Universiteit Hasselt
Agoralaan – gebouw D
B 3590 Diepenbeek

GENETIC VARIATION AND ENVIRONMENTAL FACTORS IN BIOLOGICAL AND ARTERIAL AGEING

by

T. S. NAWROT and J. A. STAESSEN

Laureates of the Prize Dr. Luc Broeckaerrt and Miss Annie Depreeuw, period 2008

1. Introduction

Ageing is a complex physiological phenomenon, which is associated with cardiovascular morbidity and mortality. The question why some subjects grow old why remaining free from cardiovascular disease, whereas others prematurely die remains largely unanswered. The future of epidemiology should be directed towards the slowing of the ageing process at the population level. (1) We focused on the role of genetic variation and environmental factors in biological and arterial ageing.

2. Prognostic significance of pulse pressure

Pulse pressure is an established index of arterial stiffness. It reflects the age-related deterioration of the elastic properties of the large arteries (2, 3). To clarify the prognostic significance of cardiovascular ageing and to place the findings of this dissertation in perspective, we investigated to what extent pulse pressure

Keywords: *ageing, telomere length, oxidative stress, selenium, arterial ageing.*

predicts cardiovascular risk in the general population. In a Belgian population study, the mean follow-up time was 7 years. During follow-up 55 subjects developed myocardial infarction. Blood pressure fluctuates with time. Analyses based on a single baseline reading will tend to underestimate the effect of blood pressure on subsequent health outcomes (4). One way to overcome this regression dilution bias is to base analyses on participants' "usual" or long term average blood pressure. In our study, the baseline blood pressure was the average of 10 readings measured at two home visits one to three weeks apart. Our results demonstrates that pulse pressure in middle aged and older subjects significantly improved the prognostic accuracy of the well-established Framingham risk score (5). Indeed, a pulse pressure of 70 mmHg or more was associated with an approximately 5-fold greater risk of future cardiovascular events, irrespective of whether the classical Framingham risk score was low or high. A sensitivity analysis showed that if we excluded the cases of pulmonary emboli, the results did not change.

Our interpretation of the above finding was that arterial stiffness, as reflected by pulse pressure, is an index of arterial as well as biological ageing. This interpretation is supported by recent work of Benetos and co-workers (6). They noticed in 193 French subjects with mean age of 56 years an inverse association between pulse pressure and telomeric chromosome length, which is a better indicator of biological ageing than chronological age (figure 1).

3. Telomere length in the process of arterial ageing

Telomeres are the extreme ends of chromosomal DNA, made up of a large number of tandem repeats of the sequence TTAGGG. Their presence allows complete replication of chromosomal DNA. DNA polymerases use the information in one DNA strand to synthesize another, but need an RNA primer at the 5' end of the DNA strand being copied to start off the new strand. Without telomeres, there would be

a loss of genetic information each time that the DNA replicates, since the most distal RNA primer is removed from the lagging strand and the information in the DNA to which it was bound will not be coded in the new DNA strand. In 1973, Olovnikov (7) was the first to suggest that telomere length therefore provides a marker of biological age, at least at the cellular level, with shorter telomeres indicating more advanced ageing. More recently several publications suggested that clinical markers of biological ageing, such as pulse pressure[6] and longevity (8), were associated with telomere length. In an age stratified sample (n=143), subjects whose telomere length was below the median had a poorer survival (figure 4.1.), attributable in part to a three-fold higher mortality rate from heart disease (95% CI: 1.36-7.45, p=0.0079) and an eight-fold higher mortality rate from infectious disease (1.52-47.9, p=0.015).[8] These were the first epidemiological results, which supported that telomere shortening in human beings predicts death from chronic age-related diseases.

