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Air Pollution Stress and the Aging Phenotype: The Telomere Connection

Dries S. Martens[1], Tim S. Nawrot[1,2]

[1] Centre for Environmental Sciences, Hasselt University, 3500 Hasselt, Belgium

[2] Department of Public Health & Primary Care, Leuven University, 3000 Leuven, Belgium

Corresponding author: Tim S. Nawrot, PhD., Centre for Environmental Sciences, Hasselt University, Agoralaan gebouw D, 3590 Diepenbeek, Belgium. Phone: 0032-11 26 83 82, fax: 0032-11 26 82 99 e-mail: tim.nawrot@uhasselt.be

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Abstract

Aging is a complex physiological phenomenon. The question why some subjects grow old while remaining free form disease whereas others prematurely die remains largely unanswered. We focus here on the role of air pollution in biological aging. Hallmarks of aging can be grouped into three main categories: genomic instability, telomere attrition, and epigenetic alterations leading to altered mitochondrial function and cellular senescence. At birth, the initial telomere length of a person is largely determined by environmental factors. Telomere length shortens with each cell division and exposure to air pollution as well as low residential greens space exposure are associated with shorter telomere length. Recent studies show that the estimated effects of particulate air pollution exposure on the telomere mitochondrial axis of aging may play an important role in chronic health effects of air pollution. The exposome encompasses all exposures over the entire life. As telomeres can be considered as the cellular memories of exposure to oxidative stress and inflammation, telomere maintenance may be a proxy for assessing the "exposome". If telomeres are causally related to the aging phenotype and environmental air pollution is an important determinant of telomere length, this might provide new avenues for future preventive strategies.

Introduction

Extensive epidemiological studies support the link between ambient air pollution and adverse health outcomes, including cardiovascular and respiratory disease, both with short-term [1-3] and chronic exposure [4-8]. The WHO estimated the effects of combustion related particulate matter on life expectancy [9]. The results of this analysis indicate that current exposure to particulate matter from anthropogenic sources leads to an average loss of 8.6 months of life expectancy in Europe. The impacts vary from around 3 months in Finland to more than 13 months in Belgium. Along similar lines, cleaner air has contributed to increased life expectancy in the US [10]. A decrease of 10 $\mu g/m^3$ in the concentration of fine particulate matter was associated with an estimated increase in life expectancy of 7.3 months. Fifteen percent of the overall increase in life expectancy over a 2 decade period was attributed to reductions in air pollution. Public health impacts of nine major environmental exposures were evaluated in 6 European countries, and findings indicated that particulate air pollution clearly caused the greatest burden of disease of the nine factors investigated [11]. Based on the recent update of the Global Burden of Disease, Injuries, and Risk Factor study, air pollution is ranked 12^{th} of a list of the most influential factors influencing health worldwide [12].

The Aging Phenotype

Although aging is universal and unavoidable, the aging process does not occur in a uniform way. It is a complex phenotype responsive to a plethora of drivers. In the general population, aging represents a continuously distributed phenotype, in which genetic, behavioral and environmental factors interact with each other [13, 14]. Given the biological complexity of the aging process, there is no single, simple and reliable measure of an individual healthy aging process. Arbeev and coworkers [15] analysed the relation between the risk of onset of ‘‘unhealthy life’’ (defined as the onset of cancer, cardiovascular disease, or diabetes) and longitudinal changes in body mass

index, diastolic pressure, hematocrit and pulse pressure, in the Framingham Heart Study. This process can be quantified already in young adults. Indeed, blood pressure, fasting glucose and glycated hemoglobin (HbA1C), bone mineral density, and blood lipids, appear to be predictive of biological age and of the rate of aging in younger healthy subjects [16]. Some of these factors including blood pressure [17-19], glucose [20] and diabetes risk [21] have been associated with exposure to air pollution. Further, the trajectory from healthy to unhealthy aging often comprises the microcirculation, which plays a role in diverse age related conditions such as hypertension, left ventricular dysfunction and chronic kidney disease [22]. Few studies of air pollution addressed the role of microcirculation. Among 4,607 participants of the Multi-Ethnic Study of Atherosclerosis (MESA), the retinal arteriolar diameter narrowed by 0.8 µm in response to an interquartile increase in the 2-year exposure to $PM_{2.5}$ (3 µg/m³). This effect size was equal to a 7-year increase in age [23]. In a repeated measure study, including 84 healthy volunteers, each 10-µg/m^3 increase in PM_{10} was associated with 0.93 and 0.86 µm decrease in the arteriolar and venular diameters, respectively [24].

