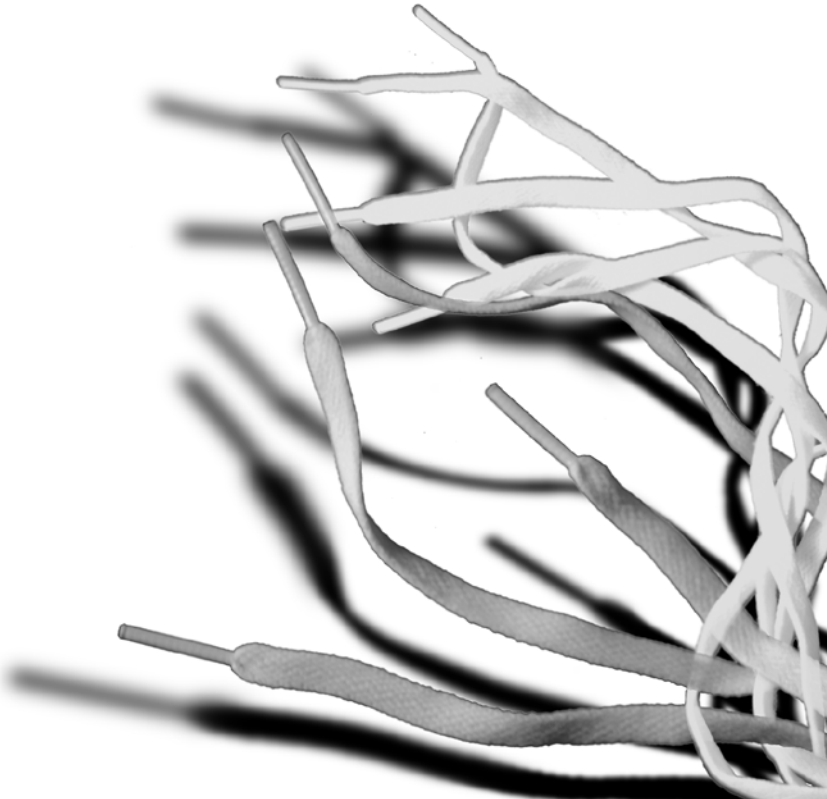
Chronic oxidative stress and telomere shortening

Chapter 7

Summary and general discussion



The major objective of the studies presented in this thesis was to examine the relationship between chronic oxidative stress and telomere shortening and the possible use of telomere length (TL) as a biological marker of aging, disease progression, and mortality.

To investigate this, we focussed in **chapter 2 and 3** on the general population, whereas in **chapter 4 and 5** the differences between healthy individuals and patients with chronic obstructive pulmonary disease (COPD) were studied. In **chapter 6** we evaluated the effects of interventions with fisetin and n-acetylcysteine on telomere shortening and markers of oxidative stress in a diabetic rat model.

This general discussion will integrate the most important findings of this thesis and potential areas for future research will also be discussed.

Main findings

TL is influenced by several factors and besides the end-replication problem, oxidative stress appears to play an important role. High levels of oxidative stress increase the rate of shortening of the ends of the chromosomes and a good antioxidant status and antioxidant defence are hypothesized to decrease the rate of telomere shortening [1-3]. The most common sources of oxidative stress and inflammation are unhealthy nutrition, little exercise, obesity, chronic emotional stress and chronic diseases, such as atherosclerosis, inflammatory bowel disease, and diabetes, in which telomeres were hypothesized and confirmed to be shorter in patients suffering from such chronic inflammatory diseases [4-8]. Previous research showed that the incidences of several aging-related chronic diseases were lower in Southern than Northern Europe, indicating a potential role for a higher antioxidant status and lower levels of oxidative stress in people living around the Mediterranean Sea [9-12]. For measurement of TL in DNA we applied a quantitative PCR technique. This technique is high-throughput, time-saving and requires only small amounts of DNA. We studied leukocyte TL (LTL) in elderly men from two different regions in Europe, from Zutphen (the Netherlands) and Crete (Greece). These groups were previously identified to differ with respect to lifestyle, and more specifically, they consume a different diet. We found that Greek elderly men had significantly longer LTL compared to their Dutch counterparts. The endogenous antioxidants albumin and uric acid were positively associated with longer

telomeres. Dietary antioxidants including carotenoïds and tocopherols were not observed to be associated with LTL.

