White Blood Cells Telomere Length Is Shorter in Males With Type 2 Diabetes and Microalbuminuria

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OBJECTIVE — To examine differences in telomere (terminal restriction fragment [TRF]) length and pulse wave velocity (PWV)—an index of arterial stiffness—in patients with type 2 diabetes with and without microalbuminuria (MA).

RESEARCH DESIGN AND METHODS — A total of 84 men with type 2 diabetes, 40 with MA and 44 without MA (aged 63.5 ± 9.0 vs. 61.2 ± 9.8 years), were studied. TRF length was determined in white blood cells. MA was defined as albumin excretion rate (AER) in the range of 30–300 mg/24 h in at least two of three 24-h urine collections. PWV was assessed using applanation tonometry. Markers of oxidative stress were also measured.

RESULTS — TRF length was shorter in patients with MA than in those without MA (6.64 ± 0.74 vs. 7.23 ± 1.01 kb, respectively, $P = 0.004$). PWV was significantly higher in the patients with MA. Multivariate linear regression analysis in the total sample demonstrated an independent association between TRF length and age ($P = 0.02$), MA status ($P = 0.04$) or AER ($P = 0.002$), and plasma nitrotyrosine levels ($P = 0.02$). AER was associated significantly with PWV ($P < 0.01$).

CONCLUSIONS — Subjects with type 2 diabetes and MA have shorter TRF length and increased arterial stiffness than those without MA. Additionally, TRF length is associated with age, AER, and nitrosative stress. As shorter TRF length indicates older biological age, the increased arterial stiffness in patients with type 2 diabetes who have MA may be due to the more pronounced "aging " of these subjects.

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Microalbuminuria (MA) is a common complication of diabetes, affecting almost 30–50% of the patients with type 2 diabetes (1,2). MA is a strong predictor of cardiovascular morbidity and mortality in individuals with diabetes (3,4). Although a number of abnormalities have been described in patients with MA, including high blood pressure, dyslipidemia, increased oxidative stress, inflammation, endothelial dysfunction, left ventricular hypertrophy, and hypercoagulation (rev. in 5), they do not seem adequate to explain the increased cardiovascular risk in this group of patients.

Telomeres, the tandem repeats of TTAGGG of the DNA sequence at the ends of eukaryotic chromosomes, undergo attrition with each cell division, and their length is an indicator of the replicative potential of somatic cells (6). Telomere length reflects the biological age of humans, which may differ from the chronological age (6). Inflammation and oxidative stress accelerate the rate of telomere attrition in different cell types (6–8). Telomere attrition has been associated with hypertension, endothelial dysfunction, arterial stiffening, atherosclerosis, and cardiovascular mortality (9–14). Diabetes is marked by increased oxidative stress and low-grade inflammation (15,16), phenomena which are further enhanced in the presence of MA (5,17,18).

The hypothesis we tested herein is that the mean length of telomeres, expressed as the mean length of the terminal restriction fragments (TRFs) of white blood cells (WBCs), is shorter in subjects with type 2 diabetes who have MA in comparison with diabetic subjects without MA. In addition, we looked for differences in pulse wave velocity (PWV)—an index of arterial stiffness—between the studied groups. Furthermore, potential relationships between TRF and PWV, low-grade inflammation, and markers of oxidative stress were also examined.

RESEARCH DESIGN AND METHODS

Subjects, blood pressure, and PWV measurements

A total of 84 subjects with type 2 diabetes (40 with MA and 44 without MA), consecutively attending the outpatient diabetes clinic of our hospital, were recruited in the study. To avoid the confounding effect of sex on TRF length (11), only men were included. Inclusion criteria required that their A1C was <8.5%, estimated glomerular filtration rate (eGFR) was >60 ml/min per 1.73 m^2, serum urea and creatinine concentrations were in the normal

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Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; AER, albumin excretion rate; ARB, angiotensin II receptor blocker; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; MA, microalbuminuria; PWV, pulse wave velocity; PWVcf, PWV between the carotid-femoral segment; PWVcr, PWV between the carotid-radial segment; TRF, terminal restriction fragment; WBC, white blood cell.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

See accompanying Editorial on p. 2974.

range, and participants were free of clinically apparent macrovascular disease, malignancy, or other chronic diseases. Subjects with proteinuria, hematuria, or nephropathy from other causes were excluded.

