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The impact of chronic and structured exercise on telomerase activity and telomere length: a systematic review

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Zusammenfassung

Ziel: Die Telomerlänge in Leukozyten scheint ein wichtiger Marker für das biologische Altern zu sein. Die Telomerase-Aktivität könnte dabei ein wesentlicher Aspekt bei der Aufrechterhaltung der Telomerlänge sein. Aktuelle Literatur deutet darauf hin, dass körperliche Aktivität und Sport eine wichtige Rolle bei der Erhöhung der Telomerase-Aktivität und der Erhaltung der Telomerlänge spielen könnten. Ziel dieses systematischen Reviews war es daher, kontrollierte Interventionsstudien zu finden, die die Wirkung eines strukturierten Trainingsprogramms über einen Zeitraum von mindestens sechs Wochen auf die Telomerase-Aktivität und die Telomerlänge in Leukozyten untersuchen.

Methoden: Die Datenbank PubMed wurde genutzt, um aktuelle Literatur zum Thema zu finden. Es wurden nur kontrollierte Interventionsstudien mit einem strukturierten Kraft- oder Ausdauertraining über einen Zeitraum von mindestens sechs Wochen mit gesunden Teilnehmenden im Alter von 18 Jahren und älter berücksichtigt. Alle Gewichtsklassen der Teilnehmenden wurden inkludiert. Studien, die ProbandInnen mit Krankheiten oder Teilnehmende unter 18 Jahren untersuchten, wurden ausgeschlossen. Weitere Ausschlusskriterien waren Studien ohne Interventionen, Studien, in denen nur das körperliche Fitnessniveau bewertet wurde und Studien mit Bewegungsprotokollen mit einer Dauer von weniger als sechs Wochen oder Bewegungsinterventionen, die mit anderen Interventionen, wie z. B. Diäten, kombiniert wurden. Außerdem wurden in diesen systematischen Review nur Studien inkludiert, in denen die Telomerlänge in Leukozyten oder Muskelzellen oder die Telomerase Aktivität vor und nach der Intervention gemessen wurde.

Ergebnisse: Insgesamt wurden sieben kontrollierte Interventionsstudien in den systematischen Review einbezogen. Alle Studien zusammen zählten 1130 Teilnehmende mit einem Durchschnittsalter von 46.1 bis 72.0 Jahren. 94 Prozent (n=1062) aller Teilnehmenden waren weiblich. Insgesamt wurden sieben Ausdauer- und zwei Krafttrainingsinterventionen einbezogen. Bei vier von sieben Ausdauerinterventionen wurde eine signifikante Zunahme der Leukozyten-Telomerlänge nach einer Ausdauerintervention festgestellt. In keiner der Studien mit Krafttraining wurden signifikante Unterschiede in der Leukozyten-Telomerlänge festgestellt. Zwei Studien untersuchten die Auswirkung von körperlichem Training auf die Telomerase-Aktivität, wobei in einer Studie ein signifikanter Anstieg der Telomerase-Aktivität nach einem aeroben Ausdauer- und Hochintensitätstraining, nicht aber nach einem Krafttraining beobachtet wurde. In der anderen Studie wurde kein signifikanter Unterschied in der Telomerase-Aktivität nach einem aeroben Ausdauertraining festgestellt. Es wurden keine randomisierten kontrollierten Interventionsstudien zur Messung der Telomerlänge in Muskelzellen gefunden.

Conclusio: Die Ergebnisse der Studien dieses systematischen Reviews führen zu keiner eindeutigen Schlussfolgerung. Es gibt jedoch Hinweise dahingehend, dass Ausdauertraining mit aeroben und anaeroben Intensitäten die Telomerlänge in Leukozyten erhalten oder sogar verlängern und die Telomerase-Aktivität erhöhen könnte. Im Gegensatz dazu hat Krafttraining keinen Einfluss auf die Telomerase-Aktivität und die Telomerlänge.

Abstract

Aim: Telomere length in leukocytes seems to be an important marker for biological and healthy ageing. Telomerase activity appears to be an essential aspect in maintaining telomere length. Current literature suggests that physical activity and exercise could play a vital role in increasing telomerase activity and maintaining telomere length. Therefore, the goal of this systematic review was to find controlled intervention studies that investigate the effect of a structured exercise program over a minimum period of six weeks on telomerase activity and telomere length in leukocytes.

Methods: The database PubMed was used to find current literature on the topic. Only controlled interventions studies following a structured strength or endurance training over a minimum period of six week including healthy participants at the age of 18 years and older were included. All weight categories of the participants were included. Studies that explicitly investigated subjects with diseases or that included participants younger than 18 years were excluded. Other exclusion criteria were studies, where no intervention was implemented and the physical fitness level was assessed, exercise protocols lasting less than six weeks or exercise interventions that were combined with other interventions, such as diet interventions. Moreover, only studies that measured telomere length in leukocytes or muscle cells or telomerase activity before and after the intervention were eligible to this systematic review.

Results: In total seven controlled intervention studies were included to the systematic review. All studies together counted 1130 participants ranging from mean age of 46.1 to 72.0 years. Ninety-four percent (n=1062) of all participants were females. In total seven endurance interventions and two resistance training interventions were included. Four out of seven endurance interventions detected a significant increase in leukocyte telomere length after an endurance intervention. No significant differences in leukocyte telomere length were found in any of the studies with resistance training interventions. Two studies investigated the impact of exercise training on telomerase activity, whereby one study observed a significant increase in telomerase activity after an aerobic endurance and high intensity training intervention, but not after a resistance training intervention. The other study did not find a significant difference in telomerase activity after an aerobic endurance intervention. No randomized controlled intervention studies measuring telomere length in muscle cells were found.

Conclusion: The results of the studies of this systematic review are inconclusive. However, there are indications that endurance training at aerobic and anaerobic intensities seem to maintain or even elongate telomere length in leukocytes and increase telomerase activity. On

the contrary, resistance training does not affect telomerase activity and leukocyte maintenance or elongation.

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1 Introduction

According to the World Health Organization (2021) there is a shift in the distribution of the worldwide population towards an older age, which is also known as population ageing. People live longer and it is estimated that the world's population older than 60 years will double from 12% to 22% between the years from 2015 to 2050. However, an older growing population does not necessarily mean that their health span increases with increasing age. There is evidence that the proportion of living a healthy life has remained constant, which implies that the additional years of life expectancy are related to poor health and diseases. This is due the fact that increasing age goes hand in hand with various conditions, such as diabetes, back and neck pain, osteoarthritis, chronic obstructive pulmonary disease, or dementia (WHO, 2021; Puterman et al., 2018). Moreover, ageing is not a linear process and is not always associated with an individual's age in years. It is rather the biological mechanisms that take place within a person and that are related to the ageing process. Looking at the biological process, ageing is caused by the "accumulation of a wide variety of molecular and cellular damage over time" (WHO, 2021). These changes cause decreased functions of the physical as well as mental capacity, which in turn increases the risk for developing diseases. Looking at the factors influencing health at an older age, there are many aspects, such as lifestyle, social environment, or genetics, that have to be considered (WHO, 2021).

Since the life expectancy of world's population is increasing and some diseases are more commonly seen in older population, it is important to find a way of increasing the healthy life expectancy to promote a better quality of life in the older population. Therefore, healthy ageing, which is defined as "the process of developing and maintaining the functional ability that enables wellbeing in older age" (WHO, 2020), becomes increasing importance as a part of maintaining health as long as possible across lifespan. It is known that "maintaining healthy behaviours throughout life, particularly eating a balanced diet, engaging in regular physical activity and refraining from tobacco use, all contribute to reducing the risk of non-communicable diseases, improving physical and mental capacity and delaying care dependency" (WHO, 2021).

1.1 Aging

Biological ageing is a complex process resulting from genetics, environmental factors, and time. These ageing processes are very heterogenous and can vary across different cell and tissue types. These age-associated changes in the human body occur on different levels and can be divided into the following categories: normal aging, somatic diseases, chronic conditions, and psychological and social changes. Muscle weakness, changes in body

composition and sensory changes, such as vision and hearing capacity, are part of the normal aging process. Moreover, the aging process is often accompanied by common diseases including cardiovascular diseases, diabetes, cancer, osteoarthritis, osteoporosis, and neurological disorders. (Dodig et al., 2019). Since biological ageing is more important than the age in years itself with regards to a good quality of life, it is interesting to look at factors that are involved in the cellular mechanisms causing biological ageing. The rate of ageing can vary within different individuals and there are many factors, such as diet, physical activity, sleep, or smoking, influencing the speed and development of ageing (Güneşliol et al., 2021; Haupt et al., 2022, Dodig et al., 2019).

1.1.1 Aging Theories

Since aging is a very complex process, several theories have evolved over the years in order to explain the reasons for aging. Most of the established theories can be classified into three different categories: program theories, error or damage theories and combined theories (figure 1) (da Costa et al., 2016).

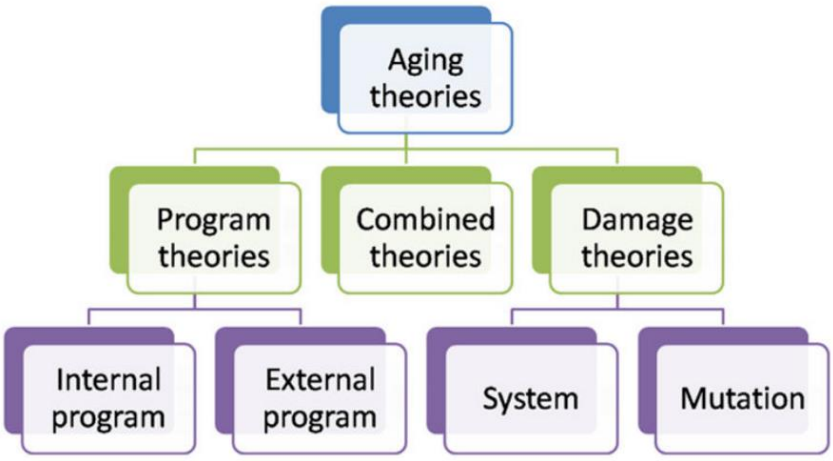

Figure 1: Aging theories (da Costa et al., 2018)

Program Theories

Program theories are often also known as active or adaptive aging theories. These theories suggest that aging occurs deliberately because a limited life span is associated with evolutionary benefits (da Costa et al., 2016). Moreover, programmed theories are based on the function of genes that contribute about 20-30% of aging and longevity. According to these theories, genes are switched on and off by an internal biological clock that regulates development, growth, and aging (Lipsky & King, 2015).

Damage Theories

According to damage theories, or also known as error theories, aging is not programmed. Instead, aging is the absence of maintenance. These theories suggest that there is a cure for aging. However, the accumulation of damage is modulated by genes and environmental factors, which results is a wide variability in lifespans. Among all damage theories, the predominant damage is oxidative damage. Reactive oxygen species (ROS) are produced during the metabolic process and lead to the accumulation of DNA, protein, and lipid damage (da Costa et al., 2016).

Combined Theories

Combined theories suggest that multiple theories of aging interact with each other. Therefore, an integrative analysis of different biological levels it necessary in order to fundamentally understand why and how we age. However, none of the attempts to combine different theories of aging were able to establish a comprehensive view and explanation for the process of aging. Nevertheless, combined theories indicate that aging should be seen as a complex network that is regulated through feedback loops between different biological levels (da Costa et al., 2016).

1.1.2 Aging Mechanisms

It is known that inflammation, immune aging, and senescence are important factors that are involved in and contribute to the aging process. These mechanisms will be explained in the following chapters.

Inflammation

Acute or transient inflammation is an important and protective mechanism for removing agents and curing damaged tissue. Chronic inflammation on the other hand persists for a long period of time and is associated with age-related changes and diseases. In general, anti-inflammatory cytokines neutralize pro-inflammatory cytokines and therefore also decrease chronic inflammation. If the activity of these two types of cytokines is balanced, healthy aging is possible. However, if there is a disturbance of this balance, aging-processes are accelerated and the development of age-related diseases can be enhanced (Dodig et al., 2019).

Immune Aging

The immune system is responsible for clearing infectious antigens, infected and senescent cells, and malignant transformed cells. However, the functions of the congenital and acquired immune system decrease with increasing age. These immunological changes in older individuals lead to a reduced resistance to infections, an increased occurrence of autoimmune

diseases due to an increased production of antibodies, and an increased development of neoplasm. Therefore, infectious diseases are seen more frequently in elderly (Dodig et al., 2019).

Senescence

Cellular senescence, also known as biological aging or cellular aging, is one of the main parts involved in complex aging mechanisms. This accumulation of senescent cells with increasing age can be a result of an increased production of senescent cells or a decrease of the capacity to eliminate senescent cells (López-Ótin et al., 2013). In general, senescence is an “irreversible form of long-term cell cycle arrest, caused by excessive intracellular extracellular stress or damage” (Dodig et al., 2019). The function of senescent cells is to limit the proliferation of damaged cells, to restrict potential malignant transformation and to remove accumulated harmful factors. However, the accumulation of senescent cells that occurs with aging shows deleterious effects on tissue homeostasis. The process of senescence can be accelerated by factors, such as loss of proteostasis, telomere shortening, DNA damage, mitochondrial dysfunction, or epigenetic alterations (figure 2). However, there are also factors, such as high levels of telomerase activity, contributing to slowing down the process of senescence (Dodig et al., 2019; López-Ótin et al., 2013; Haupt et al., 2022).

