Shortened Telomeres in Circulating Leukocytes of Patients with Chronic Obstructive Pulmonary Disease

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***Rationale*: Telomere length is considered a marker for biological aging. Chronic obstructive pulmonary disease (COPD) may be associated with premature aging.**

***Objectives*: To test the hypothesis that patients with COPD experience accelerated telomere shortening and that inflammation is linked to this process.**

***Methods*: We measured telomere length, using a quantitative polymerase chain reaction–based method, and plasma levels of various cytokines in 136 patients with COPD, 113 age- and sex-matched smokers, and 42 nonsmokers with normal lung function.**

***Measurements and Main Results*: Median (range) telomere length ratio was significantly lower in patients with COPD (0.57 [0.23–1.18]) than in control smokers (0.79 [0.34–1.58]) or nonsmokers (0.85 [0.38–1.55]) ($P < 0.001$). The difference remained highly significant when using logistic regression analysis adjusted for age, sex, and tobacco exposure. Both females and males with COPD had shorter telomere length than same-sex control subjects. Telomere length was related to age in patients and control subjects but was shorter in patients than in control subjects in all age groups. No relationship was found between telomere length and tobacco exposure in patients or control subjects, with no difference between control smokers and nonsmokers. In patients with COPD, telomere length was related to Pa_{O_2} ($P < 0.001$) and Pa_{CO_2} ($P < 0.001$) but not to lung function parameters or the BODE Index. Patients with COPD also had elevated plasma levels of various cytokines, interleukin-6 correlating negatively with telomere length ($P < 0.001$).**

***Conclusions*: Given that *in vivo* telomere length reflects cellular turnover and exposure to oxidative and inflammatory damage, our data support accelerated aging in COPD.**

Keywords: telomere length; chronic obstructive pulmonary disease; inflammation; hypoxemia

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Patients with chronic obstructive pulmonary disease (COPD) develop many age-related complications that substantially affect outcome. Telomere length is considered a marker for biological aging.

What This Study Adds to the Field

Excess telomere shortening was found in patients with COPD, supporting accelerated aging in COPD. Decreased telomere length, used as a marker for cellular turnover, exposure to oxidative and inflammatory damage, and biological age, may have clinical significance in COPD.

Chronic obstructive pulmonary disease (COPD) is becoming increasingly prevalent in most countries and is predicted to become the third leading cause of death worldwide by 2020 (1, 2). Although COPD is primarily a respiratory disease, systemic complications contribute substantially to the prognosis. Most of these systemic complications, including weight loss, skeletal muscle dysfunction, osteoporosis, and atherosclerosis, are considered age-related abnormalities (3–5). Osteoporosis and stiffness of the central arteries are increased in patients with COPD compared with age-matched control subjects, suggesting that COPD may be associated with premature aging related to amplification of the mechanisms that underlie aging (4, 6).

Telomere length is considered a biomarker for aging (7, 8). Telomeres are DNA sequences and associated proteins that cap and stabilize the ends of linear chromosomes, thereby maintaining genome integrity and stability. Telomere length is not only related to the basic biology of aging as a trigger of cellular senescence but also reflects the balance between oxidative stress and antioxidant defense mechanisms (9–12). Thus, telomere attrition in circulating white blood cells has been proposed as a marker for cumulative oxidative stress and inflammation and, therefore, as an indicator of the pace of biological aging. Several studies also suggest that telomere shortening may be a risk factor for the development of cancer (13, 14), particularly lung cancer (15, 16). Moreover, telomere shortening in leukocytes was associated with the development of coronary artery and cerebrovascular disease, and shortening of the age-corrected telomere length was associated with a substantial additional risk

(*Received in original form September 5, 2008; accepted in final form January 22, 2009*)

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Supported by grants from INSERM and the Délégation à la Recherche Clinique de l'AP-HP. Financial support was also received from the European Commission under the 6th Framework Program (contract no. LSHM-CT-2005-018725, PULMOTENSION). This publication reflects only the authors' views, and under no circumstances is the European Community liable for any use that may be made of the information it contains.