Primary hallmarks of the aging process cause damage to cellular functions including genomic instability, telomere attrition, and epigenetic alterations. These are followed by critical responses including altered mitochondrial function and cellular senescence. Finally, these integrative hallmarks of the cellular aging process are possible culprits which ultimately contribute to the clinical effects of aging as seen in physiological loss of reserve, organ decline and reduced function. Air pollution might influence the aging phenotype through its action on the primary molecular hallmarks of aging.

Biological Underpinnings of Aging

Aging begins at the very beginning of life, to accelerate at middle-age. The biological underpinnings of aging may begin before birth. Indeed, complications in adults often find their origin in risk factors operative in early life [13]. Some recently defined patterns of diseases that

begin in childhood include metabolic syndrome and cognitive aspects [14]. It is suggested that an adaptive response in the fetus to *in utero* exposures could result in persistent changes that influence health later in life [25].

Telomeres

Telomeres are ribonucleoprotein complexes that cap the end of chromosomes and thereby protect it from degradation and end-to-end fusion to ensure genome stability and to prevent the loss of genetic information [26]. Human telomeres consists of several kb of tandem repeated TTAGGG sequences with a 3' G-rich single stranded overhang up to 200 nucleotides [27]. Telomeres shorten after each cellular division due to the end-replication problem and they are considered as a marker of biological aging [28]. During DNA replication on the lagging strand, DNA polymerase is not able to fully replicate the DNA strand, as the last RNA primer cannot be removed and fully replicated. Once telomeres reach a critical length (also known as the Hayflick limit), end-to end fusions are formed and genomic instability increases, leading to replicative senescence (mortality phase 1, M1) [29-31]. If human cells circumvent the M1 phase, more and more telomeres continue to shorten and reach a critical length, resulting in crisis (mortality phase 2, M2), characterized by end-to end fusions, chromosome breakage-fusion-bridge cycles, mitotic catastrophe and extensive genomic instability, eventually leading to cell death [32, 33] To compensate for telomeric DNA loss, telomere length is maintained by the enzyme telomerase which is capable of adding the telomeric repeat sequences to the ends of the chromosomes [34]. Telomerase is a ribonucleoprotein containing a RNA template (TERC) and a reverse transcriptase (TERT) and is mainly active in germ, stem and immortal cells and is mainly repressed in somatic cells [35, 36]. DNA binding proteins are able to bind with telomeres to form the shelterin complex. Proteins of the shelterin complex that directly binds to the TTAGGG repeats includes the telomeric repeat binding factor 1 (TRF1) and 2 (TRF2) and protein protection of telomeres 1 (POT1). Other proteins of the shelterin complex that are connected are

the repressor activator protein 1 (RAP1), tripeptidyl peptidase 1 protein (POT1) and the TRF1 interacting nuclear factor 2 (TIN2) [37, 38]. This shelterin complex is able to affect the structure of telomeric DNA by the formation of a large duplex loop referred to as T-loop that protects the telomeres and regulates telomerase [39]. The single-stranded overhang invades into the DNA duplex of telomeric repeats and forms a displacement loop (D-loop) [40]. The amount of shelterins on telomeres can influence telomerase inhibition [37].

Telomere research in humans has explored whether telomere length provides information, over and above chronological age. Most of the large population based studies focused on leukocyte telomere lengths [41]. Leukocytes consists of the most replicative cell types, resulting in shorter telomeres compared with somatic tissues and results obtained from leukocyte telomere lengths research might not be generalized to other tissues [42]. Although leukocyte telomere length have showed to be highly correlated with other somatic tissues from the same individual such as muscle, fat, skin, synovial tissue , indicating that a clear intra-individual synchronization in telomere length exists in adults. Interestingly, Daniali *et al.* [43] showed that during adulthood the rate of telomere shortening in different tissues is equal regardless of their replicative activity. These studies suggest that leukocyte telomere length might be a proxy for telomere length in most somatic tissues.[44].

Studies among populations show that persons with shorter mean telomere length in leukocytes have increased risk for cardiovascular disease, [45-50] indices of obesity and insulin resistance [51, 47, 48, 52]. Excessive telomere shortening is associated with higher risk of age related diseases, such as heart failure [53] and cancer [46, 38]. However, whether mortality in elderly is associated with shorter telomere length is still a question with conflicting results among studies [54, 46, 55]. Telomere length is a complex trait, it is heritable (estimated between 36% and 82%) [56-60], longer in women than in men [61], and longer in offspring of older fathers [62]. At birth telomere length is highly variable among newborns [63], but as observed in adults, even at birth telomere lengths are synchronized across different cell types and tissues within the