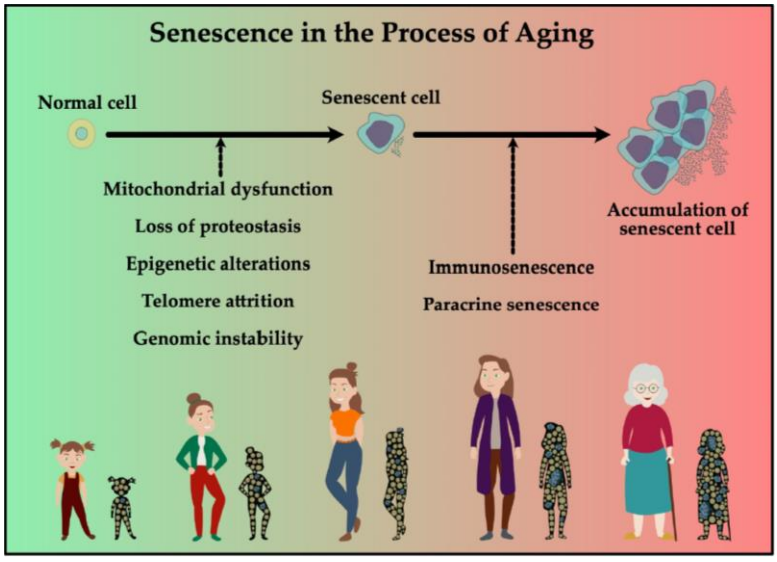

Figure 2: Development of senescent cells (Haupt et al., 2022)

Cellular senescence can be differentiated between acute or transient and chronic cellular senescence. Acute senescence is a normal biological process and may be a programmed mechanism. In young tissues the function of senescent cells is beneficial and important for tissue repair and wound healing. Chronic senescence on the other hand is the result of cellular stress for a long period of time or slow macromolecular damage and shows harmful effects on

cells and tissues. Senescent cells in older individuals are not able to maintain the physiological functions of tissue and to repair tissue, which in turn leads to age-associated diseases (Dodig et al., 2019).

1.1.3 Hallmarks of Biological Aging

Since chronological aging is not always associated with biological aging, it is necessary to find specific hallmarks or biomarkers that can measure the biological process of aging of an individual. These biomarkers will help in explaining how and why people age with different rates (Dodig et al., 2019). Biomarkers are defined as “a substance, structure or process that can be objectively measured in the body or its products and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to therapeutic intervention” (Dodig et al., 2019). These biomarkers have to meet certain criteria in order to predict the physiological ageing rate. The American Federation for Aging and Research (AFAR, 2016) suggests that biological markers for aging should fulfil the following criteria:

1. predict remaining life expectancy better than chronological age,
2. monitor mechanisms underlying the aging process but not a specific disease,
3. be subject to repeated tests without harming the individual,
4. be testable in both laboratory animals and humans.

Currently, there are no biomarkers that meet all the above-mentioned criteria and that could serve as standardized biomarkers for cellular aging or healthy ageing. Moreover, most of the aging biomarkers measure age-related illness instead of healthy aging and a combination of multiple biomarkers should be used in order to assess process of aging. Therefore, the authors of the review “Hallmarks of senescence and aging” recommend referring to terms, such as hallmarks of senescent cells, hallmarks of aging or possible biomarkers of senescence (Dodig et al., 2019).

In a review of López-Otín et al. (2013) hallmarks of biological aging should meet the following three criteria: they should manifest during the normal ageing process, and they should be able to increase or decrease the aging rate with experimental interventions. Based on this definition of hallmarks, the authors summarized nine potential hallmarks (figure 3) for biological ageing on a cellular and molecular level.

Figure 3: Nine potential hallmarks of aging (López-Ótin et al., 2013)

These hallmarks can be again divided into the following three categories, which are shown in figure 4: primary hallmarks, antagonistic hallmarks and integrative hallmarks. These three categories show a hierarchical relation and seem to influence each other. According to this hierarchical structure, primary hallmarks act as initiating triggers that result in damaging consequences that accumulate over time. Antagonistic hallmarks, which usually serve as beneficial hallmarks, are accelerated by primary hallmarks. As a result, the protective function of antagonistic hallmarks turns into a detrimental function. Eventually, integrative hallmarks appear when the damage caused by primary and antagonistic hallmarks cannot be compensated by the mechanisms of tissue homeostasis anymore (López-Ótin et al., 2013).

The main characteristic of primary hallmarks is that all of them are causes of damage. These hallmarks include genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis. Antagonistic hallmarks include deregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence. Antagonistic hallmarks are responses to cellular damage and can show beneficials as well as deleterious effects depending on the intensity of their response. Low levels of activation seem to induce beneficial effects, whereas high levels cause deterioration and consequently accelerate aging. Therefore, these hallmarks are designed to protect the organism form damage. However, if these hallmarks are impaired or chronic, they cause further damage. Stem cell exhaustion and altered intercellular

communication are considered as integrative hallmarks. These hallmarks directly affect the function and homeostasis of tissues (López-Ótin et al., 2013; Anaun et al., 2016).

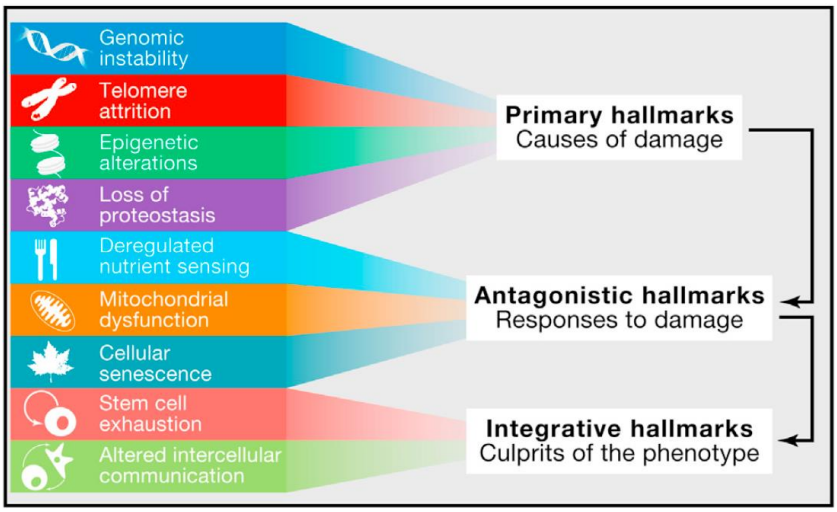

Figure 4: Categories of hallmarks for aging (López-Ótin et al., 2013)

1.1.3.1 Genomic Instability

It was shown that genomic damage is related to aging. Therefore, one predictor of aging is the accumulation of genetic damage. Genetic damage occurs due to the exposure of the DNA to exogenous and endogenous threats. Exogenous damages can by caused by physical, chemical or biological agents, whereas endogenous damages occur due to DNA replication errors, reactive oxygen species (ROS) or spontaneous hydrolytic reaction. These genetic damages caused by intrinsic or extrinsic factors can result in point mutations, chromosomal gains and losses, telomere shortening and gene disruption. In order to reduce DNA damage, complex mechanisms to repair DNA have evolved. These so called “genomic stability systems” include specific mechanisms to protect nuclear DNA, mitochondrial DNA and telomere length. These mechanisms support genomic stability and further interventions have to be explored in order to reinforce this stability in order to positively influence normal aging and extend longevity (López-Ótin et al., 2013; Annaun et al., 2016).

1.1.3.2 Telomere Attrition

The normal aging process is accompanied by telomere attrition in humans and animals. Additionally, pathological telomere dysfunction is associated with accelerated aging, and it has been shown that the stimulation of telomerase can delay the aging process. Although, the exact mechanisms behind biological ageing are not entirely understood, telomerase activity and telomere length seem to play an important role regarding the ageing process, the maintenance of health or developing various diseases. Therefore, telomeres are considered as an important marker for biological aging (López-Ótin et al., 2013; Haupt et al., 2022).

The process of telomere shortening is part of natural ageing but can be negatively influenced by several factors, such as obesity or a sedentary lifestyle, that promote oxidative stress and inflammation. Moreover, telomere attrition is associated with a decreased life expectancy and an increased risk of developing chronic diseases, such as diabetes, cancer, cardiovascular diseases, and psychological disorders (Haupt et al., 2022; Marques et al, 2020; Saretzki, 2018; Balan et al. 2018).

1.1.3.3 Epigenetic Alterations

Current literature suggests that aging is caused by epigenetic changes and that dysfunctions of epigenetics can accelerate the aging process (Lopez et al., 2016). On the other hand, in a review of Anaun et al. (2016), the authors claim that is not clear whether epigenetic alterations are the cause or a consequence of ageing. Epigenetic alterations involve changes in “DNA methylation patterns, posttranslational modification of histones, and chromatin remodeling” (López-Ótin et al., 2013). The enzyme SIRT6 seems to be a very important epigenetic factor that can influence longevity. A study showed that a reduced function of this enzyme decreased lifespan, whereas a greater function is associated with extended longevity (Kanfi et al, 2012). Moreover, increased levels of histone H4K16 acetylation, H4K20 trimethylation, or H3K4 trimethylation and decreased H3K9 methylation or H3K27 trimethylation can serve as age-associated epigenetic markers. However, there are several enzymatic systems that are responsible for the maintenance of epigenetic patterns. These systems include “DNA methyltransferases, histone acetylases, deacetylases, methylases, and demethylases, as well as protein complexes implicated in chromatin remodeling” (López-Ótin et al., 2013). Therefore, it can be assumed that influencing or manipulating epigenetics can improve age-related diseases and increase a healthy lifespan (López-Ótin et al., 2013, Anaun et al., 2016).

1.1.3.4 Loss of Proteostasis

Aging and age-related pathologies are accompanied by impaired protein homeostasis or proteostasis. In general, proteostasis involve mechanisms to stabilize correctly folded proteins by heat-shock proteins and mechanisms that are responsible for the destruction of proteins by the proteasome or the lysosome. These mechanisms are important when endogenous or exogenous stress results in the unfolding of proteins. If these mechanisms fail to refold or destruct unfolded proteins, their accumulation results in proteotoxic effects (figure 6) (López-Ótin et al., 2013).

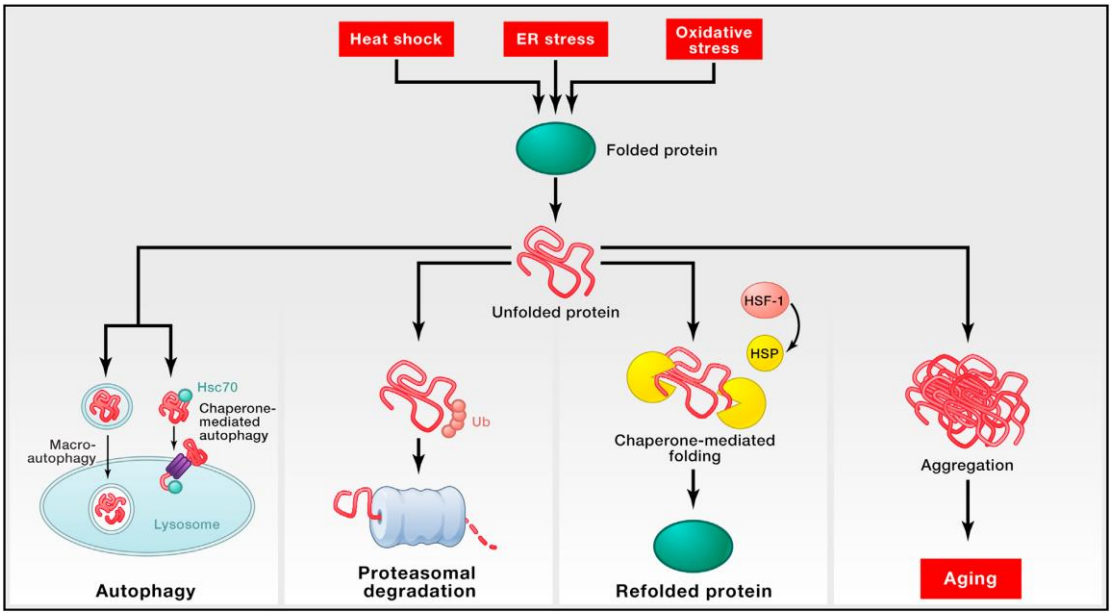

Figure 5: Loss pf Proteostasis (López-Ótin et al., 2013)

Literature has shown that proteostasis is altered with aging and that a chronic manifestation of unfolded or aggregated proteins can lead to the development of age-related diseases, such as Parkinson or Alzheimer (Powers et al., 2009). Therefore, aging is associated with a dysfunction of proteostasis. In addition, studies have demonstrated that the intentional impairment of proteostasis can result in pathologies that usually occur with aging. However, genetic manipulations could potentially improve proteostasis and therefore delay the aging process (López-Ótin et al., 2013; Anaun et al., 2016).

1.1.3.5 Deregulated Nutrient Sensing

Evidence shows that deregulated nutrient sensing can serve as a hallmark of aging. In general, nutrient sensing describes the pathways that regulate metabolism based on nutrient levels within the body. There are four key proteins that are involved in this process: insulin growth factor (IGF-1), mTOR, AMPK and sirtuins. The activity of these proteins is influenced by the nutrient levels and are therefore also known as nutrient-sensing proteins. However, if the process of nutrient sensing is disturbed, the aging process can be accelerated. Literature has shown that increased nutrient signals increase the aging rate, whereas decreased nutrient signals increase longevity. Moreover, it has been demonstrated that dietary restrictions can increase a healthy lifespan (López-Ótin et al., 2013).

1.1.3.6 Mitochondrial Dysfunction

Functional mitochondria regulate cellular homeostasis by maintaining the balance between oxygen uptake, ATP production, membrane potential and generation of reactive oxygen species (ROS). Mitochondrial dysfunction, which can result in accelerated aging, is caused by

mitochondria that accumulate in senescent cells (Dodig et al., 2019). The mitochondrial free radical theory suggests that the progressive mitochondrial dysfunction associated with aging causes an increased production of ROS which results in further mitochondrial and global cellular damage. Moreover, as chronological age increases, cellular damage and ROS increase at the same time in order to maintain the survival of cells. If a certain threshold is reached, ROS eventually rather aggravate age-associated damage than preventing it. However, it is not clear if improving mitochondrial function can increase a healthy lifespan (López-Ótin et al., 2013).

1.1.3.7 Cellular Senescence

As already mentioned, the number of senescent cells increases with increasing age (Dodig et al., 2019; López-Ótin et al., 2013; Haupt et al., 2022). Therefore, it has been thought that senescence contributes to the aging process. However, in young organisms, cellular senescence seems to have a beneficial effect because it contributes to tissue homeostasis and it prevents the proliferation of damaged cells, which in turn leads to the prevention of cancer. On the other hand, the accumulation of senescent cells in aged organisms aggravates damage and contributes to aging. Based on this information, the authors of the review “The Hallmarks of Aging” (López-Ótin et al., 2013) suggest that cellular senescence is a protective mechanism in response to damage. However, as soon as tissues reach their regenerative capacity, that mechanism becomes deleterious and accelerates the aging process. Moreover, studies showed that senescence-inducing tumor suppressor pathways extend longevity, whereas the elimination of senescent cells also delays age-associated pathologies (Mathew et al., 2007, 2009; Baker et al., 2011). Therefore, it is still not clear, how interventions on the cellular senescence could influence the process of healthy aging (López-Ótin et al., 2013).