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Am J Respir Crit Care Med Vol 179. pp 566–571, 2009
Originally Published in Press as DOI: 10.1164/rccm.200809-1398OC on January 29, 2009
Internet address: www.atsjournals.org

of mortality from cardiovascular and infectious diseases (17, 18). Patients with COPD are at increased risk for cardiovascular disease and lung cancer, which are the leading causes of death in COPD. Evaluation of telomere length in COPD may therefore be of considerable usefulness, not only to gain further insight into the process of premature aging, but also potentially as a biomarker for disease severity.

A previous study suggested that telomere length might be shorter in patients with COPD (19), although another found no difference between patients with and without COPD (20). This discrepancy may be ascribable to the small number of patients studied and to the absence of an appropriate control group matched for age and sex (19, 20). Smoking may also affect telomere length (21). However, most comparisons of smokers and nonsmokers failed to include lung function measurements, so that specific effects of smoking could not be assessed independently from those of airflow limitation (21).

In the present study, we measured telomere length in circulating leukocytes from 136 patients with COPD and in 155 age- and sex-matched smokers and nonsmokers with normal lung function. Our goal was to determine whether COPD was associated with reduced telomere length independently from sex, age, and tobacco consumption. We also determined whether lung function parameters and circulating inflammatory cytokine levels were linked to telomere length in patients with COPD and, for some of these factors, in control subjects.

METHODS

Study Population

We studied 136 unrelated patients with COPD recruited at two French centers located in Strasbourg (n = 60) and Créteil (n = 76), respectively. Inclusion criteria included an at least 10–pack-year history of tobacco smoking and FEV_1/ FVC ratio less than 70%. Patients were in a stable phase of their disease, defined as no requirement for antibiotic or oral corticosteroid therapy and no change in respiratory symptoms within the last month. Patients were excluded if they had known heart disease, malignancy, or other inflammatory or metabolic conditions. The BODE (*b*ody mass index, airflow *o*bstruction, *d*yspnea, and *e*xercise capacity) Index score was calculated according to Celli and colleagues (22).

We also recruited 155 healthy control subjects (using announcements in local newspapers), including 113 smokers and 42 nonsmokers, who were free of acute or chronic illness except for mild systemic hypertension, whose FEV_1 values were greater than 80% of predicted, and whose FEV_1/FVC ratios were greater than 70%. Screening of control subjects for study inclusion involved a medical history, clinical examination, electrocardiogram, and comprehensive set of lung function tests. Our initial goal was to recruit 1 age-matched (±2 yr) and sex-matched control per patient, but we then included additional individuals to have at least 50 control subjects with a smoking history greater than 20 pack-years. Nonsmokers were defined as individuals who had never regularly smoked one or more cigarettes a day or who had smoked one or more cigarettes a day for less than 1 year. Smokers were individuals who reported regularly smoking one or more cigarettes a day for at least 1 year. Former smokers were individuals who reported smoking one or more cigarettes a day regularly in the past but who had not smoked during the last year.

This study was approved by the institutional review board of the Henri Mondor teaching hospital (Créteil, France). All patients and control subjects signed an informed consent document before study inclusion.

Laboratory Investigations

Measurements of plasma cytokine concentrations in patients and control subjects. Plasma samples for measurements of IL-6, IL-8, IL-1β, monocyte chemotactic protein-1 (MCP-1), transforming growth factor-β (TGF-β), and tumor necrosis-α (TNF-α) were obtained from all control subjects and from 103 patients with COPD. Plasma levels of these cytokines were determined with an ELISA (R&D Systems, Lille, France).

Telomere length assay. All patients and control subjects underwent blood sampling for genotype determination. Telomere length was assessed in a real-time quantitative polymerase chain reaction (PCR)–based assay (23). Briefly, the telomere repeat copy number to single-gene copy number (T/S) ratio was determined with a 7900HT thermocycler (Applied Biosystems, Foster City, CA) in a 96-well format, using the comparative C_t method ($T/S = 2^{-\Delta\Delta C_t}$). Genomic DNA was extracted from blood with a DNeasy blood kit (Qiagen, Courtaboeuf, France) and quantified with a spectrophotometer. Each sample was run in triplicate, using the SYBR green method (Invitrogen, Cergy-Pontoise, France) and 30 ng of DNA. The sequences and final concentrations of the primers for the telomere and 36B4 (acidic ribosomal phosphoprotein PO, a single-copy gene for normalization) were as follows: Tel F, 5′-CGGTTTGT TTGGGTTTGGGTTTGGGTTTGGGTT-3′, 300 nM; Tel R, 5′-GGCT TGCCTTACCCTTACCCTTACCCTTACCCTTACC CT-3′, 300 nM; 36B4F, 5′-CAGCA AGTGGGAAGGTGTAATCC-3′, 300 nM; and 36B4R, 5′-CCCATTCTATCATCAACGGGTA CAA-3′, 300 nM.