newborn [64, 63, 65]. Telomere length during the *in utero* life and at birth sets the aging phenotype and might predict overall life expectancy. Data provided by telomere length assessment across different time-points during the entire life of zebra finches, indeed indicate that telomere length during early life predicts lifespan and these findings underline the importance of unravelling early life factors that sets telomere length [66]. During the first years of life, telomeres shortens at a much higher rate compared to adulthood [67, 68]. So in general, the main factors that influences telomere length are factors operative during the *in utero* life and/or childhood, and these early life influences on telomere length remain persistent later in life, whit the least contribution during adulthood [43].The environmental factors during *in utero* life that affect telomere length variability are largely unidentified. The natural shortening of telomeres associated with aging may be accelerated through oxidative stress and inflammation induced by environmental factors [69, 70].

Mitochondria

Decreased mitochondrial function as exemplified by impaired ATP generation and increased reactive oxygen species (ROS) production are associated with aging, whereas conservation of mitochondrial function is an important mechanism of extending lifespan [71]. Mitochondria are involved in a variety of critical cell functions, including oxidative energy production, programmed cell death, growth, and redox signaling. By-products of mitochondrial electron transfer reactions in aerobic cells result in the production of reactive oxygen species (ROS), *e.g.* superoxide and hydrogen peroxide. Compared with nuclear DNA, mitochondrial DNA is more vulnerable to damage due to the lack of protective histones and insufficient repair capacity [72]. Thus, it has been proposed that mitochondrial damage, from any of a number of causes, might result in increased ROS that are causative agents in the development of genomic instability as observed in aging and cancer [73]. In this regard, genes that impact upon longevity have recently been characterized in *S. cerevisiae* and *C. elegans*, and the human homologs include the Sirtuin

family of protein deacetylases. Interestingly, three of the seven sirtuin proteins are localized into the mitochondria suggesting a connection between the mitochondrial sirtuins, the free radical theory of aging, and carcinogenesis [74, 75]. Based on these results it has been hypothesized that Sirt3 functions as a mitochondrial localized tumor suppressor [75]. Mitochondria have been linked with environmental interaction to an array of metabolic and age-related diseases, including cancer [76, 77], diabetes [78, 79] and cardiovascular illness [80, 81]. Endogenous mitochondrial oxidative stress is an important cardiovascular risk factor that can modulate atherogenesis by environmental inputs [82]. Mitochondrial superoxide dismutase 2 (SOD2)-specific activity declines in mice exposed to environmental tobacco smoke [83]. Until now, the focus has been on experimental research. However, biomarkers of mitochondria might be important in disease prediction and so far its association with environmental factors has barely been studied [84]. Moreover, observational studies in newborns indicate that low birth weight infants were prenatally subjected to conditions of oxidative stress and inflammation and this might be involved in vascular dysfunction [85] and atherosclerosis development and progression later in life [86].

Assessment of telomere length and mitochondrial DNA content

Different methods to assess telomere length have been extensively reviewed before [87-89].The first available method to measure telomere lengths was by the use of terminal restriction fragment (TRF) length estimation using southern blotting. Throughout the years it became and remained the ‘gold standard’ to measure telomere lengths, but other methods to measure telomeres have been developed throughout recent years. TRF estimation is a robust method with low coefficients of variation, that can express average telomere lengths in absolute values (kb). Major disadvantages of this method is the high quantity of DNA that is required (up to several ug of DNA) which might be a major limitation in some epidemiological studies, besides it captures subtelomeric DNA which might bias absolute telomere length estimation [90]. In 2002 a

real-time qPCR method was described to measure telomere length, which was later adapted to a multiplex version with adapted telomere specific primers to generate a PCR product of a fixed length compared with the older version which generated PCR products of various lengths [91, 92]. Major advantages of the qPCR method over the TRF method is that it can be used for high-throughput measurements, it is less labor intensive, limited amount of DNA is required (less than 100ng), and relative low cost [90]. Disadvantages of the qPCR assay are that telomeres are expressed as average relative telomere length, by determining the ratio of telomere repeat copy number to a single-copy gene copy number (T/S ratio), and it has in general a higher coefficient of variation. Studies have shown modest to high correlations between the TRF and qPCR method (R^2 up to 0.91) and both techniques are commonly used in epidemiological settings [93, 91, 92, 94]. Other methods available includes quantitative fluorescence *in situ* hybridization (Q-FISH), flow-FISH, and a PCR based method for single telomere length analysis (STELA), which are in general more labor intensive and are applied in context of specific research questions [95-97]. Mitochondrial DNA content is assessed by the use of a real-time qPCR method [98]. A ratio of a mitochondrial gene copy number to a single-copy nuclear gene copy number is determined to estimate the relative mtDNA content of human samples [99, 100].

