

REVIEW  
ARTICLE

## Smoking and health: association between telomere length and factors impacting on human disease, quality of life and life span in a large population-based cohort under the effect of smoking duration

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biological aging,  
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in white blood cells),  
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tobacco smoking

**ABSTRACT**

Reactive oxygen species (ROS) are of primary importance as they cause damage to lipids, proteins, and DNA either endogenously by cellular mechanism, or through exogenous exposure to environmental injury factors, including oxidation insult factors, such as tobacco smoke. Currently 46.3 million adults (25.7 percent of the population) are smokers. This includes 24 million men (28.1 percent of the total) and more than 22 million women (23.5 percent). The prevalence is highest among persons 25–44 years of age. Cigarette smokers have a higher risk of developing several chronic disorders. These include fatty buildups in arteries, several types of cancer and chronic obstructive pulmonary disease (lung problems). As peripheral leukocytes have been the main target of human telomere research, most of what is known about human telomere dynamics in vivo is based on these cells. Leukocyte telomere length (TL) is a complex trait that is shaped by genetic, epigenetic, and environmental determinants. In this article, we consider that smoking modifies leukocyte TL in humans and contributes to its variability among individuals, although the smoking effect on TL and its relation with other metabolic indices may accelerate biological aging and development of smoking-induced chronic diseases in a large human population-based cohorts with smoking behavior. Recent studies confirmed that individuals with shorter telomeres present a higher prevalence of arterial lesions and higher risk of cardiovascular disease mortality. This study originally suggests that efficient therapeutic protection of TL and structure in response to stresses that are known to reduce TL, such as oxidative damage or inflammation associated with tobacco smoking, would lead to better telomere maintenance. Recently, we have discovered the potential use of telomere-restorative imidazole-containing dipeptide (non-hydrolyzed carnosine, carbinine) based therapy for better survival of smokers. We conclude that a better therapeutic or nutritional maintenance of TL may confer healthy aging in smokers and exceptional longevity in regularly ROS-exposed human survivors.

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## INTRODUCTION

Recent studies have found that nonsmoking women are more susceptible to DNA damage than nonsmoking men when exposed to an environmental carcinogen. Secondhand smoke is a known risk factor for lung cancer, which still kills more women and men than any other single cancer. *PARADE* – page 16 – October 9, 2005

Globally, tobacco use is associated with five million deaths per annum and is regarded as one of the leading causes of premature death [1]. Cigarette smoking is the most important risk factor for young men and women. It produces a greater relative risk in persons under age 50 than in those over 50 (Figure 1) [2].

Currently 46.3 million adults (25.7 percent of the population) are smokers [3]. This includes 24 million men (28.1 percent of the total) and more than 22 million women (23.5 percent). The prevalence is highest among persons 25–44 years of age. Cigarette smoking is the most important preventable cause of premature death in the United States [4]. It accounts for more than 440 000 of the more than 2.4 million annual deaths [4]. It is estimated that over 500 000 EU citizens die each year from smoking-related ailments [2]. The nationwide study comparing different control groups in a population-based case–control study, to assess the association between smoking and death from various cancers in Chinese men. It shows that tobacco smoking is associated with a moderate, but highly significant, increase in the risk of death from various cancers [5]. The frequency of smoking has decreased in the US population, whereas it has increased in Eastern Europe and parts of the developing world [6,7].

In viewing the problems and complications related to tobacco use, recognition of the contributions of passive smoking is increasing, as described in a 1992 report by the Environmental Protection Agency (EPA) [8]. Reports indicate that environmental tobacco smoke is composed of mainstream smoke exhaled by the smoker and sidestream smoke emitted from the burning tobacco between puffs [8,9]. Sidestream smoke is the main component of environmental tobacco smoke. The great majority of smoke emitted from a lit cigarette is sidestream smoke rather than smoke that is actively inhaled. Sidestream and mainstream smoke contain many of the same air contaminants. Sidestream smoke has more particles with smaller diameters, and these particles are therefore more likely to be deposited in the most distant regions of the lungs [9]. Environmental tobacco smoke is a major source of indoor-air contam-



### Tobacco and Disease

- Tobacco use causes—
  - Cancer
  - Heart disease
  - Lung diseases (including emphysema, bronchitis, and chronic airway obstruction)
- Cigarette smoking increases the length of time that people live with a disability by about 2 years.
- For every person who dies from a smoking-related disease, 20 more people suffer with at least one serious illness from smoking.

### Tobacco and Death

#### Worldwide

- Tobacco use causes more than 5 million deaths per year.
- Current trends show that tobacco use will cause more than 8 million deaths annually by 2030.
- On average, smokers die 13 to 14 years earlier than nonsmokers.

#### In the United States

- Cigarette smoking is the leading preventable cause of death.
- Cigarette smoking is responsible for about one in five deaths annually, or about 443,000 deaths per year.
- An estimated 49,000 of tobacco-related deaths are the result of secondhand smoke exposure.

### Tobacco-related costs and expenditure in the US

#### Costs of Smoking

- Annually, in the United States, **cigarette smoking** costs more than \$193 billion: \$193 billion = \$97 billion in lost productivity + \$96 billion in health care expenditures

Overall data are reviewed from Ref. [28].

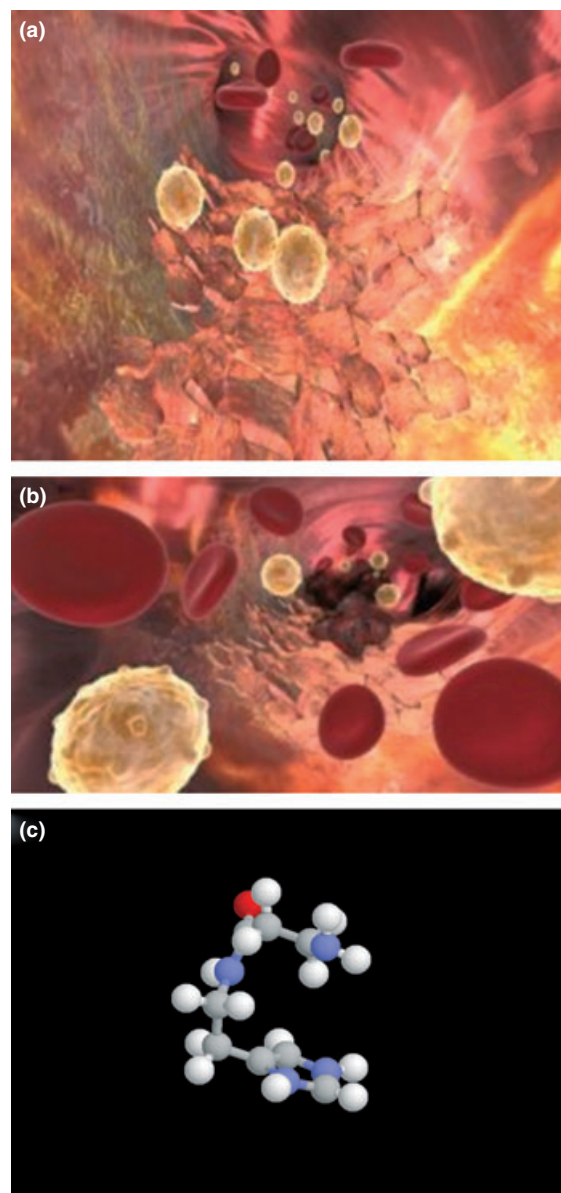
**Figure 1** Morbidity and mortality (related to tobacco use).

inants, and thus some unintentional inhalation by nonsmokers is virtually unavoidable. As there appears to be no evidence of a safe threshold level, nonsmokers exposed to environmental tobacco smoke appear to be at increased risk for the same problems and complications recognized in smokers. Environmental tobacco smoke has been classified as a known human lung carcinogen, or a 'group A' carcinogen, under the EPA's system of carcinogen classification.

