White Blood Cells Telomere Length is Shorter in Males with Type 2 Diabetes and Microalbuminuria

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Short title: Telomere shortening in microalbuminuria

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Abstract

Objective: To examine differences in telomere (TRF) length and pulse wave velocity (PWV)-an index of arterial stiffness-in patients with type 2 diabetes mellitus (T2DM) with and without microalbuminuria (MA).

Research Design and Methods: Eighty four males with T2DM, 40 with MA and 44 without MA (age 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 (AER) rate in the range of 30-300 mg/24 hours in at least two out of three 24 hours 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 T2DM 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 T2DM who have MA may be due to the more pronounced ‘aging’ of these subjects.
Microalbuminuria (MA) is a common complication of type 2 diabetes mellitus (T2DM) affecting almost 30-50% of the patients with T2DM (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 (reviewed 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 (TRF) of WBC, is shorter in subjects with T2DM who have MA in comparison with diabetic subjects without MA. In addition, we looked for differences in PWV—an index of arterial stiffness—between the studied groups. Furthermore, potential relationships between TRF and pulse wave velocity (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 T2DM (40 with MA and 44 without MA), consecutively attending the outpatient diabetes clinic of our hospital were recruited in the study. In order to avoid the confounding effect of sex on TRF length (11) only males were included. Inclusion criteria required that their GHB A1C (A1C) was < 8.5%, estimated glomerular filtration rate (eGFR) > 60 ml/min/1.73 m², serum urea and creatinine concentrations were in the normal 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; ex-smokers who had given up smoking for a period of at least three years were considered as non-smokers. Retinopathy was reported from the medical records. BMI, waist circumference, and waist-hip ratio (WHR) were measured and calculated. Measurements of blood pressure and pulse wave velocity PWV were performed under constant temperature (20°C to 22°C). Blood pressure was measured in the non-
dominant arm three consecutive times, two minutes 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 minutes using the SphygmoCor pulse wave analysis system (AtCor Medical, 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 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. Afterwards, 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 hours. Serum glucose, lipids (total cholesterol, HDL cholesterol, triglycerides) and creatinine were measured enzymatically on a Technicon RA-XT analyzer (Technicon Ltd, 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, Germany) with a non-diabetic reference range of 4.1-6.0%. Microalbuminuria was diagnosed when albumin excretion rate (AER), measured by radioimmunoassay (RIA) (Pharmacia and Upjon Diagnostics AB, Sweden), was in the range of 30-300 mg/24-hours in at least two out of three 24-hours urine collections over a three 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, Germany). Plasma insulin concentrations were measured by radioimmunoassay (Biosure, Belgium). Homeostatic 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 measured as previously described (9,11). Briefly, DNA samples were digested overnight with restriction enzymes Hinf I and Rsa I (40 U) and resolved on a 0.8% agarose gel (15 cm x 25 cm) at 40 V (PowerPac Basic, Biorad). After 20 hours, the DNA was depurinated, denatured and neutralized, and then transferred for 1.5 hour to a positively charged nylon membrane (Amersham). The membranes were hybridized with the telomeric probe [digoxigenin 3'-end labeled 5'-(CCTAAA)] overnight and washed in SSC 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 company, USA). Protein carbonyls content was expressed in nanomoles per milligram (nmol/mg) of total protein.

Nitrotyrosine in plasma was measured with a specific enzyme immunoassay (EIA) (Cayman Chemical Company, USA).

To access lipid peroxidation, we analyzed the levels of thiobarbituric acid reactive substances (TBARS) in plasma by colorimetric assay ($\lambda = 535$ 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 ng/ml), serum samples were thawed, applied to spin filters (10,000 Mw cut-off, Sartorius AG, Germany) and centrifuged at 12,000 g in a benchtop centrifuge for 20 min. Flow-through was applied to Gentaur highly sensitive competitive ELISA kit according to the manufacturer's instructions (Gentaur, Belgium).

**Statistical analysis**

Analyses were performed using the SPSS 10.0 (SPSS, USA) statistical package. All variables were tested for normal distribution of the data. Data are shown as mean $\pm$ SD, or as median (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 non-parametric data, respectively, while a chi-square 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-sided) were considered statistically significant.

