Telomere Length as an Indicator of Biological Aging

The Gender Effect and Relation With Pulse Pressure and Pulse Wave Velocity

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Abstract—Chronological age is the primary determinant of stiffness of central arteries. Increased stiffness is an independent indicator of cardiovascular risk. The aim of this study was to determine whether telomere length, a possible index of biological aging, provides a better account than chronological age for variation in arterial stiffness, evaluated by measuring pulse pressure and aortic pulse wave velocity. The study population included 193 French subjects (120 men, 73 women), with a mean age of 56±11 years, who were not on any antihypertensive medications. Telomere length was evaluated in white blood cells by measuring the mean length of the terminal restriction fragments. Age-adjusted telomere length was longer in women than in men (8.67±0.09 versus 8.37±0.07 kb; $P=0.016$). In both genders, telomere length was inversely correlated with age ($P<0.01$). Multivariate analysis showed that in men, but not in women, telomere length significantly contributed to pulse pressure and pulse wave velocity variations. In conclusion, telomere length provides an additional account to chronological age of variations in both pulse pressure and pulse wave velocity among men, such that men with shorter telomere length are more likely to exhibit high pulse pressure and pulse wave velocity, which are indices of large artery stiffness. The longer telomere length in women suggests that for a given chronological age, biological aging of men is more advanced than that of women. **(*Hypertension*. 2001;37[part 2]:381-385.)**

Key Words: blood pressure ■ aorta ■ genetics ■ aging ■ sex

Telomeres, the TTAGGG tandem repeats at the ends of mammalian 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.[1] This feature of telomere biology is at the core of the concept that, at the cellular level, telomeres serve as a mitotic clock.[1] In human beings, telomere length is heritable,[2,3] relatively short, highly variable, and with regard to replicating somatic cells, inversely related with donor age.[2,4–6] Because of the long lifespan of humans and their short telomeres, attrition in telomere length may be a major determinant of human aging not only at the cellular level, but also at the organ and perhaps the systemic levels.

Pulse pressure (PP), a clinical marker of large artery stiffness, increases with chronological age.[7,8] An increase in PP is a strong predictor of cardiovascular mortality, especially coronary mortality, independently of mean blood pressure (MAP).[7] Also, large artery stiffness, assessed by pulse wave velocity (PWV) measurements, greatly increases with age[9] and could be an independent cardiovascular risk factor.[10] Collectively, these observations suggest that large artery stiffness and its clinical manifestations (PP, PWV) are phenotypes of biologic aging of the arterial system.

In a previous work, using telomere length as an indicator of biologic aging of a person, we found that, in young subjects (mean age, 37 years), after age-adjustment, PP was inversely related to telomere length, measured by the mean length of the terminal restriction fragments (TRF), in white blood cells.[3] However, in young subjects, brachial PP is an inadequate indicator of large artery stiffness, because it overestimates central aortic PP.[11] The aim of the present study was to assess whether TRF length explains variability in aortic stiffness evaluated by PP and PWV, in a large cohort of French men and women with an age distribution of 6 decades.

Methods

Subjects, Blood Pressure, and PWV Measurements

The French national health care system (Securité Sociale-CNAM) provides all working and retired persons and their families with a free medical examination every 5 years. The Center d'Investigations Préventives et Cliniques (IPC) is one of the medical centers of this healthcare system. Subjects participating in the study were from a

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Parisian cohort that had a health checkup at the IPC Center. All clinical and arterial parameters were evaluated in an hemodynamic laboratory before the standard examination at the IPC Center. All subjects signed an informed consent approved by an ethical committee.

We assessed, using a questionnaire, family medical history (diabetes, hypertension, cardiovascular disease, and sudden death before 60 years), personal medical history, drug intake, and lifestyle. Menopausal status and hormone replacement therapy were also recorded. None of the subjects participating in this study were on antihypertensive medications.