Statistical Analysis

The variables compared in patients with COPD, control smokers, and nonsmokers are listed in Table 1. Qualitative variables are reported as numbers and percentages, and compared by Chi-square or Fisher exact test, as appropriate. Quantitative variables are reported as the median (range), except where otherwise indicated, and compared by nonparametric Kruskal-Wallis test. Most of the quantitative variables were not categorized; however, telomere length was divided according to tertile values defined in the control population and coded 1 when greater than the third tertile, 3 when less than the first tertile, and 2 otherwise. When analysis indicated significance, patients with COPD were compared with smokers and smokers with nonsmokers. Patients and control subjects were also stratified into age groups to evaluate whether telomere length differences between patients and control subjects varied with age. To take into account potential associations linking telomere length and/or plasma cytokine levels to pack-year exposure and patient characteristics, multivariate logistic regression models comparing patients with COPD and control subjects were built. In these models, telomere length and plasma cytokine levels were $\log_{10}$ transformed and pack-year exposure was expressed by 5–pack-year increments. Multivariate odds ratios were estimated with their 95% confidence intervals. Potential associations between telomere length and physiological or biological variables were separately assessed in patients with COPD or control subjects, using the nonparametric Spearman's rank correlation (Rho). All tests were two-tailed; *P* values not greater than 0.05 were considered significant. Data were analyzed with Stata statistical software (release 8.0; StataCorp, College Station, TX).

RESULTS

Clinical Characteristics of the Study Population

The characteristics of the 136 patients with COPD and 155 control subjects are reported in Tables 1 and 2. Patients and control subjects did not significantly differ regarding age, sex, body mass index, or systemic arterial pressure. All patients were smokers or former smokers, and pack-year exposure was significantly greater in the patients than in the control smokers. Among the 155 control subjects, 49 were current smokers (pack-year exposure, 30 [range, 2–72]), 64 were former smokers (pack-year exposure, 15 [range, 0.5–66]), and 42 were nonsmokers. Lung function tests were normal in all control subjects. Airflow limitation was moderate to severe in the patients (Table 2), and 24 patients were receiving long-term oxygen therapy at the time of the study.

Telomere Length and Cytokine Plasma Levels in Patients with COPD and in Control Subjects

Telomere length ratios were significantly lower for the patients with COPD (0.57 [0.23–1.18]) than in the overall control population (0.82 [0.34–1.58]) and for the control smokers (0.79 [0.34–1.58]) (Table 1). Telomere shortening in the patients

TABLE 1. COMPARISON OF PHYSIOLOGICAL AND BIOLOGICAL VARIABLES BETWEEN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND CONTROL SUBJECTS (SMOKERS AND NONSMOKERS)