**Results**

**Main clinical, biological, and hemodynamic parameters in subjects with and without microalbuminuria**

Patients with and without MA did not differ significantly in terms of age. BMI, waist circumference, WHR, 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 (ACE-I) and/or angiotensin II receptor blockers (ARBs), and had lower total and LDL cholesterol levels than those without MA. Pulse pressure (PP), duration of diabetes, glycaemic 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 ($6.64 \pm 0.74$ vs. $7.23 \pm 1.01$ kb, respectively, $P=0.004$). This difference
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Persisted after adjustment for BMI \((P=0.006)\), WHR \((P=0.01)\), smoking status \((P=0.005)\), hypertension status \((P=0.01)\), use of statins \((P=0.01)\), use of ACE-I and/or ARB \((P=0.005)\), treatment with diet alone \((P=0.007)\), treatment with antidiabetic 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 \((10.2 \pm 1.9 \text{ vs. } 9.1 \pm 2.3 \text{ m/sec, respectively, } P=0.01)\); the same was valid for the PWVcr \((7.7 \pm 1.3 \text{ vs. } 6.6 \pm 1.4 \text{ m/sec respectively, } P<0.001)\) (Figure 1).

Plasma hsCRP values did not differ significantly between participants with and without MA \((1.40 \pm 0.72 \text{ vs. } 1.41 \pm 0.83 \text{ mg/dl, respectively } P=0.98)\). Similarly, no significant difference was found between the two groups in plasma nitrotyrosine \((1140.8 \pm 70.1 \text{ vs. } 1127.0 \pm 59.4 \text{ nM, } P=0.35)\), 8-OHdG \((0.42 \pm 0.12 \text{ vs. } 0.44 \pm 0.13 \text{ ng/ml, } P=0.62)\), and TBARS concentrations \((2.86 \pm 0.80 \text{ vs. } 2.75 \pm 0.56 \mu \text{M, } P=0.48)\). Plasma levels of protein carbonyls were higher in the patients with MA but the difference did not reach the statistically significant level [median (interquartile range)] \([0.82 (0.65-0.98) \text{ vs. } 0.70 (0.51-0.91) \text{ nmol/mg, } P=0.07]\).

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 PP \((r = 0.20, P=0.06)\).

### 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 \([\text{unstandardized regression coefficient } (b) = -0.023 \pm 0.011, P=0.03]\), WHR \((b= -3.761 \pm 1.691, P=0.02)\), triglycerides \((b= -0.003 \pm 0.001, P=0.02)\), AER \((b= -0.007 \pm 0.002, P=0.007)\), MA status \((b= -0.605 \pm 0.204, P=0.004)\), PWVcr \((b= -0.248 \pm 0.069, P=0.001)\), and plasma nitrotyrosine concentrations \((b= -0.004 \pm 0.002, P=0.03)\). 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 glycaemia, type of antidiabetic treatment, eGFR, HOMA-IR, use of ACE-I and/or ARB, use of statins, PWVcf, PP, hsCRP, protein carbonyls, 8-OHdG, as well as TBARS. 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 \((b= -0.021 \pm 0.010, P=0.02)\), AER \((b= -0.007 \pm 0.002, P=0.002)\) or MA status \((b= -0.421 \pm 0.202, P=0.04)\), PWVcr \((b= -0.149 \pm 0.064, P=0.02)\) as well as plasma nitrotyrosine levels \((b= -0.004 \pm 0.002, P=0.02)\).

### Determinants of the TRF length in the subjects without microalbuminuria

Univariate linear regression analysis in the normoalbuminuric subjects showed significant relationships between TRF length and age \((b= -0.035 \pm 0.013, P=0.01)\), and a suggestive association with eGFR \((b= 0.010 \pm 0.006, P= 0.08)\). No significant associations were found between TRF length and PWVcr, PWVcf, PP as well as demographic, clinical or laboratory parameters. Multivariate linear regression analysis, after adjustment for all these associated factors and for PWVcf as well as PWVcr, showed a significant independent association only between TRF length and age \((b= -0.031 \pm 0.014, P=0.02)\).
Determinants of the TRF length in the subjects with microalbuminuria