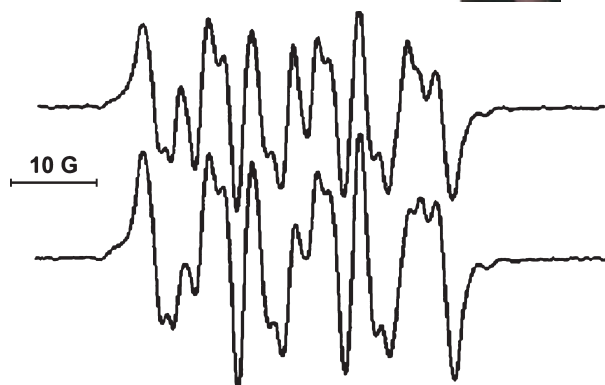
Workplace smoking bans were reported less often among those in technical positions (OR = 0.64, CI: 0.50, 0.82) and among skilled workers (OR = 0.53, CI: 0.32, 0.88) than among professional workers. Workplace smoking bans are in place for most workers in these countries. Having a home smoking ban was based on smoking behavior, demographics, beliefs, and personal preference [10].

Cigarette smokers have a higher risk of developing several chronic disorders (*Figure 2a-c*). These include fatty buildups in arteries, several types of cancer and chronic obstructive pulmonary disease (lung problems). According to World Health Organization figures, 30% of all cancer deaths, 20% of all coronary heart diseases and strokes, and 80% of all chronic obstructive pulmonary disease are caused by cigarette smoking (CS) [11,12]. Compared with nonsmokers, smokers are 15 times more likely to develop lung cancer, 11 times more likely to develop chronic lung disease, and twice as likely to have acute myocardial infarctions (AMIs) [13]. Atherosclerosis (buildup of fatty substances in the arteries) is a chief contributor to the high number of deaths from smoking [13,14]. Many studies detail the evidence that CS is a major cause of coronary heart disease, which leads to heart attack [11-18]. Cigarette smoking increases the risk of coronary heart disease by itself. When it acts with other factors, it greatly increases risk. Smoking increases blood pressure, decreases exercise tolerance, and increases the tendency for blood to clot. Smoking also increases the risk of recurrent coronary heart disease after bypass surgery [19].

The mechanisms involved in the pathological consequences of smoking are still under intensive study. Cigarette smoke is a complex dynamic mixture of more than 4800 chemicals distributed between the particulate and vapor phases [20,21]. Cigarette smoke contains a large variety of compounds, including many oxidants and free radicals that are capable of initiating or promotes oxidative damage (*Figure 3*). Also, oxidative damage may result from ROS generated by the increased and activated phagocytes following CS [20]. It is widely



**Figure 2** Atherosclerosis disease associated with smoking. (a) When LDL cholesterol enters the weakened artery wall, it changes and can lead to inflammation. Over time, this creates 'a fatty deposit' known as an 'arterial plaque' by a process called atherosclerosis, a sort of 'furring up of the arteries'. (b) A stable plaque can continue to grow, slowly reducing blood flow over time, leading to the chest pain of angina, but does not necessarily completely block the artery. Sometimes even a small plaque can become unstable and rupture. A clot may form and this can completely block the flow of blood. (c) Lesions associated with smoking can be prevented and therapeutically treated with the oral supplements including carnosine or nonhydrolyzed carnosine. 3D ball-and-stick claw-like molecular structure of carnosine is presented, able to bind and scavenge heavy metals, lipid peroxidation products and act as a telomerase inducer agent (for details, see Refs. [28,131]).

**(a) Electron spin resonance technique (ESR)**

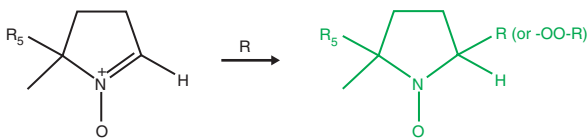
Free radical species generated in tobacco smoke condensate per puff monitored with ESR technique using DMPO as a spin trapping agent.

Spin trapping EPR

Catching short-lived radical species

Spin trap catch the radical and change into stable nitroxide radical

Typical spin traps: DMPO and DEPMPO

**(b) Chemiluminescence technique**

**Figure 3** Monitoring systems of free radical species generated in tobacco smoke condensate. Cigarette smoke is a complex dynamic mixture including in the range of 4800 chemicals distributed between the particulate and vapor phases with most reactive oxygen species (ROS) generated per puff. (a) Electron spin resonance technique (ESR). Free radical species generated in tobacco smoke condensate per puff monitored by ESR technique using DMPO as a spin trapping agent. (b) Chemiluminescence technique employing luminol as a detector dyer. For details, see Ref. [28].

accepted that cigarette smoke is capable of causing oxidative damage in DNA, either directly or through generation of ROS (Reviewed in Reference 21).

However, information obtained from in vivo studies is inconclusive. Contrary to expectations, the levels of lipid peroxidation (LPO) products were found to be decreased or unchanged in the lungs of chronically smoked rats. Metabolic adaptation, such as accumulation of vitamin E in the lung, and increased activities of superoxide dismutase (SOD) in alveolar macrophages and pulmonary tissues of chronically smoked animals may enable smoked subjects to counteract oxidative stress and to resist further damage to smoke exposure. However, it is also possible that the metabolic adaptation may be secondary to inflammatory response and injury repair process following smoking exposure [20].

In vitro studies are generally supportive of the hypothesis that cigarette smoke can initiate or promote oxidative damage. Because of its high content of oxidants, the cigarette smoke is bound to cause a prooxidant/antioxidant imbalance in the blood plasma and tissues of smokers. The occurrence in cigarette smoke of numerous toxic compounds and oxygen reactive species has been demonstrated [22,23] and in vitro studies showed that oxidants in the gas phase of cigarette smoke induced LPO in low-density lipoproteins (LDL) [24]. Furthermore, a large body of evidence accumulated in the past 10 years or so has conclusively linked LDL oxidation in the arterial wall to the onset of the process that leads to plaque formation [25,26].