		Control Subjects (*n* = 155)			Adjusted Analysis (Patients vs. Control Subjects)*	
	Patients (*n* = 136)	Smokers (*n* = 113)	Nonsmokers (*n* = 42)	*P* Value†	OR (95% CI)	*P* Value
Females/males	33/103	32/81	15/27	0.34	—	—
Age (yr), mean ± SD	62.9 ± 6.6	62.2 ± 7.7	61.4 ± 6.1	0.07	—	—
BMI, kg/m^2	25.6 (14.2–42.7)	25.6 (19.5–47.0)	25.4 (20.7–45.3)	0.69	—	—
MAP, mm Hg	96.7 (68.3–125.3)	99.3 (75–140)	98.3 (79.3–130)	0.51	—	—
FEV_1, % pred	38.4 (13.3 – 90.7)‡	94.0 (81.0–128)	98 (81.0–135)	0.0001		
FEV_1, L	1.01 (0.3–3.12)‡	2.86 (1.24–4.42)	2.76 (0.97–4.40)	0.0001		
FVC, % pred	72.8 (29.3–131)‡	103 (75.0–118)	101 (84.0–129)	0.0001		
FVC, L	2.21 (0.80–5.4)‡	3.65 (1.75–5.77)	3.70 (1.78–5.96)	0.0001		
FEV_1/FVC	45.6 (19.7–69.0)‡	77.0 (71.0–99.4)	76.3 (71.0–99.4)	0.0001		
Pack-years	48 (10–180)‡	22 (0.5–72)	0§	0.0001	1.47 (1.35–1.61)‖	0.000
Telomere length (T/S ratio)	0.57 (0.23–1.18)‡	0.79 (0.34–1.58)	0.85 (0.38–1.55)	0.0001	0.0001 (10^{-5}–0.001)¶	0.000
IL-6, pg/ml	2.4 (0.3–30.5)‡	1.5 (0.5–15.3)	0.9 (0.1–3.6)§	0.0001	5.17 (1.73–15.47)¶	0.003
MCP-1, pg/ml	475 (126–1,274)	468 (304–769)	394 (111–779)§	0.0006	8.12 (1.05–63.06)	0.05
IL-8, pg/ml	12.4 (0.9–36.6)‡	9.6 (3.6–37.6)	8.3 (2.2–19.2)§	0.0001	3.82 (0.90–16.20)¶	0.069
IL-1β, pg/ml	0.63 (0.1–3.5)‡	0.34 (0.1–2.28)	0.24 (0.06–1.4)	0.0001	5.23 (0.91–30.21)¶	0.065
TGF-β, pg/ml	28.9 (4.3–55.5)‡	18.3 (5.9–38.2)	16.4 (9.1–24.3)§	0.0001	94.93 (8.74–1,030.5)¶	0.000
TNF-α, pg/ml	1.8 (0.2–10.9)‡	1.5 (0.1–12.2)	1.6 (0.5–5.0)	0.08	1.59 (0.33–7.68)¶	0.56

Definition of abbreviations: % pred, percentage of the predicted value; BMI, body mass index; CI, confidence interval; MAP, mean arterial pressure; MCP-1, monocyte chemotactic protein-1; OR, odds ratio; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

Data are presented as medians (range) unless stated otherwise.

* Odds ratios, with 95% confidence intervals estimated by unconditional logistic regression analysis adjusted for age, sex, and number of pack-years.

† *P* value of the Chi-square test, Fisher exact test, or nonparametric Kruskal-Wallis test, as appropriate, comparing the three populations (patients, smokers, and nonsmoker control subjects).

‡ $P \leqslant 0.05$ for the comparisons between patients and smoker control subjects (Kruskal-Wallis test).

§ $P \leqslant 0.05$ for the comparisons between smoker and nonsmoker control subjects (Kruskal-Wallis test).

‖ OR expressed for an increase of 5 pack-years.

¶ OR expressed for a change of 1 unit of measurement after $\log_{10}$ transformation.

remained significant after adjusting for age, sex, and tobacco exposure, using a multivariate logistic model (Table 1). To better assess whether telomere shortening was an effect of COPD, as opposed to smoking, we performed an additional analysis among smokers (⩾10 pack-years) by matching patients with COPD to control subjects on age (±2 yr), sex, and smoking history (±3 pack-years). This comparison of 50 patients with COPD and 50 matched control subjects showed a highly significant decrease in telomere length in the patients (Table 3). Telomere length within the second tertile had an odds ratio of 15.0 (95% confidence interval, 2.8–81.7) and within the first tertile of 28.3 (95% confidence interval, 5.0–159.1). As shown in Figure 1, both females and males with COPD had shorter telomere length values than their same-sex control subjects. Interestingly, telomere length was longer in female control subjects than in male control subjects, whereas no difference was noted between male and female patients (Figure 1). Compared with control smokers, patients had significantly increased plasma levels of IL-6, IL-8, IL-1β, TNF-α, and TGF-β but no significant differences in plasma levels of MCP-1 (Table 1). Within the control group, plasma levels of IL-6, IL-8, MCP-1, and TGF-β were higher in smokers than in nonsmokers. In contrast, plasma levels of TNF-α and IL-1β did not differ between smokers and nonsmokers.