All subjects gave written informed consent before entering the study, which was conducted according to the principles of the declaration of Helsinki. The study was approved by the ethics committee of the Laiko Hospital, Athens, Greece.

All participants underwent complete physical examination in the morning of the study. They were questioned about previous and current diseases, use of medications and their smoking habits; exsmokers who had given up smoking for a period of at least 3 years were considered as nonsmokers. Retinopathy was reported from the medical records. BMI, waist circumference, and waist-to-hip ratio were measured and calculated.

Measurements of blood pressure and PWV were performed under a constant temperature (20–22°C). Blood pressure was measured in the nondominant arm three consecutive times, 2 min apart in the sitting position, using an appropriate cuff size. The mean value of the last two measurements was used in the statistical analysis. Arterial hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or use of antihypertensive medications. After anthropometric and blood pressure determination, PWV was measured by the same experienced person who was blind to the albuminuric status of the subjects, after they had remained in the supine position for 20 min using the SphygmoCor pulse wave analysis system (AtCor Medical, Sydney, Australia). This automatic device records online pulse wave and calculates PWV with two transducers—one positioned at the base of the neck for the common carotid artery and the other over the femoral artery for determination of PWV between the carotid-femoral segment (PWVcf) first, and subsequently over the right radial artery for determination of PWV between the carotid-radial segment (PWVcr) (19). PWVcf reflects aortic stiffness, while PWVcr reflects the stiffness of the arteries of the right upper limb (19). The intra-observer coefficient of variation of the SphygmoCor device tested in 30 subjects in our department was 6.4% for the PWVcf and 7.2% for the PWVcr. Afterward, fasting blood samples were collected, centrifuged, and either used immediately for measurement of biochemical parameters or stored in −80°C until determination of TRF length and plasma oxidative markers.

Analytical methods

Blood was collected after an overnight fast of at least 12 h. Serum glucose, lipids (total cholesterol, HDL cholesterol, and triglycerides), and creatinine were measured enzymatically on a Technicon RA-XT analyzer (Technicon, Dublin, Ireland). LDL cholesterol was calculated using the formula of Friedwald et al. and eGFR using the four-variable Modification of Diet in Renal Disease Study equation. A1C was measured by HPLC (Roche Diagnostics, Mannheim, Germany) with a nondiabetic reference range of 4.1–6.0%. Microalbuminuria was diagnosed when albumin excretion rate (AER), measured by radioimmunoassay (Pharmacia and Upjon Diagnostics, Upsala, Sweden), was in the range of 30–300 mg/24 h in at least two of three 24-h urine collections over a 3-month period. The average value of at least two determinations of AER was used in the analysis and is shown in tables. Plasma concentrations of high-sensitivity C-reactive protein (hsCRP) were determined by ELISA (IMTEC, Berlin, Germany). Plasma insulin concentrations were measured by radioimmunoassay (Biosure, Brussels, Belgium). Homeostasis model assessment was used to calculate insulin resistance (HOMA-IR) (20).

Measurement of the TRF length

DNA samples were extracted from white blood cells, and TRF length was measured as previously described (9,11). Briefly, DNA samples were digested overnight with restriction enzymes *Hinf*I and *Rsa*I (40 units) and resolved on a 0.8% agarose gel (15 × 25 cm) at 40 V (PowerPac Basic, Bio-Rad). After 20 h, the DNA was depurinated, denatured, and neutralized and then transferred for 1.5 h to a positively charged nylon membrane (Amersham). The membranes were hybridized with the telomeric probe [digoxigenin 3′-end labeled 5′-$(CCTAAA)_3$] overnight and washed in a sodium chloride and sodium citrate buffer. The digoxigenin-labeled probe was detected by the digoxigenin luminescent detection procedure (Roche) and exposed on X-ray film. Each DNA sample was measured in duplicate.

Oxidative stress determination

Total protein carbonyls in plasma were determined by using the Cayman assay kit according to the manufacturer's instructions (Cayman Chemical, Ann Arbor, MI). Protein carbonyls content was expressed in nanomoles per milligram of total protein. Nitrotyrosine in plasma was measured with a specific enzyme immunoassay (Cayman Chemical).

To access lipid peroxidation, we analyzed the levels of thiobarbituric acid–reactive substances in plasma by colorimetric assay (λ = 535 nm) (21).