1.1.3.8 Stem Cell Exhaustion

In general, stem cells have the potential to renew themselves and to develop into different cell types. However, it is known that the function of stem cells is affected by the aging process. The exhaustion of stem cells is a result of several aging processes and is therefore one of the main reasons for tissue and organismal aging. The most important factors contributing to stem cell exhaustion seem to be telomere shortening and increased levels of fibroblast growth factor 2 (FGF2). Therefore, it can be assumed that strategies aimed at slowing down telomere attrition and inhibiting FGF2 can reduce stem cell exhaustion during aging (López-Ótin et al., 2013).

1.1.3.9 Altered Intercellular Communication

One of the nine hallmarks of aging defined by López-Ótin et al. (2013) is the altered intercellular communication. This involves endocrine, neuroendocrine as well as neuronal communication.

Inflammation is one of the most important factors resulting in changes in intercellular communication. This aging-associated process is also known is “inflammaging” and is a result of several causes, such as the accumulation of proinflammatory tissue damage, a dysfunctional immune system to clear pathogens and damaged cells or the tendency of senescent cells to release proinflammatory cytokines. Inflammation processes are also involved in obese individuals or patients with type 2 diabetes. These pathologies contribute to and correlate with aging. In addition, defective inflammatory responses have been found to play an important role in developing atherosclerosis. Next to the inflammation processes, evidence suggests that aging-associated changes in on tissue can lead to deterioration in other tissues. This process is known as contagious aging and is also seen in senescent cells that cause senescence in neighbouring cells via processes that involve ROS and gap-junction-mediated cell-cell contacts (Nelson et al., 2012). These findings provide new opportunities to influence aging at the level of intercellular communication (López-Ótin et al., 2013).

1.2 Telomere Length

Telomere length is currently recognized as an important biomarker of aging and aging-related pathologies. The advantaged of telomere length serving as biomarker include the correlation with the chronological age, the prediction of several diseases and mortality and the responsiveness to harmful or beneficial exposures. Moreover, it could also act as an important marker for the aging rate over the course of lifestyle interventions that could potentially influence the progression of aging. However, since age-related telomere attrition is a very complex mechanisms, it remains still unclear whether telomere length is a reliable and valid tool to evaluate the aging rate in humans. In that sense, it is not established yet whether telomere length can either be the reason for aging or the result of aging. Despite this uncertainty, telomere length is one of the most common used biomarkers for biological aging in clinical studies as well as in personalized medicine (Vaiserman & Krasnienkov, 2021).

1.2.1 Mechanisms of Telomere Maintenance

As already mentioned, cellular senescence is a result of telomere shortening. Therefore, it is important to take a closer look to the exact function and the complex mechanisms behind telomere biology that could potentially influence the aging process. In general, telomeres are repetitive deoxyribonucleic acid (DNA) sequences located at the ends of chromosomes (figure 6). They are built up by six nucleotides (G-strand: 50-TTAGGG-30; C-strand: 30-AATCCC-50) that are repeated several thousand times (Haupt et al., 2022).

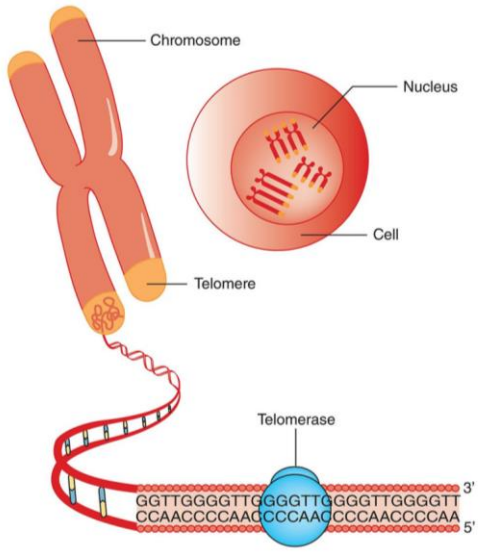

Figure 6: Telomeres (Vaiserman & Krasnienkov, 2021)

The role of telomeres is to protect the end of chromosomes and to maintain genomic integrity. With each cell division, its chromosomes have to be doubled first. This process makes sure that each daughter cell receives the complete complement of genetic material. The enzyme telomerase is responsible for completely replicating the terminal ends of DNA molecules. However, most cells do not express telomerase, which leads to the loss of telomere sequences at the ends of chromosomes with each cell division. This telomerase deficiency in humans is associated with premature development of various diseases. Moreover, oxidative damage can also contribute to telomere shortening. As soon as telomere length reaches a critical length, it cannot replicate anymore. This stage of telomere length is called replicative senescence or Hayflick limit (figure 7) (López-Ótin et al., 2013; AFAR, 2016).

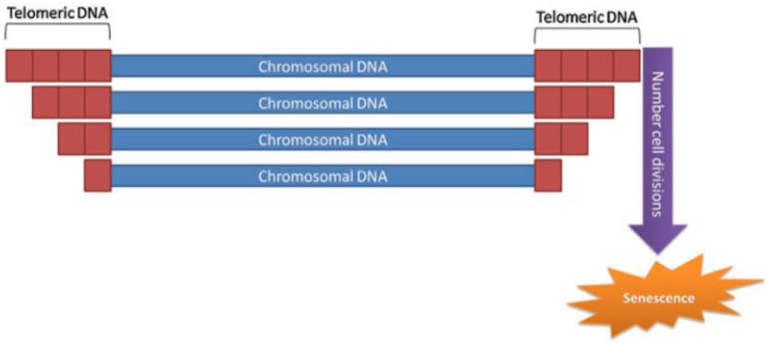

Figure 7: Telomere attrition leading to senescence (da Costa et al., 2016)

The regulation of telomere length and their integrity is a very complex process that involves several mechanisms at many different levels. One important underlying mechanism in maintaining telomere length is the enzyme telomerase, which plays an essential role in

counteracting telomere attrition. Moreover, telomere length and telomerase activity are regulated independently and their interaction with each other is not completely understood yet and it is still not entirely clear to what extend telomere length can assist in serving as a biological marker of ageing. However, there are indications that high levels of telomerase activity can potentially result in longer telomeres and delay the occurrence of cellular senescence (Saretzki, 2018; Haupt et al., 2022).

Another important aspect for maintaining telomere length is the protein complex named shelterin. This protein complex is important to prevent excessive telomere shortening and consists of six subunits including telomere repeat binding factor 1 (TRF1), telomere repeat binding factor 2 (TRF2), protection of telomere 1 (POT1), repressor/activator protein 1 (RAP1), TRF1- and TRF2-interacting nuclear protein 2 (TIN2) and tripeptidyl peptidase 1 (TPP1). The 3´end of the G-strand is longer than the 5´end of the C-strand. If telomere length is sufficient, a t-loop is being formed, which overlaps with the double-stranded 5´end and is building the D-loop, which protects telomeres (figure 7). During replication, the G- and C-strands open and the enzyme telomerase can bind to the G-strand. In the next step, the enzyme telomerase adds telomeric repeats to prevent the cell from damage. However, the protective function of the shelterin complex declines during aging and leads to DNA damage, which in turn stops the cell from dividing (Haupt et al., 2022).

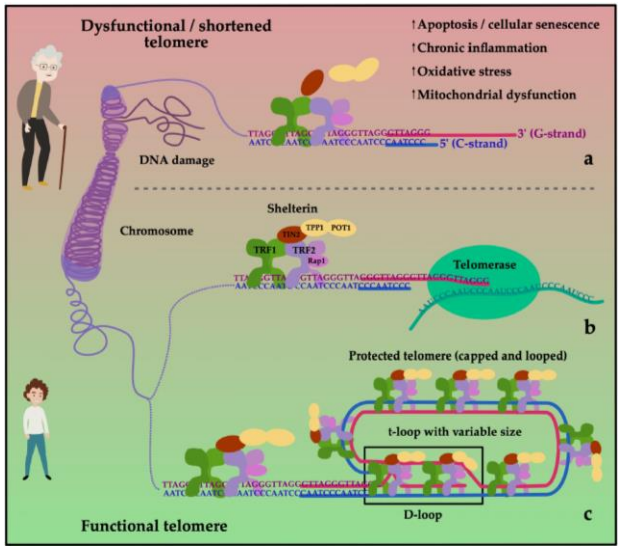

Figure 8: Telomere length maintenance (Haupt et al., 2022)

1.2.2 Measurement of Telomere Length

The measurement of telomere length is possible through several different methods. However, it is important to know that the results of different methods are not comparable between each

other. Some protocols measure the length of single telomeres, whereas others measure the average telomere length (Haupt et al., 2022).

The most widely used method for quantifying telomere length is the quantitative polymerase chain reaction (qPCR). It determines the ratio of telomere length to a single copy gene (T/S) which is proportional to the average telomere length. This method is a very easy way to determine telomere length. However, the lack of standardized protocols leads to big variations between different laboratories. Other methods such as the terminal restriction fragment analysis (TRF) or quantitative fluorescence in-situ hybridization (Q-FISH) follow more standardized protocols and are therefore better comparable between different laboratories (Haupt et al., 2022).

Telomere length can be measured in any cell. However, whole blood is the most common way of determining telomere length. Moreover, it seems that leukocyte telomere length reflects the overall senescence state within a person very accurately as there is a positive correlation between leukocyte telomere length and telomeres of other tissues. Therefore, measuring telomeres in leukocytes seems to be the best way of assessing biological ageing (Haupt et al., 2022). However, individuals with longer leukocyte telomere length also seem to show longer telomeres in muscle cells. Therefore, it can be assumed that there might be a shared mechanism for regulating telomere biology in leukocytes and skeletal muscle cells (Hiam et al., 2020).

1.3 Telomerase Activity

As already described in the previous chapters, telomerase is an important enzyme that contributes to the maintenance of telomere biology. Telomerase is required for cell proliferation and is mainly expressed in tumor cells but less in normal somatic cells. Therefore, telomerase can also serve as an important marker for cancer. The enzyme consists of the enzymes telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC). Both are necessary to maintain and elongate telomeres and play an important role in slowing down telomere attrition (Haupt et al., 2022; Zheng et al., 2020). Telomerase activity in combination with the measurement of telomere length can serve as a molecular marker of aging (Schellnegger et al., 2022).

1.3.1 Measurement of Telomerase Activity

Compared to the variety of different methods for determining telomere length, quantifying telomerase activity is more challenging. Telomerase activity cannot be measured in many cell types. Most somatic cells have low or even no measurable telomerase activity. Only stem cells and tissues with a high proliferation rate show high levels of telomerase activity. In human

blood lymphocytes also show high and measurable telomerase activity levels and are therefore often used to quantify telomerase (Haupt et al., 2022).

The most commonly used method to detect telomerase activity are modified versions of the telomere repeat amplification protocol (TRAP). Similar to the results of measuring telomere length, the results of telomerase activity are not comparable among different laboratories. The main challenge of lymphocyte telomerase activity is the preanalytical sample preparation. In order to measure telomerase activity, peripheral blood mononuclear cells (PBCM) have to be separated and isolated from freshly drawn blood samples. Specific tools are needed for this process, which explains why telomerase activity is not measured as often as leucocyte telomere length (Haupt et al., 2022).

1.4 Telomere Biology & Physical Activity

1.4.1 General Physical Activity Recommendations

In general, it is well known that physical activity and participating in regular exercise promote overall health and mental wellbeing. Leading an active lifestyle can reduce the risk of developing various diseases such as cardiovascular diseases, obesity, diabetes and depression (Sellami et al., 2021). The American College of Sports Medicine (ACSM, 2018) provided physical activity recommendations in order to maintain a health-related quality of life and functional capability (figure 9).

Table 1: Exercise recommendations for elderly (Rebelo-Marques et al., 2018)

Type of exercise	Frequency	Intensity (0–10)	Duration/volume
Aerobic exercise	≥5 days/week for moderate intensity	5–6	30–60 min/day in bouts of at least 10 min each to total 150–300 min/week
	≥3 days/week for vigorous intensity	7–8	20–30 min/day to total 75–100 min/ week
	3–5 days/week for combination of moderate and vigorous intensity	5–8	Combination of both
Muscle strengthening	≥2 days/week	Start with light (40–50% of 1RM) Progress to moderate (60–70% of 1RM)	8–10 exercises involving the major muscle groups with 1 set of 10–15 repetitions each
Flexibility	≥2 days/week	Until tightness or slight discomfort	Hold for 30–60 s

According to these guidelines aerobic endurance training, muscle strength and muscle endurance training and flexibility training should be included into an exercise program for older

individuals. The progression of the difficulty and intensity of the exercises should be tailored to the person's needs and preferences in order to achieve beneficial health effects (Rebelo-Marques et al., 2018).

Based on the information in the previous chapters, it seems that telomere length and telomerase activity have an important impact on the process of biological and healthy ageing. It is known that there are several factors that influence telomere length as well as telomerase activity. For example, an appropriate diet and certain types of physical activity seem to affect telomere length and could potentially serve as suitable lifestyle interventions to promote health (Broghini et al., 2015).

Over the past years, several studies were able to show an association between physical activity and telomere length. Besides these findings, also different levels of physical activity and certain types of exercise may have different effects on telomere maintenance (Günesliol et all, 2021).

1.4.2 Physical Activity Levels

While regular physical activity seems to improve antioxidant activity, it was shown that a sedentary lifestyle increases oxidative stress within the body (Marques et al., 2020). Since oxidative stress was shown to negatively influence telomere length, it can be assumed that participating in regular exercise could have an important impact in slowing down the process of telomere attrition. However, the impact of physical activity and exercise on telomerase activity and telomere length is still unclear (Sellami et al., 2021; Arsenis et al., 2017).

Regarding the effects of an inactive vs. an active lifestyle, there is evidence that individuals with a sedentary lifestyle have shorter telomeres compared to physical active individuals. In that sense, some studies suggest that even simple activities of daily life can already be associated with an increased telomere length. For example, a study of Soares-Miranda et al. (2015) showed that greater walking distance and better chair test performance were significantly associated with longer telomers in older adults. Another study was able to show that physically active women had longer leukocyte telomeres than sedentary individuals. These outcomes suggest that higher levels of physical activity could delay the process of telomere shortening (Günesliol et all, 2021; Ludlow & Roth, 2010).

