory functions (Peake et al., 2005) and/or telomerase activity (see Section 3.2.1).

Based on these findings and interpretations, a general qualitative model is shown in Fig. 2, illustrating the described dynamic equilibrium between telomere shortening effects (cellular turnover, oxidative stress, inflammation) and telomere lengthening effects (telomerase activity, telomerase recruitment). Also depicted in Fig. 2 is the stabilizing negative feedback-loop for momentary TL (short vs. long) which is mediated by telomerase recruitment. The color codes for arrows indicate that TL increasing (green), TL decreasing (red), and TL increasing or decreasing (black) effects may be dominating. In Fig. 2a the model for leukocyte TL is shown, while Fig. 2b depicts the model for satellite cell TL by also taking into account the effects of eccentric muscle contractions.

The multitude of competing parameters and mechanisms in Fig. 2 together with their potential interactions may of course help to explain why at least on first sight seemingly contradictory results have been published in the literature. At the same time they underscore that the question, if endurance training under specific conditions may shorten or lengthen telomeres, apparently can only be answered by taking all these variables into account in a quantitative mathematical model that accurately reflects TL dynamics. In this context, a proposal for future research is provided in the final chapter of this paper.

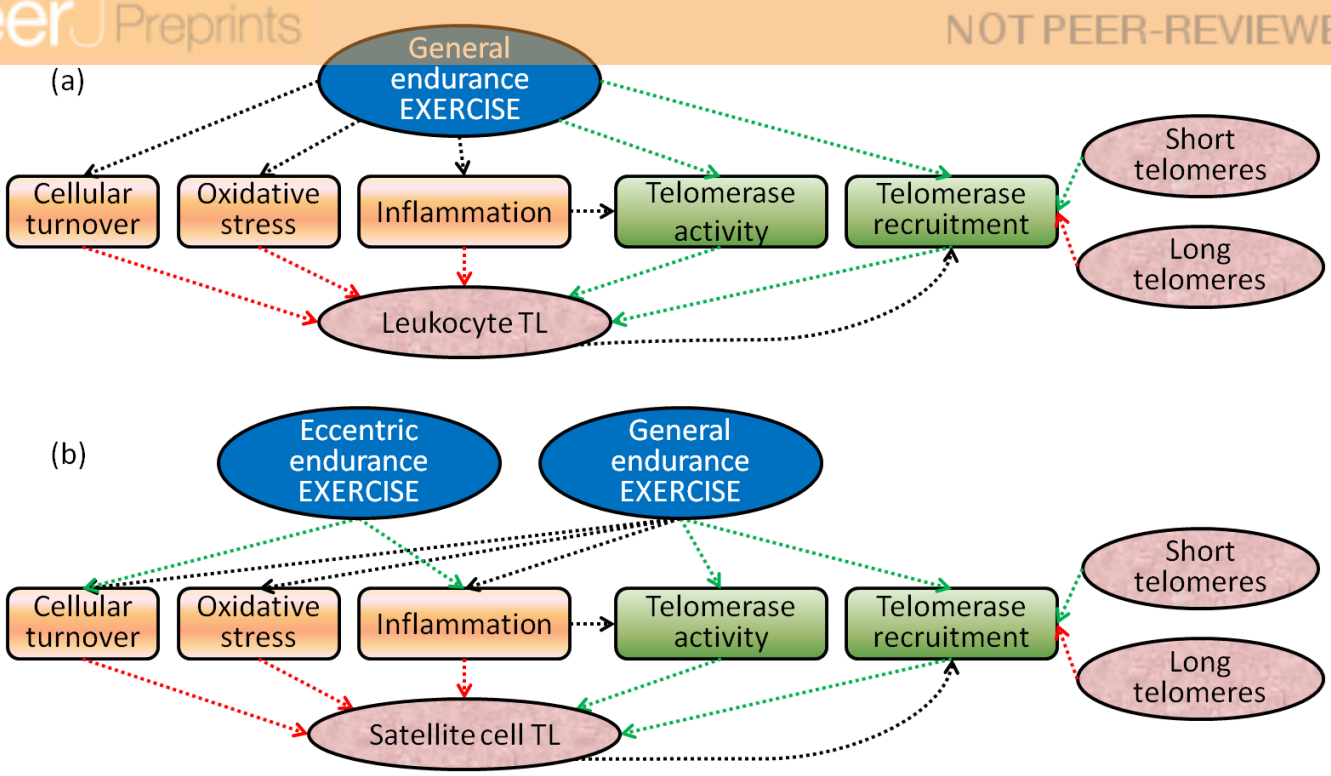

Fig. 2: A general qualitative model for endurance exercise related TL dynamics: (a) in leukocytes and (b) in satellite cells (see text for details)

(a) leukocytes; note: not all circulating leukocytes are able to express telomerase, and exercise induced stimuli may differ between haematopoietic stem cells in the bone marrow and their circulating descendants (i.e. leukocytes). *(b) satellite cells;* note: the model depicts satellite cell TL dynamics (not effects in muscle fiber nuclei).