Aging and Inflammation

Long-term inflammation is a cause of aging-associated diseases [101]. A mechanistic role for immune activation and inflammation in the development of a plethora of age related disease emerged over the last decade. Vascular inflammation contributes to the pathogenesis of hypertension, heart failure and renal disease. An early step in the development of these diseases is the local production of cytokines that attract immune cells to the vascular wall. These immune cells subsequently cross the endothelial lining and migrate into the underlying tissue, where they produce an inflammatory response. Circulation levels of pro-inflammatory cytokines (e.g. TNF-α, IL1, IL6) are increased during the early onset of cardiovascular disease. Numerous

causes may contribute to aging-associated inflammation, such as pro-inflammatory tissue damages, a dysfunctional immune system [102], pro-inflammatory cytokines secreted by senescent cells. Mean leukocyte telomere length reflects the senescent status of circulating cells of the immune system and systemic influences on telomere maintenance in other tissues. Because of the pro-inflammatory processes instigated by immune cell senescence, telomere attrition in immune cells is relevant in inflammatory induced diseases.

Relation between inflammation, oxidative stress and air pollution

Particulate air pollution can trigger multiple cellular responses in the lung such as cytotoxicity, inflammation, and mutagenesis. The adverse effects of particulate matter are not confined to the lung, but there are also extrapulmonary manifestations of exposure to particulate matter. The systemic consequences of particulate matter induced health effects may be due to a 'spill-over effect' of pulmonary inflammation or to translocation of the nano-sized particles into the circulation. Particulate matter has been described to induce oxidative stress, by the generation of reactive oxygen species (ROS). Indeed particles are able to generate ROS in different ways as reviewed by Risom and colleagues [103]. Firstly, ROS can directly be formed at the surface of particles. At the particle surface, soluble transition metals such as iron can be present that generates reactive hydroxyl radicals via a Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + {}^{-}OH$) [104]. Higher levels of ROS can induce mitochondrial DNA damage and dysfunction leading towards more additional production of ROS by mitochondria itself. Exposure to $PM_{2.5}$ has been associated with increased mitochondrial ROS production via the site III of the mitochondrial electron transport chain and subsequent produced ROS can activate apoptotic pathways through apoptosis signal-regulating kinase 1 (ASK1), c-Jun N-Terminal kinase (JNK) and tumor suppressor (p53) [105]. Other indirect sources of PM generated ROS are due to altered NADPH function and activation of inflammatory cells that are able to generate ROS [103].

The Air Pollution Telomere-Mitochondrial Connection

Telomere Shortening and Oxidative Stress

Besides cellular replication that imply constant telomere shortening, external factors and stress can interact with telomeres and may influence telomere attrition rates throughout the life course [68]. Interestingly, von Zglinicki was the first to show in 1995 experimentally, that cultivating human fibroblasts under hyperoxia conditions (represented as a state of oxidative stress) indeed shortened telomeres [106]. The underlying mechanism by which ROS induces DNA damage by which telomeres shorten is by the accumulation of single-strand nicks in telomeres which are less well repaired compared to other regions in the genome [107]. Mores specific, the G-rich parts of the telomere sequence (TTAGGG) and not the G-rich single stranded overhang are highly sensitive for DNA damage in human fibroblasts induced by oxidative stress conditions (after exposure to H_2O_2, an NO-generating agent and a O_2^- generating system) [108, 109]. Accelerated shortening of telomeres, and as such, senescence of cells may be an important pathway by which oxidative stress may accelerate biological aging and the resultant development of aging-related morbidity, including cardiovascular disease [47, 49].

The air pollution induced telomere-mitochondrial aging hypothesis

Direct and indirect formation of ROS by air pollution as described previously, can explain a cascade by which both telomeres and mitochondria are targeted, leading to telomere shortening as well as mitochondrial dysfunction and this might explain the mechanism by which air pollution alters the aging phenotype (Fig 1).

Recently, Sahin *et al.* [71] unveiled a fascinating connection between the nuclear and mitochondrial aging processes. Telomere-deficient mice showed p53 activation which results in suppression of peroxisome proliferator-activated receptor gamma coactivator 1 alpha and beta (PGC-1) genes thereby revealing a direct link between telomere and mitochondrial biology [110]. Further evidence of the telomere-mitochondrial axis of aging was shown in sirtuin1 (SIRT1)

knock-out mice. SIRT1 functions as a metabolic sensor and its deacetylase activity is controlled by the cellular NAD+ /NADH ratio [111]. SIRT1 expression was found to play a pivotal role in mitochondrial biogenesis [112, 113]. SIRT1 affects longevity in humans by influencing telomerase activity [114] and its function by inactivation of the 'guardian of the genome', TP53 [115]. In addition, SIRT1 stimulates PPARGC1A, a regulator of mitochondrial biogenesis [116]. Overexpression of SIRT1 in mice reduces the incidence of several aging related diseases, such as cardiovascular disease, metabolic disease and cancer [117].