Measurements of blood pressure and PWV were performed under constant temperature (19°C to 21°C) by two specialized physicians. Supine blood pressure was measured in the right arm using a manual sphygmomanometer. After blood pressure determination, the PWV was measured using the Complior (Colson). This automatic device records online pulse wave and calculates PWV with 2 transducers, one positioned at the base of the neck for the common carotid artery and the other over the femoral artery. The validation of the Complior device has been previously described, with an intraobserver repeatability coefficient of 0.935 and an interobserver reproducibility of 0.890.[12] After the PWV measurements, fasting blood samples were collected.

DNA samples, extracted from white blood cells as previously described,[13] were coded in France and shipped to the USA for TRF analysis. No clinical information (eg, gender, age, blood pressure, etc.) was revealed by the code. After completion of TRF measurements, the code was broken for data analysis.

Measurements of the TRF Length

TRF length was measured in DNA samples by a modification of a method described earlier.[3] The samples were digested overnight with restriction enzymes *Hin*fI (10 U) and *Rsa*I (10 U) (Boehringer Mannheim). DNA samples and DNA ladders were resolved on a 0.5% agarose gel (20 ×20 cm) at 50 V (GNA-200 Pharmacia Biotech). After 16 hours, the DNA was depurinated for 30 minutes in 0.25 N HCl, denatured 30 minutes in 0.5 mol/L NaOH/1.5 mol/L NaCl and neutralized for 30 minutes in 0.5 mol/L Tris, pH 8/1.5 mol/L NaCl. The DNA was transferred for 1 hour to a positively charged nylon membrane (Boehringer Mannheim) using a vacuum blotter (Appligene, Oncor). The membranes were hybridized at 65°C with the telomeric probe [digoxigenin 3′-end labeled 5′-$(CCTAAA)_3$] overnight in 5×SSC, 0.1% Sarkosyl, 0.02% SDS and 1% blocking reagent, washed 3 times at room temperature in 2×SSC, 0.1% SDS each for 15 minutes and once in 2×SSC for 15 minutes. The probe was detected by the digoxigenin luminescent detection procedure and exposed on x-ray film. Each DNA sample was measured in triplicate.

Statistical Analysis

Mean values of TRF length, blood pressure measurements, body mass index (BMI), and PWV in men and women were compared by analysis of covariance to adjust for age. Pearson correlation coefficients were computed separately for men and women to estimate the pairwise correlations between TRF length and age, PP and PWV. The joint effect of age, MAP, TRF length, and gender on PP and PWV was assessed in a sequence of multiple regression models. PP and PWV were regressed on MAP and TRF length, adjusted for age, inclusive of both genders and then stratified by gender. PP and PWV were then modeled as the dependent variable, with MAP, TRF length, and age as independent variables. Multivariate analyses were also performed including heart rate (HR), total cholesterol, HDL cholesterol, and glycemia. All reported probability values are global or two-tailed; the criterion for significance is $P<0.05$. Statistical analyses were performed using the SAS statistical software package.

TABLE 1. Age-Adjusted Mean Values (±SEM) of Blood Pressure, PWV, and TRF Length in Men and Women

Parameter	All	Men	Women
Number	193	120	73
Age (years)	56±1	55±1	56±1
BMI (kg/m^2)	24.9±0.2	25.4±0.2	24.2±0.4*
DBP (mm Hg)	83.7±0.8	85.2±0.9	81.3±1.2*
SBP (mm Hg)	133.5±1.3	134.8±1.5	131.2±1.9
MAP (mm Hg)	100.3±0.9	101.8±1.0	97.9±1.3*
PP (mm Hg)	49.8±1.0	49.7±1.1	50.0±1.4
HR (b/min)	67.0±0.7	67.0±0.8	67.1±1.0
PWV (m/sec)	11.0±0.2	11.4±0.2	10.4±0.3*

SBP, systolic blood pressure.
*Significant at $P<0.05$.

Results

Table 1 describes general characteristics of the cohort. Men and women did not differ in mean age. Age-adjusted BMI, diastolic blood pressure (DBP), MAP, PP, HR, and PWV were higher in men than women ($P<0.05$). After age-adjustment, telomere length in women was significantly longer than in men by 0.28 kb (8.67±0.09 versus 8.37±0.07 kb for women and men, respectively; $P=0.016$).