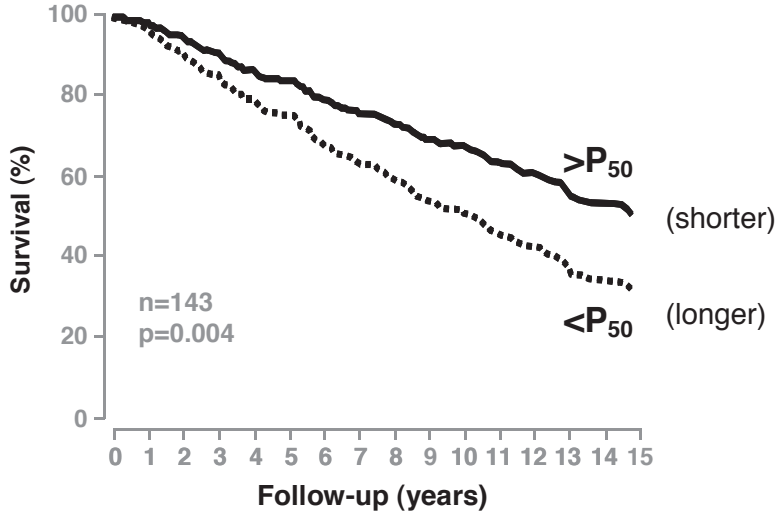

Figure 1

Survival data for age adjusted telomere length after age 60 years. Longer identifies individuals from the top half of the telomere length distribution and shorter those from the bottom half of the distribution. From Cawthon et al. (8) [with permission]

Furthermore, Benetos and colleagues (9) observed that telomere length was shorter in hypertensive men with carotid artery plaques compared to hypertensive men without plaques (8.17+/-0.07 kb versus 8.46+/-0.07 kb; P<0.01). These findings support the concept that shorter telomeres are a biomarker associated with atherosclerosis.

3.1. Heritability of telomere length

In twin studies, the heritability coefficient of telomere length was 80% (10). However, the mode of inheritance of telomere length remained to be elucidated. We observed no correlation in gender- and age-adjusted telomere length between spouses or between fathers and sons. In contrast, we noticed robust correlations in telomere length between fathers and daughters, between mothers and both sons and daughters, and among siblings (11). X-linked inheritance of telomere length is the most likely explanation for these findings. Pending confirmation, our observations also suggested that the ageing process might be a X-linked trait. Sensitivity analysis with outliers excluded or involving fictive parent-off-spring pairs, respectively, were confirmatory or excluded that random variability might explain our observations.

Furthermore, adjustments for age, smoking and gender (if applicable) did not alter our findings. The X-chromosome harbours two candidate genes, which might influence telomere length. The *DKC1* gene encodes the protein dyskerin, implicated in telomerase activity (12). This enzyme is a specialized reverse transcriptase that copies a region within its integral RNA component to extend chromosome 3□ ends by synthesis of telomeric sequence repeats to ensure telomere maintenance. Telomerase RNA must interact with dyskerin and probably with other unknown proteins to accumulate in cells as stable and fully functional RNA. Cells from patients with X-linked dyskeratosis congenita, caused by a missense mutation in the *DKC1* gene, have lower telomerase activity and shortened telomere length (12, 13). Thus, polymorphisms in the *DKC1* gene with less profound effects than those noted in dyskeratosis congenita might be determinants of telomere length in the general population.

Oxidative stress plays a key role in cellular senescence and ageing. Nitric oxide stimulates telomerase activity and delays senescence of endothelial cells (15). It is conceivable that nitric oxide reacts with intracellular radicals, that it decreases oxidative stress, and that by doing so it attenuates telomere attrition. Of interest in this regard is that the gene encoding the angiotensin II type-2 receptor, whose stimulation leads to increased nitric oxide production, also maps to the X-chromosome (16).

Mitochondrial DNA represents only a tiny fraction of the human genome. Mitochondria DNA is completely from maternal origin (19). Therefore, transmission of mitochondrial DNA cannot explain the high father-daughter concordance in telomere length.

3.2. Role of smoking, oxidative stress and inflammation in biological and vascular ageing

In keeping with previous reports, we identified sex and age as major determinants of telomere length. In addition, our analyses revealed that independent of age (mean age 43 years) and sex telomere length was on average 190 bp shorter in smokers than non-smokers. The rate of telomere attrition in the entire cohort was 24 bp/year. Thus, in telomeric year equivalents, smokers were biologically older than non-smokers by roughly 8 years. This effect of smoking on telomere length corresponds with Doll's prospective observations (17) on mortality and smoking showing that smokers on average die about 10 years earlier than lifelong non-smokers (figure 2). To the best of our knowledge, our report is the first published account of an inverse association between telomere length and smoking. Of interest in this regard is the observation that homocysteine, a known risk factor for human atherosclerosis, enhances the rate of telomere attrition per replicative cycle in cultured human vascular endothelial cells (18). To a large extent, the effect of homocysteine appears to be mediated by reactive oxygen species.