The current accepted experimental model proposes that DNA damage to telomeres activates several signaling pathways and alters SIRT1 gene expression, which leads to mitochondrial dysfunction [110, 118]. This experimental model is in line with human observations. Among 166 elderly, telomere length and SIRT1 gene expression were found to be intermediate mechanisms between particulate matter air pollution exposure and mtDNA alterations. Formal mediation analysis indicated that the effect of long-term particulate matter air pollution on mtDNA content was mediated by SIRT1 expression [119].

Current evidence of air pollution induced telomere-mitochondrial aging

To date, epidemiological studies examining the effects of PM exposure and traffic related exposure on telomere length and mitochondrial DNA content have reported different responses after long-term or short-term exposure to PM. As the main focus here is on telomere biology, we summarized occupational and population based studies investigating the association between air pollution exposure and telomere length in Table 1 and Table 2 respectively. In several occupational studies a positive association is observed between PM exposure and telomere length. In 57 steel workers increased telomere length has been found in association with short-term (3 days of work) exposure to high levels of PM_1 and PM_{10} [120]. This observation is in line with observations made by Hou *et al.* [121] in truck drivers compared to office workers in Beijing, China. In this repeated measure study, the participants were examined on two different work

days with 1 to 2 weeks interval. Exposure to $PM_{2.5}$ and elemental carbon during the last 24 hours were positively associated with blood telomere length [121]. In accordance with these studies at high exposure, variation in relatively low short-term exposure (1 month prior blood drawn) was also associated with a 17.5% increase in telomere length for a 5 $\mu g/m^3$ increase in $PM_{2.5}$ [119]. Although PM is hypothesized to induce oxidative stress which causes telomere shortening, the observed increase in telomere length due to short-term exposure might be explained by acute inflammatory processes that has been linked with increased telomerase activity in B-cells [122]. However, this observation could not yet be confirmed based on gene expression data of the human telomerase reverse transcriptase (*hTERT*) component of telomerase [120], and this lengthening of telomeres should be further evaluated by investigating both the involvement of telomerase and components of the shelterin complex.

Observations on long term-exposure to PM and short-term traffic related exposure in both occupational and population based studies, indicates shortening of telomeres and supports the role of PM induced oxidative stress in increased DNA damage at telomeres. Traffic officers compared with office workers had much lower leukocyte telomere length which corresponds to a telomeric year equivalence of 13 years [123]. Among 165 never-smoking men from the Normative Aging Study [124], an IQR increase in annual black carbon (0.25$\mu g/m^3$) was associated with a 7.6% decrease in telomere length. Besides black carbon, it was shown in 166 elderly, that a 5$\mu g/m^3$ increment in annual $PM_{2.5}$ was associated with 16.8% decrease in telomere length, and a 25.7% decrease in mitochondrial content [119]. The estimated decrease in telomere length for a 5$\mu g/m^3$ higher annual $PM_{2.5}$ corresponded to a telomere-age equivalence loss of 4 years. Recently, in 211 newborn twins from the East Flanders Prospective Twin Survey, placental telomere lengths were inversely associated with traffic intensity within a buffer of 100m surrounding maternal residence, while placental telomeres were longer in association with maternal residential surrounding greenness [125].

Similar with the observed associations between telomere length and air pollution exposure, both positive and negative associations between short-term and chronic exposure to PM and mtDNA content have been reported. Hou *et al*. [84] found an increase in mtDNA content in relation to personal PM_{10} and PM_1 in steelworkers while they found a decrease in mtDNA content in office workers and truck drivers in association with short-term PM_{10} and elemental carbon exposure. These opposite results indicate that mtDNA may react in a different way under different environmental circumstances [99]. A study in newborns [100] revealed that a 10 μg/m³ increment in PM_{10} exposure during the last trimester of pregnancy was associated with a 17.4% decrease in placental mtDNA content. A significantly decrease in mtDNA content was observed after long-term (1 year) exposure to $PM_{2.5}$ whereas a significantly increase in mtDNA was observed for short-term (1 month and 1 week) exposure windows of $PM_{2.5}$ in an elderly population [119]. Recently, in 675 elderly men from the Normative Aging Study an increase of mtDNA was observed in association with BC exposure (5days to 28days) [126].