Bivariate analysis showed that in both genders, TRF length was negatively correlated with age (Figure 1). The rate of telomere attrition across the age range was 0.038 kb per year for men and 0.036 kb per year for women. PP also correlated positively with PWV; however, this correlation was substantially stronger in men ($r=0.53$, $P=0.001$) than in women ($r=0.25$, $P=0.025$). TRF length was significantly correlated

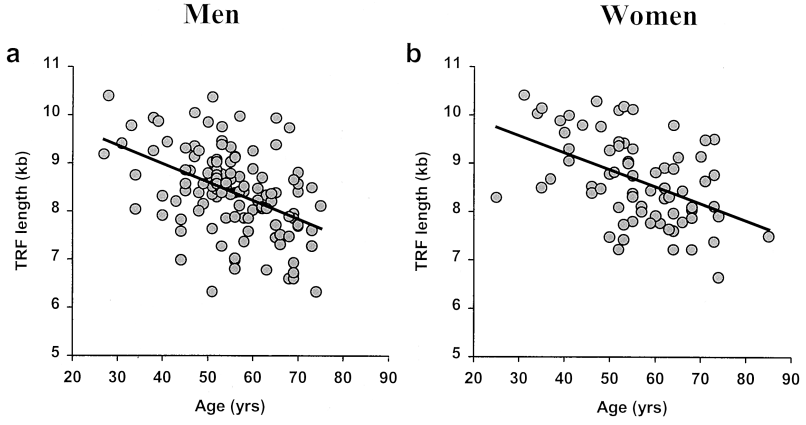

Figure 1. Relation between age and TRF length in white blood cells of men (a) and women (b). For men, $r=-0.45$, $P=0.0001$; for women, $r=-0.48$; $P=0.0001$.

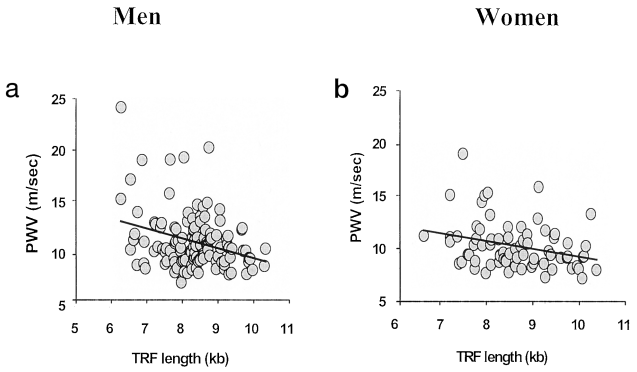

Figure 2. Relation between TRF length and PWV in men (a) and women (b). For men, PWV (m/sec) =19.28 to 0.95 TRF ($r=-0.31$, $P=0.001$); for women, pulse wave velocity=16.69 to 0.72 TRF ($r=-0.29$, $P=0.02$).

with PWV in both men and women (Figure 2). TRF length was also significantly correlated with PP in men but not in women (Figure 3). TRF length was not correlated with MAP, body mass index, total cholesterol, heart rate, HDL, or glycemia.

In a preliminary model for men and women combined, the independent variables age, MAP, and TRF length accounted for 34% of the variability in PP. However, this model masked gender differences in the influence of these variables. Addition of gender and gender-covariate interaction terms significantly increased the proportion of explained variability in PP to 37% ($F_{4,186}=2.68$, $P<0.05$), indicating the need for a gender-specific model.