Oxidative DNA damage

Reactive oxygen species alter deoxyguanosine, one of the constituents of DNA, into 8-hydroxy-2′-deoxyguanosine (8-OHdG), which is excised from DNA by the repair enzyme system and ultimately released into blood. Before 8-OHdG measurement (expressed in nanograms per milliliter), serum samples were thawed, applied to spin filters (10,000 Mw cutoff; Sartorius, Mannheim, Germany), and centrifuged at 12,000*g* in a benchtop centrifuge for 20 min. Flow-through was applied to a Gentaur highly sensitive competitive ELISA kit according to the manufacturer's instructions (Gentaur, Brussels, Belgium).

Statistical analysis

Analyses were performed using the SPSS 10.0 (SPSS) statistical package. All variables were tested for normal distribution of the data. Data are shown as means ± SD or as medians (interquartile range), as appropriate. Differences between the groups of patients with and without MA were examined using the Student's *t* test or the Mann-Whitney *U* test for parametric and nonparametric data, respectively, while a χ^2 test was used for categorical data. Analysis of covariance was used to test differences in TRF length between the studied groups adjusted for the effect of various confounding factors. Bivariate correlations were performed using the Pearson or the Spearman correlation coefficient, as appropriate. Univariate linear regression analyses were performed to look for relationships between TRF length and the studied parameters. Variables that were found to have a significant association in univariate analyses and PWVcr as well as PWVcf were entered in the multivariate analyses models (stepwise backward method). *P* values <0.05 (two-

Table 1—*Demographic, clinical, and biochemical parameters of the study subjects according to the presence (MA$^+$) or absence (MA$^-$) of microalbuminuria*

	MA$^-$	MA$^+$	*P*
n (%)	44 (52.5)	40 (47.6)	—
Age (years)	61.2 ± 9.8	63.5 ± 9.0	0.27
BMI (kg/m^2)	27.08 ± 3.70	29.48 ± 4.54	0.009
Waist circumference (cm)	99.57 ± 9.93	106.85 ± 11.38	0.002
Waist-to-hip ratio	0.97 ± 0.063	1.00 ± 0.05	0.05
Mean arterial blood pressure (mmHg)	95.1 ± 12.2	100.2 ± 11.6	0.053
Pulse pressure (mmHg)	59.3 ± 14.1	63.6 ± 12.3	0.14
Duration of diabetes (years)	6.0 (3.0–13.0)	8.0 (4.0–16.5)	0.42
A1C (%)	6.86 ± 0.86	7.24 ± 1.38	0.14
Glucose (mg/dl)	145.9 ± 45.0	159.1 ± 53.3	0.22
Total cholesterol (mg/dl)	215.34 ± 38.81	196.87 ± 35.36	0.02
HDL cholesterol (mg/dl)	41.02 ± 9.46	39.87 ± 6.71	0.53
LDL cholesterol (mg/dl)	147.12 ± 32.91	125.27 ± 31.09	0.003
Triglycerides (mg/dl)	128.02 ± 64.83	161.40 ± 93.11	0.05
Estimated GFR (ml/min per 1.73 m^2)	84.6 ± 18.2	86.7 ± 19.1	0.60
HOMA-IR	2.91 (1.75–5.49)	4.40 (3.50–7.03)	0.01
Current smokers	3 (6.8)	4 (10.0)	0.70
Hypertension (yes)	17 (38.6)	27 (67.5)	0.008
Use of ACE inhibitors and/or ARBs (yes)	15 (34.1)	23 (57.5)	0.03
Use of statins (yes)	8 (18.2)	18 (45)	0.008
Any retinopathy (yes)	7 (15.9)	8 (20.0)	0.62
Albumin excretion (mg/24 h)	5.5 (0.0–13.5)	88.6 (44.0–150.0)	<0.001
Treatment for diabetes			
Diet alone	7 (15.9)	3 (7.5)	
Antidiabetes tablets	31 (70.5)	30 (75.0)	
Insulin	6 (13.6)	7 (17.5)	0.47

Data are means ± SD, *n* (%), or median (interquartile range) unless otherwise indicated.

sided) were considered statistically significant.

RESULTS

Main clinical, biological, and hemodynamic parameters in subjects with and without MA

Patients with and without MA did not differ significantly in terms of age. BMI, waist circumference, waist-to-hip ratio, mean arterial pressure (MAP), and HOMA-IR values were higher in the patients with MA (Table 1). More patients with MA had hypertension. Participants with MA were more often on treatment with lipid-lowering medications and with ACE inhibitors and/or angiotensin II receptor blockers (ARBs) and had lower total and LDL cholesterol levels than those without MA. Pulse pressure, duration of diabetes, glycemic control, eGFR, and treatment for diabetes was not significantly different between the two groups (Table 1).