Telomeres and the Exposome

The exposome encompasses all exposures over the entire life [127, 128] Aside from air pollution, telomere length is associated with a broad spectrum of environmental factors including life stress [129], mediterranean diet [130], education [131, 132], smoking status [48, 52], obesity [133], sleep quality [134], childhood maltreatment [135]. Therefore, telomere maintenance may be a proxy for assessing the “exposome”. Telomere research has largely been focused on studies in adults, although, even at birth, newborn cord and placental telomere lengths have been associated with environmental factors such as prenatal stress [136, 137], maternal residential traffic exposure [125], intrauterine tobacco exposure [138], and *in utero* cadmium

exposure [139], indicating that from early-life (even intrauterine life) onwards these environmental factors change telomere biology. Since factors of the exposome are already present during the intrauterine life and may contribute to telomere length setting of newborns, this might have a large impact on later life expectations and may impact overall life quality and the development of age-related diseases as well.

Conclusions

In addressing the epidemic of non-communicable disease, identification of factors that influence healthy or unhealthy aging is pivotal in prevention. Aging is a complex phenotype. Cellular aging, as exemplified by shorter telomere length, is associated with higher exposure to particulate air pollution during adulthood and in newborns with lower maternal residential greenness.

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Compliance with Ethics Guidelines

Conflict of Interest

Dries S. Martens and Tim S. Nawrot declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Table 1. Occupational studies describing the association between air pollution exposure and telomere length

Authors	Study Population	n	Age, y	% Men	Telomere method and tissue	Exposure time	Exposure, concentration in µg/m³	% change or β, (95%CI), p value	Adjustments
Wong et al.,2014 [140]	Boilermakers Study: Population of males, Massachusetts, USA	48	39.3 ±12.8[a] at baseline	100%	qPCR, WBC	Career exposure, last year and last month exposure	Career cumulative $PM_{2.5}$: 713.7 ± 1457.9[a] mg/m³hr Year cumulative $PM_{2.5}$: 74.0 ± 77.3[a] mg/m³hr Month cumulative $PM_{2.5}$: 8.5 ±10.2[a] mg/m³hr	Career: β:-0.021 (-0.048 to 0.006), p>0.05 Year: β:-0.022 (-0.053 to 0.009), p>0.05 Month: β:-0.04 (-0.08 to 0.001), p≤0.05	% neutrophils, % lymphocytes, % monocytes, % eosinophils, current smoking status, age at baseline blood drawn, BMI, years as boilermaker
Hou et al., 2012 [121]	The Beijing Truck Driver Air Pollution Study: Population of truck drivers and indoor office workers, Beijing, China	120	Truck drivers: 33.5±5.7[a] Office workers: 30.27±7.96[a]	67%	qPCR, WBC	Personal $PM_{2.5}$ and EC exposure for 8-h Ambient PM_{10} exposure: Examination day, 1day, 1-2 days, 1-5 days, 1-7 days, 1-10 days, 1-14 days	Personal $PM_{2.5}$ for truck drivers *vs* office workers: 126.8±68.8[a] *vs* 94.6±64.9[a] Personal EC for truck drivers *vs* office workers: 17.3±6.7[a] vs 13.1±4.0[a] Ambient PM_{10} for truck drivers *vs* office workers: - *Examination day*: 116.7±50.2[a] *vs* 123.5±50.1[a] - *1-day*: 121.5±47.8[a] *vs* 119.5±51.2[a] - *1-2 days*: 121.6±38[a] *vs* 119.3±40.3[a] - 1-5 days: 119.5±26.9[a] *vs* 118.2±25.6[a] - 1-14 days: 119.9±18.7[a] *vs* 121.7±17.8[a]	$PM_{2.5}$: 5.2% (1.5% to 9.1%), p=0.007 for each IQR increase Personal EC: 4.9% (1.2% to 8.8%), p=0.01 for each IQR increase PM_{10}: - *Examination day*: 7.7% (3.7% to 11.9%), p<0.001 - *1-day*: 8.4% (4.0% to 13.0%), p<0.001 - *1-2 days*: 8.1% (3.1% to 13.3%), p=0.002 - 1-5 days: -1.3% (-6.3% to 4.1%), p=0.64 - 1-14 days: -9.9% (-17.6%, -1.5%), p=0.02	Age, gender, BMI, number cigarettes smoked during examination time, day of the week, usage central heating, time used for commuting to work, temperature, dew point
Dioni et al., 2011 [120]	Population of steel workers, Brescia, Italy	57	44 (27-55)[b]	100%	qPCR, WBC	PM_{10} and PM_1 exposure for 3 days	PM_{10}[a]: 262±272 PM_1[a]: 8.0±7.7	PM_{10}: β: 0.30 (0.11 to 0.49), p=0.002 for each increase of 285 µg/m³ (10^{th}-90^{th}perc) PM1: β: 0.29 (0.01 to 0.57), p=0.042 for each increase of 11.05 µg/m³ (10^{th}-90^{th}perc)	Age, BMI, pack-years of smoking, % lymphocytes