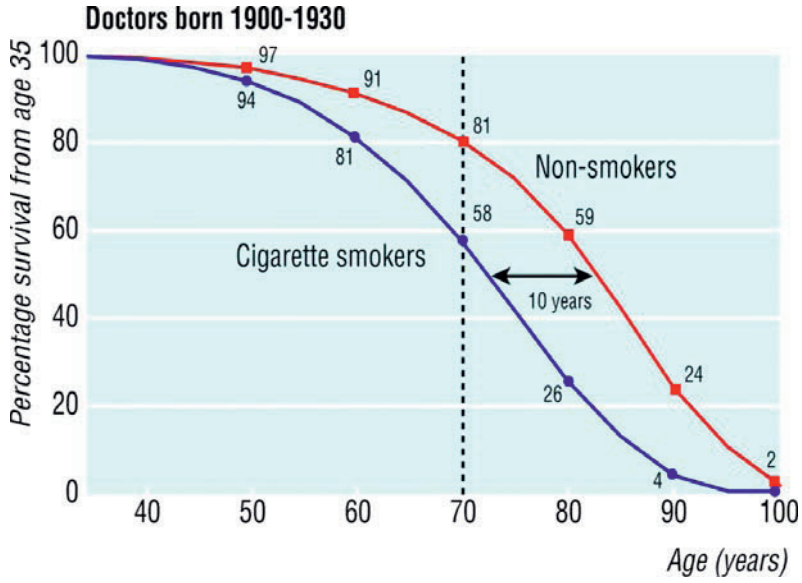

Figure 2.

Survival from age 35 for continuing cigarette smokers and lifelong non-smokers among 34 439 UK male doctors born 1900-1930, with percentages alive at each decade of age. From Doll R et al. (17) [with permission]

Until now, the possible interaction between telomere attrition and reactive oxygen species has not been studied in humans. We tested in a random population sample the hypothesis that increased oxidative stress and inflammation may promote biological and vascular ageing and that smoking further accelerates this process. We estimated vascular ageing from the distensibility of the common carotid artery. In addition, we explored the effect of the –174 G→C polymorphism of interleukin-6 on these variables in smokers and non-smokers.

With adjustments for sex and age applied, smokers had shorter telomere length and higher plasma levels of oxidized-LDL than non-smokers. In all subjects, the sex- and age- adjusted telomere length was independently and inversely correlated with plasma oxidized-LDL. In smokers, telomere length and carotid distensibility decreased, whereas plasma oxidized LDL increased with the number of copies of the

interleukin-6 –174 C allele. These associations were not significant in non-smokers.

In keeping with the literature, plasma levels of interleukin-6 were higher in smokers carrying the –174 C allele (19, 20). Furthermore, our findings are in line with the observations that there exists strong interaction between the G→C polymorphism and smoking in relation coronary heart disease risk (20). Men who smoked and carried the –174 C allele had the greatest risk. The relative risk was 2.66 (1.64-4.32) in this group compared to men homozygous for the –174 G allele.

What might be the mechanism behind the inverse association between telomere length and oxidative stress? Oxidative stress may exert a direct effect on telomere dynamics by increasing the rate of telomeric erosion per replicative cycle, or alternatively, an indirect effect mediated by enhancing the turnover rate (replication) (21). The increase in telomeric erosion per replicative cycle might be due to oxidation of G bases in the telomere sequence (21). In epidemiological studies, such as ours, we can not address these mechanistic issues. However, we showed for the first time that in the population at large telomere length and carotid distensibility are inversely associated with plasma levels of oxidized-LDL. Thus, oxidative stress and inflammation likely play an important role in biological and arterial ageing, a process which may be accelerated in smokers, particularly in those carrying the C allele of the –174 G→C interleukin-6 polymorphism.

4. Environmental determinants of oxidative stress

A Medline search using the term “cardiovascular risk factor” currently yields about 18,000 original articles on several hundreds of different cardiovascular risk factors. The question might be asked whether we still have to search for new risk factors. The answer likely is “yes”. Clinical trials showed that in tightly managed patients, who were exposed to interventions with various risk reductions, the relative reduction in cardiovascular events at best ranges from 20 to 40%, leaving 80% to 60% of the cardiovascular risk unexplained. Recently, we investigated the influence selenium on blood pressure (22).

4.1. Blood pressure and selenium

To further study the clinical significance of oxidative stress, we investigated in a prospective population study the relation between blood pressure and selenium. Selenium is a key component of a number of functional selenoproteins required for normal health, including the antioxidant glutathione peroxidase enzyme, which prevents oxidation of lipids and phospholipids (figure 3).