TRF length was significantly shorter in the patients with MA than in those without MA (6.64 ± 0.74 vs. 7.23 ± 1.01 kb, respectively, $P = 0.004$). This difference persisted after adjustment for BMI ($P = 0.006$), waist-to-hip ratio ($P = 0.01$), smoking status ($P = 0.005$), hypertension status ($P = 0.01$), use of statins ($P = 0.01$), use of ACE inhibitors and/or ARBs ($P = 0.005$), treatment with diet alone ($P = 0.007$), treatment with antidiabetes tablets other than glitazones ($P = 0.005$), treatment with glitazones ($P = 0.004$), and use of insulin ($P = 0.004$). PWVcf was significantly higher in the patients with than in those without MA (10.2 ± 1.9 vs. 9.1 ± 2.3 m/s, respectively, $P = 0.01$); the same was valid for the PWVcr (7.7 ± 1.3 vs. 6.6 ± 1.4 m/s, respectively, $P < 0.001$) (Fig. 1).

Plasma hsCRP values did not differ significantly between participants with and without MA (1.40 ± 0.72 vs. 1.41 ± 0.83 mg/dl, respectively, $P = 0.98$). Similarly, no significant difference was found between the two groups in plasma nitrotyrosine (1,140.8 ± 70.1 vs. 1,127.0 ± 59.4 nmol/l, $P = 0.35$), 8-OHdG (0.42 ± 0.12 vs. 0.44 ± 0.13 ng/ml, $P = 0.62$), and thiobarbituric acid reactive substance concentrations (2.86 ± 0.80 vs. 2.75 ± 0.56 μmol/l, $P = 0.48$). Plasma levels of protein carbonyls were higher in the patients with MA, but the difference did not reach the statistically significant level (0.82 [0.65–0.98] vs. 0.70 [0.51–0.91] nmol/mg, median [interquartile range], $P = 0.07$).

No significant correlations were found between AER and plasma hsCRP or the studied oxidative markers ($P > 0.05$). AER was associated significantly with both PWVcr ($r = 0.26$, $P = 0.01$) and PWVcf ($r = 0.38$, $P < 0.001$), and there was a suggestive association with pulse pressure ($r = 0.20$, $P = 0.06$).

Determinants of TRF length in the total sample population

Univariate linear regression analysis in the total sample population showed significant relationships between TRF length and age [unstandardized regression coefficient (b) ± SE (b) = −0.023 ± 0.011, $P = 0.03$)], waist-to-hip ratio (b = −3.761 ± 1.691, $P = 0.02$), triglycerides (b = −0.003 ± 0.001, $P = 0.02$), AER (b = −0.007 ± 0.002, $P = 0.007$), MA status (b = −0.605 ± 0.204, $P = 0.004$), PWVcr (b = −0.248 ± 0.069, $P = 0.001$), and plasma nitrotyrosine concentrations (b = −0.004 ± 0.002, $P = 0.03$). No significant associations were found between TRF length and BMI, waist circumference, hypertension or smoking status, MAP, the other plasma lipids, duration and degree of glycemia, type of antidiabetic treatment, eGFR, HOMA-IR, use of ACE inhibitors and/or ARBs, use of statins, PWVcf, pulse pressure, hsCRP, protein carbonyls, 8-OHdG, and thiobarbituric acid–reactive substances. Multivariate linear regression analysis, after adjustment for all these associated factors and for PWVcf, showed a significant and independent association only between TRF length and age (b = −0.021 ± 0.010, $P = 0.02$), AER (b = −0.007 ± 0.002, $P = 0.002$) or MA status (b = −0.421 ± 0.202, $P = 0.04$), PWVcr (b = −0.149 ± 0.064, $P = 0.02$), and plasma nitrotyrosine levels (b = −0.004 ± 0.002, $P = 0.02$).