Relationships between Telomere Length and Physiological and Biological Variables in Patients with COPD and in Control Subjects

In both patients with COPD and control subjects, telomere length correlated negatively with age (Table 4). However, telomere length was shorter in the patients than in the control subjects within each age group (Figure 2). No relationship was found between telomere length and pack-year history in the patients (Rho = 0.02, $P = 0.82$) or the control subjects (Rho = −0.09, $P = 0.25$) whereas a negative correlation was found when patients and control subjects were combined (Rho = −0.37, $P < 0.00001$). Moreover, telomere length in the control subjects did not differ between smokers and nonsmokers (Table 1). To evaluate whether telomere length was related to COPD severity, we examined the relationships between telomere length and gas exchange parameters, lung function test results, and functional indices in the patients. As shown in Table 4 and Figure 3, telomere length showed a strong positive correlation with both Pa_{O_2} and Sa_{O_2}, as well as a negative correlation with Pa_{CO_2}. No relationship was found between telomere length and FEV_1, FVC, $D_{LCO}/\dot{V}A$ (ratio of the diffusion capacity of the lung for carbon monoxide to alveolar ventilation), or the

TABLE 2. OTHER CHARACTERISTICS OF THE 136 PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

	n	Median Value	Range
Dyspnea score	107	3	0–5
6-Minute walking distance, m	115	400	80–620
BODE Index	96	4	0–10
Hb, g/dl	135	14.1	9.9–21.3
$D_{LCO}/\dot{V}A$, % pred	82	67.2	20.4–135
Pa_{O_2}, mm Hg	136	65.1	46.4–103
Pa_{CO_2}, mm Hg	136	42.1	30.8–66.0
Sa_{O_2}, %	136	93.4	76.2–98.5

Definition of abbreviations: BODE = *b*ody mass index, airflow *o*bstruction, *d*yspnea, and *e*xercise capacity; D_{LCO} = diffusion capacity of the lung for carbon monoxide; Dyspnea score = Medical Research Council Dyspnea Scale score; Hb, hemoglobin; Sa_{O_2} = oxygen saturation of arterial blood; $\dot{V}A$ = alveolar ventilation.

Blood gases were analyzed from arterial blood samples.

TABLE 3. COMPARISON OF TELOMERE LENGTH IN 50 PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND 50 CONTROL SUBJECTS MATCHED FOR SEX, AGE (±2 YR), AND SMOKING HISTORY (±3 PACK-YEARS)

				Adjusted Analysis*	
	Patients (*n* = *50*)	Control Subjects (*n* = *50*)	*P* Value†	OR (95% CI)	*P* Value
Male/female	35/15	35/15	1		
Age, yr	59 (49–76)	59 (50–78)	0.69		
Pack-years	35 (10–75)	34 (10–72)	0.79		
Telomere length (T/S ratio)	0.58 (0.23–1.18)	0.76 (0.42–1.19)	0.0001	0.00001 (0.8×10^{-7}–0.002)‡	0.000
⩾3rd tertile (0.85), n (%)	2 (4.0)	17 (34.0)		1	
3rd–1st tertile (0.63–0.85), n (%)	18 (36.0)	16 (32.0)		15.0 (2.8–81.7)	0.002
<1st tertile (0.63), n (%)	30 (60.0)	17 (34.0)	0.000	28.2 (5.0–159.1)	0.000

Definition of abbreviations: OR = odds ratio; T/S ratio = ratio of telomere repeat copy number to single-gene copy number.
Data are presented as medians (range) unless stated otherwise.
* Odds ratios, with 95% confidence intervals estimated by unconditional logistic regression analysis adjusted for age, sex, and number of pack-years.
† *P* value of the nonparametric Kruskal-Wallis test or the Fisher exact test, as appropriate.
‡ Odds ratio expressed for a change of 1 unit of measurement after $\log_{10}$ transformation.

BODE Index. Among BODE components, the 6-minute walking distance, which correlated negatively with age ($P < 0.001$), also correlated positively with telomere length. We found no significant difference in telomere length between patients with FEV_1 values less than or greater than 50% (0.56 [0.28–1.18] vs. 0.60 [0.23–1.16], respectively). Telomere length correlated negatively with IL-6 levels but did not correlate with other cytokine levels in the patients (Table 4). In the control subjects, only the relationship between telomere length and IL-8 was statistically significant.