Legend to arrows: dotted green arrows show TL increasing, dotted red arrows TL decreasing effects; dotted black arrows show that a mixture of both can be present; the curved dotted black arrows in particular indicate that short telomeres increase telomerase recruitment in *cis*. (note: the same arrow color code is used in Fig. 3 below).

4.2 Potential Pathways for Exercise Dependent Telomerase Activity Regulation

In Section 3.2.1 it was shown that PA can be associated with increased telomerase activity. Referring back to Fig. 2, a closer and more detailed look will now be taken on specific pathways interlinking exercise and telomerase activity (also accounting for effects of inflammation). Since only little is known about any details and specifics of such pathways, in attempt is made in the following to propose 'hypothetical' pathways linking exercise as stimulus to telomerase transcription or activity as response, integrating findings of numerous studies in the literature for different cell types. In this regard it must be kept in mind that different cell types might be exposed to different ambient conditions and the involved regulatory components might differ in gene expression, biomolecule localization and stability, post-translational modifications, etc. Nonetheless, for this integration it was assumed that common features between the various cell types predominate. Thus again, most pathways postulated in this section are hypothetical and not yet validated. They will be shown in summarized graphical form commencing the following overview of the relevant literature.

One central element in exercise induced TL regulation is endothelial NO synthase (eNOS). eNOS is positively regulated through phosphorylation by adenosine monophosphate activated protein kinase (AMPK) at S617, AKT at S617 and S1177, and protein kinase A (PKA) at S635 and S1177. Extracellular signal regulated kinases

1 and 2 (ERK1/2) negatively regulate eNOS via T495 phosphorylation. All of these kinases are activated by increased shear forces at the cell membrane of endothelial cells due to increased blood flow during exercise, with ERK1/2 exhibiting only a transient response and returning to the baseline within 30-60 min of increased blood flow (Zhang et al., 2009). Furthermore, exercise induced increase of AMP/ATP ratio also activates AMPK (Zhang et al., 2009), and strength exercise induced insulin-like growth factor I (IGF-I) increment (Vissing et al., 2013) activates the AKT pathway in skeletal muscle (Fernandes et al., 2012) and endothelial cells (Kim et al., 1999; Sowers, 2004; Standley et al., 1993; Standley et al., 1995). The resulting active NO signaling is required for eNOS to be co-localized together with estrogen receptor alpha (ERα; bound ligand required) in the nucleus, where the eNOS/ERα complex binds to estrogen receptor elements (ERE) in the hTERT promoter. The eNOS/ERα complex also methylates histone 3 at lysine 79 (H3K79). Additionally NO, which is possibly synthesized locally by the hTERT promoter bound eNOS, inactivates histone deacetylase 2 (HDAC2). Both leads to chromatin unfolding and together with the promoter binding of the eNOS/ERα complex enhances hTERT transcription in endothelial cells (Grasselli et al., 2008). Parts of this pathway are supported by a study on running mice (5,100±800 m/day for 21 days) that revealed increased cardiac expression of IGF-1 and a more than 2-fold upregulation in cardiac telomerase activity compared to sedentary control. Furthermore, treatment with IGF-1 upregulated myocardial telomerase activity more than 14-fold and increased the expression of phosphorylated AKT and phosphorylated eNOS (Werner et al., 2008). Besides activating hTERT transcription via eNOS, AKT also activates hTERT post-transcriptionally via phosphorylation as discussed in Section 2.2. Since endothelial NO synthase is a central element of these pathways, they appear to be restricted to endothelial cells. However, other isoforms of NO synthase are expressed in skeletal muscle (nNOS), macrophages (iNOS) and other cell types (Furfine et al., 1993; Silvagno et al., 1996). It is therefore conceivable that these NO synthases are similarly involved in exercise mediated TL regulation in tissues other than endothelium.