Determinants of the TRF length in the subjects without MA

Univariate linear regression analysis in the normoalbuminuric subjects showed

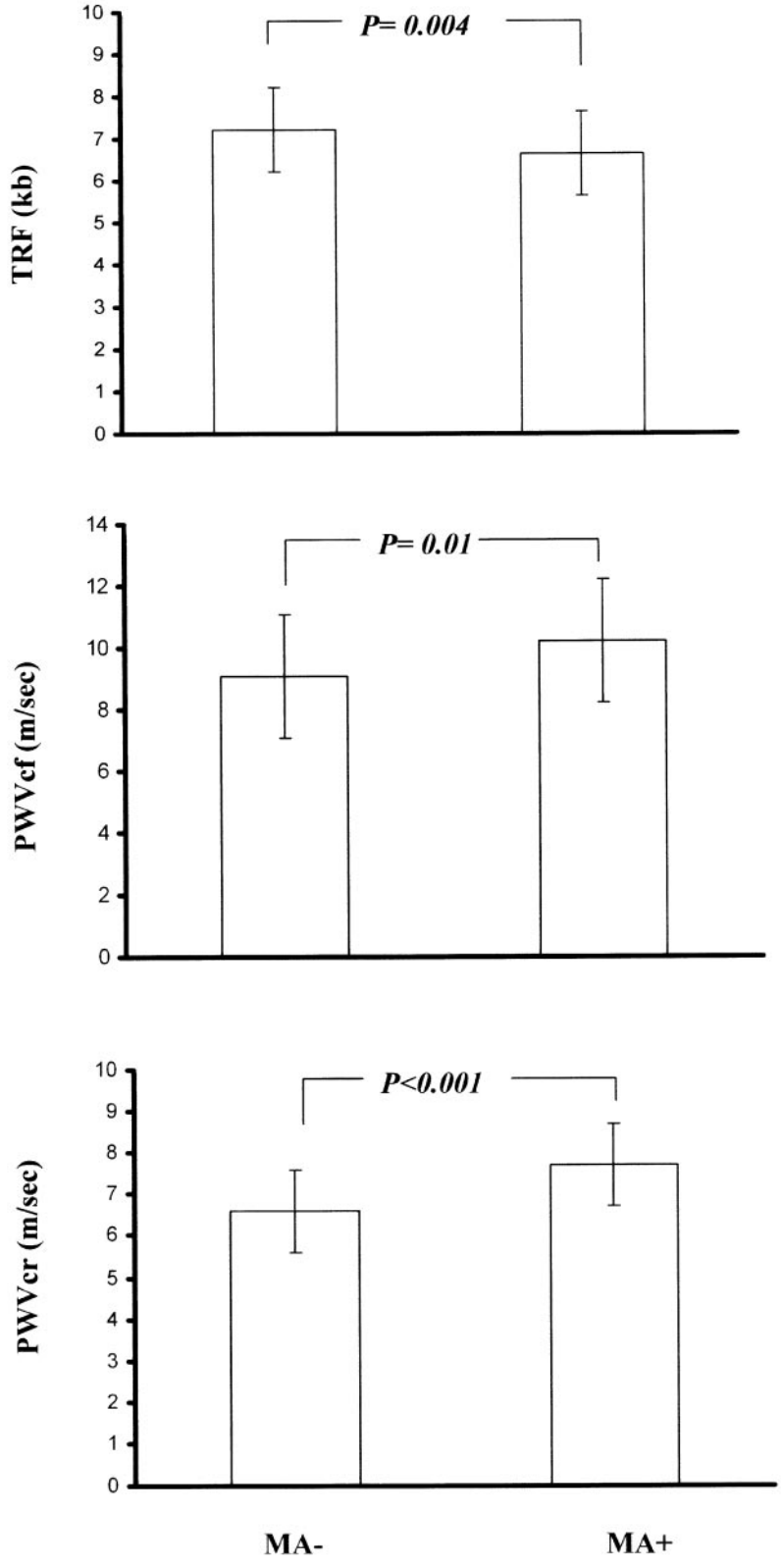

Figure 1—*Bars represent mean values ± SD of TRF and PWV in the subjects according to the presence (MA^+) or absence (MA^-) of microalbuminuria.*

significant relationships between TRF length and age (b = −0.035 ± 0.013, P = 0.01) and a suggestive association with eGFR (b = 0.010 ± 0.006, P = 0.08). No significant associations were found between TRF length and PWVcr, PWVcf, pulse pressure, and demographic, clinical, or laboratory parameters. Multivariate linear regression analysis, after adjustment for all these associated factors and for PWVcr as well as PWVcf, showed a significant independent association only between TRF length and age (b = −0.031 ± 0.014, P = 0.02).