Hoxha et al., 2009 [123]	Population of street traffic officers and office workers (referents), Milan, Italy	134	< 30 y: 39% 30-40 y: 37% > 40 y: 24%	63%	qPCR, WBC	One work shift (7 hours)	Airborne benzene: Referents: 13.0 (2.0-115.1)[b] Traffic officers: 31.8 (9.0-315.7)[b] Airborne toluene: Referents: 43.4 (6.0-368.0)[b] Traffic officers: 128.7 (24.4-1710.7)	Benzene: -6.4% (-10.4% to -2.1%), p=0.004 for each IQR (11.2 µg/m³) increase Toluene: -6.2% (-10.4% to -1.7%), p= 0.008 for each IQR (25.7 µg/m³) increase	Age, gender, smoking, pack years of smoking

[a] Mean±SD
[b] Mean (range)

Table 2. Population-based studies describing the association between air pollution exposure and telomere length

Authors	Study Population	n	Age, y	% Men	Telomere method and tissue	Exposure time	Exposure, concentration in µg/m³	% change or β (95%CI), p value	Adjustments
Pieters *et al.* 2015 [119]	Population of elderly, all non-smokers, Flanders, Belgium	166	70.6 ± 4.7[a]	46%	qPCR, WBC	One year exposure, last month and last week	Annual $PM_{2.5}$: 21.1±1.76[a]	Last year: -16.8% (-26.0% to -7.4%), p=0.0005 for each 5µg/m³ increment Last month: 17.5% (9.6% to 26.0%), p=0.0001 for each 5µg/m³ increment Last week: 1% (-2.4% to 4.9%), p=0.5 for each 5µg/m³ increment	Gender, age, BMI, socio-economic status, statin use, past smoking status, WBC count, % neutrophils
Xia et al., 2015 [141]	Type 2 diabetes patients, Shangai, China	35	65 ±8[a]	49%	qPCR, WBC	Lag 0, 1, 2, 3 and 4-7 days prior blood drawn	24-h PMC: PMC< 1µm: 39.6±21.7[a] PMC 1-2.5µm: 6.6±4.5[a] PMC 2.5-10µm: 21.1±18.5[a]	All lags not significant $PM_{2.5}$: 0.11% (-0.97% to 1.19%), p=NS for each IQR increment in 24-h average air pollutant	Temperature and relative humidity on concurrent day and previous 3 days, day of the week, month, age, gender, BMI, income, duration T2DM, statin use, random intercept for correlation among multiple measurements
Shan et al., 2014 [142]	Population of females, Sichuan, China	21	59 (38-85)[b]	0%	qPCR, Buccal cells	24-h exposure	24-h personal $PM_{2.5}$ in low *vs* high exposed: 39±11[a] *vs* 101±37[a] 24-h personal BC in low *vs* high exposed: 2.6±1.8[a] *vs* 14.9±11.2[a]	Low *vs* high exposed group -43% (-113% to 28%) p=NS	Age, BMI
McCracken et al., 2010 [124]	Normative Aging Study (NAS): Population of males, all never smokers, Massachusetts, USA	165	73.6 ±7.1[a]	100%	qPCR, WBC	365 days before each blood drawn	Annual BC[a]: 0.32[a] ± 0.2[a]	-7.6 % (-12.8% to -2.1%) for each IQR (0.25 µg/m³) increase. p= 0.008	Age at baseline, age change between measurements, year, BMI, WBC count, % neutrophils, % lymphocytes, statin use, diabetes, fasting blood glucose, education, urbanity, census-tract socioeconomic status, inverse probability of follow-up response weighting

[a] Mean±SD

[b] Mean (range)