In one published study, subject cohorts were selected from an apparently healthy population living in urban areas, comprising 200 subjects aged 18–80 years, half of whom were smokers [27]. In smokers aged 18–45 years, the changes of the plasma pro-oxidant parameters (i.e., lipid peroxides, leukocyte activation, and the antioxidant ones [thiol concentration, total antioxidant capacity]) were not significantly different from those of the age-matched controls, whereas in the 46–80 age group they

were. In smokers, both antioxidant erythrocyte enzymes, glutathione peroxidase (GSH-Px) and SOD, exhibited increased activity in the 18–45 age group and decreased activity in the 46–80 age group. The differences in enzyme activity between the smoking and nonsmoking groups were highly significant for SOD in all ages, whereas for GSH-Px the difference in activity was significant only in the case of older smokers. These findings would suggest that a process of adaptation takes place in younger smokers, in whom the antioxidant systems are able to counteract the oxidant factors, whereas in older smokers this process is no longer occurring and the plasma and tissues are under permanent oxidative stress. The results of this study clearly demonstrated [27] that a prooxidant/antioxidant imbalance exists in the blood of smokers, and the determination of leukocytes stimulation index may be a useful and simple way of assessing the oxidative stress status of these individuals. A hypothesis regarding a possible mechanism linking CS to the development of coronary heart disease is presented [27].

We have previously demonstrated that smoking individuals in the clinical studies experience the significantly reduced longevity and have generally been spared earlier with age-related diseases associated with oxidative stress, such as cardiovascular disease, lung diseases, and cancer, which are largely responsible for mortality in the elderly, and that these features are heritable [28]. Because studies on individuals with a normal life span suggest that common age-related diseases and a shorter life span are associated with shorter telomeres (reviewed in Reference 29), we consider in this review article the information about the telomere length (TL) in blood leukocytes among subjects under smoking duration to investigate whether the smoking population can survive during therapeutic strategies targeting protection or elongation of telomeres.

We now consider a TL maintenance as an indicator of life span in smoking, survival, and/or reflecting chronologically old age. In addition, we review the approaches to assess family history, metabolic factors of longevity associated with protection against cumulative oxidative stress and genetic basis of TL maintenance. We considered the TL as a trait that would be inherited by the offspring of the smoker survivors and/or associated with the status of aging-related diseases. Our overall hypothesis is that therapeutic maintenance of TL may indicate generalized genomic integrity, which, in turn, may have a profound influence on survival and life span of smokers, health, and aging in humans.

## **CIGARETTE SMOKING, CUMULATIVE OXIDATIVE STRESS AND CHRONIC-ASSOCIATED DISEASES**

This review alerts that CS is a major health hazard particularly for the cardiovascular system and cancer. The mechanisms involved in CS-related cardiovascular dysfunction have been largely debated. Components of cigarette smoke have been shown to damage vascular endothelium [30], and endothelial injury is considered a primary antecedent to atherosclerosis [30,31]. The adverse effects of smoking are also related to its effects on coronary vaso-occlusive factors, such as platelet aggregation, vasomotor reactivity, and a prothrombotic state, [32] and factors such as carbon monoxide production, increased plasma viscosity, and fibrinogen levels [31]. Smoking is a major risk factor for coronary vasospasm [33].

Cigarette smoking increases inflammation, thrombosis, and oxidation of LDL. Oxidative stress is caused by an imbalance between ROS production and a biological system's ability to readily detoxify these reactive intermediates or easily repair the resulting damage. Recent experimental and clinical data support the hypothesis that cigarette smoke exposure increases oxidative stress as a potential mechanism for initiating cardiovascular dysfunction. Cardiac myocytes and other long-lived postmitotic cells show dramatic smoke-related alterations that mainly affect the mitochondria and lysosomal compartment [34].

Atherosclerosis is a chronic inflammatory disease of the arterial wall [35–38] with enormous epidemiological relevance [39,40]. Sound evidence has been generated that oxidative stress is one of the most potent inductor of vascular inflammation in atherogenesis [41]. ROS are known to change the oxidation-reduction (redox) state of the exposed cells, and it is known that several inflammatory genes and the related transcription factors are regulated through redox-sensitive mechanisms [36]. Nuclear factor (NF)- $\kappa$ B was the first eukaryotic transcription factor shown to respond directly to oxidative stress. A huge amount of experimental data supports the activation of the transcription factor NF- $\kappa$ B as a key redox-sensitive event associated with vascular dysfunction (reviewed in [42]). NF- $\kappa$ B intervenes in the transcription of a large number of inflammatory genes coding for cytokines, chemokines, and adhesion molecules [43].

Cigarette smoke can be divided into two phases: tar- and gas-phase smoke. Both phases contain high

concentrations of ROS, nitric oxide, peroxyxynitrite, and free radicals of organic compounds [44,45]. In addition to these short-lived, highly reactive substances, previous studies have shown that aqueous cigarette tar extracts also contain pro-oxidant substances that have the potential to increase cellular production of ROS [46]. Thus, it has been hypothesized that water-soluble components of cigarette smoke that are likely to reach the systemic circulation can directly promote oxidative stress in vasculature and blood cells (*Figure 3*) [44,46]. Modified low-density lipoprotein (LDL) induces ROS production by vascular cells. It is unknown whether specific oxidized components in these LDL particles such as oxidized-1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (ox-PAPC) can stimulate ROS production [35,47,48]. The phospholipid 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (PAPC) is a major component of cell membranes and lipoproteins. Oxidation products of PAPC (oxPAPC) are found in cells during inflammation, in membranes of apoptotic cells, as well as in oxidized low-density lipoprotein and are considered sensitive markers of oxidative stress (reviewed in References 34,46). Furthermore, ox-PAPC have been shown to induce ROS production in vascular cells that appears to be mediated largely by NADPH-oxidase activity [35,48].

Oxygen-centered free radicals generated from CS have been known to trigger lung inflammation and, thereby, progression of airway disease [22,49]. The airway epithelium is the primary target for inhaled oxidants. Epithelial lining fluid is the first point of contact between the lung and inhaled environmental oxidants, such as CS and ozone. Oxidant challenge to the airway and alveolar epithelium is normally neutralized by the antioxidants in the epithelial lining fluid.

Reactive oxygen species, either directly or via the formation of (LPO) products, may play a role in respiratory diseases in enhancing inflammation through the activation of stress kinases (c-Jun activated kinase, extracellular signal-regulated kinase, p38) and redox-sensitive transcription factors, such as NF-kappaB and activator protein-1 [49]. This results in increased expression of a battery of distinct pro-inflammatory mediators. Oxidative stress activates NF-kappaB-mediated transcription of pro-inflammatory mediators either through activation of its activating inhibitor of kappaB-alpha kinase or the enhanced recruitment and activation of transcriptional co-activators. Enhanced NF-kappaB-co-activator complex formation results in targeted increases in histone modifications,

such as acetylation leading to inflammatory gene expression.