DISCUSSION

The main finding from this study is that telomere length, as determined in peripheral leukocytes, is markedly decreased in patients with COPD compared with control subjects, independently from age, sex, and smoking history. Interestingly, telomere length was greater in female control subjects than in male control subjects, whereas no sex-related difference was found in the patients. In patients with COPD, telomere length correlated negatively with Pa_{O_2} and circulating IL-6 levels. Assuming that *in vivo* telomere length reflects cellular turnover and exposure to oxidative and inflammatory damage, these findings support accelerated aging in patients with COPD and suggest that telomere length may serve as a biomarker in this disease.

We found shorter telomeres in patients with COPD compared with age-matched control subjects with normal lung function. Telomere length decreased with age in both the patients and the control subjects but remained shorter in the patients after adjustment for age. The difference between patients and control subjects also persisted after stratification into age groups, indicating that COPD influenced telomere length at all ages. Telomere length was decreased in both males and females, and telomere length was not greater in the females than in the males of the COPD group, in contradistinction to what was seen in our control group and reported in other studies (24). Thus, COPD was associated with decreased telomere length and also with elimination of the sex-related difference seen in healthy individuals. Because tobacco use may be among the environmental factors that promote telomere shortening and cellular senescence, an important point was whether smoking affected telomere shortening and contributed to the decreased telomere length measured in patients with COPD. We found no relationship between telomere length and tobacco use in either group. Moreover, in the control group, telomere length did not differ between smokers and nonsmokers, or between current smokers, former smokers, and nonsmokers. The multivariate analysis indicated clearly that tobacco use did not affect the relationship between telomere length and COPD and the additional analysis comparing 50 patients and 50 control subjects strictly matched on sex, age, and smoking history revealed a highly significant telomere length decrease in the patients. Although these data do not rule out a contribution of smoking to telomere shortening, they indicate that the impact of smoking on telomere length may be small compared with that of COPD. In a previous study including patients with COPD and control smokers, telomere length was affected by smoking but not by COPD (20). The discrepancy with our results may be ascribable to the small number of individuals evaluated in this earlier study and also,

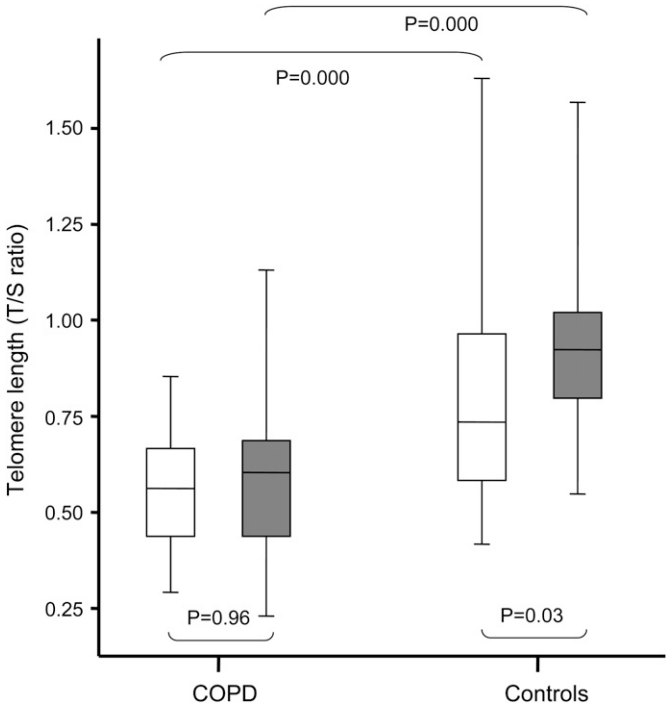

Figure 1. Telomere length in females and males among control subjects and patients with chronic obstructive pulmonary disease (COPD). Comparisons were adjusted for age and pack-years. Values represent median (interquartile range). *Bars* represent extreme values. *Shaded boxes* represent females; *open boxes* represent males. T/S ratio = ratio of telomere repeat copy number to single-gene copy number.