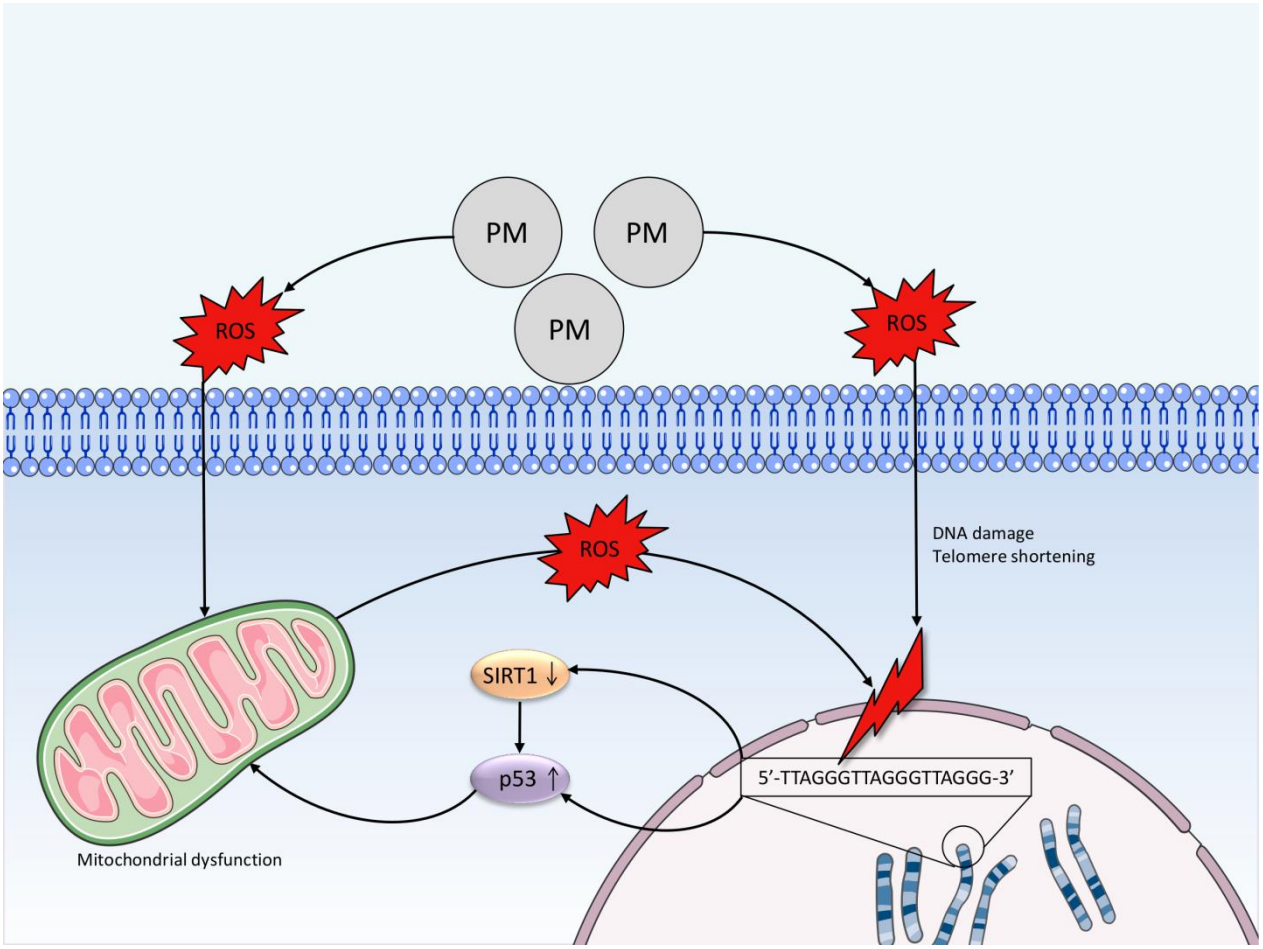

Fig 1. The air-pollution induced telomere-mitochondrial aging hypothesis. Particulate matter exposure induces the formation of ROS at particle surfaces by Fenton like reaction. Besides direct ROS formation from the particle surface, PM induces elevated ROS levels due to altered function of NADPH-oxidase, mitochondria and activation of inflammatory cells. The cellular presence of ROS induces DNA damage and induces single-strand breaks at the G rich telomeres leading to telomere shortening and induces cellular aging. Subsequently DNA damage and telomere shortening is associated with the increase of p53 production. Elevated levels of p53 leads to increased mitochondrial dysfunction, leading to accelerated cellular aging. Under normal conditions high levels of SIRT1 reduces the production of p53. Telomere damage and shortening have been associated with the suppression of SIRT1 which is associated with high p53 levels. Besides the direct effects of ROS production by particulate matter on telomeres and subsequently the p53 pathways, particulate matter has also direct effects on mitochondria by inducing mitochondrial dysfunction, which leads to mitochondrial ROS production. This mitochondrial induced ROS production may alternatively also influences telomere shortening and again inducing the p53 pathway cascade. These mechanisms indicates a close relationship between mitochondrial and telomere function in the aging phenotype affected by exposure to particulate matter.