Emerging evidence suggests the glutathione redox couple may entail dynamic regulation of protein function by reversible disulfide bond formation on kinases, phosphatases, and transcription factors. Oxidative stress also inhibits histone deacetylase activity and in doing so further enhances inflammatory gene expression and may attenuate glucocorticoid sensitivity. The antioxidant/anti-inflammatory effects of thiol molecules (glutathione, N-acetyl-L-cysteine and N-acetylcystein, erdoesteine), dietary polyphenols (curcumin-diferuloylmethane, catechins/ quercetin and resveratrol), specific spin traps, such as alpha-phenyl-N-tert-butyl nitron, a catalytic antioxidant (extracellular SOD mimetic, SOD mimetic M40419 and SOD, and catalase manganic salen compound, eukarion-8), porphyrins (AEOL 10150 and AEOL 10113) and theophylline have all been shown to play a role in either controlling NF-kappaB activation or affecting histone modifications with subsequent effects on inflammatory gene expression in lung epithelial cells [49].

Oxidative stress occurs if antioxidant levels in the epithelial lining fluid are inadequate to neutralize inhaled oxidants/free radicals. Reduced glutathione (GSH), the most abundant cellular thiol antioxidant, plays a critical role in the maintenance of intracellular redox balance in epithelial lining fluid and is involved in the detoxification reaction through direct conjugation or by enzyme-catalyzed reactions [50]. This essential antioxidant has been reported to be depleted in the airways in several pulmonary disorders, such as chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome, and cystic fibrosis, suggesting a role for oxidative stress in the pathogenesis of these chronic inflammatory lung diseases [50,51]. Both GSH and gamma-glutamylcysteine synthetase (gamma-GCS) expression are modulated by oxidants, phenolic antioxidants, and inflammatory and anti-inflammatory agents in lung cells. Gamma-GCS is regulated at both the transcriptional and the post-transcriptional levels [50,51]. Knowledge of the mechanisms of GSH regulation in the lungs could lead to the development of novel therapies of CS-induced damages based on the pharmacological or genetic manipulation of the production of this important antioxidant in lung inflammation and injury [51].

Oxidative stress is accepted as a critical pathophysiological mechanism in different frequent human pathologies, including cancer. In fact, ROS can cause protein,

lipid, and DNA damage, and malignant tumors often show increased levels of DNA base oxidation and mutations. Different lifestyle- and environmental-related factors (including, e.g., tobacco smoking, diet, alcohol, ionizing radiations, biocides, pesticides, viral infections) and other health-related factors (e.g. obesity or the aging process) may be procarcinogenic. In all these cases, oxidative stress acts as a critical pathophysiological mechanism. Nevertheless, it is important to remark that, in agreement with present knowledge, oxidative/nitrosative/metabolic stress, inflammation, senescence, and cancer are linked concepts that must be discussed in a coordinated manner [52].

Genome instability is a hallmark of most human cancers. Although a mutator phenotype is not required for tumorigenesis, it can foster mutations that promote tumor progression. Indeed, several inherited cancer-prone syndromes are because of the mutations in DNA repair pathways. However, sporadic tumors are usually proficient in DNA repair, making it unlikely that unrepaired lesions are a major source of genome instability in sporadic cancers [53]. Collapse in telomere function can explain a significant portion of the genetic instability in tumors [54].

Normal human cells undergo a finite number of cell divisions and ultimately enter a nondividing state called replicative senescence. It has been proposed that telomere shortening is the molecular clock that triggers senescence [55].

Telomeres are DNA capping structures that protect the ends of eukaryotic chromosomes. *In vitro* studies in mammalian cells suggest that telomere shortening triggers cellular senescence or apoptosis, depending on cell type [56–59]. Studies in humans have shown that telomeres shorten with aging in various mitotic tissues and cell types [60–62]. The rate of telomere attrition is slower in long-lived mammals compared with short-lived ones [63]. Senescent cells accumulate with increasing age *in vivo* [64] and are thought to play an important role in organismal aging [65], which is characterized by physiologic and metabolic decline [59] and increasing susceptibility to several diseases associated with death [66]. Thus, it is likely that telomere shortening may be mechanistically linked to organismal life span, especially in population of smokers. The published results support the hypotheses that telomere attrition may be related to diseases of aging through mechanisms involving oxidative stress associated with CS, inflammation, and progression to cardiovascular disease (CVD) [66].

Since original publications time, the structure of mammalian telomeres has been analyzed, the consequences of telomere dysfunction have been determined, a mouse model for cancer-relevant aspects of telomere biology has been developed, and the nature and magnitude of cancer genome rearrangements have been revealed. In light of these developments, this is an opportune time to revisit the conjecture that telomere dysfunction contributes to genome instability in human cancer.

### **TELOMERE LENGTH AND CUMULATIVE OXIDATIVE STRESS ASSOCIATED WITH CIGARETTE SMOKING**

Telomeres consist of the TTAGGG tandem repeats at the ends of chromosomes and are known to protect these regions from degradation and DNA repair activities [67]. A complex formed by six telomere-specific proteins associates with this sequence and protects chromosome ends (*Figures 4 and 5*) [67]. During normal aging, the gradual loss of telomeric DNA in dividing somatic cells can contribute to replicative senescence, apoptosis, or neoplastic transformation [68].

A tight link exists between TL and both population doublings of a cell culture and age of a given organism. The more population doublings of the cell culture or the higher the age of the organism, the shorter the telomeres. The proposed model for telomere shortening, called the end replication problem, explains why the telomere erodes at each cellular turnover. TL is regulated by a number of associated proteins through a number of different signaling pathways [69].

Three shelterin protein subunits, TRF1, TRF2, and POT1 directly recognize TTAGGG repeats [67]. They are interconnected by three additional shelterin proteins, TIN2, TPP1, and Rap1, forming a complex that allows cells to distinguish telomeres from sites of DNA damage. Without the protective activity of shelterin, telomeres are no longer hidden from the DNA damage surveillance and chromosome ends are inappropriately processed by DNA repair pathways. How does shelterin avert these events? The current data argue that shelterin is not a static structural component of the telomere. Instead, shelterin is emerging as a protein complex with DNA remodeling activity that acts together with several associated DNA repair factors to change the structure of the telomeric DNA, thereby protecting chromosome ends [67].