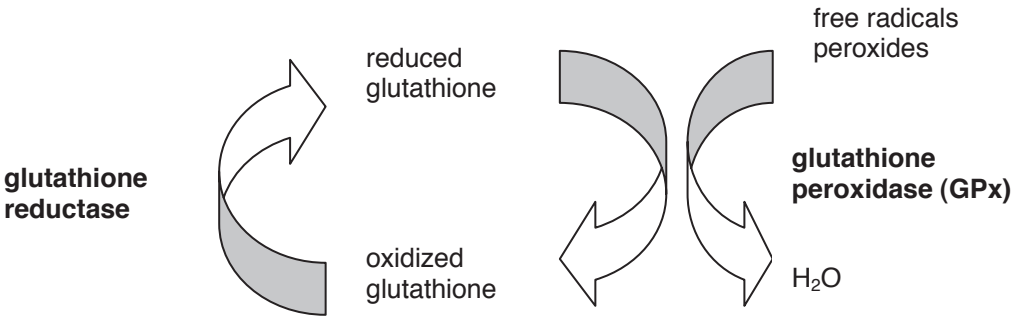

Figure 3

Glutathion peroxidase (GPx), reduces H_2O_2 to H_2O. The reaction catalyzed by GPx, requires reduced glutathione. Oxidized glutathione is reduced by glutathione reductase. GPx has four atoms of selenium bound as selenocysteine

Glutathion peroxidase (GPx), reduces H_2O_2 to H_2O. The reaction catalyzed by GPx, requires reduced glutathione. Oxidized glutathione is reduced by glutathione reductase. GPx has four atoms of selenium bound as selenocysteine which are required for the enzymatic activity. Furthermore, in selenium deficiency, an accumulation of hydroperoxides inhibits the enzyme prostacyclin synthase, which in endothelial cells generates the vasodilatory prostacyclin (23). In selenium-deficient hypercholesterolaemic rats, selenium supplementation enhances endothelium-dependent relaxation (24).

Taken up from the soil, selenium enters the food chain. Dietary intake in large parts of Europe is considerably lower than in the United States, mainly because the selenium content of the soil is low in Europe (<0.5 ppm) so that smaller amounts of this mineral enter the human food chain. According to the British Reference Nutrient a daily dietary allowance of 60 µg in women and 75 µg in men is required to attain a plasma selenium concentration of nearly 95 µg/L (range 89-114 µg/L) and to maximize the activity of the antioxidant selenoenzyme glutathione peroxidase. Current European intakes (Table 1) are only about half of the Nutrient Reference intake. In keeping with the literature, we found that in our random population sample the 95th percentile of the blood selenium concentration was 125 µg/L, which is approximately the median of the distribution in the United States (25). Because selenium intake is sub- optimal in Belgium and large parts of Europe, we and others (26, 27) hypothesized that selenium depletion might be a cardiovascular risk factor. In this doctoral dissertation we particularly investigated the relation between blood pressure and the concentration of selenium in blood, for the first time using a prospective approach.

In our cross-sectional analysis, we found that each increment in the blood selenium concentration by one standard deviation was associated with a 2/1 mmHg lower systolic/diastolic blood pressure in men, but not women. More importantly, in prospective analysis spanning 5.2 years of follow-up (median) the risk of men to get higher than normal blood pressure decreased by 37% for each standard deviation increase in the blood selenium concentration at baseline.

A previous cross-sectional study in 722 middle-aged Finnish men showed a negative relation between systolic blood pressure and serum selenium (28). Furthermore hypertensive patients compared to normotensive controls may have a 25% lower intra-renal selenium concentration (29). Rat experiments revealed that administration of sodium selenite via drinking water attenuated the increase in blood pressure in response to an infusion of angiotensin II, which has a pro-oxidant activity (30).

To the best of our knowledge we demonstrated for the first time in a prospective population study that selenium deficiency might be a risk factor for hypertension in men but not women. This sex difference was not due to a larger range of the blood pressure or

selenium values neither in men nor to the presence of a non-linear relation between blood pressure and blood selenium in women. Why the blood pressure of women is less sensitive to selenium deficiency remains to be elucidated. However, estrogens exert a strong antioxidant activity by direct reduction of free radicals and via the stimulation of enzymes, which are crucial for the scavenging of free radicals (31, 32). Thus, the more elaborate protection against oxidative stress in women might explain why we failed to observe any association between blood pressure and blood selenium in this gender.