Table 2 shows the results of 3 different models of the stepwise multivariate analysis on the influence of age, MAP, and TRF length on PP variability. In men, after adjustment for MAP, age explained 10.3% of PP variability (model 1). After adjustment for MAP, TRF length accounted for 11.9% variability in PP (model 2). The contribution of TRF length to PP variability remained unchanged after adjustment for both age and MAP (model 3). In model 3, TRF length was a more significant determinant of PP (11.9%, $P<0.005$) than chronological age (3.1%, $P<0.02$). In women, after adjustment for MAP, age, significantly explained 5.6% of the variability in PP (model 1). TRF length did not account significantly for PP variation in women after adjustment for MAP (model 2)

TABLE 2. Different Models of Multivariate Analysis on the Influence of TRF Length, Age, and Mean Arterial Pressure on PP in Men and Women

	Men			Women		
Parameter	Beta	R^2	P	Beta	R^2	P
Model 1						
Age	0.415	0.103	0.0001	0.332	0.056	0.01
MAP	0.356	0.190	0.0001	0.683	0.380	0.0001
Total		0.293			0.436	
Model 2						
TRF length	−5.076	0.119	0.0001	−1.550	0.008	Ns
MAP	0.459	0.190	0.0001	0.816	0.379	0.001
Total		0.309			0.387	
Model 3						
Age	0.261	0.031	0.02	0.349	0.056	0.02
TRF length	−3.661	0.119	0.005	0.433	0.005	Ns
MAP	0.396	0.190	0.001	0.680	0.379	0.001
Total		0.341			0.440	

Ns, not significant.

or age plus MAP combined (model 3). After adjustment for age and MAP, multivariate analysis showed that total cholesterol, HDL cholesterol, heart rate, and glycemia were not significant determinants of PP in either men or women.

Similar analyses were performed to explain PWV variations (Table 3). In men, after adjustment for MAP, age explained 26.6% of PWV variability (model 1), whereas TRF accounted for 9.8% variability in PWV (model 2). When all 3 parameters were considered together (model 3), age was the strongest determinant for PWV variations (26.6%, $P<0.0001$). TRF length remained a weak but significant determinant (2%, $P<0.05$) of PWV variations. In women, age accounted for 31.8% of PWV variability (model 1). TRF length significantly accounted ($P<0.05$) for 4.9% variations in PWV after adjustment for MAP (model 2). However, in women, TRF length did not contribute significantly to PWV variations after adjustment for age and MAP (model 3).

Multivariate analysis showed that after adjustment for age and MAP, only heart rate (but not total cholesterol, HDL cholesterol, or glycemia) was a significant determinant of

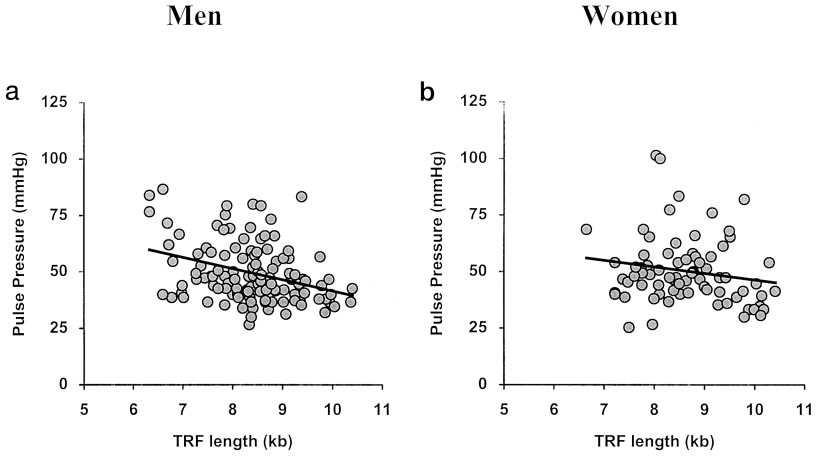

Figure 3. Relation between TRF length and pulse pressure in men (a) and women (b). For men, pulse pressure (mm Hg) =91.38 to 4.98 TRF ($r=-0.34$, $P=0.0002$); for women, pulse pressure=75.2 to 2.89 TRF ($r=-0.17$, $P=0.15$).