TABLE 4. CORRELATIONS BETWEEN TELOMERE LENGTH AND OTHER VARIABLES IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND IN CONTROL SUBJECTS WITH NORMAL LUNG FUNCTION

	Patients with COPD		Control Patients	
	Spearman's Rho	*P* Value	Spearman's Rho	*P* Value
Age, yr	−0.175	0.042	−0.29	0.0003
Pack-years	0.02	0.82	−0.09	0.25
FEV_1, %	0.03	0.75	0.04	0.66
FEV_1, L	0.08	0.38	0.03	0.71
FVC, L	0.11	0.22	0.01	0.90
FEV_1/FVC	0.009	0.91	0.10	0.24
Pa_{O_2}, mm Hg	0.27	0.002	—	
Pa_{CO_2}, mm Hg	−0.26	0.002	—	
Sa_{O_2}	0.25	0.004		
BODE Index	−0.07	0.48	—	
6-Minute walking distance	0.24	0.009	—	
IL-6, pg/ml	−0.27	0.005	−0.11	0.18
IL-8, pg/ml	−0.02	0.84	−0.21	0.012
IL-1β, pg/ml	−0.07	0.56	−0.13	0.22
TGF-β, pg/ml	0.023	0.84	−0.14	0.11
TNF-α, pg/ml	0.005	0.96	−0.03	0.67
MCP-1, pg/ml	−0.12	0.21	0.12	0.14

Definition of abbreviations: BODE = *b*ody mass index, airflow *o*bstruction, *d*yspnea, and *e*xercise capacity; COPD = chronic obstructive pulmonary disease; Hb, hemoglobin; MCP-1, monocyte chemotactic protein-1; Sa_{O_2} = oxygen saturation of arterial blood; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

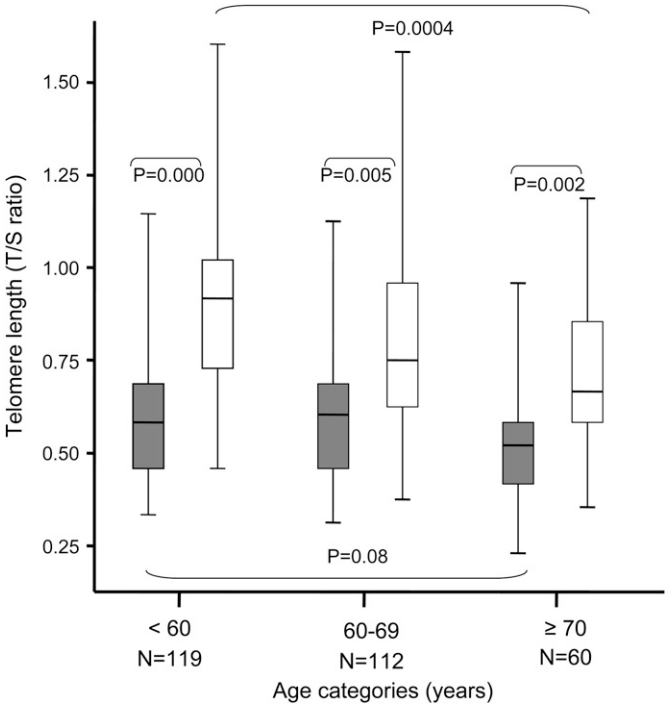

Figure 2. Telomere length in control subjects and patients with chronic obstructive pulmonary disease (COPD) in each 10-year age group. Comparisons were adjusted for sex and pack-years. Values represent median (interquartile range). *Bars* represent extreme values. *Shaded boxes* represent patients with COPD; *open boxes* represent control subjects. T/S ratio = ratio of telomere repeat copy number to single-gene copy number.

possibly, to the higher level of tobacco exposure in the control subjects, compared with our control group. A negative correlation between pack-years exposure and telomere length was also reported in a large cohort of women (21). In these studies, however, lung function test results were not available and, therefore, the specific effects of tobacco exposure could not be assessed independently from those of airflow limitation. In our study, when both patients and control subjects were pooled, a significant negative correlation was found between pack-years and telomere length. Thus, our data emphasize the importance of COPD as an independent risk factor for accelerated aging as assessed by telomere length, but they do not rule out a contribution of tobacco exposure.

Among physiological variables, Pa_{O_2} showed the strongest link to telomere length in patients with COPD. No direct relationship was found between telomere shortening and the severity of airway obstruction, and some of our patients with mild airway obstruction ($FEV_1 > 50\%$) had shorter telomeres than did control subjects with normal lung function. The relationship between Pa_{O_2} and telomere length may be of particular significance, because hypoxia is a known cause of cellular oxidative stress; and patients with COPD experience frequent episodes of oxygen desaturation during sleep, exercise, episodes of acute bronchitis, and COPD exacerbations. Whether treatment aimed at preventing oxygen desaturation and improving arterial oxygenation in patients with COPD may delay this process deserves evaluation.