Some studies suggest that cardiorespiratory fitness could be important for maintaining telomere length. A systematic review concluded that telomere length was better preserved in people with better cardiorespiratory fitness and in endurance athletes. Since cardiorespiratory fitness is an outcome of physical activity, it could be an indication that regular exercise acts as an important factor in maintaining telomere length (Marques et al., 2020). For example, the

results of a study of LaRocca et al. (2010) showed that leukocyte telomere length is better preserved in healthy older adults who regularly perform aerobic exercise and is positively related to the maximal aerobic capacity. These results indicate a positive relationship of telomere length with higher levels of cardiorespiratory fitness. Another study of Tucker (2017) found a strong association between the weekly amount of physical activity levels and leukocyte telomere length. A study of Borghini et al. (2015) showed similar results when assessing leukocyte telomere length in elder endurance athletes compared to a control group. Endurance athletes had longer leukocyte telomeres than their age matching controls. These results indicate a positive relationship of telomere length with higher levels of cardiorespiratory fitness, higher physical activity levels, and regular engagement in endurance training, supporting the hypothesis that chronic endurance exercise could potentially serve as an “anti-aging” intervention (Marques et al., 2020; LaRocca et al., 2010; Borghini et al., 2015).

On the other hand, a study of Hiam et al. (2020) found contradicting results. The authors found no association between aerobic capacity and telomere length in leukocytes as well as in muscle cells. Moreover, they also found a correlation between telomere length in leukocytes and muscle cells, indicating that these cells share similar mechanisms that regulate telomere length (Hiam et al., 2020).

1.4.3 Acute Exercise

Moreover, there may be a difference between the effects of acute and chronic exercise on telomere length. Different types of acute exercises could possibly affect telomere length and increase the healthy lifespan of an individual. However, there are still inconsistencies about the exact effects of acute exercise. Simpson et al. (2010) found an immediate increase in telomere length after an acute aerobic endurance session. Similar results are shown in a study of Werner et al. (2019), where they found that a single bout of endurance training increases telomerase activity. However, no changes in telomerase activity were detected after acute resistance training (Werner et al., 2019). Ludlow et al. (2017) also reported an increase of telomere-protective genes after acute exercise, which could indicate the activation of protective mechanisms that contribute to telomere maintenance (Ludlow et al., 2017; Sellami et al., 2021).

On the other hand, research showed that exhaustive acute bouts of exercise, such as extreme endurance running, are associated with accelerated oxygen radical generation and oxidative stress, which in turn leads to shorter telomeres. Although, it is important to mention that these outcomes were based on the effects of an acute and intense endurance session on telomere length. However, it could be an indication that high intensities of exercise could have a negative impact on telomere length (Borghini et al., 2015).

Regarding the effect of exercise on telomerase activity, endurance athletes were also shown to have higher telomerase activity (Sellami et al., 2021). However, current literature suggests that exercise-induced higher levels of telomerase activity could be transient and only last for a few hours after an acute bout of exercise. Therefore, the effect of regular exercise on telomerase activity over a longer period of time remains unclear (Banal et al., 2018).

1.4.4 Different Exercise Types

Based on the results of the studies that investigated the impact of acute exercise on telomere biology, it can be assumed that different types of exercise show different effects on telomere length or telomerase activity. For example, Werner et al. (2019) found an increase in telomerase activity after acute endurance training but not after acute resistance training. Another study found that telomere length was longer in elite master sprinter than their untrained peers, indicating that high intensity training programs have a beneficial effect at a biomolecular level. The authors therefore suggest that high intensity is not only useful to improve muscle power but should also be encouraged because of its anti-aging effects (Simoes et al., 2017). Additionally, it is also important to mention that previously discussed studies found positive effects of long-term endurance training on telomere biology (LaRocca et al., 2010; Borghini et al., 2015). Compared to the effects of aerobic exercise training, the available literature on effects of resistance training on telomere length and telomerase activity is still limited (Sellami et al., 2021) However, a study of Kadi et al. (2007) showed no difference in telomere length in muscle cells between powerlifters and age-matching controls with no experience in strength training. These results could indicate that strength training does not affect telomere biology (Kadi et al., 2007).

1.4.5 Summary & Research Question

Based on the current literature it can be assumed that exercise training might preserve telomere length, although most of the studies only showed an association between physical activity levels or cardiorespiratory fitness levels and telomere length or telomerase activity (Sellami et al., 2021). Moreover, studies that investigated the effect of physical activity on telomerase activity were mainly based on acute bouts of exercise (Banal et al., 2018). Additionally, there are indications that different intensities as well as types of exercise may have different effects on telomere length and telomerase activity (Borghini et al., 2015). Since telomere shortening is a very slow process and there are several factors contributing to this process, it is difficult to isolate the influence of exercise on telomere. Long-term exercise intervention studies are needed to study the effect of chronic and structured exercise training on telomere length and telomerase activity (Sellami et al., 2021).

Since telomere length and telomerase seem to be important aspects in maintaining a healthy life and preventing diseases, it is important to investigate how to slow down the process of telomere attrition to promote healthy aging. Physical activity seems to be one of many factors that may influence telomere biology. However, it is not entirely clear how different types of exercise influence telomere length (Sellami et al., 2021). Therefore, the goal of this systematic review is to provide an overview of current literature that investigates the impact of chronic, structured exercise interventions, such as endurance training or strength training, on telomerase activity and telomere length over a minimum period of 6 weeks. Since telomere length in leukocytes seems to be the best way of assessing biological ageing and to make the outcomes easier to compare, studies that investigated the impact of chronic exercise on telomere length in leukocytes will be included to this systematic review. In order to compare telomere length across different tissues, studies that measured telomere length in muscle cells will also be included (Haupt et al., 2022).

2 Methods

2.1 Search Strategy

The database PubMed (PubMed, 1996) was used to find controlled intervention studies that investigate the impact of a structured strength training or endurance training over a minimum period of 6 weeks on the telomere length and the telomerase activity in healthy adults. The following search string was created in order to find intervention studies that are related to this topic:

(sports OR exercise OR “physical exercise” OR “physical activity” OR “strength training” OR resistance training OR “endurance training” OR “running” OR “cycling”) AND (telomere OR “telomere length” OR “telomerase activity”) NOT (review[Publication Type]) NOT (cancer[Title]).

Different terms that are related to exercise and physical activity were used to find different types of training as interventions. The terms running and cycling were explicitly mentioned to cover studies that do not mention the words “exercise”, “sports” or “training”. The terms “telomere length” and “telomerase activity” were used to find studies with the desired outcomes. Only controlled intervention studies on healthy people were included in this systematic review. Therefore, the term “review” as well as the term “cancer” were explicitly excluded to reduce the number of studies. The search on PubMed was done on the 9th of April, 2022. Furthermore, the reference lists of the included studies were manually assessed for additional suitable studies. Full texts were obtained from the University in Vienna, the University of Applied Sciences Wiener Neustadt and the Medical University of Vienna. Articles that were not available online were obtained directly from the authors upon request by the supervisor.

2.2 Eligibility and exclusion criteria

The eligibility criteria for the studies of this systematic review were formulated based on the framework of PICOS. This framework provides a base to select eligible studies by defining the most important inclusion and exclusion criteria regarding the population, intervention, control or comparison groups, outcome, and study design. (Amir-Behghadami & Janati, 2020) (table 1).

The population included men and women who are aged 18 years and older. Only healthy subjects were included to avoid any influences of diseases on telomere length and telomerase activity. All weight categories were included to the systematic review.

The goal of this systematic review is to investigate the long-term effects of structured exercise on the telomere length and telomerase activity. Therefore, only studies with a structured exercise intervention of any type over a minimum period of 6 weeks were included. Combined interventions, such as exercise interventions combined with nutrition interventions or interventions consisting of strength training and endurance training, were excluded to avoid effects of other interventions. Studies in which no exercise interventions were performed were excluded from the systematic review. Only studies with an explicit control group (no intervention) were included. Therefore, only controlled intervention studies were included in the final analyses.

Studies, in which telomere length in leukocytes or telomerase activity before and after the intervention were measured, were included. Studies, in which the telomere length was measured only in other cell types, were excluded.

Table 2: Inclusion and exclusion criteria according to the PICOS framework (Amir-Behghadami & Janati, 2020)

	Inclusion criteria	Exclusion criteria
Population	- Healthy men and women (≥ 18 years) - All weight categories included	- Studies explicitly investigating diseased subjects (e.g., cancer, COPD, ...) - Subjects < 18 years
Intervention	Structured strength or endurance training over a period ≥ 6 weeks	- Exercise protocols <6 weeks - Exercise interventions in combination with other interventions (e.g., diets) - Studies without interventions - Studies in which physical fitness level was assessed and no intervention was implemented
Control	Control group in which no interventions were implemented	Control groups in which other interventions were implemented
Outcome	- Telomere length in leukocytes and/or muscle cells before and after intervention - Telomerase activity before and after intervention	Studies in which telomere length or telomerase activity were not measured
Study design	Controlled intervention studies	All other study designs

2.3 Study selection

Suitable references that were found on PubMed were exported to Covidence (Covidence, 2022) which is “a web-based collaboration software platform that streamlines the production of systematic and other literature reviews” (Covidence, 2022). A free trial version of Covidence was used where only 500 studies can be screened. The titles of the studies exceeding this number had to be downloaded and screened manually by the author.

The platform was used to screen the titles and abstracts of all found studies. Title- and abstract-screenings applying the mentioned inclusion and exclusion criteria were independently performed by the author and the supervisor of this systematic review. Studies which were related to the topic of the impact of exercise interventions on the telomere length in leukocytes and the telomerase activity were included in the full text review on Covidence.

The study selection of the full text review was based on the previous explained inclusion and exclusion criteria (table 1), which was executed by the author. Studies, in which it was unclear to the author whether the eligibility criteria were fulfilled or not, were discussed in a meeting with the supervisor and, ultimately, included to or excluded from the systematic review.

After the full text review on Covidence was completed, the references of the included studies were reviewed to check if more intervention studies could be found on that topic. Titles that were related to the topic were again forwarded for abstract screening and full text review where they were screened for eligibility and exclusion criteria.

2.4 Data Items

The most relevant data on the characteristics of the study, population, intervention, and the key outcomes were determined before extracting the data from the included studies. These variables were elaborated by the author and discussed together with the supervisor to check if the most important details were included.

Regarding to the total population, data on the age, BMI, and the ratio of males to females were collected. In addition, the details of the types, intensities, durations, and frequencies of the interventions were recorded.

To assess the outcomes of the studies, the results of telomere length and telomerase activity before and after the intervention, as well as the measurement method used, were collected. To see if there were any significant changes in telomere length and telomerase activity within the groups the level of significance was extracted. Additionally, p-values for significant differences between the control and intervention groups were collected if present.

2.5 Data Collection

After determining the main variables which are used as the results of this systematic review, data from each study were collected by the author. The mean and the standard deviation for each outcome of the telomere length and telomerase activity were extracted. Data which were presented as graphs and no exact numbers were given were collected using the online app "PlotDigitizer" (PlotDigitizer, 2022). This application allows to extract the exact data from the graphs given in the studies. In some cases the standard deviation had to be calculated using the following formulas:

$\sigma d = \mathrm{sqrt}(\sigma 1^2 2 / n1 + \sigma 2^2 / n2)$ (Berman, 2022)

$SD = \sqrt{N}x \frac{(upperlimit\ C - lower\ limit\ CI)}{3{,}92}$ (Cochrane Training, 2022)

2.6 Risk of Bias

The PEDro scale (PEDro, 1999) was used to assess the risk of bias of each study. This scale is mainly used to evaluate the quality of physiotherapeutic interventions but can also be applied in the field of sport scientific research. It consists of 11 items which serve to assess randomized clinical trials regarding their internal validity and interpretability of the results. The total score of the PEDro scale was calculated from the items 2 to 11 (PEDro, 1999). Telomere length and the telomerase activity were used as the primary key outcomes regardless of the actual primary key outcome of the study. The risk of bias assessment was done for each study by the author. Unclear aspects were discussed in a meeting with the supervisor.

3 Results

3.1 Study Selection

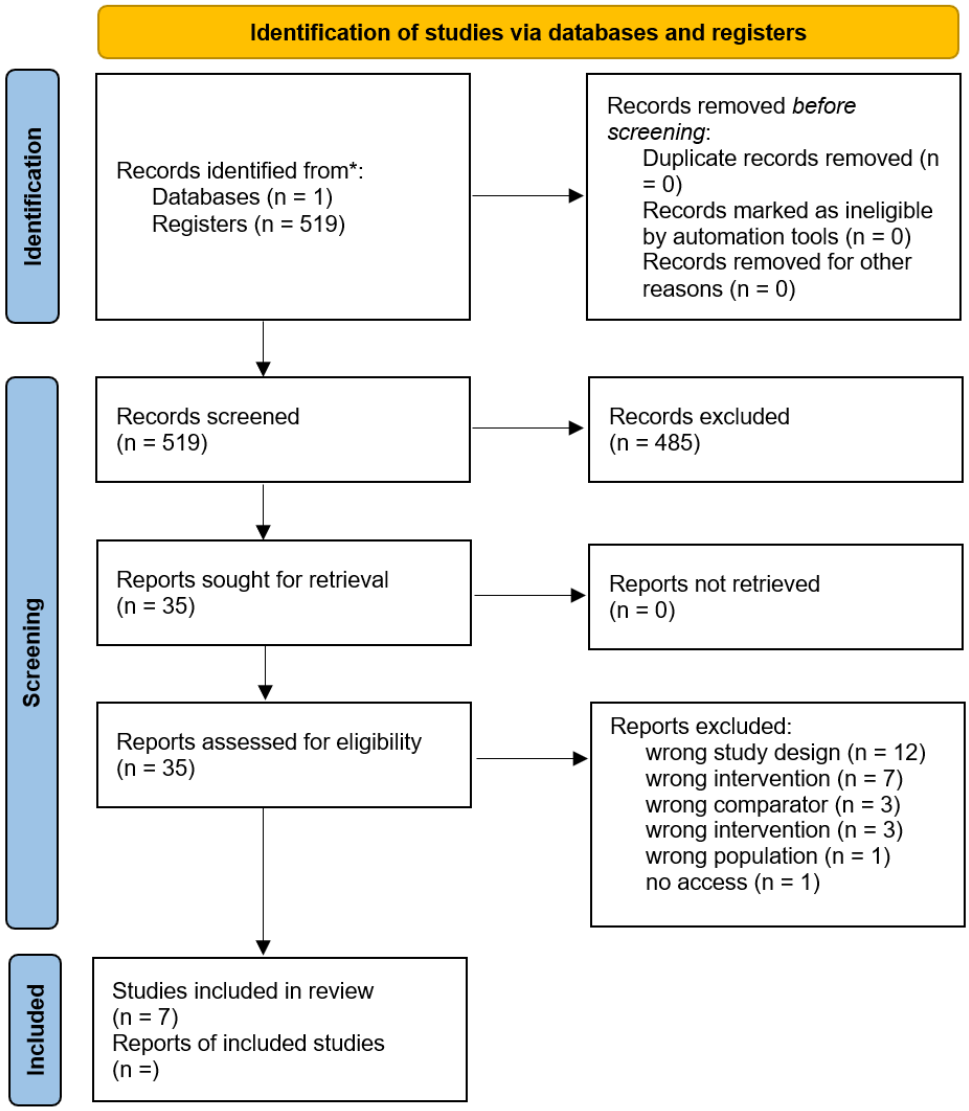

Figure 9: Flow Diagram (PRISMA, 2020)

In total 519 relevant articles were found using the search strategy mentioned in the methods in chapter 2. No duplicates were found. After the title- and abstract screening, 485 studies were excluded because they were not relevant to the research question of this systematic review. After this step, 34 studies were considered potentially eligible and forwarded to the full text review. Most of the studies (n=12) were excluded because of a wrong study design. Other reasons were wrong interventions (n=7) or wrong control groups (n=3). Only one study was excluded because of the wrong population. One other study had to be excluded from the systematic review because the full text was not accessible to the author and the supervisor.