The next step was to measure LTL in the general elderly male population over a period of time. This would give us information on the individual rate of telomere shortening and the potential use of LTL as a marker for all-cause and disease specific mortality. We found that LTL declined with a mean of 40.2 bp per year. We observed a good correlation between LTL in 1993 and 2000 for 75 men, but we could not confirm previous research showing that LTL is a marker for mortality. LTL was neither associated with the risk of cardiovascular disease mortality nor with cancer mortality. After studying the general population, we performed two different COPD - control studies. COPD is characterized by incompletely reversible airflow obstruction. Chronic systemic oxidative stress and inflammation play a major role in the pathophysiology of COPD [13-16]. In the first study we observed shorter LTL in COPD patients compared to healthy controls and also found a positive association between LTL and blood SOD activity and in COPD patients a modest positive association between LTL and fat mass. In the second study we additionally measured the cytokines IL-8, IL-6 and TNF-α as well as CRP, SOD and plasma homocysteine. Coffee, tea and alcohol consumption were also assessed in relation to inflammation and TL in lymphocytes. We found that COPD patients had shorter telomeres than their healthy counterparts, after adjusting for age. The best predictors of TL in the total study population (healthy controls as well as COPD patients included) were the presence of COPD, coffee consumption and IL-8. This study gives a first indication for the potential beneficial effects of coffee consumption and for negative effects of alcohol consumption on TL.

Finally we investigated the effects of dietary interventions on TL in a diabetic rat model. Rats were injected with streptozotocin to induce diabetes, a disease that is also accompanied by chronic oxidative stress [17,18]. The effect of fisetin, a flavonoid, and n-acetylcysteine, an antioxidant, administered via the food, was investigated. Several markers of oxidative stress were measured, TL was determined and gene expression of relevant genes was measured by quantitative PCR. We observed significantly shorter telomeres in the diabetic rat hearts, and both fisetin and NAC seemed to partially counteract this accelerated telomere shortening. The diabetic status increased the expression of antioxidant, DNA repair and inflammation related genes in heart and kidneys.

Moreover, dietary intervention with fisetin and NAC reduced/attenuated these responses.

Discussion and future directions

Several aspects of the potential application of TL as a marker for disease progression and mortality and the potential protective effects of antioxidants, either taken as supplements or consumed with the diet, since these are present mostly in fruits and vegetables, require further discussion.

Telomere length: marker for mortality?

Cross-sectional studies have indicated that TL might be a relevant biomarker of disease progression and survival. However, this needs to be confirmed by longitudinal studies. To address these questions, we measured LTL in a prospective study (**chapter 3**) in male subjects from the Zutphen Elderly Study. TL was measured in 203 men in 1993 and 75 surviving subjects in 2000. We found that LTL declined with a mean rate of 40.2 bp per year. We could not confirm previous research showing that TL is a marker for mortality. LTL was neither associated with the risk of cardiovascular disease mortality nor with cancer mortality in our study population. Likewise, three studies with similar study populations, e.g. elderly and the oldest old and similar follow-up periods, also found no association between LTL and mortality (bisschoff, martin-ruiz and nordfjall). A possible explanation for these negative findings might be that there is a higher degree of TL instability in the oldest old compared to younger populations, as we discussed in **chapter 3**, which makes elderly men over 70 years not representative of the younger population. Furthermore, as humans reach advanced age, a survival effect may modify associations with LTL [19]. Recent research confirms that people older than 86 years of age exhibit longer telomeres than younger individuals, suggesting that the rate of telomere attrition may be an important determinant of overall survival in the general population. The relatively older group may be affected by survival bias that selected out individuals with aging-related disease or those who would have died before reaching the age of 85 years, leaving the survivors with relatively longer telomeres [20]. Investigation into cellular mechanisms involved in telomere attrition across age groups might ultimately unravel the underlying mechanisms causing this difference.

Protection of telomeres by antioxidants?

In **chapter 2** we compared LTL in 109 Greek elderly men and in 143 Dutch elderly men. We aimed to compare LTL between these two European regions and to investigate the possible relationship between antioxidant status/oxidative stress levels and LTL within these two European regions that were previously found to have marked differences in oxidative stress status [9-12].