Wnt/β-catenin signaling, a pathway involved in regulation of cell proliferation (Alberts et al., 2007b), is probably also involved in linking exercise stimuli to TERT transcription. Voluntary wheel running in mice converts satellite cells to the activated state due to accelerated Wnt signaling (Fujimaki et al., 2014). In addition to activating satellite cells, Wnt/β-catenin signaling has been found to induce hTERT mRNA expression and telomerase activity in various tumor cell lines (Zhang et al., 2012). Also, in human colorectal cancer, β-catenin directly enhances hTERT expression (Jaitner et al., 2012). Furthermore, in (Zhang et al., 2012) an endogenous function of hTERT as a cofactor of the β-catenin transcriptional complex is discussed, which would theoretically result in a positive feedback loop between hTERT and the Wnt pathway. However, other studies did not confirm the function of hTERT as a cofactor of the β-catenin transcriptional complex (Strong et al., 2011; Vidal-Cardenas and Greider, 2010; Zhang et al., 2012).

In contrast to the local mediator Wnt, the myokine irisin acts more globally since it is released into the plasma by exercised skeletal muscle fibers. A recent study found TL to be predicted by plasma levels of irisin in PBMCs of 81 healthy individuals (Rana et al., 2014). At least in adipocytes *"irisin activates signaling pathways associated with the regulation of cellular proliferation including"* p38 mitogen-activated protein kinases (p38 MAPK) (Rana et al., 2014; Zhang et al., 2014). Exercise can also lead to elevated levels of pro-inflammatory

cytokines (Pedersen, 2000), which have been shown to activate p38 MAPK in HeLa cells (Raingeaud et al., 1995). The idea that exercise indeed activates p38 MAPK has been supported by the finding of transiently increased p38γ (but not isoform p38α) MAPK phosphorylation in skeletal muscle after marathon running (Boppart et al., 2000). There is a lot of evidence that p38 MAPK regulates hTERT transcription, however, whether this regulation is positive or negative depends on the mediators downstream of p38 MAPK (Alfonso-De Matte et al., 2002; Goueli and Janknecht, 2004; Maida et al., 2002; Matsuo et al., 2012; Takakura et al., 2005; Wang et al., 2000). Additionally, elevated levels of pro-inflammatory cytokines have been found to trigger NFκB binding to the NFκB response element of the TERT promoter in macrophages and consequently transcriptional activation of TERT (Gizard et al., 2011).

As for irisin, plasma levels of the stress hormone cortisol are also increased by physical exercise (Hill et al., 2008). Noteworthy is, that T lymphocytes exhibit reduced human telomerase activity if incubated for 3 days in a medium to which cortisol was added (Choi et al., 2008). On the other hand, one hour after exposure to an acute stressor the leucocytes of 44 probands showed 18% elevated telomerase activity despite concomitant cortisol increase (Epel et al., 2010). Other studies have shown that chronic stress, which is associated with elevated cortisol levels (Schulz et al., 1998), is linked to shorter telomeres (Damjanovic et al., 2007; Epel et al., 2004; Epel et al., 2010; Parks et al., 2009). Chronic stress has also been related to both dampened telomerase activity (Epel et al., 2004; Epel et al., 2010) and, paradoxically, enhanced telomerase activity (Damjanovic et al., 2007; Epel et al., 2010). This phenomenon is difficult to explain, especially without any knowledge on the mechanism by which cortisol affects telomerase activity. It is conceivable, however, that cortisol might actually decrease telomerase activity; but if an acute stressor directly precedes cortisol sampling, the cortisol effect is overcompensated by other immediate stress responses.

To conclude, an overview of the pathways postulated linking exercise as stimulus to telomerase transcription or activity as response is shown in Fig. 3. The proposed hypothetical signaling pathways suggest increased TERT transcription as response to exercise with only two weak objections. A first exception are p38 MAPK dependent pathways in that they might either increase or decrease TERT transcription depending on the downstream mediators (Alfonso-De Matte et al., 2002; Goueli and Janknecht, 2004; Maida et al., 2002; Matsuo et al., 2012; Takakura et al., 2005; Wang et al., 2000). Second, for cortisol there is also some uncertainty whether it increases or decreases telomerase activity. Also unknown is whether this regulation is achieved on transcriptional or other level (Damjanovic et al., 2007; Epel et al., 2004; Epel et al., 2010).