Table 1

Country	Intake (μg/day)	Reference
Belgium	28-61	Robberecht and Deelstra. 1994 (33)
Denmark	49	Larsen, et al. 2002 (34)
France	29-43	Lamand, et al. 1994 (35)
Germany	43	Oster and Prellwitz. 1989 (36)
Greece	100	Bratakos, et al. 1991 (37)
Italy	51	Amodio-Cocchieri, et al. 1995 (38)
Lithuania	100	Golubkina, et al. 1992 (39)
The Netherlands	70	Van Dokkum, et al. 1989 (40)
Poland	30-40	Wasowicz, et al. 2003 (41)
Slovakia	38	Kadrabova, et al. 1998 (42)
Spain	38	Schroder, et al. 2001 (43)
Sweden	44	Becker and Kumpulainen. 1991 (44)
United Kingdom	34	Rayman. 2000 (45)
United States	109	Zhou, et al. 2003 (46)

Values are mean or range

Daily selenium intakes in some European countries and the US

Our findings might have important implications for public health. Indeed, a small downward shift of the blood pressure distribution in the general population, for instance by 2 mmHg systolic may account for appreciable reductions in risk, amounting to 7% reduction for coronary heart disease and to 10% for stroke (47). On the assumption that the association between blood pressure and blood selenium in men is reversible, such a cardiovascular protective effect could be obtained by

an increase in the blood selenium concentration by 20 µg/L. This would require an increase in the daily dietary selenium intake by 13 µg from an estimated 50 µg (48, 49).

5. Conclusions and perspectives

Population studies, which account for gene-environment interactions, are increasingly being used to study complex phenotypes, such as biological ageing and arterial ageing. This approach, termed molecular epidemiology, attempts to merge highly sensitive laboratory methods, many of them developed during the current revolution in molecular biology, with a classical epidemiological approach and demographic statistics. The most commonly held view is that molecular epidemiology represents a natural convergence between molecular biology and population sciences.

Through integration of molecular with epidemiological research we showed that biological ageing as reflected by telomere length is probably a X-linked mechanism. Further research should identify the genetic loci underlying this association. We demonstrated that the elasticity of the carotid artery was related to telomere length. We admit that our findings do not provide an insight in the pathophysiological pathways linking arterial ageing with telomere length. Until now, the dynamics of telomere attrition and its role in the biology of human ageing remains poorly understood. In our study, telomere length simply served as a biomarker of biological rather than chronological age. However, our findings set the stage for further clinical and experimental studies involving this new biomarker.

Our results suggest that oxidative stress might explain variation between individuals in cardiovascular ageing and the age-dependent telomere attrition in humans. By undergoing erosion with each mitosis, telomere length represents a life-time record of the replicative history of human somatic cells. In white blood cells, this history not only reflects cellular turnover but also inflammation. Therefore, telomere length might be an index of the cumulative, additive or synergistic effects of oxidative stress and inflammation, integrating human susceptibility of these effects over the lifetime of each individual. We strongly believe that telomere length will become an important biomarker in future epidemiological research of cardiovascular disorders. One sidetrack

to this concept might be that telomere length might be considered as a potential new biomarker of oxidative stress in toxicology or environmental medicine to study the potential effects of environmental pollutants which may cause oxidative stress.

One of the factors, which might reduce oxidative stress and pro-inflammatory responses, is the essential trace element selenium, of which the intake in Western Europe is sub optimal (table 1). Our prospective population study demonstrated that deficiency of selenium leads to an increase in blood pressure in men. At present, soil fortification with selenium is a topic of discussion in the European union (50, 51). However, careful consideration needs to be given to the potential consequences of increasing the soil concentration of selenium, because of the narrow safety margin between the selenium levels which are beneficial or toxic for humans. Our study might provide essential information to feed the cost-benefit calculations which should guide policy makers in their decisions.

Overall, standardized epidemiological observations such as presented in this dissertation, reflect the experiment of nature and help to distinguish what is clinically relevant at the level of the general population. There are new challenges ahead for epidemiology and epidemiologists. First, there is the need to evaluate the benefits and adverse consequences of exposures as for instance in our studies on the effects of selenium and high outdoor temperature. Second, new technologies and developments in molecular biology create a window of opportunity to test hypotheses at more fundamental biological levels in an epidemiological context as for instance our studies on biological ageing. Hence, population science will increasingly become a multidisciplinary enterprise. Therefore, the future of epidemiology is bright.