Greek elderly men had significantly longer LTL compared to their Dutch counterparts. In Greek men a positive association was found between LTL and plasma zeaxanthin and albumin, whereas in Dutch men a positive relationship was found with uric acid. When both groups were combined, albumin and uric acid levels remained positively correlated with LTL. Oxidative stress parameters could not be linked to LTL, except for hydroperoxides which were positively associated with LTL in Zutphen. High levels of the enzyme gamma-glutamyl transferase (GGT) are associated with an increased turnover of glutathione, indicating an increased antioxidant status [21]. However, when we determined the ratio between hydroperoxides and GGT we found that the increase in hydroperoxides was more pronounced than the increase in GGT in the men from Zutphen, indicating higher levels of oxidative stress. Although this was not expected, it is known that oxidant species can act as signalling molecules promoting cell survival [22]. If the concentration of hydroperoxides is high, oxidation processes may lead to irreversible damage, followed by cell death. However, hydroperoxides are also capable of inhibition of phosphatases that negatively regulate cell survival pathways [23], promoting cell survival. The data of our study indicate that endogenous antioxidants play an important role in protecting telomeres from shortening. In Greek men, also zeaxanthin was positively associated with LTL, indicating that dietary antioxidants additionally contribute to telomere stability. Several studies have reported positive associations between telomere length and the use of nutritional supplements such as vitamin C, E and folic acid [24-27]. Recently, omega-3 fatty acids have also been found to be inversely associated with the rate of telomere shortening over time [28].

Supplementation with omega-3 fatty acids has been associated with lower levels of F2-isoprostanes, an established standard for measurement of systemic oxidative stress, and with higher levels of the antioxidant enzymes catalase and superoxide dismutase [29]. Furthermore, daily supplementation

with omega-3 fish oil, was associated with a significant increase in telomerase activity in normal adult human leukocytes [7].

To answer the question whether these supplements are also capable of reducing the mortality risk, prospective intervention studies with these supplements are required.

TL as a marker of disease progression in COPD: what about nutrition?

In **chapter 4** we investigated LTL in COPD patients in relation to pulmonary function and disease severity, i.e. cachexia. Furthermore, based on experimental evidence suggesting the effects of oxidative stress on telomere shortening, we studied the association of LTL with the antioxidant enzyme superoxide dismutase (SOD). 102 COPD patients with moderate to severe COPD were studied and compared with 19 healthy age-matched controls. Patients were characterized by elevated levels of inflammatory markers (CRP, sTNF-receptors) and lower SOD-activity than healthy controls, irrespective of SOD genotype. LTL was negatively associated with age and was significantly shorter in COPD patients than in controls. Within the patient group age-adjusted LTL variability could not be explained by lung function and smoking history, but a modest association was found with the percentage of fat mass. These data provide evidence for a relationship between a disturbed oxidant/antioxidant balance and telomere shortening and indicate that preservation of fat mass may be protective in delaying telomere shortening in COPD patients.

In **chapter 5** we investigated TL in COPD patients and healthy (ex-)smoking controls in relation to oxidative stress and inflammation. The second aim of this study was to investigate the potential protective effects of caffeine, coffee and tea intake on TL in COPD patients and healthy (ex) smokers. For this purpose, we measured TL in PBMCs of 89 COPD patients and compared these with 93 healthy controls. In addition, the cytokines IL-8, IL-6 and TNFα were determined as well as CRP, SOD and plasma homocysteine. Coffee consumption, tea consumption, caffeine intake and alcohol consumption were assessed by a questionnaire.

We found that COPD patients had shorter TL than healthy controls, even after controlling for age. This study gives a first indication for the potential beneficial effects of coffee consumption and for negative effects of alcohol consumption on TL. Coffee consumption has been associated with reduced risks

for type 2 diabetes [30,31] and the occurrence of diabetic complications, and it was proposed that coffee contains compounds with antioxidant and anti-inflammatory activity [32-34]. Recently, Cassidy et al. showed that a diet high in cereal fiber was positively associated with LTL. They also concluded that a diet high in plant-based foods may favourably influence TL via anti-inflammatory and antioxidant mechanisms [35]. Other studies also indicated that a high intake of plant-based diets and whole grains is inversely associated with risk factors for chronic disease [36,37]. Interestingly, Ornish et al. showed that a healthy lifestyle (lifestyle modifications included a lowfat, plant-based diet high in fruits, vegetables, unrefined grains, legumes, and low in refined carbohydrates; moderate exercise and stress management) was associated with an increase in telomerase activity in PBMCs and that a decrease in LDL cholesterol was associated with an increase in telomerase activity [7]. In addition, statins, which exhibit anti-inflammatory effects, were also found to be capable of decreasing the cardiovascular risk and inhibit the rate of telomere shortening in vitro [38,39]. This indicates that a reduction of inflammation may be associated with a decrease the rate of telomere shortening. In our study, we also found that cytokine plasma levels as well as CRP and homocysteine plasma levels were increased in COPD patients and SOD activity was decreased, indicating elevated inflammation and oxidative stress in COPD patients. Future studies should elucidate whether interventions with dietary antioxidants or anti-inflammatory compounds, for instance with bioactive substances found in coffee, are able to reduce inflammation and oxidative stress, and reduce disease progression in patients with chronic inflammatory diseases.