Telomeres progressively shorten with each cell division in cultured primary human cells [70] until a



**Figure 4** The concept of life span studies in smokers based on the telomere biology. Telomeres (telomeric-FISH), the TTAGGG tandem repeats at the ends of human chromosomes, undergo attrition with each division of somatic cells in culture and their length is, hence, an indicator of replicative history and replicative potential of these cells affected by oxidation. This feature of telomere biology is at the core of any smoker's survival and, at the cellular level, telomeres serve as a mitotic clock. In human beings, telomere length (TL) is heritable, relatively short, highly variable, and with regard to replicating somatic cells, inversely related to donor age. Because of the attempt to prolong the life span of humans and their short telomeres, attrition in TL may be a major determinant of human aging not only at the cellular level, but also at the organ and at the systemic levels. TL is a target of therapeutic protection with carbinine and nonhydrolyzed carnosine supplements as telomerase inducers. These nutritional formulations are effective to increase the life span and survival of smokers. For details, see Ref. [28] and text of the current study.

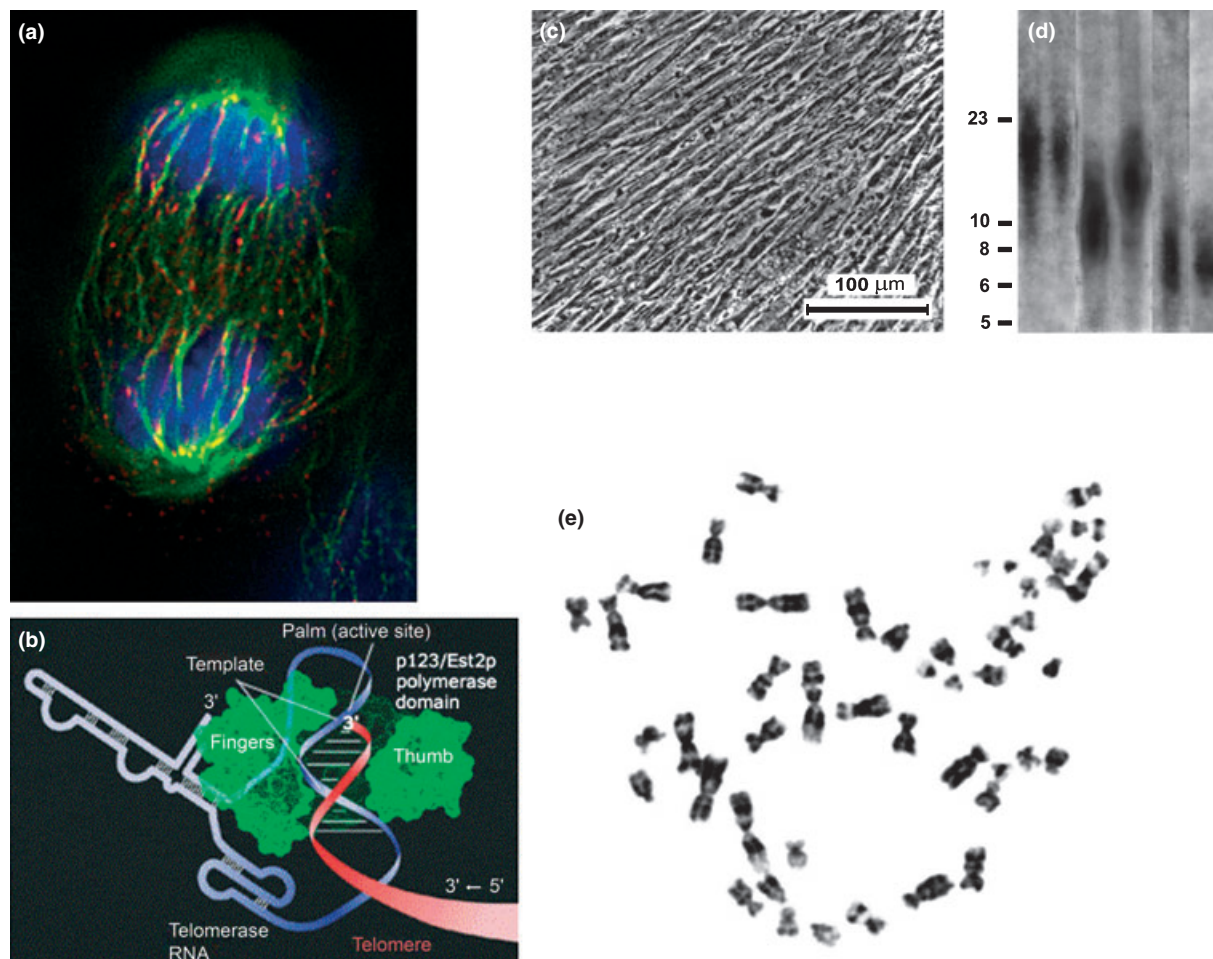
critically shortened length is achieved, upon which the cells enter replicative senescence [71]. Telomeres prevent fusion of chromosomal ends, nucleolytic decay, and atypical recombination [72]. Telomeric repeats in normal somatic tissue shorten by approximately 30–200 bp after each mitotic division eroding chromosomal termini [73]. Telomere erosion below a certain length can trigger crisis. The rate of telomere shortening per cell division is not constant and may be a function of oxidative stress and antioxidant defenses [74]. Cells continually experience stress and damage from exogenous and endogenous sources, and their responses range from complete recovery to cell death. Proliferating cells can initiate an additional

response by adopting a state of permanent cell cycle arrest that is termed cellular senescence. Understanding the causes and consequences of cellular senescence has provided novel insights into how cells react to stress, especially genotoxic stress, and how this cellular response can affect complex organismal processes such as the development of cancer and aging [71].

The cell phenotypes of senescence and crisis operate to circumscribe the proliferative potential of mammalian cells, suggesting that both are capable of operating *in vivo* to suppress the formation of tumors.

Telomere length is emerging as a biomarker for aging and survival [75,76]. TL varies between individuals of





**Figure 5** (a, b) Telomeres and telomerase structure at work. The telomerase enzyme complex consists of an RNA component and a protein component. The protein component's catalytic subunit acts as a reverse transcriptase (green), using telomerase RNA (blue/white) as a template for the addition of telomeric repeat sequences to the telomere DNA strand (red). (c, d, e) The consequences of telomerization: c- bar, 100  $\mu\text{m}$ . High-density monolayer of telomerase-immortalized human skin fibroblasts; d- telomere length (TL) of various clones of telomerase-immortalized human fibroblasts: right column- original fibroblasts; other columns- different clones. e- preservation of diploid karyotype of telomerase-immortalized human fibroblasts. The patented family of antioxidant imidazole-containing dipeptides has been developed targeting the induction of telomerase and protection from telomere attrition. The dipeptides were formulated in nutritional supplements to increase survival rate and for the health care protection in smokers under the terms of smoking duration (see Refs. [28,131]).

the same age, is influenced by DNA-damaging factors such as oxidative stress, and is heritable. The studied sample included 356 men and 551 women, aged 18–92 years, from large Amish families. Mean TL in leukocytes was measured by quantitative PCR (mean:  $6198 \pm 1696$  bp). The  $h^2$  of TL was  $0.44 \pm 0.06$  ( $P < 0.001$ ), after adjusting for age, sex, and TL assay batch. As expected, TL was negatively correlated with age ( $r = -0.40$ ;  $P < 0.001$ ). The published data, which are based on one of the largest family studies of human TL, support a link between TL and aging and life span

and suggest a strong genetic influence, possibly via an imprinting mechanism, on TL regulation [75]. Factors influencing telomere homeostasis are not fully known; however, it is likely that both environmental and biological factors play roles. Among the biological factors, a growing body of evidence suggests that genes play a very important role.