Summary

Arterial ageing is a complex continuously distributed phenotype, which comes about through the interaction between inherited susceptibility, life-style and environmental factors. We used an integrated approach combining methods from genetics, molecular biology and population sciences to study the role of genetic variation and environmental factors in biological ageing. The discussed work comprises four population based

studies of which two had a prospective design and two integrated recently developed biomolecular markers of ageing with classical epidemiological tools.
The striking variability in the age of the manifestation of cardiovascular diseases is not fully explained by conventional risk factors. Variation in biological age has been suggested. The initial telomere length of a person is mainly determined by genetic factors. In this regard, we noticed robust correlations in telomere length between fathers and daughters, between mothers and both sons and daughters, and among siblings. X-linked inheritance of telomere length is the most likely explanation for these findings. Telomere length shortens with each cell division, and exposition to harmful environmental factors results in shorter telomere length as we observed in smokers. Telomere length correlated with the distensibility of the carotid artery and oxidative stress and inflammation are major determinants of arterial and biological ageing. In this context, selenium a component of antioxidant enzymes such as glutathione peroxidase, correlated inversely with blood pressure in the population at large.
Oxidative stress and inflammation are major determinants of arterial and biological ageing. If telomeres are indeed causally involved in the pathogenesis of arterial ageing, this might provide new avenues for future preventive and therapeutic strategies.

Samenvatting

Arteriële veroudering is een fenotype waarbij men redelijkerwijze kan vermoeden dat zowel genetische variatie als omgevingsfactoren een interactieve rol spelen. Epidemiologische studies kunnen worden geoptimaliseerd door gebruik te maken van een geïntegreerde benadering die gebaseerd is op epidemiologische, genetische en moleculair biologische methodes. Het bediscussieerde werk omvat vier epidemiologische studies waarvan twee een prospectief karakter hadden en twee studies die gebruik maakten van recent ontwikkelde biomoleculaire merkers van veroudering.
Er bestaat een grote variatie in chronologische leeftijd waarop hart- en vaatziekten zichtbaar worden. Deze variatie kan niet geheel verklaard worden door conventionele risicofactoren. Wij verouderen niet alleen chronologisch, maar ook biologisch, waarschijnlijk met grote interindividuele verschillen. Onze hypothese is dat de biologische leeftijd mede de kans bepaalt op het manifest worden van hart- en vaatziekten en dat de telomeerlengte hiervoor een goede maat is. De telomeerlengte is in belangrijke mate genetisch bepaald. Bij een representatief bevolkingsstaal, (119 mannen en 152 vrouwen) vonden we geen significante correlatie in de telomeerlengte tussen vader en zoon maar wel sterk significante correlaties in telomeerlengte gevonden tussen vader en dochter, moeder en zoon en moeder en dochter. X-gebonden overerving van telomeerlengte is het meest waarschijnlijke mechanisme om dit patroon te verklaren. De telomeerlengte verkort bij elke celdling, en blootstelling aan schadelijke omgevingsfactoren zoals tabaksrook resulteert in een kortere telomeerlengte. De telomeerlengte was negatief gecorreleerd met de distensibiliteit van de arteria carotis, en oxidatieve stress en inflamatie bleken belangrijke deterimentanten van zowel de arteriële als de biologische veroudering te zijn. Om de associatie tussen oxidatieve stress en arteriele veroudering verder te onderzoeken, bestudeerden we de relatie tussen bloeddruk en selenium, een

noodzakelijke co-factor van het antioxiderende glutathione peroxidase. De bloeddruk bij mannen was negatief geassocieerd met de bloedseleniumwaarde.
Oxidatieve stress en inflammatie zijn belangrijke determinanten van arteriële en biologische veroudering. Indien telomeren een causale relatie met hart- en vaatziekten blijken te hebben, dan vormen ze een potentieel nieuw aangrijpingspunt voor toekomstige preventieve en therapeutische strategieën.