It has to be taken into account that the studies described in **chapter 4 and 5** differ in several aspects. First of all, in **chapter 4**, we measured TL in blood leukocytes whereas in **chapter 5**, we determined TL in PBMCs. Measurement of TL in leukocytes has been reported to be less accurate than in PBMCs, because PBMCs showed a greater age-related decline in TL [40,41] and subpopulations of lymphocytes displayed differences in TL and in telomerase activity [42]. However, a very recent study demonstrated that there is synchrony among leukocyte subsets throughout the human lifespan. It was found that individuals with long telomeres in one subset also have long telomeres in other leukocyte subsets. Therefore it was concluded that LTL is a useful index of TL in other subsets of blood cells [43].

Secondly, we did not find a relationship between fat mass and TL in **chapter 5**, whereas we did in **chapter 4**. Importantly to note here is that we studied quite different groups of COPD patients in both studies. In **chapter 4**, we studied TL in severely ill COPD patients with many of them having cachectic symptoms. In chapter 5, the COPD group studied consisted of a more heterogeneous sample of the COPD population, with only few patients showing cachectic symptoms. Previous studies investigating the association between fat mass/obesity and COPD also found contradictory results. On the one hand it has been hypothesized that fat mass has a negative effect on the prognosis of COPD, but on the other hand it has also been speculated that fat mass may be protective [44-47]. Adipose tissue was found to produce over 50 adipokines, including TNF-α and IL-6, causing an enhanced inflammatory state [48], which is associated with the development of cardiovascular diseases and diabetes [49]. On the other hand, it has to be taken into account that body fat can be subdivided in visceral fat and subcutaneous fat. Unlike visceral fat, subcutaneous fat may confer survival advantages in patients subject to catabolic events such as infectious complications. It may therefore be hypothesized that subcutaneous fat provides greater energy stores, which protects against the risk of complications [50].

In both **chapters 4 and 5** we did not find a relationship between smoking and TL, although this was expected since smoking is a major source of ROS. Previous studies also showed contradictory results. In some studies smoking was associated with shorter telomeres [51], but not in all [52], which may be explained that these studies may have been under powered to detect an association (n<1000 participants). In studies with larger numbers of participants a small significant correlation has been observed between TL and smoking, and appears therefore likely that this relationship is only detectable in studies with sufficiently large sample sizes [53].

Can dietary interventions with flavonoids or antioxidants attenuate the rate of telomere shortening?

In **chapter 6** the effects of the flavonoid fisetin and the antioxidant n-acetylcysteine on telomere shortening in a diabetic rat model was described. Diabetes mellitus and hyperglycaemia cause severe damage to cells and tissues, especially the arteries and vital organs such as the kidneys and the heart. The

disease is also marked by low grade inflammation and an increased production of reactive oxygen species leading to oxidative stress [17,18].

Since diabetes is associated with chronic oxidative stress and accelerated aging, dietary interventions with antioxidants and/or anti-inflammatory agents were therefore hypothesized to decrease the levels of oxidative stress and slow the rate of aging. We also hypothesized that chronic oxidative stress and systemic inflammation in diabetes lead to an increased rate of telomere shortening and investigated the effects of dietary interventions with the antioxidant n-acetylcysteine (NAC) and the anti-inflammatory flavonoid fisetin, on oxidative stress, inflammation and the rate of telomere shortening. The effects of 24-week dietary interventions with fisetin and NAC on oxidative stress, inflammation and telomere shortening were evaluated in streptozotocin-induced diabetic Wistar rats. We observed significantly shorter telomeres in the diabetic rat hearts, and both fisetin and NAC seemed to partially counteract this accelerated telomere shortening in the heart. The diabetic status increased the expression of antioxidant, DNA repair and inflammation related genes in heart and kidneys. Moreover, dietary intervention with fisetin and NAC attenuated these responses. Interestingly, in addition to its antioxidant and PARP-1 inhibiting activity, fisetin has also been identified as a sirtuin activator [54]. Sirtuins are deacetylases that are dependent on NAD+ for their activity. Calorie restriction, which increases lifespan and is beneficial in age-related disorders, was capable of activating sirtuins [55]. The nuclear sirtuin SIRT1 has been found to promote longevity mammalian cells in several ways [55]. SIRT1 was reported to down-regulate p53 activity, thereby increasing lifespan, cell survival, and neuroprotection [55]. This implies that not only the antioxidant and PARP-inhibiting properties of fisetin may have an effect on TL and disease progression, but also its sirtuin activating property warrants further investigation.