Out of the 34 studies, 7 randomized controlled studies met the eligibility criteria and were included to the systematic review.

3.2 Study Characteristics

The main characteristics of the included studies are listed in table 2. All studies were conducted as randomized controlled trials. Five out of the seven studies focused on the impact of aerobic endurance interventions on the telomere length or telomerase activity (Mason et al., 2013; Puterman et al., 2018; Shin et al. 2008; Eigendorf et al., 2019; Friedenreich et al., 2018). One study included three intervention groups that observed the impact of aerobic endurance training, high intensity interval training and resistance training on leukocyte telomere length and telomerase activity (Werner et al., 2018). Another study only observed the impact of resistance training on leukocyte telomere length (Dimauro et al., 2016). No studies that investigated the effect of chronic exercise on telomere length in muscle cells were found.

Table 3: Study Characteristics

Authors	Study Design	Exercise Intervention	Duration Intervention	Cells Telomere Length	Method Telomere Length	Cells Telomerase Activity	Method Telomerase Activity
Mason et al. (2013)	RCT	AE	12 months	Leukocytes	qPCR (T/S)		
Puterman et al. (2018)	RCT	AE	24 weeks	Leukocytes	qPCR (T/S)	BMC	Droplet Digital PCR
Shin et al. (2008)	RCT	AE	6 months	Leukocytes	qPCR (T/S)		
Dimauro et al. (2016)	RCT	EMRT	12 weeks	Leukocytes	qPCR (T/S)		
Eigendorf et al. (2019)	RCT	AE	6 months	Leukocytes	qPCR (T/S)		
Friedenreich et al. (2018)	RCT	AE	12 months	Leukocytes	qPCR (T/S)		
Werner et al. (2018) *	RCT	AE, HIIT & RT	6 months	Leukocytes	qPCR (T/S)	BMC	TRAP

*Same study with different intervention groups; RCT: randomized controlled trial; AE: aerobicendurance; HIIT: high intensity interval training; EMRT: explosive type resistance training; RT: resistance training; qPCR: real-time quantitative PCR; BMC: blood mononuclear cells; droplet digital PCR; TRAP: telomeric repeat amplification protocol

The longest exercise intervention was executed over a period of 12 months whereas the shortest intervention was done over a period of 12 weeks. Most of the studies (n=3) investigated the impact of 6 months of exercise on the telomere length and/or telomerase activity. The characteristics of the interventions are described in chapter 3.3.

In all studies, telomere length was measured in leukocyte cells using the real-time quantitative polymerase chain reaction method (qPCR). Only two studies investigated the effect of exercise interventions on the telomerase activity. Both studies measured the telomerase activity in blood mononuclear cells using the telomeric repeat amplification protocol and droplet digital PCR (Puterman et al., 2018; Werner et al., 2018).

Table 4: Population Characteristics

Author	Participants	Age (±SD)(years)	BMI(±SD)(kg/m²)	Females (%)
Mason et al. (2013)	399	58.00 (5.00)	30.90 (4.00)	399 (100%)
Puterman et al. (2018)	68	61.30 (-)	28.15 (-)	55 (80.81%)
Shin et al. (2008)	16	46.81 (6.43)	28.55 (2.80)	16 (100%)
Dimauro et al. (2016)	20	72.00 (1.00)	24.00 (-)	10 (50%)
Eigendorf et al. (2019)	291	52.90 (4.50)	25.80 (4.50)	291 (100%)
Friedenreich et al. (2018)	212	-	29.00 (-)	212 (100%)
Werner et al. (2018)	124	49.05 (-)	24.33 (-)	79 (60.71%)

The mean of the age, body mass index and the ratio of female and male participants of the total population of each study are presented in table 3. The study with the highest number of subjects counted 399 participants, whereas the lowest number included 16 participants. In total, all studies together counted 1130 participants consisting of 94% female participants and 6% male participants. Four studies included only women as participants (100%), whereas the study with the lowest percentage of women shows 50 participants counted 50% female participants (Mason et al., 2013; Shin et al., 2008; Eigendorf et al., 2019; Friedenreich et al., 2018; Dimauro et al., 2016). The two remaining studies showed a higher percentage of women than men as participants with a percentage of 60,71% and 80,81% females (Werner et al., 2018; Puterman et al., 2018).

The average age of all studies together, excluding one of them because no mean age was given in the data, is 56.7 years. The highest mean age is 72 (± 1) years, whereas the youngest mean age is 46.8 (± 6.4) years (Dimauro et al., 2016; Shit et al., 2008). The study of Friedenreich et al. (2018) did not give any information about the mean age of the population. However, the age of the participants ranged from 50 to 74 years (Friedenreich et al., 2018).

The highest mean of the body mass index of the population in one of the studies is 30,9 (± 4,0) kg/m², the lowest values are 24 kg/m² (Mason et al., 2013; Dimauro et al., 2016).

3.3 Intervention Characteristics

Most of the studies (n=6) investigated the effect of aerobic endurance training on the telomere length and telomerase activity. The study of Werner et al. (2018) included aerobic endurance, high intensity interval training as well as resistance training as separate interventions. One study only focused on the effect of resistance training on the telomere length. None of the control groups of any of the studies received any type of intervention over the period of the study.

3.3.1 Endurance Training

The exact characteristics of the various endurance exercise interventions of each study are given in table 4.

Table 5: Endurance Exercise Intervention Characteristics

Author	Type	Intensity	Sessions/Week	Duration of 1 session (minutes)
Mason et al. (2013)	AE	70-85% HRmax	5	45
Puterman et al. (2018)	AE	40% HRR	3-5	40
Shin et al. (2008)	AE	60% VO_2R	3	45
Eigendorf et al. (2019)	AE	individual training HR*	≥ 3	≥ 20
Friedenreich et al. (2018)	AE	70-80% HRR	5	45
Werner et al. (2018)	AE	60% HRR	3	45
	HIIT	4x4: 80-90% HRR	3	45

*Based on initial exercise; MET: metabolic equivalent of task; HRmax: maximum heart rate; HRR: heart rate reserve; VO_2R: $VO_2\,\max - VO_2rest$

The intensities of the aerobic endurance exercise interventions were mainly given as a percentage of the heart rate reserve and varied from 40% to 80% HRR. One endurance intervention focused on anaerobic endurance using the 4x4 method with an intensity at 80-90% HRR. Other studies used the maximum heart rate and VO_2R as measures of the intensities.

Most of the endurance interventions were performed with a minimum frequency of 3 times a week. The maximum frequency was five times a week. Five out of the seven endurance interventions lasted for 45 minutes. The minimum duration of one endurance session was 20 minutes.

3.3.2 Resistance Training

Table 6: Resistance Training Intervention Characteristics

Author	Type	Intensity	Sets	Repetitions	Sessions/Week
Dimauro et al. (2016)	EMRT	70% of 1RM	3-4	10-12	2
Werner et al. (2018)	RT	20RM	2	16-20	3

EMRT: explosive-type resistance training; RT: resistance training; RM: repetition maximum

As already mentioned, two out of the seven studies investigated the impact of resistance training on the telomere length and telomerase activity. The exact characteristics of the interventions are shown in table 5. Dimauro et al. (2016) prescribed a higher intensity than Werner et al. (2018) using 70% of the one-repetition maximum of every individual. The test subjects were instructed to do three to four sets of 10-12 repetitions. The participants in the study of Werner et al. (2018) were instructed to do a circuit training of eight exercises. The circuit was performed twice with 16-20 repetitions. The intervention group in the study of Dimauro et al. (2016) trained with a frequency of two times a week, whereas the intervention group in the study of Werner et al. (2018) trained three times a week.

3.4 Risk of Bias

Table 7: Risk of Bias Assessment - PEDro (1999)

Items	Mason et al. (2013)	Puterman et al. (2018)	Shin et al. (2008)	Dimauro et al. (2016)	Eigendorf et al. (2019)	Friedenreich et al. (2018)	Werner et al. (2018)
Eligibility criteria	1	1	1	1	1	1	1
Randomized groups	1	1	1	1	1	1	1
Concealed allocation	0	0	0	0	0	1	0
Similar groups baseline	0	0	0	1	1	0	1
Blinding subjects	0	0	0	0	0	0	0
Blinding therapists	0	0	0	0	0	0	0
Blinding assessors	1	1	0	0	1	1	0
Outcomes 85% of all initial subjects	1	1	1	1	1	0	0
Intention to treat / all subjects received intervention	1	1	1	1	1	1	1
Between-group comparison	1	1	0	1	1	1	1
Point measure and measure of variability	1	1	1	0	1	1	0
Total Score	6	6	4	5	7	6	4

The risk of bias assessment with the scores for each item as well as the total score of the PEDro scale (1999) is given in table 6. The lowest score out of all studies was 4. The highest score was achieved by one study with a total of 7 out of 10 possible points. Most of the studies (n=3) achieved a score of 6 points. Criteria 5 (“there was blinding of all subjects”) and criteria 6 (“there was blinding of all therapists who administered the therapy) were absent in every single study of this review, whereas criteria 1 (“eligibility criteria were specified”) and criteria 2 (“subjects were randomly allocated to groups”) were present in every study.

3.5 Results of Individual Studies

3.5.1 Telomere Length

Table 8: Telomere Length & Changes in Telomere Length: Endurance Training - Intervention Group vs. Control Group

Author	Intervention Pre Mean (±SD)	Intervention Post Mean (±SD)	Change (±SD)	Change in %	Control Pre Mean (±SD)	Control Post Mean (±SD)	Change (±SD)	Change in %	p-value (for between group changes)
Mason et al. (2013)	1.027 (0.213)	1.025 (0.192)	-0.002(0.027)	-0.19%	1.042 (0.197)	1.015 (0.171)	-0.027 (0.029)	-2.59%	-
Puterman et al. (2018)	0.96 (0.11)	-	+0.03 (0.04) *	+3.13%	0.98 (0.16)	-	-0.002 (0.06)	-0.20%	-
Shin et al. (2008)	0.9962 (0.0159)	0.9959 (0.0146)	-0.0003 (0.0013)	-0.03%	1.0135 (0.1277)	0.9991 (0.0075)	-0.0144 (0.1202)	-1.42%	-
Eigendorf et al. (2019)	1.07 (0.30)	-	+0.0553(0.0256) *	+5.17%	1.10 (0.32)	-	+0.0209 (0.0267)	+1.90%	0.44
Friedenreich et al. (2018)	1.08 (1.17)	0.95 (1.02)	-0.13 (0.16)	-12.04%	1.10 (1.30)	1.00 (1.36)	-0.10 (0.18)	-9.09%	0.74
Werner et al. (2018): AE	0.47 (0.20)	0.65 (0.25)	+0.18 (0.06) *	+38.30%	0.46 (0.26)	0.48 (0.23)	+0.02 (0.06)	+4.35%	-
Werner et al. (2018): HIIT	0.44 (0.20)	0.62 (0.23)	+0.18 (0.06) *	+40.91%	0.46 (0.26)	0.48 (0.23)	+0.02 (0.06)	+4.35%	-

*Denotes significance within group changes (p<0,05)

Six studies investigated the impact of endurance training on the telomere length in leukocytes. The means of the telomere length in the intervention group and control group before and after the intervention, as well as the mean change of the telomere length are given in table 7.

After the endurance intervention, telomere lengthening was found in four of the intervention groups whereas telomere shortening was detected in three of the intervention groups. The biggest change was measured in Werner et al. (2018) in the high intensity intervention group (+40.9%) as well as in the aerobic endurance intervention group (+38.3%). The smallest change was observed in Shin et al. (2008) with a change in telomere length of -0.03% before and after the intervention. Significant changes in telomere length ($p<0.05$) before and after the intervention were found in all studies that observed telomere lengthening. No significant changes ($p>0.05$) were found in the intervention groups where telomere length shortened over time.

Regarding the control groups, four control groups showed telomere shortening at the second point of telomere length measurement (Mason et al., 2013; Puterman et al., 2018; Shin et al., 2008; Friedenreich et al., 2018). In two control groups telomere lengthening was found (Eigendorf et al., 2019; Werner et al., 2018). The biggest change in telomere length was observed in the study of Friedenreich et al. (2018) with a decrease by 9.1%, whereas the smallest change was observed in Puterman et al. (2018) with a decrease by 0.2%. However, none of the changes that were measured in the control groups were considered significant ($p>0.05$).

Additionally, the p-values for the changes in telomere length between the groups are presented in table 7. Only two studies mentioned the p-values for differences between groups where no significant differences could be found ($p>0.05$) (Eigendorf et al., 2019; Friedenreich et al., 2018).