Remarkable progress has been made during the last two decades in understanding telomere biology at the molecular and cellular levels. Clinical epidemiology research of human telomeres, in contrast, is a discipline

just coming into its own. The most important observation in studying human telomere biology is that TL is highly variable among humans [76]. TL varies among individuals and families and follows the polygenic mode of inheritance pattern typical of most quantitative traits [77]. Heritability estimates for TL vary from 35 to 80% [69,78–80]. Although several candidate genes have been identified as potential modulators of TL in humans [80,81], none of these genes seem to play a direct role in maintenance of TL [82]. Recently, one of the most obvious candidate genes of telomere maintenance, telomerase, has been shown to play a direct role in the maintenance of TL in humans [66].

Several genes that influence TL have been identified in model organisms [83,84]. In humans, shelterin, the protein complex that shapes and safeguards telomeres is made up of six subunits: *TRF1*, *TRF2*, *TIN2*, *Rap1*, *TPP1*, and *POT1* [67]. Other genes, such as *TERT*, *UPL*, *Tankyrase*, *EST1*, *EST2*, and *EST3* are known to influence telomere homeostasis, and other genes such as *YKU70*, *SIR4*, and *RIF2* encode proteins that bind specifically to the telomeres [84]. Previous reports have indicated that TL and chromosome-specific telomere-length patterns partly are inherited. In humans, the reported heritability of TL ranges from 36 to 90% [80,81]. Two genomewide linkage studies have shown significant evidence of linkage to autosomal regions [80,81]. On the other hand, one study [85] has suggested that TL is an X-linked trait, and another recent study [86] provides evidence for influence by paternally transmitted genes.

Cellular TL is linked to replicative life span. Telomere loss has been postulated to be a cause of cell senescence [87,88]. The relevance of replicative senescence to in vivo aging was investigated, and numerous reports confirm that telomere shortening may be associated with organismal aging, with concomitant metabolic decline and increased risk for disease and death [73,74]. Mean leukocyte TL may be an indicator of biological age, and as such it appears to provide information over and above chronological age of the risk for developing diseases of aging in humans.

It has been proposed that the mean leukocyte TL is an index of 'somatic fitness,' a concept that breaks down the artificial boundary between aging and diseases of aging [89]. Telomere repeats are lost in peripheral blood cells in vivo by age, and women show less telomere attrition than men. Several cross-sectional studies in humans have shown that TL in white blood cells is inversely related to the age of the cell donor [79,85,87,88].

Likewise, shorter TL has been shown to be associated with age-related disease including coronary heart disease, hypertension, and dementia, as well as general risk factors for disease such as insulin resistance and obesity [89]. TL provides an additional account to chronological age of variations in both pulse pressure and pulse wave velocity among men, such that men with shorter TL are more likely to exhibit high pulse pressure and pulse wave velocity, which are indices of large artery stiffness. The longer TL in women suggests that for a given chronological age, biological aging of men is more advanced than that of women [87]. Telomeric DNA in the skin cells of 21 human subjects aged between 0 and 92 years was quantified by determining the length of the telomeric smear and the relative amount of TTAGGG repeat sequences. Both TL and quantity of telomeric repeat sequences were found to decrease significantly with age [88]. In exceptionally old humans, ultrashort leukocyte telomeres might be a determinant of life span [89].

Furthermore, oxidative stress and inflammation, two major postulated causal factors of aging, are known to accelerate telomere shortening, suggesting that TL may be an important biomarker of aging because it reflects the cumulative burden of oxidative stress and inflammation [77,90]. Telomeres in telomerase-negative cells shorten during DNA replication in vitro owing to numerous causes including the inability of DNA polymerases to fully copy the lagging strand, DNA end processing and random damage, often caused by oxidative stress. Short telomeres activate replicative senescence, an irreversible cell cycle arrest. Thus, TL is an indicator of replicative history, of the probability of cell senescence, and of the cumulative history of oxidative stress. Telomeres in most human cells shorten during aging in vivo as well, suggesting that TL could be a biomarker of aging and age-related morbidity [90]. In addition, most [68,69,86] but not all studies [91,92] have shown a positive association between TL and overall survival in humans. These results indicate that telomere shortening could be used as a biomarker of disease risk and progression as well as early mortality (Figure 4). However, biological mechanisms responsible for these associations are not known.

The proliferative capacity of human cells is regulated by telomerase, an enzyme uniquely specialized for telomeric DNA synthesis [55]. Telomerase is a specialized ribonucleoprotein enzyme complex that adds telomere repeats to the ends of chromosomes and has two essential components: a catalytic component encoded by the human telomerase reverse transcriptase (hTERT)

and a human telomerase RNA component (hTERC). The latter component provides the template for nucleotide addition by hTERT (*Figure 5*). Heterozygote mutations in the hTERT and hTERC genes lead to short telomeres and are the major risk factors for rare hematopoietic disorders of bone marrow failure, including aplastic anemia and dyskeratosis congenita. These results indicate that the levels of functional telomerase are critical for telomere maintenance [93]. Environmental, oxidation insults and genetic modifiers that accelerate telomere shortening and increase cell turnover may exaggerate the effects of telomerase haploinsufficiency, contributing to the variability of age of onset as well as tissue-specific organ pathology. A central still unanswered question is whether telomerase dysfunction and short telomeres are a much more prominent factor than previously suspected in other adult-onset, age-related diseases. Understanding the biological effects of these lesions may ultimately lead to novel therapeutic treatments for these patients [93]. Telomerase is expressed at high levels in specific germline cells, proliferating stem-like cells, and many cancers, whereas in normal adult cell types, it is either not expressed or is expressed at very low levels that are not sufficient to maintain TL. However, telomerase can be unregulated in these cells under certain conditions to maintain TL [94]. In most instances, cells become senescent before they can become cancerous; thus, the initial growth arrest induced by short telomeres may be thought of as a potent anti-cancer protection mechanism. When cells can be adequately cultured until they reach telomere-based replicative senescence, introduction of the telomerase catalytic protein component (hTERT) into telomerase-silent cells is sufficient to restore telomerase activity and extend cellular life span (*Figure 5*). Cells with introduced telomerase are not cancer cells, because they have not accumulated the other changes needed to become cancerous. This indicates that therapeutic telomerase-induced TL manipulations may have utility for tissue engineering and for dissecting the molecular mechanisms underlying genetic diseases, including cancer [94].