Determinants of the TRF length in the subjects with MA

Univariate linear regression analysis in the microalbuminuric group showed significant associations between TRF length and AER (b = −0.005 ± 0.002, P = 0.01) as well as triglycerides (b = −0.003 ± 0.001, P = 0.04). No significant relationships were found between TRF length and PWVcr, PWVcf, pulse pressure, demographic, clinical, or laboratory parameters. Multivariate linear regression analysis, after adjustment for triglycerides and for PWVcf and PWVcr, showed a significant association only between TRF length and AER (b = −0.004 ± 0.001, P = 0.03).

CONCLUSIONS — The main findings of the present study are *1*) subjects with type 2 diabetes and MA have shorter TRF length and increased arterial stiffness than diabetic individuals without MA and *2*) in addition to age, AER and nitrosative stress are associated with TRF length.

Telomere shortening is emerging as an important molecular mechanism of vascular aging (6,22). Telomeres are involved in the maintenance of cellular stability (23). As DNA polymerases cannot fully repair the replication of the 3′-end of a linear DNA molecule to its very end, telomeres shorten with repeated cell division. Thus, in cultured somatic cells, telomeres act as a mitotic clock registering the number of cell divisions; when the telomere length reaches a critical value, senescence occurs (23). Shorter TRF length in either WBCs or monocytes has been described in patients with type 1 diabetes (24) and type 2 diabetes (25,26). In addition, recent data showed that the TRF length in patients with type 2 diabetes is associated with oxidative DNA damage (25) and insulin resistance (26). Collectively, these data suggest that in patients with diabetes, the enhanced telomere attrition could serve as a biomarker of advanced biological aging.

The present study demonstrates for the first time that patients with type 2 diabetes who have MA have shorter TRF length than diabetic individuals without

this complication. One previous study reported no difference in TRF length in WBCs between subjects with and without MA in either individuals with type 1 or type 2 diabetes (24). However, that study was designed to look for differences in TRF length between subjects with and without diabetes, and it was underpowered to confirm differences in TRF length according to microalbuminuric status, since a small number of the participants (10 with type 1 diabetes and 22 with type 2 diabetes) had MA.

Excessive production of reactive oxygen species has been described in the mononuclear cells of microalbuminuric hypertensive patients proportional to the degree of albuminuria (27). In addition, lower plasma antioxidant concentrations have been demonstrated in adults with MA (18). Increased oxidative stress is strongly associated with enhanced telomere attrition in WBCs (7,12,24,25), vascular smooth muscle cells (8), and endothelial cells (28). Additionally, low-grade inflammation has also been associated with shorter WBC telomeres (29).

We did not find significant differences in the oxidative markers studied or low-grade inflammation between patients with and without MA. Moreover, with the exception of plasma nitrotyrosine levels, oxidative markers or hsCRP were not associated with TRF length in WBCs. This finding may have several explanations. There is strong evidence that oxidative stress is responsible for accelerated telomere attrition in cultured human fibroblast and endothelial cells in vitro (23,28). Only one clinical study has found an association between TRF length and urinary isoprostane so far; this was described in male subjects from the Framingham study and suggests that oxidative stress may accelerate telomere erosion not only in vitro but also in vivo (7). However, other studies have failed to show an association between TRF length and oxidative stress in vivo, probably due to the complexity of the regulation of TRF length in this condition (23). Moreover, most of our patients have been treated with medications like statins and ACE inhibitors/ARBs, all of which possess anti-inflammatory and antioxidant properties (30–34). To the best of our knowledge, no data exist to suggest an effect of ACE inhibitors/ARBs on TRF length, while clinical and experimental data suggest that treatment with statins may have a protective effect on TRF attrition in human progenitor endothelial cells (35). Thus, it is possible that these associated treatments may have masked relationships between oxidative or inflammatory markers and TRF length.

The present study demonstrated a significant independent association between plasma nitrotyrosine levels with TRF length in the total sample population but not in the patients with MA. Increased nitrotyrosine concentrations accelerate apoptosis of myocytes, endothelial cells, fibroblasts, and chondrocytes (36). In addition, nitrosative stress is considered as a major pathogenetic mechanism in the development and progression of diabetic nephropathy, and previous reports described intense immunohistochemical staining for nitrotyrosine in both human and experimental diabetic renal disease (37,38). On the other hand, statins and ACE inhibitors/ARBs may reduce plasma and urine nitrotyrosine levels and attenuate the expression of nitrotyrosine in renal tissue (39–41). The high percentage of the microalbuminuric patients treated with such medications in our study probably explains the lack of association between TRF length and plasma nitrotyrosine levels in the patients with MA.