Future studies should focus on evaluation of the effects of prolonged interventions with higher doses or measures that increase the biological availability of the administered compounds. Trials longer than 24 weeks in diabetic rats are probably not achievable, and not ethically approvable, since the health of these animals deteriorates significantly during 24 weeks. Although in human studies TL is mainly studied in white blood cells (WBC), animal studies usually only focus on target organs. To our knowledge we are the first to have measured TL in the WBC of rats, but we did not observe any effects on TL. A

possible explanation may be that the effects of the diabetic status are more pronounced in target tissues such as heart and kidney as compared to WBC, which are for the greater part directly derived from the hematopoietic stem cells. Since these hematopoietic cells have been shown to possess telomerase activity, TL in WBC may be less sensitive for the effects of hyperglycemia [42]. Previous research also suggests a tissue-specific regulation of telomeres during aging in the rat [56]. Cherif et al. showed that the percentage of short telomeres increased with age in the kidney, liver, pancreas and lung, but not in the brain. The liver, kidney, pancreas and lung are expanding cell populations while the brain is a more static cell population [56]. Previous human as well as animal studies, showed that increased levels of oxidative stress, as in hypertension and hemodynamic stress, induced accelerated telomere shortening in arterial tissue, the aorta and in the myocardium [57-61]. In leukocyte DNA as well as in monocyte DNA, accelerated telomere shortening was found in type 2 diabetes patients [8,62]. Few studies have investigated telomere shortening in patients with type 1 diabetes and these results are not conclusive. Jeanclos et al. showed that TL in patients with diabetes type 1 was significantly shorter than TL in healthy control subjects [63]. In a study by Fyhrquist et al. no difference was found in TL between healthy controls and patients with diabetes type 1, but patients with progressive nephropathy had the shortest telomeres [64]. On the contrary, in a study by Astrup et al., no difference was found in TL between diabetes type 1 patients with or without diabetic nephropathy [65]. Although we confirmed in our study the increased (tissue-specific) rate of telomere shortening in diabetes type 1 in a rat model, our knowledge on telomere shortening in patients with type 1diabetes is still limited.

Does lifestyle matter?

As discussed in **chapter 2**, Cretan men were approximately 5 years younger, in terms of LTL, when compared to their Dutch peers. Additionally in **chapter 5** we reported that coffee consumption was associated with increased LTL, whereas alcohol consumption was associated with shorter telomeres. These data provide evidence for the hypothesis that lifestyle and dietary factors do influence TL and possibly also life expectancy. Besides nutritional factors [35] that are involved in the rate of telomere shortening, other environmental and lifestyle factors seem to play a pivotal role in telomere biology. Shortened

telomeres have been associated with psychological stress [66], low physical activity levels [67], BMI [68,69], smoking [68,69] and socioeconomic status [51]. In addition to lifestyle, genetic background also plays a role in telomere maintenance as will be discussed in the last section of this discussion.

Telomerase in (accelerated) aging and gene-environment interactions

Although we did not measure telomerase activity in our studies, it may also play a role in healthy ageing as well as in accelerated aging.

It has been shown that stress and senescence can influence telomerase levels in lymphocytes [70,71]. Changes in telomerase activity in cell types such as lymphocytes, endothelial cells and tissue stem/progenitor cells could influence processes relevant for healthy ageing. Importantly, even lifestyle factors known to promote cancer and cardiovascular disease could affect telomerase function. Recently, it has been shown in a longitudinal study that improvements in nutrition and lifestyle were associated with increases in telomerase activity which were associated with decreases in low-density lipoprotein cholesterol and psychological distress [7]. Thus, comprehensive lifestyle changes could significantly increase telomerase activity, stabilise telomeres and decrease oxidative stress within tissues and organs [72]. In addition to telomerase activity, it is likely that many of the proteins that are important in regulating TL and function, such as the telomere repeat binding factors, and proteins involved in DNA repair, will also have an important role in healthy/accelerated aging since they can regulate the action of telomerase at telomeres [73].