The critical role of telomerase activation in tumor progression and tumor maintenance has been well established in studies of cancer and of oncogenic transformation in cell culture. New evidence suggests that telomerase activation has an important role in normal somatic cells, and that failure to activate sufficient telomerase also promotes disease. Bone marrow and peripheral blood leukocytes from 19 leukemia patients were found to contain telomerase activity

detectable by a PCR-based assay. Telomerase was also detectable in nonmalignant bone marrow and peripheral blood leukocytes from normal donors, including fractions enriched for granulocytes, T lymphocytes, and monocytes/B cells. Semiquantitative comparison revealed considerable overlap between telomerase activities in samples from normal subjects and leukemia patients, confounding evaluation of the role of telomerase in this disease. These data indicate that human telomerase is not restricted to immortal cells and suggest that the somatic expression of this enzyme may be more widespread than was previously inferred from the decline of human telomeres [53].

This suggests that efficient regulation of telomerase expression in response to stresses that are known to reduce TL such as oxidative damage or inflammation would lead to better telomere maintenance. Recently, we have discussed the potential use of telomere-restorative imidazole-containing dipeptide (non-hydrolyzed carnosine, carbinine) therapy for survival of smokers [28]. The published findings demonstrate a critical role for TL in the overall fitness, reserve, and well-being of the aging organism. Cells with critically truncated telomeres exhibit chromosomal rearrangements and undergo senescence and eventually apoptosis [74,95]. Mouse models indicate an increased incidence of tumor formation with shorter telomeres [96–101].

Altered functioning of both telomerase and telomere-interacting proteins is present in some human premature aging syndromes and in cancer, and recent findings indicate that alterations that affect telomeres at the level of chromatin structure might also have a role in human disease [100]. Telomere shortening with repeated cell divisions may lead to genomic instability and carcinogenesis. Studies suggest that shorter telomeres in constitutional DNA are associated with bladder, breast, lung, and renal cancer. Ovarian cancer tissues also have shortened telomeres and increased telomerase activity, suggesting that telomere abnormalities may be related to ovarian cancer. The recent study shows that short leukocyte telomeres are associated with serous ovarian adenocarcinoma [102]. Short telomeres appear to be associated with increased risks for human bladder, head and neck, lung, and renal cell cancers [103]. These findings suggest that truncated telomeres are associated with an increased risk of cancer.

These published findings have inspired a number of potential therapeutic strategies that are based on telomerase and telomeres (*Figure 5*).

Cigarette smoking is a risk factor for cancer [76,104–106] and may cause oxidative stress [17,107], which enhances telomere shortening [74]. Case–control studies observed shorter telomeres in peripheral blood leukocytes (PBL) in both men and women, and buccal cells from patients with bladder cancer than in controls [104–106]. Patients with bladder cancer displayed significantly shorter telomeres than control subjects ( $P = 0.001$ ). Median TL ratio was 0.95 (range 0.53–3.2) for cases and 1.1 (0.51–2.4) for controls. Moreover, the adjusted odds ratio (OR) for bladder cancer was significantly increased in the quartile with the shortest TL OR = 4.5 [95% confidence interval (CI) 1.7–12] [76,105–107].

It is known that oxidative stress, alkylation, or UV radiation increases shortening of telomeres. Therefore, the authors also analyzed whether environmental and genetic factors associated with DNA damage, i.e. smoking and polymorphisms in the genes involved in the metabolism of genotoxic carcinogens (EPHX1, GSTA1, GSTM1, GSTP1, GSTT1, NAT1, NAT2 and NQO1) or DNA repair (APE1, NBS1, XPC, XPD, XRCC1, XRCC3 and XRCC4), could modify the association between TL and cancer risk. A clear effect of smoking and TL could be observed. Current smokers with short telomeres had more than six times as higher risk as nonsmokers/former smokers with long telomeres (OR = 6.3, 95% CI 1.7–23) [105]. The authors observed a statistically significant difference in TL among men and women ( $P < 0.001$ ); however, the interaction between gender, TL, and bladder cancer risk was not significant [106]. The authors also observed a significant difference in TL across categories of pack-years of smoking ( $P = 0.01$ ) [106].

The most important observation in studying human telomere biology is that TL is highly variable among humans [76]. Biological age may be distinct from chronological age and contribute to the pathogenesis of age-related diseases. Mean telomeres lengths provide an assessment of biological age with shorter telomeres, indicating increased biological age. White blood cells (WBCs) have been used as the primary model in attempts to decipher links between aging, aging-related disorders, and telomere dynamics in humans. The WBC model may be appropriate in clinical settings, provided that we fully appreciate its drawbacks and limitations. The consistent findings of a negative correlation between TL and replicative potential of cultured cells, as well as a decreasing TL in a number of different tissues in humans with age, have led to the suggestion that telomeres play

a role in cellular aging in vivo and ultimately even in organismal aging [91]. On the basis of WBC telomere data, it is evident that age-adjusted TL is highly variable, highly heritable, longer in women than men, and shorter in people who harbor a host of age-related disorders, whose common denominators may prove to be increased oxidative stress and inflammation [108]. It appears that shorter age-adjusted WBC TL augurs a greater risk of morbidity and premature mortality in the elderly [68]. However, whether mortality in the elderly is also associated with shortened TL is still an open question [68,91,92]. Recent studies confirmed that individuals with shorter telomeres present a higher prevalence of arterial lesions and higher risk of cardiovascular disease mortality [87].

The innovative telomere research has moved rapidly from the laboratory to clinical and cross-section epidemiology population-based studies, which have observed that shorter mean TL in leukocytes is associated with cardiovascular disease [66,68,87,109–111], indices of obesity and insulin resistance [66,110,112–114], dementia [115,116], CS [85,112], and a host of other maladies. These observations are highly relevant, yet in an increasing number of studies little attention has been paid to potential biases and problems leading to discrepant results. The authors of the study [106] evaluated the effect of smoking on TL and found significantly shorter telomeres in healthy individuals who smoked than in those who did not smoke. Age-adjusted TL was approximately 5 bp shorter for every pack-year smoked in the Valdes et al. [112] study, with 40 pack-years of smoking corresponding to 7.4 years of age-related shortening in TL. Similarly, Morla et al. [117] observed a dose–response relationship between cumulative lifetime exposure to tobacco smoking and TL.

The gender effects of smoking on TL are important in several studies. In the study [106], women had longer relative telomeres when compared with those in men, which is consistent with prior studies demonstrating a similar relation [58,69,76,91,118]. In the matched published works, TL was evaluated in WBCs by measuring the mean length of the terminal restriction fragments. Age-adjusted TL was longer in women than in men ( $8.67 \pm 0.09$  vs.  $8.37 \pm 0.07$  kb;  $P = 0.016$ ) [87]. The longer TL in women suggests that for a given chronological age, biological aging of men is more advanced than that of women. TL has been observed to be similar in male and female newborns [119], yet TL in adults may have potential differences because of

gender differences and exposures to oxidative stress [69,120].