REFERENCES

1. Kesteloot H. Epidemiology: past, present and future. Verh K Acad Geneeskd Belg. 2004;66:384-405.
2. Nawrot T, Den Hond E, Thijs L, Staessen JA. Isolated systolic hypertension and the risk of vascular disease. Curr Hypertens Rep. 2003;5(5):372-9.
3. Safar ME, Levy BI, Struijker-Boudier H. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. Circulation. 2003;107(22):2864-9.
4. Staessen JA, Den Hond E, Celis H et al. Antihypertensive treatment based on blood pressure measurement at home or in the physician's office: a randomized controlled trial. JAMA. 2004;291(8):955-64.
5. Nawrot TS, Staessen JA, Thijs L et al. Should pulse pressure become part of the Framingham risk score? J Hum Hypertens. 2004;18(4):279-86.
6. Benetos A, Okuda K, Lajemi M et al. Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. Hypertension. 2001;37(2 Part 2):381-5.
7. Olovnikov AM. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. J Theor Biol. 1973;41(1):181-190.
8. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet. 2003;361(9355):393-5.
9. Benetos A e.a. Short telomeres are associated with increased carotid atherosclerosis in hypertensive subjects. Hypertension. 2004;43(2):182-5.
10. Slagboom PE, Droog S, Boomsma DI. Genetic Determination of Telomere Size in Humans - A Twin Study of 3 Age-Groups. American Journal of Human Genetics. 1994;55(5):876-82.
11. Nawrot TS, Staessen JA, Gardner JP, Aviv A. Telomere length and possible link to X chromosome. Lancet. 2004;363(9408):507-10.
12. Bessler M, Wilson DB, Mason PJ. Dyskeratosis congenita and telomerase. Curr Opin Pediatr. 2004;16(1):23-8.
13. Bessler M, Wilson DB, Mason PJ. Dyskeratosis congenita and telomerase. Curr Opin Pediatr. 2004;16(1):23-8.
14. McCarty MF. Optimizing endothelial nitric oxide activity may slow endothelial aging. Med Hypotheses. 2004;63(4):719-23.
15. Volpe M, Musumeci B, De Paolis P, Savoia C, Morganti A. Angiotensin II AT2 receptor subtype: an uprising frontier in cardiovascular disease? J Hypertens. 2003;21(8):1429-43.

16. Vina J, Sastre J, Pallardo F, Borras C. Mitochondrial theory of aging: importance to explain why females live longer than males. Antioxid Redox Signal. 2003;5(5):549-56.
17. Doll R, Peto R, Boreham J, Sutherland I. Mortality in relation to smoking: 50 years' observations on male British doctors. BMJ. 2004;328(7455):1519.
18. Richards JB e.a. Homocysteine levels and leukocyte telomere length. Atherosclerosis. 2008;200(2):271-7
19. Kaessmann H, Heissig F, von Haeseler A, Pääbo S. DNA sequence variation in a non-coding region of low recombination on the human X chromosome. Nat Genet. 1999;22(1):78-81.
20. Humphries SE, Luong LA, Ogg MS, Hawe E, Miller GJ. The interleukin-6 -174 G/C promoter polymorphism is associated with risk of coronary heart disease and systolic blood pressure in healthy men. Eur Heart J. 2001;22(24):2243-52.
21. von Zglinicki T. Role of oxidative stress in telomere length regulation and replicative senescence. Ann N Y Acad Sci. 2000;908:99-110.
22. Nawrot TS, Staessen JA, Roels HA, Den Hond E, Thijs L, Fagard RH, Dominiczak AF, Struijker-Boudier HA. Blood pressure and blood selenium: a cross-sectional and longitudinal population study. Eur Heart J. 2007;28(5):628-33.
23. Weaver JA, Maddox JF, Cao YZ, Mullarky IK, Sordillo LM. Increased 15-HPETE production decreases prostacyclin synthase activity during oxidant stress in aortic endothelial cells. Free Radic Biol Med. 2001;30(3):299-308.
24. Raij L, Nagy J, Coffee K, DeMaster EG. Hypercholesterolemia promotes endothelial dysfunction in vitamin E- and selenium-deficient rats. Hypertension. 1993;22(1):56-61.
25. Niskar AS e.a. Serum selenium levels in the US population - Third National Health and Nutrition Examination Survey, 1988-1994. Biological Trace Element Research. 2003;91(1):1-10.
26. Kok FJ e.a. Decreased selenium levels in acute myocardial infarction. JAMA. 1989;261(8):1161-4.
27. Neve J. Selenium as a risk factor for cardiovascular diseases. J Cardiovasc Risk. 1996;3(1):42-7.
28. Salonen JT e.a. Relationship of serum selenium and antioxidants to plasma lipoproteins, platelet aggregability and prevalent ischaemic heart disease in Eastern Finnish men. Atherosclerosis. 1988;70(1-2):155-60.
29. Perry H, Masironi R, Parr R, Miller J. Concentration of trace metals (Cd, Zn, Se, Cu, Cr, and Fe) in organs (heart, kidney and liver) of subjects with myocardial infarction or hypertension: WHO/IAEA myocardial infarction and hypertension autopsy study. J Trace Elem Exper Med. 1991;4:109-28.
30. Hilse H, Oehme P, Krause W, Hecht K. Effect of sodium selenite on experimental hypertension in rat. Acta Physiol Pharmacol Bulg. 1979;5(3):47-50.
31. Lacy F, Kailasam MT, O'Connor DT, Schmid-Schonbein GW, Parmer RJ. Plasma hydrogen peroxide production in human essential hypertension - Role of heredity, gender, and ethnicity. Hypertension. 2000;36(5):878-4.
32. Romer W, Oettel M, Menzenbach B, Droescher P, Schwarz S. Novel estrogens and their radical scavenging effects, iron-chelating, and total antioxidative activities: 17 alpha-substituted analogs of Delta(9(11))-dehydro-17 beta-estradiol. Steroids. 1997;62(11):688-94.