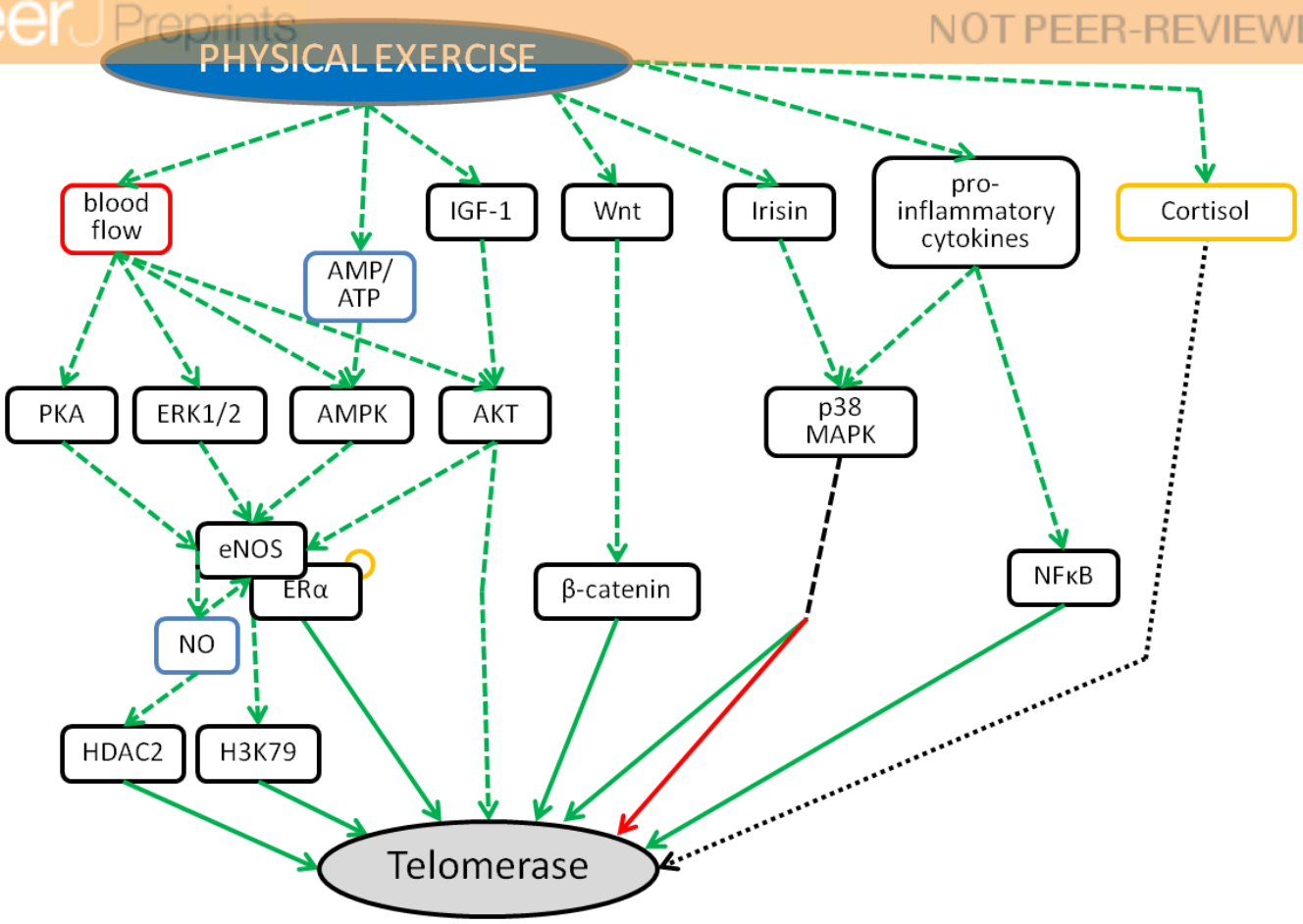

Fig. 3: Hypothetical pathways linking exercise as stimulus to telomerase transcription or activity as response. Most of the pathways shown were obtained by consolidating findings of different studies in different cells. In some cases it has not yet been determined, however, if a certain pathway indeed uninterruptedly exists in any one single cell (see text for further details).

Legend to arrows: solid arrows indicate transcriptional regulation, dashed arrows post-transcriptional regulation, and dotted arrows an unknown regulation mechanism; green color symbolizes upregulation, red color downregulation, and black color that both effects may be present (note: the same color arrow code is used in Fig. 2).

Legend to framings: black framing indicates proteins; orange framing indicates steroid hormons; red framing indicates mechanical stimuli; blue framing indicates other.

5 Conclusions and Outlook

In view of the multiple individual and biobehavioral factors affecting TL, the many complex interactions of partially competing bioregulatory mechanisms together with the additional methodological difficulties and limitations, it is not surprising that the current state of scientific knowledge/understanding of the influence of physical exercise on telomere length (TL) is still rather incomplete. Perhaps especially noteworthy, up to now no study seems to be available involving a sufficient number of professional endurance athletes with their substantial higher exercise volume.