Table 9: Telomere Length & Changes in Telomere Length: Resistance Training - Intervention Group vs. Control Group

Author	Intervention Pre Mean (±SD)	Intervention Post Mean (±SD)	Change (±SD)	Change in %	Control Pre Mean (±SD)	Control Post Mean (±SD)	Change (±SD)	Change in %
Dimauro et al. (2016)	4.70 (0.40)	5.80 (0.30) *	+1.10 (0.20)	+23.40%	4.90 (0.30)	3.90 (0.20) *	-1.00 (0.10)	-20.41%
Werner et al. (2018)	0.52 (0.27)	0.54 (0.27)	+0.02 (0.07)	+3.85%	0.46 (0.26)	0.48 (0.23)	+0.02 (0.06)	+4.35%

*Denotes significance between groups post intervention

Two out of the seven included studies investigated the impact of resistance training on the telomere length (Dimauro et al., 2016; Wenter et al., 2018). The results of the main outcomes of these studies are given in table 8.

After the resistance intervention both studies observed a positive change in telomere length. The biggest change was seen in the intervention group of Dimauro et al. (2016) with an increase by 23.4%. Although there was an increase, none of the studies observed significant changes in telomere length ($p>0.05$).

In the control group of Dimauro et al. (2016) a decrease in telomere length by 20.4% was measured whereas Werner et al. (2018) showed an increase by 4.4%. None of the changes of the control groups were significant.

In the study of Dimauro et al. (2016) the telomere length of the intervention group and the control group showed a significant difference ($p<0.05$) at the measurement after the intervention.

3.5.2 Telomerase Activity

Table 10: Telomerase Activity & Changes in Telomerase Activity: Intervention Group vs. Control Group

Author	Intervention	Intervention Pre Mean (±SD)	Intervention Post Mean (±SD)	Change (±SD)	Change in %	Control Pre Mean (±SD)	Control Post Mean (±SD)	Change (±SD)	Change in %
Puterman et al. (2018)	AE	4.43 (4.78)	-	-0.14 (0.97)	-3.16%	9.38 (9.01)	-	+0.12 (0.95)	+1.28%
Werner et al. (2018)	AE	281.63 (287.75)	691.84 (673.47)	+410.21 (143.63) *	+145.66%	236.73 (273.47)	257.14 (244.90)	+20.41 (62.05)	+8.62%
Werner et al. (2018)	HIIT	216.33 (200.00)	473.47 (422.45)	+257.14 (86.79) *	+118.86%	236.73 (273.47)	257.14 (244.90)	+20.41 (62.05)	+8.62%
Werner et al. (2018)	RT	277.55 (330.71)	355.10 (310.20)	+77.55 (77.76)	+27.94%	236.73 (273.47)	257.14 (244.90)	+20.41 (62.05)	+8.62%

*Denotes significance within group changes ($p<0.05$)

Two studies investigated the effect of exercise on the telomerase activity. Puterman et al. (2018) used aerobic endurance training as an intervention. Werner et al. (2018) studied the impact of aerobic endurance training, high intensity interval training and resistance training on the telomerase activity. The outcomes of these studies are presented in table 9.

After the intervention periods, the outcomes show that three out of the four intervention groups showed an increase in telomerase activity (Werner et al., 2018). Only one study showed a slight decrease by 3.2% (Puterman et al., 2018). The biggest change in telomerase activity was observed in the aerobic endurance intervention group in the study of Werner et al. (2018) with an increase by 145.7%. Moreover, the high intensity interval intervention group showed an increase by 118.9%. Significant changes ($p<0.05$) in telomerase activity were detected within the aerobic endurance intervention group and within the high intensity intervention group in the study of Werner et al. (2018).

Control groups in both studies show a slight, but not significant increase after the intervention period ($p>0.05$). Telomerase activity of the control group of Werner et al. (2018) increased by 20.4%, whereas telomerase activity increased by 0.12% in the control group of Puterman et al., 2018.

4 Discussion

In total seven studies were found that studied the effect of chronic physical exercise on the leukocyte telomere length or telomerase activity. Five studies only investigated the impact of aerobic endurance exercise whereas one study observed the effect of resistance training on the telomere length in leukocytes. One study included three intervention groups which were asked to perform aerobic exercise, high intensity training or resistance training. two out of the seven studies, which were included to this systematic review, studied the impact of exercise on the telomerase activity. In these studies, the effect of aerobic endurance training, high intensity training and resistance training were observed.

4.1 Telomere length

The results of the studies show significant telomere lengthening in four of the seven endurance exercise intervention groups. As already mentioned, two studies investigated the impact of resistance training. Whereas some of the endurance intervention groups were able to detect significant changes in telomere length, none of the resistance training interventions could provide any evidence for significant telomere lengthening or shortening.

4.1.1 Endurance Training

Some of the outcomes of the selected studies in this review suggest that endurance training could be an important aspect in the maintenance or elongation of telomeres in leukocytes. This hypothesis can be supported with the conclusion of the systematic review of Marques et al. (2020) in which they investigated the correlation between cardiorespiratory fitness and telomere length by reviewing 20 studies. Compared to this systematic review, they included cross-sectional, observational, comparative studies as well as controlled intervention trials. The conclusion of the review was that a higher cardiorespiratory fitness level is associated with longer telomeres among healthy older people but not in young participants (Marques et al., 2020). The relationship between a high cardiorespiratory fitness level was not only observed by Marques et al. (2020), but also by a study of LaRocca et al. (2010), where they found a positive association between regular aerobic exercise, maximal aerobic capacity (VO2max) and telomere length in older adults (LaRocca et al., 2010). Cardiorespiratory fitness can mainly be improved by aerobic endurance exercise. Therefore, it can be assumed that endurance training leads to a better cardiorespiratory fitness and is therefore associated with longer telomeres (Buckely & Doherty, 2008).

To show an example that structured endurance training potentially attenuates biological aging, Denham et al. (2015) compared the telomere length of endurance athletes with the telomere length of active controls. The endurance athletes were individuals who followed a

structured training with a minimum frequency of three times a week for at least one year. Although the control group included active individuals, they did not follow any structured endurance or resistance training. The results of the study show that endurance athletes had longer leukocyte telomeres than the controls. Moreover, the study investigated the impact of volume of exercise per week on telomere length by dividing the individuals into tertiles. The results show that participants of the middle and higher tertiles had significant longer telomeres than individuals of the lowest tertile. These outcomes suggest that the volume of exercise can have a significant influence on the maintenance or elongation of telomeres (Denham et al., 2015). Similar results were found in another study where telomeres of 67 ultra-marathon runners were compared to 63 healthy controls. All endurance runners had an average training distance of 40-100 km per week for at least two consecutive years and participated in two or more ultra-marathons. Endurance athletes showed significantly longer telomeres than age-matching controls (Denham et al, 2013). Borghini et al. (2015) also studied the effect of chronic endurance training on telomere length. They found that telomere length was better preserved in endurance athletes than in sedentary individuals of the control group. Moreover, another study observed that, besides ultra-endurance athletes showing longer telomeres than sedentary controls, it also seems that endurance exercise is more beneficial in older people than in younger individuals. Based on this information it can be assumed that aerobic endurance training has a positive influence on preserving telomere length especially in elderly people (Marques et al., 2020; Hernando et al. 2020). This conclusion is also in line with the study outcomes of Werner et al. (2009). They found that long term endurance training is related to reduced leukocyte telomere attrition compared to untrained controls (Werner et al. 2009).

In this review, one study found a significant positive effect of high intensity interval training on telomere length. This could be an indication that the intensity of endurance training can play an important role in maintaining telomere length (Werner et al., 2018). This outcome can be confirmed by the study of Simoes et al. (2017) in which the telomere length of 11 elite master sprinters was compared to the telomeres of 10 age matching inactive controls. The main characteristics of training protocols of top sprinters are high-intensity and low-volume protocols with anaerobic training intensities (Simoes et al., 2017). Therefore, the assumption that anaerobic endurance training can preserve telomere length can be made. However, the studies that investigate the impact of anaerobic exercise on telomere length are still limited (Sellami et al. 2021).

Taking the information from before into account, endurance training at an aerobic as well as an anerobic intensity can potentially result in preserving telomere length or even telomere elongation (Eigendorf et al., 2019; Puterman et al., 2018; Werner et al., 2018;

Simoes et al. 2017; Denham et al., 2016; Borghini et al., 2015). Indeed, there are several studies which indicate that different activity levels and intensities play an important role in maintaining telomere length. Friedenreich et al. (2018) and Sellami et al. (2021) report an inverted U-shape relationship between telomere length and chronic exercise. This assumption is supported by a cohort study that investigated the association between physical activity levels and leukocyte telomere length. Activity levels were assessed using a questionnaire and were divided into categories of low, middle and high levels of activity. The results show that low and high physical activity levels are associated with telomere shortening in leukocytes. Individuals of the group with moderate activity levels showed the longest telomeres (Savela et al., 2013). Another study detected similar results. They observed the relationship of different levels of weekly exercise energy expenditure with telomere length. They found that telomere length was longer in individuals with moderate physical activity levels compared to individuals with high and low physical activity levels (Ludlow et al., 2008).

Based on that information, it seems that shorter telomeres are seen in individuals with low and high activity levels, whereas subjects with moderate activity levels seem to have longer telomeres (Savela et al. 2013; Ludlow et al. 2008; Friedenreich et al., 2018; Sellami et al., 2021). According to Sellami et al. (2021) endurance exercise is beneficial for leukocyte telomere length. Although, they mention the possibility that these beneficial effects can decrease if the volume and intensity of training exceed a certain limit. This assumption is in line with the study of Collins et al. (2003). They observed that endurance athletes, who were diagnosed with “fatigued athlete myopathic syndrome”, showed significant shorter telomeres than the control group which included healthy, age-matching athletes with the same level of training. However, telomere length was measured in human skeletal cells of the vastus lateralis (Collins et al., 2003). Thus, it is not clear whether overtraining in athletes also leads to shorter telomeres in leukocytes. However, there are indications that acute long distance running also leads to telomere shortening in leukocytes which could be caused by oxidative damage. Although, it is important to mention that this was only observed in acute exposure to intense endurance exercise (Borghini et al., 2015). Since there are more studies that investigated the impact of acute bouts of exercise on telomere length, there may be a difference between the effects of acute exercise and chronic exercise on telomeres. In this context, Arsenis et al. (2016) suggest that measures of leukocyte telomeres should not be taken immediately after vigorous activity because “acute periods of exercise can lead to lower mean estimates of telomere length” (Arsenis et al., 2016). This is in line with the outcomes of Mastaloudis et al. (2001; 2004). The authors reported that acute endurance exercise leads to an increase of free radicals and oxidative stress.

Especially high intensity acute exercise seems to induce oxidative DNA damage after a few hours (Mastaloudis 2001; 2004; Borghini et al., 2015).

On the contrary, current literature still seems to be contradicting regarding the effects of acute bouts of exercise on telomere length. Another study reported an immediate increase in telomere length after acute aerobic exercises. Although is important to mention that telomere length was determined in T-Cells (Simpson et al., 2010; Arsenis et al. 2016). Therefore, further research is needed to determine the effects of acute exercise on telomere length in leukocytes and telomere-regulating molecules, such as telomerase activity. This research will be useful to establish whether acute exercise result in shortened telomeres or if telomeres are maintained by telomerase activating mechanisms caused by acute bouts of exercise (Arsenis et al., 2016).

On the other hand, there are many studies which provide evidence that chronic exercise and periods of intensive endurance training are associated with better preserved telomeres than their inactive controls (Werner et al., 2009; Borghini et al., 2015; Denham et al., 2013; Denham et al., 2015). Therefore, it seems that there are differences in the effects of acute bouts of exercise and chronic exercise on telomeres. It remains unclear how and to what extent repeated endurance exercise positively affects leukocyte telomere length (Borghini et al., 2015).

Moreover, the outcomes of this review show that three out of the seven endurance intervention groups did not show any significant changes in telomere length. They even detected a slight decrease in telomere length which is not in line with the assumption that endurance training could have a beneficial effect in maintaining telomere length. Even though the study by Shin et al. (2008) did not show any significant changes in telomere length, the authors observed that telomere attrition was greater in the control group than in the exercise group. Therefore, the authors suggest that endurance exercise may slow down the process of telomere attrition (Shin et al., 2008). Other authors of the studies in which telomere length shortened over time argue that the duration or dose of the exercise prescription was insufficient to detect any beneficial effects of exercise on telomere length (Mason et al., 2013; Friedenreich et al., 2018). However, this is in contradiction with the studies of Werner et al. (2018) and Eigendorf et al. (2019) in which they observed significant telomere elongation after an intervention that was carried out over a period of six months, whereas the studies of Friedenreich et al. (2018) and Mason et al. (2018) were performed over 12 months. As already mentioned before, a higher volume of endurance exercise per week is associated with longer telomeres (Denham et al, 2015). Comparing the duration and frequency of the studies that detected a slight decrease in telomere length over time with the studies that observed significant telomere elongation, they show the same or even

a higher frequency as well as duration of exercise per week (Shin et al., 2008, Friedenreich et al., 2018; Mason et al., 2018; Werner et al., 2018; Puterman et al., 2018; Eigendorf et al.,2019). In this context, it is interesting to take a look at the outcomes of Eigendorf et al. (2019). They observed significant telomere elongation in subjects with the lowest levels of cardiorespiratory fitness which suggests that a higher training intensity is needed in people with higher cardiorespiratory fitness levels. This in turn could be an indication that the chosen training intensity for the populations that did not show significant telomere lengthening was too low. Since the fitness levels of the populations of the studies were not assessed in this review, it remains unclear if the intensities of the implemented interventions were appropriate to their cardiorespiratory fitness levels. However, it seems that “frequent and vigorous intense training, exceeding activity levels of ≥300 min/week” (Eigendorf et al., 2019) leads to longer telomeres.