Furthermore, variations in the two major genes associated with telomerase activity, hTERT and hTERC, have been described. Atzmon et al. identified a common hTERT haplotype that is associated with both exceptional longevity and longer telomeres. They concluded that variations in the telomerase gene are associated with a better maintenance of the telomeres which may be associated with healthier aging [20]. On the other hand, there have also been several mutations (hTERT, hTERC and in dyskerin gene 1) described that cause telomerase deficiencies, which results in failure to elongate or maintain telomeres and induce progressive shortening [40].

Not only telomerase activity, but also TL appears to be partly genetically determined [74,75]. In a study by Nordfjäll et al., TL was investigated in PBMCs

of 132 individuals from 49 unrelated families using quantitative PCR. A statistically significant association between TL comparing father-son (P = 0.011, n = 20) and father-daughter (P = 0.005, n = 22) pairs was found while this was not found in mother-offspring pairs. In this study a relationship between TL and genetics was demonstrated, and evidence for a father-to-offspring heritage was obtained [75]. More evidence for genetic factors influencing TL was obtained from a study by Okuda et al. In this study, TL was determined by Southern blot analysis in white blood cells from newborns. TL was not different between male and female newborns, but there was a high variability among donors. This indicates that variations among adults may be in large part attributed to genetic determinants that probably start exerting their effect in utero [76]. In a twin-study by Slagboom et al., TL was determined by Southern blot analysis in 123 twin pairs, both dizygous and monozygous twins of 2-95 years of age. Statistical analysis in 115 pairs, 2-63 years of age, indicated a 78% heritability for mean TL in this age cohort. Telomeric restriction fragment length variation among unrelated individuals was higher than variation within twin pairs, and the variation within monozygous twins was observed to be least [77]. In conclusion, TL appears to be a highly heritable trait.

Since there is quite some variation in TL among individuals of the same age, polymorphisms in genes associated with telomere maintenance, oxidative stress and DNA repair were hypothesized to be involved in TL regulation. In a study by Broberg et al., polymorphisms in genes involved in the metabolism of genotoxic carcinogens and DNA repair were determined in the DNA of patients with bladder cancer and in the DNA of control subjects. In this study no association was found between cancer risk, TL, smoking and susceptibility genotypes [78]. In a recent study by Starr et al., it was found that some polymorphisms related to oxidative stress are also linked to increased telomere shortening [79]. The related genes in which polymorphisms were found included: 2 mitochondrial genes, heme oxygenase 2, vimentin (stress response), ceruloplasmin (involved in the catalyzation of Fe2+ to Fe3+), methionine sulfoxide reductase A, lipoprotein carriage gene and nitric oxide synthase 3 [79]. It can be concluded that the clinical manifestation of inflammation/oxidative stress related diseases depends on several factors, among which inherited TL and telomerase activity, exposure of specific tissues to pathogens (i.e. oxidative damage), and tissue-specific genetic alterations that result in an increase in cellular turnover.

Conclusions

Based on the results presented in this thesis, it can be concluded that telomeres shorten faster under conditions of chronic inflammation and oxidative stress. Furthermore, we showed that endogenous antioxidants, exogenous antioxidants as well as foods containing antioxidants are associated with reduced telomere shortening. This was found in elderly male populations, in COPD patients and in diabetic rats. We conclude that lifestyle factors do not only contribute to disease risk, but also affect TL. To confirm our results and to investigate the potential beneficial effects of antioxidants and anti-inflammatory compounds, either administered as supplements, or consumed with the diet, on the rate of telomere shortening, future studies should focus on interventions with these compounds. Preferably these studies should be prospective, and genetic determinants should also be taken into account. Since beneficial effects may be expected specifically in patients with chronic oxidative stress, such as COPD and diabetes patients, these are considered relevant target populations. If future studies can confirm that TL is causally involved in the development and progression of these age-associated diseases, it will pave the way for new therapeutic or preventive strategies.