TABLE 3. Different Models of Multivariate Analysis on the Influence of TRF Length, Age, and Mean Arterial Pressure on PWV in Men and Women

	Men			Women		
Parameter	Beta	R^2	*P*	Beta	R^2	*P*
Model 1						
Age	0.107	0.266	0.0001	0.075	0.318	0.001
MAP	0.081	0.128	0.0001	0.077	0.124	0.001
Total		0.394			0.442	
Model 2						
TRF length	−0.967	0.098	0.0001	−0.554	0.049	0.024
MAP	0.108	0.239	0.0001	0.105	0.310	0.0001
Total		0.337			0.359	
Model 3						
Age	0.086	0.266	0.0001	0.069	0.318	0.01
TRF length	−0.502	0.020	0.047	−0.162	0.003	Ns
MAP	0.087	0.128	0.0001	0.078	0.124	0.001
Total		0.414			0.445	

Ns, not significant.

PWV in women ($P<0.01$); in men, the same trend was observed but was not significant ($P=0.067$). In men, when heart rate was added in model 3, TRF length remained a significant determinant of PWV variability.

Discussion

The main findings of this study are: (1) for a given chronological age, men with shorter telomere length are more likely to exhibit a high PP and a high PWV; this finding does not hold for women; and (2) age-adjusted telomere length is longer in women than in men, confirming a finding from our previous work.[3]

A limitation of this study is the small sample size, especially for women. However, the observation of gender difference in TRF length suggests that the relation between TRF and vascular parameters may not be the same in men and women. We note that in our previous study, we found that after adjustment for age and MAP, PP was a significant cardiovascular risk factor in men but not in women.[14] Given that PP reflects arterial aging, whereas telomere length is an index of biologic aging, our findings suggest that the biology of arterial aging is modified by gender.

Premenopausal women are less prone than men to cardiovascular diseases and women tend to catch up with men in the expression of these diseases during the postmenopausal period.[15] In addition, several cellular and systemic parameters that are correlated with blood pressure in men show relatively poor correlations with blood pressure in women.[16,17] Such enigmatic findings are in line with our conclusion that the biology of aging differs between men and women.

The lack of contribution, after age-adjustment, of TRF length to variations in PP and PWV among women probably results from differences between men and women in age-dependent telomere attrition. This is supported by findings that telomere length does not differ between male and female newborns (Okuda et al, unpublished data), whereas telomere length in adult women is longer than in adult men, as shown in this study and our previous work.[3] Due to the substantial scatter and the cross-population nature of the data of TRF length versus age (Figure 1), our work does not provide sufficient power to detect significant gender-related differences in the rate of age-dependent telomere attrition. This rate may not be constant throughout life,[18] although it has been a common practice to describe telomere length as a linear function of donor age.

Age-dependent increase in arterial stiffness is associated with fracture and fragmentation of elastic lamellae, with consequent dilation in the vascular lumen. Thus, some changes in arteries with aging may be explained by fatigue of the elastin fibers after repetitive stress cycles. However, it is very unlikely that aging can be simply explained by mechanical wear. This subject was the focus of debate more than 4 decades ago.[19] Arterial aging is probably related to cellular elements in the vascular wall and their role in age-dependent alterations in properties of elastin fibers. Recent investigations have shown that, in human beings, telomeres in both vascular endothelial cells and vascular smooth muscle cells undergo age-dependent attrition in vivo.[5,6] Such findings suggest that biologic aging of major blood vessels may also be linked to the cellular elements of the vascular wall.

The effect of age on arterial stiffness in various populations is influenced by environmental and genetic factors. Environmental factors such as salt intake have been reported to have independent effects on arterial wall properties and to modify the effects of age on large artery stiffness.[20] More recently, it has been shown that aortic-femoral PWV increased more rapidly in blacks than in whites.[21] How these environmental and presumably genetic factors influence cellular elements in the arterial wall is not known at present.

In conclusion, our work indicates that the biology of cardiovascular aging differs between men and women. For a given chronological age and MAP, men with shorter telomeres have increased arterial stiffness and PP. We note that our findings do not provide mechanistic links between telomere length and arterial aging, because the dynamics of telomere attrition and its role in the biology of human aging in vivo is poorly understood. In our study, telomere length merely served as an indicator of biological aging rather of chronological age. Given that aging is a multifactorial and highly variable entity, the use of telomere length provides a new dimension to the study of cardiovascular disease.

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