Another approach of explaining the different outcomes of the studies is the body mass index of the populations that were observed. The results of a systematic review that was based on cross-sectional studies suggest that the BMI and telomere length show an inverse correlation, meaning that a higher BMI is associated with shorter telomeres, which could be due to the fact that “obesity increases oxidative stress and chronic systemic inflammation” which results in telomere attrition (Shin, 2019; Müezzinler et al., 2014). Moreover, there are indications that obesity can reduce the positive effects of endurance training on telomere length (Dankel et al., 2016). If the populations of the studies that did not detect a significant change in telomere length are considered, it becomes clear that the populations fall into the BMI category of “pre-obesity” and “obesity class1” (WHO, 2010). This could be a possible explanation of why the exercise interventions were not sufficient enough to show a significant beneficial effect on telomere length. However, this assumption is again in contradiction with the study of Puterman et al. (2018), which was conducted on a population with a BMI of the "pre-obesity" category. In this study a 24-week aerobic endurance intervention led to a significant telomere elongation compared to the inactive control group (Puterman et al., 2018). Therefore, literature on the beneficial effects of physical exercise in a pre-obese or obese population still seems to be inconsistent. However, there is evidence that there is an inverse correlation between BMI and telomere length (Shin, 2019; Müezzinler et al., 2014).

Besides the association between telomere length and BMI, shorter telomeres are associated with high levels of perceived stress (Puterman et al. 2019). Friedenreich et al. (2018) mentions the fact that they did not assess the stress levels of their test subjects, which could be an explanation of why no significant changes in telomere length were found. The same applies to the studies of Shin et al. (2008) and Mason et al. (2013) where the

stress levels of their test subjects were not taken into account. On the other hand, the study of Puterman et al. (2018) was conducted on family caregivers who experienced high levels of chronic stress that were assessed using the Perceived Stress Scale. The results showed significant telomere lengthening and decreased levels of perceived stressed of the subjects after a 24-week aerobic endurance exercise intervention (Puterman et al. 2018). Another study observed that inactive individuals showed shorter telomeres than active individuals who experience chronic stress. Therefore, it seems that physical activity could potentially buffer the effect of telomere shortening or even increase telomere length in people who experience chronic stress (Puterman et al., 2010).

Looking at the control groups of the endurance intervention groups, no significant changes in telomere length were detected over time. If we compare these outcomes to the results of the intervention groups, which detected a significant change in telomere length, they support the hypothesis of a beneficial effect of endurance training on leukocyte telomere length. Since Werner et al. (2018) used the same control group to compare it with the aerobic endurance and high intensity interval intervention group, there are only six control groups compared to seven endurance intervention groups. Four out of the six control groups showed a non-significant telomere shortening over time which is in line with the current literature that telomere length decreases over time (Mason et al., 2013; Puterman et al., 2018; Shit et al., 2018; Friedenreich et al., 2018; Oeseburg et al., 2010; Hiam et al., 2020). However, there are two control groups that showed a slight but not significant increase in telomere length. A possible explanation for this outcome could be that telomere length is very dynamic feature. Therefore, telomere elongation as well as shortening can appear within a short period of time (Svenson et al., 2011).

4.1.2 Resistance Training

Compared to studies that investigate the effect of endurance training on telomere length, there are only few studies that study the impact of resistance training on telomeres (Sellami et al., 2021). In this systematic review, only two studies were found that focused on strength training as a potential aspect in preserving or elongating telomeres in leukocytes. Both studies did not find significant changes in telomere length which indicates that resistance training does not show a beneficial effect on maintaining telomere length. Although, the study of Dimauro et al. (2016) found a significant difference between the control and exercise group at the second point of telomere length measurement after the intervention period. Therefore, the authors assume that strength training could reduce telomere attrition in older people (Dimauro et al., 2016). On the contrary, Werner et al. (2018) conclude that telomere length is increased only by endurance training but not by strength training.

If searching for additional literature that investigates the impact of strength training on telomere length, there is one study that conducted a controlled intervention study on women who recovered from breast cancer. Resistance training was performed three times a week over a period of 16 weeks. The results of this study showed that strength training does not significantly influence telomere length in leukocytes in women who survived breast cancer (Hagstrom & Denham, 2018). These outcomes are again in line with the outcomes of this systematic review, although it is important to mention that the interventions in this review were only done on healthy subjects with no previous diseases. It appears that cancer causes accelerated biological aging and is associated with shorter telomeres (Wentzesen, et al. 2011). Thus, it is not clear whether the results of Hagstrom & Denham (2018) can be compared with the study outcomes of this review.

Another study looked at the impact of resistance training on the telomere length in human skeletal muscle cells of the vastus lateralis. They compared the telomere length of power lifters to the telomere length of healthy individuals with no history of strength training. No significant difference in telomere length was observed between power lifters and controls, although they observed a tendency of longer telomeres in power lifters (Kadi et al., 2008). However, telomere length in this study was measured in muscles cells which makes it difficult to compare the outcomes with telomere length in leukocytes. According to the study of Hiam et al. (2020) it seems that there is a correlation between leukocyte telomere length and human skeletal muscle telomere length. The outcomes show that longer telomeres in muscles cells are associated with longer telomeres in leukocytes. This might be an indication that changes in human skeletal muscle telomeres can result in changes of leukocyte telomeres. On the other hand, the authors discovered that telomere length in leukocytes decreases with increasing age, whereas telomere length in muscles cells remains relatively constant across the lifespan. This indicates that telomere length is variable among different tissues and makes it difficult to compare telomeres of different cells (Hiam et al., 2020).

In another study of Brandao et al. (2020), the impact of an intervention consisting of aerobic and strength exercises over a period of eight weeks in obese women was evaluated. The results revealed that telomeres were significantly longer after the intervention period. Since this study implemented a combined intervention of strength training and aerobic endurance training it is difficult to assess, whether strength training only can contribute to telomere elongation or not (Brandao et al., 2020).

Based on this information, more intervention studies on the effect of resistance training on leukocyte telomere length in a healthy population are needed. So far, it seems that there is either no effect of strength training on telomere elongation or it can slow down the process

of telomere attrition. Although, the combination of endurance training and strength training could have an impact on elongating telomeres (Werner et al., 2018; Dimauro et al., 2016; Kadi et al., 2008; Hagstrom & Denham, 2018; Brandao et al., 2020).

4.1.3 Age

In general, it is already well established that telomere length shortens with increasing age. However, there seem to be variations in telomere attrition across different life stages. Great differences in telomere length are seen in the first few years after birth, whereas relatively stable values in telomere length are observed in the years of childhood until adolescence. After the age of 20 years telomere length starts to decrease gradually (Oeseburg et al., 2010; Hiam et al., 2020). Therefore, it can also be assumed that age can influence the impact of physical activity on telomere length.

Some studies mention that a positive effect of exercise on leukocyte telomere length is mainly seen in the elderly population, but less in young individuals (Marques et al., 2020; Hernando et al., 2020; LaRocca et al., 20210). Taking these findings into consideration, the question arises how physical exercise can slow down the process of telomere attrition across the lifespan.

Hernando et al. (2020) compared telomere length of ultra-endurance runners with controls and found that telomers in elderly athletes (>40 years) were better preserved compared with their controls, whereas no significant difference was found in younger endurance athletes (<40 years) compared to their age matching control group. Another study where the test subjects were divided into younger (18-32 years), and older (55-72 years) groups found similar results. The outcomes show that leukocyte telomere length was longer in active older individuals compared to their inactive peers. No difference in telomere length was found in the active and inactive groups of younger individuals (LaRocca et al., 2010). The outcomes of these studies suggest that the effect of exercise on telomere length is mainly seen in the older population but not in the younger population. LaRocca et al. (2010) assume that this could be due to the fact that telomere length in sedentary healthy young adults is still intact and cannot be positively influenced.

The studies of this systematic review were done on healthy people with a mean age ranging from 49.1 to 72.0 years. According to the information before, the age categories of the included studies are expected to benefit from the endurance exercise interventions. However, only four interventions groups were able to find a significant telomere lengthening (Puterman et al., 2018; Eigendorf et al., 2019; Werner et al., 2018).

4.1.4 Sex

There is some literature indicating that telomere length seems to vary among gender. In general, there is evidence that women have longer telomeres than men (Oeseburg et al., 2010; Zhu et al., 2011). In a recent review it is mentioned that sex could also have an impact on the association between the physical activity level and telomere length. They found that positive effects of high levels of physical activity on telomere length was especially found in adolescent females (Güneşliol et al., 2021). Therefore, it might be the case that physical activity has a more beneficial effect on leukocyte telomere length in women than in men. This is in contradiction with the outcomes of the study of LaRocca et al. (2010) in which they analyzed leukocyte telomeres of older people (55-72 years) with regards to the sexes of the test subjects and found that telomere length was not influenced by gender.

Looking at the distribution of men and women in the population of studies in this systematic review, it is striking that the percentage of women is higher than of men. Four of the included studies only chose women as test subjects, whereas the study with the least percentage of women as participants was still 50%. Only one of the interventions that were exclusively performed on women showed a significant effect of endurance exercise on telomere length (Mason et al. 2013; Shin et al., 2008; Eigendorf et al., 2019; Friedenreich et al., 2018). The studies that included both female and male individuals in their populations did not consider the influence of gender in the presentation of their results (Puterman et al., 2018; Dimauro et al., 2016; Werner et al., 2018). Comparing the outcomes of the studies that were only done on female individuals with the results of the studies that included both sexes, it is inconclusive whether gender has an impact on the beneficial effect of exercise on telomere length or not. However, there are several studies that were only conducted on a male population and found a correlation between higher activity levels and longer telomeres (Borghini et al., 2015; Denham et al., 2013). Thus, it appears that exercise can protect telomeres from attrition in women as well as in men.

4.1.5 Measurement methods

In general, telomere length can be measured in any cells, although leukocyte telomere length “accurately reflects the overall senescence state of an individual” (Haupt et al., 2022). There are several ways of determining telomere length. While some protocols measure the length of a single telomere, other methods assess the average telomere length. The results can either be expressed as relative or absolute numbers. The most common method to for the determination of telomere length in leukocytes is the quantitative polymerase chain reaction (qPCR). This is an easy way of quantifying the ratio of telomere length to a single copy gene (T/S). This ratio is defined by a “relative measure, perfectly valid within a given

population (as it will correctly rank subjects) but more difficult to compare between populations" (Oeseburg et al., 2009). Therefore, the results of this method show great variations between different laboratories and make comparisons between different protocols difficult. Terminal restrictions fragment analysis or protocols following the quantitative fluorescence in-situ hybridization (Q-FISH) might be more suitable for assessing telomere length in a better standardized way to make the results comparable between different laboratories (Haupt et al., 2022).

Regarding the outcomes of the telomere length of the included studies, it is outstanding that the telomere length in the study of Werner et al. (2018) in all intervention groups and the controls are relatively short compared to the measurements of the other studies. On the contrary, Dimauro et al. (2016) showed relatively high values of telomere length compared to the other studies. Thus, it is important to analyze the reasons for the differences in telomere length at baseline between the different studies of this review.

Looking at the method of determining telomere length, all of the studies in this systematic review used qPCR to measure telomere length and all of them presented their data as the T/S ratio. Considering the information before, qPCR shows high variability between different laboratories. Therefore, this could be a possible reason for the different telomere length outcomes among the studies of this systematic review.

Lastly, it is also important to mention that "telomere length in leukocytes is highly variable among individuals" (Oeseburg, 2009). According to one study, "telomere length can be a highly dynamic feature" (Svenson et al., 2011). They observed that telomere elongation and shortening can appear within months and can be influenced by lifestyle, environmental and genetic factors throughout life. Therefore, it seems that telomere attrition is not a constant progress and can vary between as well as within individuals. (Svenson et al., 2011; Balan et al., 2018).

As already discussed before, age, BMI, gender, and stress can be important factors influencing telomere length (Oeseburg et al., 2010; Hiam et al., 2020; Shin, 2019; Müezzinler et al., 2014; Puterman et al. 2019). With regards to the age, the population of Werner et al. (2018) shows a mean age of 49.05 years, whereas the mean age of the test subjects Dimauro et al. (2016) is 72 years. These observations are not in line with the previous mentioned studies which show that telomere length decreases with increasing age (Oeseburg et al., 2010; Hiam et al., 2020). Therefore, there must be another reason for the different outcomes in telomere length. If the BMI of the various studies are compared, it is obvious that the population of Werner et al. (2018) and Dimauro et al. (2016) are both categorized as "normal weight", which means that it is unlikely that the body mass index is

the reason for the big differences in telomere length. Since high stress levels are associated with shorter telomeres, it would be interesting to look at the perceived levels of stress of the populations of the studies (Puterman et al., 2019). However, none of the studies, except Puterman et al. (2018), assessed this variable. Thus, it is not possible to make any assumptions whether different stress levels could be a possible cause for the difference in telomere length between the studies.

4.2 Telomerase Activity

While there are already several intervention studies investigating telomere length, less studies were found that studied the effect of exercise interventions on telomerase activity. In total two studies were found that measured telomerase activity before and after a structured exercise intervention. In the end, four intervention groups were included. The outcomes show that two out of the four interventions showed a significant increase in telomerase activity whereas two intervention groups could not detect significant changes in telomerase activity. The intervention groups with a significant increase in telomerase activity were asked to do aerobic endurance training and high intensity training. The groups where no significant changes were observed were asked to perform aerobic endurance training and resistance training (Werner et al., 2018; Puterman et al., 2018). Based on the outcomes of the selected studies of this systematic review the results are still inconclusive, whether physical activity can increase telomerase activity or not. Although, it seems like endurance training could have a beneficial effect. Since there was an increase of telomerase activity in the aerobic endurance group as well as in the high intensity interval training group in one of the studies, it could be that the intensity of endurance exercise does not play a crucial role in telomerase activity. Compared to the intervention groups, no significant changes in telomerase activity were observed in any of the control groups.