We did not find significant association between TRF length in WBCs and plasma 8-OHdG, which is a sensitive and specific indicator of oxidative DNA damage. Sampson et al. (25) showed a direct relationship between oxidative DNA damage, assessed by determination of plasma 8-oxoguanin levels and TRF length in the peripheral blood monocyte cells. The divergent results may be because the monocyte cells have a high turnover rate as their adherence to the endothelium, entrance to the vascular wall, and macrophage transformation are enhanced (25). Additionally, recent data suggest that the macrophage-mononuclear cells within vascular plaque have a senescent and apoptotic phenotype (42). Moreover, Sampson et al. (25) studied a highly selected group of patients with type 2 diabetes without MA and not treated with ACE inhibitors; therefore, the results of the two studies are not comparable.

Our findings corroborate previous reports showing that in patients with type 2 diabetes, MA is associated with increased vascular aging, manifested either as increased carotid wall thickness or PWV (43,44). The profound difference in TRF length (of 590 bp) between the two groups and the expected (as it was shown by the present study in the normoalbuminuric group and previous studies [6,9]) decline of 24–35 bp per year in TRF length implies a much older biological age (despite the similar chronological age) of microalbuminuric patients. Thus, the increased arterial stiffness in patients with MA may be due to the more pronounced "aging" of these patients. No significant independent associations between TRF length and measures of arterial stiffness were found in our study, as previously described in male hypertensive subjects without diabetes and not treated with antihypertensive medications (11). An association between PWVcf and TRF length was observed in subjects not receiving any antihypertensive treatment (11). Actually, antihypertensive treatment has profound effects on PWVcf, while, as we mentioned before, the effects of such treatment on TRF length is unknown. The high percentage of the patients treated with statins and ACE inhibitors/ARBs in our study, medications with known favorable effects on arterial stiffness (32,45), may have affected the discrepancy in the association between TRF length and indexes of arterial stiffness.

In agreement with previous reports (24–26), we did not find significant relationships between TRF and duration as well as degree of glycemia. This finding implies that the consequences of hyperglycemia, such as oxidative stress and inflammation, rather than hyperglycemia per se may be more important factor affecting telomeres. Within the constraints of the limited numbers, we showed that the type of antidiabetic treatment was not associated with TRF length.

This study does not explain the mechanisms responsible for the short TRF length in the microalbuminuric patients. A plausible explanation is that the shorter TRF length reflects an increased WBC turnover as a consequence of the chronic inflammation and oxidative stress that accompany MA. The importance of MA on telomere attrition is suggested by the independent association in the total sample between MA with TRF length. In addition, in the group of patients with MA, only AER, and not age, which is consistently associated with TRF length in previous studies (6,9,11), emerged as a predictor of TRF length, suggesting a cardinal role for MA in the enhanced telomere attrition. However, other explanations need to be considered. As TRF length is genetically determined (6), patients with type 2 diabetes and MA may be preprogrammed to both shorter TRF and

MA. Alternatively, the shorter TRF length in WBCs in the patients with MA could reflect more developmental cell divisions during the uterine or early postnatal period (6).

Because no previous data were available to allow sample size calculations at the initiation of the study, power calculations were performed at the end of the study. A sample size of 80 subjects would have 88% power at the 0.001 level to detect the observed difference of 590 bp in the mean values of the TRF length between participants with and without MA.

This study is not without limitations. Most of the participants were on treatment with medications with effect on oxidative stress, inflammation, and arterial stiffness, and these associated treatments may have blunted potential associations between these markers and TRF length as well as PWV. Moreover, the cross-sectional design of our study does not provide evidence for a cause and effect relationship between MA and telomere shortening. Furthermore, our study was underpowered to examine relationships between TRF length or AER and either low-grade inflammation or oxidative markers, all of which were secondary end points.

In conclusion, patients with type 2 diabetes who have MA have enhanced WBC telomere attrition and increased arterial stiffness than patients with type 2 diabetes without MA. In addition, TRF length is associated with age, AER, and nitrosative stress. As shorter WBC telomere length indicates an older biological age, the increased arterial stiffness in patients with type 2 diabetes and MA may be due to the more pronounced "aging " of these patients. MA should be considered in the design of studies exploring the links between WBC telomere length and diseases of aging.

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