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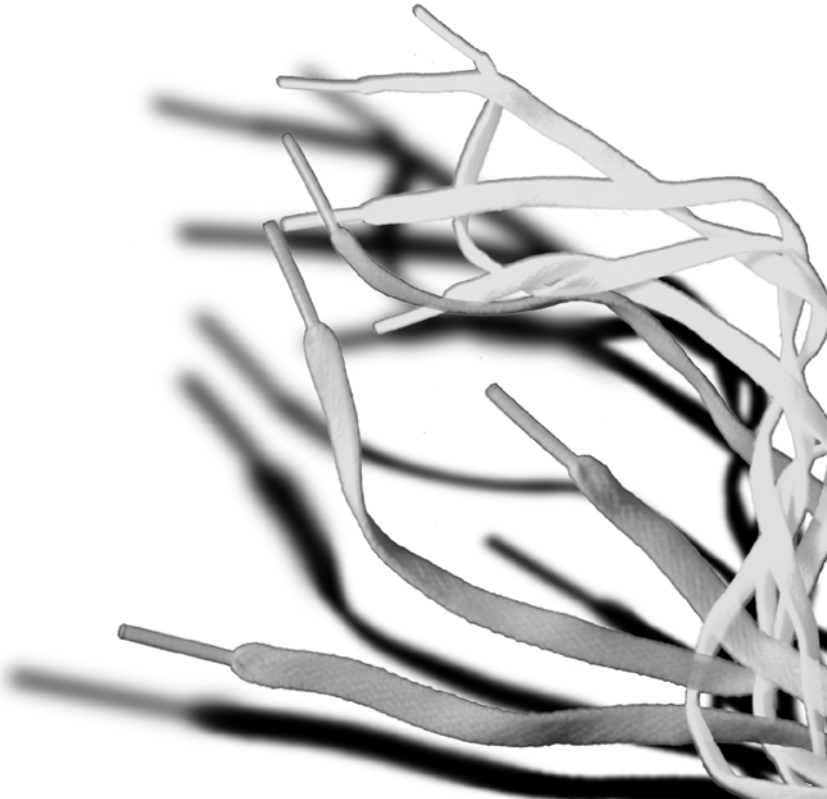
Nederlandse samenvatting



Dit proefschrift beschrijft de relatie tussen chronisch oxidatieve stress en telomeerverkorting, en de mogelijke effecten van voedingsinterventies op deze relatie. Telomeren zijn de uiteinden van onze chromosomen en ze verkorten met elke celdeling. De hypothese die in dit proefschrift is onderzocht luidt: onder condities van chronische oxidatieve stress vindt er een versnelde telomeerverkorting plaats wat leidt tot versnelde veroudering en ouderdomsgerelateerde aandoeningen.

Om dit te onderzoeken, hebben we ons in **hoofdstuk 2 en 3** gericht op de algemene (oudere) populatie. Vervolgens hebben we in **hoofdstuk 4 en 5** de verschillen tussen gezonde mensen en mensen met chronisch obstructief longlijden (COPD) bestudeerd. In **hoofdstuk 6** hebben we gekeken naar de effecten van voedingsinterventies met fisetine en n-acteylcysteine op telomeerverkorting en markers voor oxidatieve stress in een diermodel (diabete ratten).

Belangrijkste bevindingen

De telomeerlengte (TL) wordt door verschillende factoren beïnvloed. Naast het zogenaamde “end-replication problem” lijkt oxidatieve stress/schade ook een belangrijke rol te spelen. Hoge concentraties van deze oxidatieve stress, oftewel DNA beschadigende deeltjes, zorgen voor een versnelde verkorting van de uiteinden van de chromosomen. Daarom wordt er ook wel gedacht dat een goede antioxidant status en antioxidant verdediging in het lichaam deze versnelde verkorting tegen kan gaan. Aangezien oxidatieve stress betrokken is bij een heleboel chronische ziekten, zoals hart- en vaatziekten, diabetes, chronische darmziekten en COPD, werd er gespeculeerd en bevestigd dat de telomeren korter zijn bij patiënten die aan deze ziektes lijden. Uit voorgaand onderzoek is gebleken dat de incidentie van verscheidene ouderdomsgerelateerde aandoeningen lager is in het zuiden van Europa dan in het noorden. Dit impliceert een potentiële rol voor een hogere antioxidant status en lagere niveaus van oxidatieve stress in mensen die leven rond het Middellandse Zee gebied. Om de TL te kunnen bepalen in het DNA, maakten we gebruik van de zogenaamde kwantitatieve PCR techniek. Deze techniek is niet alleen snel, maar er kunnen ook veel monsters tegelijk gemeten worden en er zijn slechts kleine hoeveelheden DNA nodig. Allereerst bestudeerden we in **hoofdstuk 2** de TL in leukocyten (LTL) in oudere mannen uit 2 verschillende regio’s in Europa: Zutphen (Nederland) en Kreta (Griekenland). Van deze 2