In general, the main components of the telomerase complex are telomerase reverse transcriptase (TERT), a telomerase RNA component (TERC) and dyskerin which is a protein that provides stability for the telomerase enzyme (Oeseburg et al., 2010). Telomerase activity plays an important role in regulating telomere length and it appears that high levels of telomerase activity contribute to telomere maintenance or even telomere lengthening, which also results in a delayed cellular senescence (Haupt et al., 2021; Ludlow et al., 2008). Moreover, current literature suggests that cardiorespiratory exercise is associated with a higher activation of telomerase (Marques et al., 2020; Sellami et al.; 2021). These findings are in line with a cross-sectional study of Werner et al. (2009) where they compared telomerase activity of endurance athletes with telomerase activity of inactive controls. They observed that long-term endurance training is related to higher levels of telomerase activity and decreased rates of telomere attrition (Werner et al., 2009). Similar

outcomes were found in another study of Saki et al. (2016), which was conducted on patients with myocardial infarction. They included 20 male participants, who were randomly divided into an exercise group and control group. The intervention was performed over a period of eight weeks and included a combination of resistance training and aerobic training. The results show that telomerase activity as well as telomere length increased significantly in the intervention group compared to the control group. Therefore, it seems that a combined exercise intervention of aerobic and resistance training has a beneficial effect on telomere biology in patients with myocardial infarction (Saki et al., 2016). However, it is important to mention that the studies included in this review only include healthy subjects, whereas the study of Saki et al. (2016) was done on patients. In this context, it is important to discuss possible differences in telomere biology between a healthy subjects and patients who suffered a myocardial infarction. Brouilette et al. (2003) investigated the difference in telomere length between patients with a previous myocardial infarction to healthy individuals. They found that patients with a history of cardiac problems had shorter telomeres than controls. Even though they only observed telomere length and not telomerase activity, the outcomes suggests that telomere biology can potentially be affected in people with myocardial infarctions. Thus, it is not clear whether the findings of the study of Saki et al. (2016) can also be applied to a healthy population. Moreover, the intervention of their study was a combined intervention which makes it impossible to make a statement about the effect of different types of exercise on telomerase activity (Saki et al., 2016). Taking these outcomes into account, the assumption that regular exercise could potentially be effective in increasing telomerase activity. Based on the findings of Werner et al. (2018), it seems that aerobic endurance exercise as well as high intensity interval training play an important role in activating telomerase (Werner et al., 2018).

However, these outcomes are in contradiction with the study of Puterman et al. (2018). They observed telomere lengthening after a 24-aerobic exercise intervention but no significant increase in telomerase activity. These outcomes are supported by the findings of another study. The authors found that moderate levels of activity are associated with longer telomeres but not with greater telomerase activity (Ludlow et al., 2008). Therefore, the authors assume that telomerase activity is not the only factor that is responsible for the regulation of telomere length (Puterman et al., 2018). This assumption can be supported by a study in which the authors assessed the correlation between telomere length and telomerase activity. The outcomes show no significant relationship between those two parameters (Iwama et al., 1998).

In addition, it seems that there are differences in telomerase activity between acute bouts of exercise and long-term exercise. While the included studies of this review investigated

the impact of chronic exercise on telomerase activity, there are some studies that studied the effect of acute exercise on the levels of telomerase activity. For example, Chilton et al. (2014) investigated the impact of a single acute bout of exercise on telomerase activity by assessing TERT, which is an enzyme that can be used as a marker for telomerase activity and can counteract telomere shortening (Oeseburg et al., 2010). They measured TERT levels in white blood cells of 22 healthy male subjects (24.1 years) before, immediately after and 60 minutes after running 30 minutes on a treadmill at an intensity of 80% of their VO_2max. The results showed that TERT levels increased immediately and 60 minutes after exercise (Chilton et al., 2014). Similar results were found in a study of Cluckey et al. (2017), in which they found that TERT increased in young individuals (22 years) after 30 minutes of cycling following a high intensity interval protocol. These findings can be supported by the outcomes of another study by Zietzer et al. (2017). They also investigated the impact of a single treadmill running session on telomerase activity in 26 young healthy individuals (23.37 years) and found a significant increase 10 minutes after the exercise session (Zietzer et al., 2017). These outcomes suggest that acute exercise as well as chronic exercise cause an increase in telomerase activity (Chilton et al. 2014; Cluckey et al., 2017; Zietzer et al., 2017).

Considering the outcomes of chronic exercises in the study of Puterman et al. (2018) together with the findings of acute exercise, it seems that an increase in telomerase activity after acute bouts of exercise is transient (Balan et al., 2018). However, this contradiction with the results of Werner et al. (2018), in which they found an increase in telomerase activity after an acute endurance exercise session as well as after a long-term endurance intervention. The long-term endurance intervention and the results are presented previously in chapter two (table 3, 4 & 8) and discussed in chapter four. The impact of one single acute exercise session on telomere length included one endurance exercise group and one resistance training group. The endurance session included 45 minutes of continuous running, while resistance training included exercises that were performed on eight strength devices. The results show increased levels in telomerase after the endurance session but not after the resistance training (Werner et al., 2018) The effects of long-term exercise were also investigated in a study of Melk et al. (2014). 59 healthy middle-aged male test subjects (53 years) were asked to perform 210 minutes of aerobic endurance training each week for six months. Telomerase activity was measured before the intervention, three months after and six months after the intervention. The outcomes showed that telomerase activity levels increased gradually over the intervention period. Moreover, telomerase activity levels were significantly higher after six months of exercise intervention than at the first point of measurement (Melk et al., 2014).

Summarizing the outcomes of the studies that investigated the impact of acute as well as chronic exercise on telomerase activity, it appears that acute exercise seems to elevate the levels of telomerase activity immediately after and a few hours after the exercise session (Chilton et al. 2014; Cluckey et al., 2017; Zietzer et al., 2017; Werner et al., 2018). The results regarding the effect of chronic endurance on telomerase activity in this systematic review are still contradicting (Werner et al., 2018; Puterman et al., 2018). However, it seems that endurance training but not resistance training interventions can potentially result in a higher telomerase activity. This was observed in both, acute exercise, and chronic exercise interventions (Werner et al., 2018; Melk et al., 2014). The hypothesis that endurance training is superior to resistance training regarding telomere biology can also be supported by the studies that showed telomere lengthening after endurance exercise interventions but not after strength training interventions. However, more intervention studies are needed to assess the effects of long-term exercise on telomerase activity.

4.2.1 Age

According to the study of Iwama et al. (1998) age seems to have an impact on the telomerase activity. They assessed telomerase activity in 124 healthy individuals ranging from four to 95 years. In their outcomes they observed a decline in telomerase with aging. Taking a closer look at the results it was shown that there was a progressive decrease in telomerase activity in individuals aged 4-39 years. The decrease in telomerase activity seems to reach a plateau in individuals at the age of 40 years. Moreover, test subjects who were older than 40 years showed stable but low telomerase activity compared to individuals who were younger than 40 years (Iwama et al., 1998).

Moreover, another study investigated whether there was a difference in the response of telomerase activity to acute exercises between young and older individuals. The test subjects were 11 young (22 years) and 8 older (60 years) men and women who performed 30 minutes of high intensity interval cycling. Telomerase levels were measured by telomerase reverse transcriptase (TERT) before exercise, 30 minutes, 60 minutes and 90 minutes after exercise. The results showed that young individuals had greater increases in telomerase activity than the older group (Cluckey et al., 2017).

Looking at the mean age of the populations of the two studies in this review that investigated the impact of different types of exercise on telomerase activity, it is obvious that the population with a mean age of 61.30 years in the study of Puterman et al. (2018) was older than the population in the study of Werner et al. (2019) with a mean age of 49.05 years. If the outcomes of the previously discussed studies are considered, it could be a possible explanation that age had a significant influence on the different outcomes of these two

studies (Iwama et al., 1998; Cluckey et al., 2017). Whereas no significant change in telomerase activity was detected in the older population in Puterman et al. (2018) was detected, significant increases were measured in the middle-aged population of Werner et al. (2018) who participated in the aerobic exercise and high intensity interval intervention. However, no significant changes were observed in the resistance intervention group in the study of Werner et al. (2018). This could support the hypothesis that aerobic and anaerobic endurance training, can increase the levels of telomerase activity in a younger population but not in older individuals (Cluckey et al., 2017).

4.2.2 Sex

Next to the age as in important factor in influencing telomerase activity, it seems that sex could also have an impact on telomerase activity. The study of Cluckey et al. (2017) measured hTERT (human telomerase reverse transcriptase) levels before and after acute exercise. Men showed a significantly higher response in hTERT to acute exercise endurance than women, independently of age. This could indicate that there are sex differences in regulating telomerase activity through exercise. However, the authors of the study discuss that women, in general, show higher levels of telomerase than men. Therefore, they assume that high-intensity exercise does not induce a further increase in telomerase activity in women. As already mentioned, women also show longer telomeres compared to men. Based on this information, Cluckey et al. (2017) suggest that telomeres in women are better protected from damage, while men need higher levels of hTERT “to compensate for short telomeres that are exposed to oxidative damage” (Cluckey et al., 2017; Oeseburg et al., 2010; Zhu et al., 2011).

Looking at the populations of Puterman et al. (2018) and Werner et al. (2018), both studies included women as well as men as their test subjects. However, none of them investigated sex differences in telomerase activities. Thus, based on the outcomes of the included studies, it is difficult to make a statement about differences in responses to exercise between sex. Moreover, Cluckey et al. (2017) were the first ones to investigate sex differences in telomerase activity after an exercise intervention. Therefore, more studies are needed on that topic.

4.2.3 Measurement methods

In general, quantifying telomerase activity is more challenging than measuring telomere length. This is due to the fact that the cells in which telomerase activity can be assessed are limited. Most somatic cells show no measurable or very low levels of telomerase. However, human blood lymphocytes, next to stem cells, cancer cells and progenitor cells, show higher and measurable levels of telomerase activity and can therefore serve as a

suitable cell to quantify telomerase activity. Moreover, in order to measure telomerase activity, it is necessary to isolate peripheral blood mononuclear cells out of freshly drawn blood samples, which is a complex process and requires specific tools. Therefore, studies do not investigate telomerase activity as often as telomere length. Looking at the methods that are used for determining telomerase activity, the “telomere repeat amplification protocol” (TRAP) is a common tool. However, similar to the measurement of telomer length, it is not possible to compare telomerase activity that are measured in different laboratories (Haupt et al. 2022; Oesenburg et al., 2010).

As already mentioned, the main components of an active telomerase are TERT and TERC. Some studies that were discusses previously used TERT as a marker of telomerase activity (Clucky et al., 2017; Chilton et al., 2014), whereas the included intervention studies in this review measured telomerase activity directly. Therefore, it is not clear, whether the outcomes of studies that measured TERT can be compared and linked to the outcomes of the studies of Werner et al. (2018) and Puterman et al. (2018).

The studies in this systematic review both investigated the impact of chronic exercise on telomerase activity in blood mononuclear cells. However, the measurements were done by using different methods. Werner et al. (2018) used the Telomerase Repeat Amplification Protocol, whereas Puterman et al. (2018) used Droplet Digital PCR (ddPCR). According to Shelton et al. (2013), ddPCR is a method that is more sensitive that TRAP radiography and “extends throughput, sensitivity, and the range of biological samples that can be analyzed for telomerase activity” (Shelton et al. 2013). These different methods of quantifying telomerase activity could be a possible explanation for the great difference of the values in telomerase activity between the two studies.

5 Conclusion

5.1 Telomere Length

The outcomes of the included studies of this systematic review show contradicting results regarding the impact of different exercise types on leukocyte telomere length. However, it seems that endurance training, but not resistance training can act as a potential aspect in maintaining or even elongating telomeres. If these outcomes are interpreted in context with other literature, the hypothesis that endurance training can preserve telomere length is supported. High levels of cardiorespiratory fitness levels and high levels of physical activity are associated with longer telomeres in leukocytes (LaRocca et al., 2010; Marques et al., 2020). Although, it is important to mention that some studies observed an inverted U-shape regarding the volume and intensity of the physical activity levels. The current literature suggests that moderate intensities of endurance training are associated with longer telomeres in leukocytes. On the other hand, one study of this review detected telomere elongation after a high intensity interval intervention. However, literature on the effect of anaerobic exercise on telomere length is still very little (Sellami et al. 2021).

Moreover, it seems that age plays in important role regarding the response of telomeres to endurance training. There is evidence that the beneficial effect of endurance training is mainly seen in elderly people, but not among young subjects (Marques et al., 2020; Hernando et al., 2020; LaRocca et al., 20210).

Lastly, it is important to mention that there are several factors that contribute to the regulation of telomere length. Physical activity is only one of many factors that may influence telomere length. Other aspects, such as sex, perceived stress levels and body mass index, are related to telomere length and should be considered when interpreting the outcomes of the studies (Oeseburg et al., 2010; Svenson et al., 2011; Balan et al., 2018; Dankel et al., 2016). These different factors affecting telomeres make it impossible to fully isolate the impact of exercise interventions on telomere length.

To summarize the outcomes of exercise on telomere length of this systematic review together with current literature, it seems that moderate endurance training potentially results in telomere maintenance or lengthening, especially in older people. Additionally, high intensity training seems to serve as a potentially beneficial factor in maintaining or elongating telomeres in leukocytes. However, in order to get a clearer picture on the effect of endurance training on telomere length, more controlled intervention studies over a longer period of time are necessary.

5.2 Telomerase Activity

The outcomes of the included studies that investigated the impact of chronic exercise intervention on telomerase activity show contradicting results. However, if the studies are interpreted in context with other studies and literature on that topic, it seems that, similar to telomere length, endurance training could cause in an increase in telomerase activity in blood mononuclear cells. This was observed in aerobic and high intensity interval training intervention. On the contrary, resistance training did not show a beneficial effect on telomerase activity (Werner et al., 2018).

Moreover, it can be assumed that there is a difference in the effects of acute exercise and chronic exercise on telomerase activity. While there are already many studies investigating the effects of acute bouts of exercise, there are still very little studies that study the effect of chronic endurance training. Acute bouts of endurance exercise seem to elevate telomerase activity immediately and a few hours after the exercise session. However, it is not clear, whether those acute effects remain for a longer period of time or only last for a few hours. In contrast to acute endurance training, acute resistance training does not show increased telomerase activity after the exercise session (Chilton et al. 2014; Cluckey et al., 2017; Zietzer et al., 2017; Werner et al., 2018).

Additionally, age as well as gender can influence levels of telomerase activity. It appears that telomerase activity can mainly be increased in younger individuals and less in an older population. Looking at gender differences, it seems that telomerase activity is higher in men than in women (Cluckey et al., 2017). However, the included studies did not differentiate telomerase activity between men and women. Therefore, it is not possible to confirm this hypothesis with the results of this systematic review.

Summarizing the outcomes of this systematic review together with other literature, chronic anaerobic endurance training and high intensity interval training, but not chronic resistance training could potentially increase telomerase activity in the long term. However, the results of the studies are still inconclusive. Since there are only few studies investigating the effect of chronic exercise, more controlled interventional studies over a longer period of time are needed in order to create a more accurate hypothesis on the impact of chronic physical activity on telomerase activity.

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