COPD may affect telomere length by inducing systemic inflammation. Respiratory tract inflammation in patients with COPD is considered an exaggeration of the normal inflammatory response to chronic irritants such as cigarette smoke (1). A wide variety of inflammatory mediators is increased in the lung and blood of patients with COPD, even those whose clinical status is considered stable (25). Our data are consistent with these well-established concepts, as circulating levels of the proinflammatory cytokines IL-6, IL-8, IL-1β, and TGF-β were higher in patients with COPD than in control smokers or nonsmokers. In patients with COPD, IL-6 levels were related to the degree of telomere shortening. The association between IL-6 levels and telomere length is consistent with evidence that oxidative stress and inflammation mediate telomere attrition. In the control subjects, we also found a tendency for inflammatory

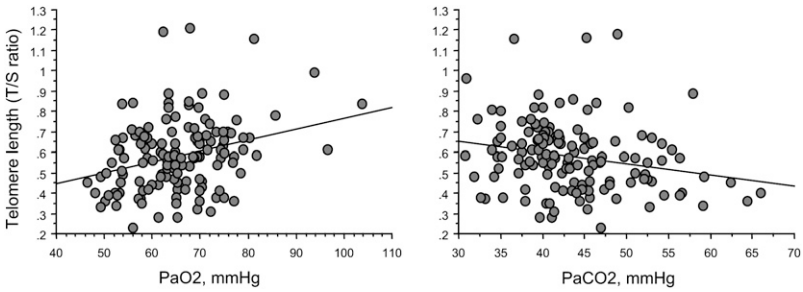

Figure 3. Correlations between telomere length, Pa_{O_2} (rho = 0.27, $P < 0.002$) and Pa_{CO_2} (rho = −0.26, $P < 0.02$) in patients with chronic obstructive pulmonary disease. T/S ratio = ratio of telomere repeat copy number to single-gene copy number.

markers to correlate with telomere length, although the relationship was significant only for IL-8. It is noteworthy that the inflammatory cytokine levels differed between smokers and nonsmokers whereas telomere length did not. Differences in cytokine levels, however, were much greater between patients with COPD and control smokers than between control smokers and nonsmokers. These results, therefore, do not disagree with the fact that inflammation may affect telomere shortening; however, they suggest a major role for inflammation severity in telomere shortening.

Telomere shortening may be of great significance in patients with COPD. Studies suggest that cell senescence may be associated with the pathological process of lung emphysema (26). Senescence of alveolar epithelial and endothelial cells has been shown to be accelerated in emphysematous lungs, and *in situ* measurements showed decreased telomere length in these cells (26). At present, it is unclear whether telomere dysfunction is a cause or a consequence of COPD. Although decreased telomere length may be viewed as a consequence of cellular turnover and exposure to oxidative and inflammatory damage, the observation that telomerase null mice, which experience accelerated aging, also exhibit structural alveolar damage suggests that telomere dysfunction may favor the development of emphysema (27). Our present findings of telomere shortening in circulating leukocytes from patients with COPD are consistent with cellular senescence in COPD being present not only in the lungs but also in other organs. This is of particular interest because cell senescence is considered part of the pathologic process of atherosclerosis (12, 17, 28). Telomere shortening is strongly associated with increased carotid intima–medial thickness and increased pulse wave velocity, two alterations closely linked to the development of atherosclerosis (24, 29). Moreover, there is growing evidence that short telomeres, by inducing chromosome instability, can promote the development of cancer (13). An association between short telomeres and lung cancer has been reported, suggesting that telomere shortening may also be a marker for susceptibility to lung cancer (15). Because lung cancer and cardiovascular events are two major causes of death in patients with COPD, our finding of decreased telomere length in patients with COPD warrants further studies to evaluate the potential prognostic usefulness of telomere length in patients with COPD.

***Conflict of Interest Statement*:** None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

***Acknowledgment*:** The authors gratefully acknowledge Prof. François Chabot for valuable discussion about the manuscript.

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