Obesity and smoking are important risk factors for many age-related diseases. Both are states of heightened oxidative stress, which increases the rate of telomere erosion per replication, and inflammation, which enhances white blood cell turnover. Together, these processes accelerate telomere erosion with age [112]. In the study [112], the authors tested the hypothesis that increased body mass and smoking are associated with shortened TL in WBC. The authors investigated 1122 white women aged 18–76 years and found that TL decreased steadily with age at a mean rate of 27 bp per year. Telomeres of obese women were 240 bp shorter than those of lean women ( $P = 0.026$ ). A dose-dependent relation with smoking was recorded ( $P = 0.017$ ). The results emphasize the pro-aging effects of obesity and CS [112].

It is likely, therefore, that the accelerated loss of TL observed is part of an oxidant-induced senescence phenomenon. If this is the case, this may have important pathogenic implications because cell senescence jeopardizes the capacity to repair tissue injury [121].

In the supporting study [117], in contrast to never-smokers, TL significantly decreased with age in smokers. There was also a dose–effect relationship between the cumulative long-life exposure to tobacco smoking (pack-yr) and TL. The presence and/or severity of chronic airflow obstruction did not modify this relationship. The results of this study confirm that smoking exposure enhances telomere shortening in circulating lymphocytes. It also demonstrates a dose–effect relationship between exposure to tobacco smoking and TL. However, the study failed to show that this phenomenon is enhanced in smokers who develop chronic obstructive pulmonary disease [117].

In this section of our review, we conclude that telomere attrition (expressed in WBCs) can serve as a significant biological marker of the cumulative oxidative stress and inflammation during CS and, consequently, indicates the pace of biological aging. TL is an independent predictor of survival and treatment requirement target in chronic disease associated with oxidative stress and smoking behavior.

## CONCLUSION

There were nearly 1.3 billion smokers worldwide in the year 2003, and this number is expected to rise to 1.7 billion (approximately 1.2 billion males and 500 million

females) by 2025, with the number of female smokers contributing most to the increase [122]. In nearly all investigated regions of the world, the ratio of female to male smokers among young people was found to be higher than the ratio among adults, suggesting a global trend for an increase in smoking habits among female adolescents and young women [123]. Smoking is associated with many serious health problems, including cancer of various organs, coronary artery disease, as well as several autoimmune disorders [124–129], and it is thus considered a leading cause of death and disability worldwide.

Although the cigarette industry has spent much of the past 50 years denying a link between smoking and disease, the industry has also dedicated a significant amount of time and money to develop a ‘safer’ cigarette. A safer cigarette that can both satisfy smokers’ demands for taste and nicotine delivery and placate public health concerns is the Holy Grail of the tobacco industry. The company that comes up with it first likely could dominate the entire industry by selling the newfangled smoke at a significant premium and grabbing market share from its competitors. Indeed, in the 1950s, Philip Morris researchers already saw the potential of a ‘healthy’ cigarette and had even begun to suggest that the company could capitalize on health concerns by admitting that cigarettes were harmful. ‘Evidence is building up that heavy smoking contributes to lung cancer,’ wrote a Philip Morris scientist in July 1958. He then suggested that the company have the ‘intestinal fortitude to jump to the other side of the fence,’ and that the company would have a ‘wealth of ammunition’ to attack competitors who did not have safer cigarettes [130].

Telomere length shortens with age in all replicating somatic cells. It has been shown that tobacco smoking enhances telomere shortening in circulating lymphocytes. This study investigated whether this effect was further amplified in smokers who develop chronic diseases associated with smoking, such as chronic obstructive pulmonary disease, cardiovascular disease, ocular diseases including cataract and age-related macular degeneration. In this work, we present multiple evidence that CS provide the important risk factors in many age-related diseases and is associated with increased cumulative and systemic oxidative stress and inflammation. Telomere attrition (expressed in WBCs) can serve as a biomarker of the cumulative oxidative stress and inflammation and, consequently, show the pace of biological aging. The longitudinal studies of the clinical population groups including elderly support

the hypothesis that TL is a predictor of survival and therapeutic treatment requirement associated with smoking behavior [28].

We propose the viability and versatility for the telomere protection-targeted therapeutic system for smokers in combination with in vitro cellular culture techniques as an investigative tool to study telomere attrition in cells induced by cigarette smoke and smoke constituents. Our working hypothesis is that imidazole-containing dipeptide-based compounds (non-hydrolyzed carnosine, carcinine, ophthalmic version of n-acetylcarnosine) can modulate the telomerase activity in the normal cells and can provide the redox regulation of the cellular function under the terms of environmental and oxidative stress and in this way protect the length and the structure of telomeres from attrition [28,132]. We have recently originally reported that patented specific oral formulations of nonhydrolyzed carnosine and carcinine provide a powerful tool for targeted therapeutic inhibition of cumulative oxidative stress and inflammation and protection of telomere attrition associated with smoking [28,132]. The proposal of universal antioxidant therapeutic strategies based on the administration of nonhydrolyzed carnosine, carcinine, and topical n-acetylcarnosine lubricant eye drops has been reported in the recently published solid research works [28,132–134].

In the previous work, a group of authors independently studied the effect of carnosine on a human fetal lung fibroblast strain (HPF), which was either kept in a continuously proliferating or proliferation-inhibited state [134]. The results indicate that carnosine can reduce telomere shortening rate possibly by protecting telomere from damage [134]. The authors suggest based on their findings that the reduction in telomere shortening rate and damages in telomeric DNA made an important contribution to the life-extension effect of carnosine [134].

The prospects of further research are proposed in this review article: the role of telomere attrition and regulation of telomerase activity in smoking, possibilities of healthy aging in smokers by therapeutic regulation the length of telomeres, telomerase activity and provision of redox regulation of the cellular physiological function and prediction of life expectancy.

In summary, this study confirms the findings of previous studies reviewed in Ref. [28], showing that tobacco smoking is significantly associated with telomere loss in circulating lymphocytes. It has been reported that this occurs in a dose–response manner (reviewed in Ref.

28). TL is rapidly becoming a new biological and pharmacological target for therapies of various metabolic and age-related disorders.

To conclude, this study raises a new avenue that the TL protection can be targeted in smokers therapeutically with the use of universal antioxidants : nonhydrolyzed carnosine, carcinine and the ophthalmic version of n-acetylcarnosine that made an important contribution to the management of a number of age-related disorders and in life-extension expectancy based on the bioavailability of the cited peptide-based compounds for therapeutic use under the physiological conditions associated with exaggerated oxidative stress apart from other social, psychological, and physicochemical environmental factors [28,132–134].

## CONFLICT OF INTEREST

Declaration of interest: The authors report no conflict of interest in this work. The authors bear primary responsibility for accuracy of made statements and for the content and writing of the paper.

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