groepen is bekend dat ze een verschillende leefstijl en voeding hebben. In deze studie vonden wij dat Griekse oudere mannen duidelijk langere telomeren hadden dan hun Nederlands leeftijdsgenoten. Verder vonden we ook dat de endogene antioxidanten, albumine en urinezuur, positief geassocieerd waren met LTL.

De volgende stap was om LTL te meten in de algemene oudere mannelijke populatie over de tijd (**hoofdstuk 3**). Dit zou ons informatie kunnen verschaffen over de individuele snelheid waarmee de telomeren verkorten, en de mogelijkheid om LTL te gebruiken als een marker voor (ziekte specifieke) mortaliteit. Uit de resultaten van deze studie bleek, dat LTL afnam met 40.2 bp per jaar en dat LTL niet was geassocieerd met cardiovasculaire mortaliteit en ook niet met mortaliteit door kanker.

In **hoofdstuk 4 en 5** hebben we TL in 2 COPD-controle studies onderzocht. COPD wordt gekenmerkt door obstructie van de luchtwegen en is een verzamelnaam voor chronische bronchitis en longemfyseem. Chronische systemische oxidatieve stress en ontstekingsreacties spelen hierin een grote rol. In **hoofdstuk 4** zagen we dat COPD patiënten kortere telomeren hadden dan de gezonde controles en we vonden een positieve associatie tussen LTL en SOD activiteit (een antioxidant die in het lichaam voorkomt) en vetmassa. In **hoofdstuk 5** hebben we een aantal extra bepalingen gedaan, waaronder de cytokines IL-8, IL-6 en TNF-α, als ook CRP, SOD en plasma homocysteine. Verder hebben we gekeken naar koffie, thee en alcoholconsumptie in relatie tot inflammatie en TL. We zagen dat er een negatieve associatie bestond tussen TL en COPD en we toonden aan dat koffie consumptie mogelijk positief geassocieerd was met TL, terwijl alcohol consumptie negatief geassocieerd was met TL. In het **laatste hoofdstuk** hebben we de effecten van voedingsinterventies op TL bestudeerd in een diabetisch ratten model. De ratten werden geïnjecteerd met streptozotocine, waardoor diabetes werd geïnduceerd, een aandoening die gekenmerkt wordt door chronisch oxidatieve stress. De effecten van fisetine, een flavonoid, en n-acetylcysteine (NAC), een antioxidant, werden onderzocht. Verschillende markers van oxidatieve stress werden gemeten, als ook TL en de genexpressie van relevante genen. In het hart van de diabete ratten vonden we significant kortere telomeren in vergelijking met de gezonde controle ratten. Zowel fisetine als NAC leken deze verkorting gedeeltelijk tegen te gaan. In de diabete ratten was de expressie van antioxidant genen, DNA herstel genen en inflammatie gerelateerde genen

verhoogd. Verder zagen we dat de interventie met fisetine en NAC deze responsen verminderden.

Samenvattend kan geconcludeerd worden dat, gebaseerd op de resultaten in dit proefschrift, de telomeren sneller verkorten onder omstandigheden van chronisch oxidatieve stress. Verder toonden we aan dat enzymatische antioxidanten, endogene antioxidanten als ook voeding die antioxidanten bevat, positief geassocieerd zijn met TL. Dit vonden we in gezonde mensen, COPD patiënten en in diabete ratten. Leefstijl lijkt dus niet alleen een effect te hebben op de gezondheid, maar ook op de telomeren. Om deze resultaten te bevestigen en om de mogelijk positieve effecten van antioxidanten, zowel in supplementen als in fruit en groenten, op TL verder te onderzoeken, zouden toekomstige studies zich moeten richten op interventies met anti-inflammatoire stoffen in patiënten met een chronische ziekte zoals COPD of diabetes. Daarnaast is het van belang dat er meer prospectieve studies worden uitgevoerd en dat er rekening gehouden wordt met de genetische determinanten die ook een rol spelen bij TL.