Nevertheless, returning to the two main questions raised in Chapter 1, the answers based on currently available data and findings may be summarized as follows:

(1) *Does physical exercise exert any verifiable effect on telomere length? If yes, is there a correlation between PA load (i.e., average exercise energy expenditure per week or accumulated total exercise energy expenditure) and TL alterations?*

- *Referring to Section 3.1.1:* There seems to be a tendency for increased endurance activity levels to be positively associated with increased mean leukocyte TL for activity levels up to roughly $2 \cdot 10^3$ kcal/week, at least in middle to advanced aged subjects. Results for activity levels beyond $2 \cdot 10^3$ kcal/week are inconclusive and range from declines back quasi the same TL_{mean} values of inactive subjects to strong increased leukocyte TL_{mean} values (i.e. up to >20% compared to inactives).
- *Referring to Section 3.1.2:* Research on effects of endurance training on skeletal muscle TL so far is fairly limited, but suggests that sports with eccentric muscle contractions rather acts to shorten telomeres, while sports with little eccentric contractions might rather act to lengthen telomeres.

(2) *What is known and can be stated about the balance between telomere lengthening and shortening effects in relation to physical exercise along with details on underlying regulatory pathways?*

- *Referring to Sections 3.2.1 - 3.2.3:* There is some experimental evidence, that exercise activates TERT transcription telomerase activity. If and how exercise also acts upon telomerase recruitment to telomeres or affects TL by influencing apoptosis is not yet clear.
- *Referring to Section 4.1:* Telomeres appear to be dynamic structures in an equilibrium between telomere shortening (e.g., cellular turnover, oxidative stress, inflammation) and lengthening (e.g., telomerase activity, telomerase recruitment) effects. A negative feedback-loop mediated by enhanced telomerase recruitment to short telomeres presumably counteracts too excessive TL alterations.
- *Referring to Section 4.2:* Telomere lengthening effects of exercise can in theory be explained in by several hypothetical exercise induced signaling pathways which result in increased TERT transcription/activation.

Considering the rather unsatisfactory overall scientific knowledge situation, which in first instance is due to the multitude of confounding variables affecting TL and its regulatory mechanisms, but is furthermore related to methodological problems (i.e., no intervention studies and hardly any longitudinal studies available, difficulties to measure/characterize specifics on mode and quantity of PA, and inference of stem cell properties from measurements on their progenitor cells), a proposal for further research activities is put forth. To deal with the complexity of the problem described, a systems biology approach centering on a quantifiable mathematical model along with measurable parameters is required. The qualitative model on how endurance exercise might influence TL, presented in Section 4.1, and the proposed potential pathways for exercise dependent telomerase activity regulation, described in Section 4.2., might serve as starting point.

To evaluate, modify and extend the proposed model concepts in order to allow for quantification, several steps should be taken. First, telomere dynamics in response to various perturbations and combinations of perturbations should be accurately modeled in cell/tissue culture of simple systems (e.g. yeast), and in the following also in human cell/tissue culture. Second, with the aid of these models, telomere dynamics should be modeled in various cell types of laboratory animals *in vivo* under strictly standardized intervention study conditions. For

studying TL in muscle tissue in particular, rodents could be exposed to sequences of different exercising regimes in true intervention studies. After each exercising regime, they could be administered different labeled nucleotides which are incorporated into newly synthesized DNA. In this manner, muscle fiber nuclei, their TL and consequently TL changes could be assigned to a specific exercise regime. Third, to gain the necessary parameters for adapting these models to humans, (a) properly designed intervention studies for short-term TL regulatory mechanisms, and (b) long-term collection and storage of a plethora of blood, muscle and other tissue samples (whenever possible from various types of adult stem cells) along with detailed biobehavioral (in particular exercise related) information are needed. In this regard, data of endurance athletes who either start or end their active sports career are of special value, as this would quasi correspond to a long-term intervention study. The general trend towards individualized medicine combined with the use of novel sensor, data storage and transmission devices (e.g. smart phones or watches) might allow for collecting a sufficient number of biological samples and biobehavioral data by the time accurate and robust models for telomere dynamics have been developed for laboratory animals.

Of course, the above procedure not only poses a significant challenge as to the security and secrecy of personal biological samples and data (i.e. information storage) but also raises numerous ethical questions. It should be emphasized, however, that these issues will increasingly be of general relevance to bio-medical studies, and thus not be limited to the study approach proposed here. To conclude with a personal remark and assessment, I believe that the benefits for human health will outweigh the risks of data abuse, if proper ethical standards for data handling are developed and implemented. It goes without saying, that even in such a future scenario every person still should decide on his/her own, whether or not he/she provides biological samples and biobehavioral information for the sake of health research and the development of more effective, individualized therapies.

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