33. Robberecht HJ, Hendrix P, Van Cauwenbergh R, Deelstra HA. Actual daily dietary intake of selenium in Belgium, using duplicate portion sampling. Z Lebensm Unters Forsch. 1994;199(4):251-4.
34. Larsen EH, Andersen NL, Moller A, Petersen A, Mortensen GK, Petersen J. Monitoring the content and intake of trace elements from food in Denmark. Food Addit Contam. 2002;19(1):33-46.
35. Lamand M, Tressol JC, Bellanger J. The mineral and trace element composition in French food items and intake levels in France. J Trace Elem Electrolytes Health Dis. 1994;8(3-4):195-202.
36. Oster O, Prellwitz W. The daily dietary selenium intake of West German adults. Biol Trace Elem Res. 1989;20(1-2):1-14.
37. Bratakos MS, Ioannou PV. Selenium in human milk and dietary selenium intake by Greeks. Sci Total Environ. 1991;105:101-7.
38. Amodio-Cocchieri R, Arnese A, Roncioni A, Silvestri G. Evaluation of the selenium content of the traditional Italian diet. Int J Food Sci Nutr. 1995;46(2):149-54.
39. Golubkina NA, Shagova MV, Spirichev VB et al. [Selenium intake by the population of Lithuania]. Vopr Pitan. 1992;(1):35-7.
40. van Dokkum W, de Vos RH, Muys T, Wesstra JA. Minerals and trace elements in total diets in The Netherlands. Br J Nutr. 1989;61(1):7-15.
41. Wasowicz W, Gromadzinska J, Rydzynski K, Tomczak J. Selenium status of low-selenium area residents: Polish experience. Toxicol Lett. 2003;137(1-2):95-101. Kadrabova J, Mad'aric A, Ginter E. Determination of the daily selenium intake in Slovakia. Biol Trace Elem Res. 1998;61(3):277-86.
43. Schroder H e.a. Use of a three-day estimated food record, a 72-hour recall and a food-frequency questionnaire for dietary assessment in a Mediterranean Spanish population. Clin Nutr. 2001;20(5):429-37.
44. Becker W, Kumpulainen J. Contents of essential and toxic mineral elements in Swedish market-basket diets in 1987. Br J Nutr. 1991;66(2):151-60.
45. Rayman MP. The importance of selenium to human health. Lancet. 2000;356(9225):233-41.
46. Zhou BF e.a. Nutrient intakes of middle-aged men and women in China, Japan, United Kingdom, and United States in the late 1990s: the INTERMAP study. J Hum Hypertens. 2003;17(9):623-30.
47. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 2002;360(9349):1903-13.
48. Neve J, Vertongen F, Peretz A, Carpentier YA. [Usual values of selenium and glutathione peroxidase in a Belgian population]. Ann Biol Clin (Paris). 1989;47(3):138-43.
49. Neve J. Human selenium supplementation as assessed by changes in blood selenium concentration and glutathione peroxidase activity. J Trace Elem Med Biol. 1995;9(2):65-73.
50. Rayman MP. Dietary selenium: time to act. BMJ. 1997;314(7078):387-388.
51. Arthur JR. Selenium supplementation: does soil supplementation help and why? Proc Nutr Soc. 2003;62(2):393-7.