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, PP, demographic, clinical or laboratory parameters. Multivariate linear regression analysis, after adjustment for triglycerides and for PWVcf as well as 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: (i) subjects with T2DM and MA have shorter TRF length and increased arterial stiffness than diabetic individuals without MA, and (ii) 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 WBC or monocytes has been described in patients with type 1 (24) and type 2 diabetes (25,26). In addition, recent data showed that the TRF length in patients with T2DM 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 T2DM who have MA have shorter TRF length than diabetic individuals without this complication. One previous study reported no difference in TRF length in WBC 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 as a small number of the participants (10 with type 1 diabetes and 22 with T2DM) 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 WBC (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 WBC. 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 males 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-I/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-I/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-I/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 WBC 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 due to the fact that 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 T2DM without MA and not treated with ACE-I; therefore, the results of the two studies are not comparable.

Our findings corroborate previous reports showing that in patients with T2DM, 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-I/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 indices 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, like 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 the 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), was 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 T2DM and MA may be pre-programmed to both shorter TRF and MA. Alternatively, the shorter TRF length in WBC in the patients with MA could reflect more developmental cell divisions during uterine or early post-natal period (6).

As 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 T2DM who have MA have enhanced WBC telomere attrition and increased arterial stiffness than patients with T2DM 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 T2DM 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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References


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stiffness independently of blood pressure lowering in hypertensive patients. *Hypertens Res* 28: 937-943, 2005
Table 1. Demographic, clinical, and biochemical parameters of the study subjects according to the presence (MA+) or not (MA-) of microalbuminuria.

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<th>MA-</th>
<th>MA+</th>
<th>P</th>
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<tr>
<td>n (%)</td>
<td>44 (52.5)</td>
<td>40 (47.6)</td>
<td>-</td>
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<tr>
<td>Age (years)</td>
<td>61.2 ± 9.8</td>
<td>63.5 ± 9.0</td>
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<td>BMI (kg/m²)</td>
<td>27.08 ± 3.70</td>
<td>29.48 ± 4.54</td>
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<td>Waist circumference (cm)</td>
<td>99.57 ± 9.93</td>
<td>106.85 ± 11.38</td>
<td>0.002</td>
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<tr>
<td>Waist-to-hip ratio</td>
<td>0.97 ± 0.063</td>
<td>1.00 ± 0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>95.1 ± 12.2</td>
<td>100.2 ± 11.6</td>
<td>0.053</td>
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<tr>
<td>Pulse pressure (mm Hg)</td>
<td>59.3 ± 14.1</td>
<td>63.6 ± 12.3</td>
<td>0.14</td>
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<tr>
<td>Duration of diabetes (years)*</td>
<td>6.0 (3.0-13.0)</td>
<td>8.0 (4.0-16.5)</td>
<td>0.42</td>
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<tr>
<td>A1c (%)</td>
<td>6.86 ± 0.86</td>
<td>7.24 ± 1.38</td>
<td>0.14</td>
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<tr>
<td>Glucose (mg /dl)</td>
<td>145.9 ± 45.0</td>
<td>159.1 ± 53.3</td>
<td>0.22</td>
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<tr>
<td>Total cholesterol (mg /dl)</td>
<td>215.34 ± 38.81</td>
<td>196.87 ± 35.36</td>
<td>0.02</td>
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<tr>
<td>HDL cholesterol (mg /dl)</td>
<td>41.02 ± 9.46</td>
<td>39.87 ± 6.71</td>
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<td>LDL cholesterol (mg /dl)</td>
<td>147.12 ± 32.91</td>
<td>125.27 ± 31.09</td>
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<td>Triglycerides (mg /dl)</td>
<td>128.02 ± 64.83</td>
<td>161.40 ± 93.11</td>
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<tr>
<td>Estimated GFR (ml/min/1.73 m²)</td>
<td>84.6 ± 18.2</td>
<td>86.7 ± 19.1</td>
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<td>HOMA-IR*</td>
<td>2.91 (1.75-5.49)</td>
<td>4.40 (3.50-7.03)</td>
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<td>Current smokers n (%)</td>
<td>3 (6.8)</td>
<td>4 (10.0)</td>
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<td>Hypertension (yes) n (%)</td>
<td>17 (38.6)</td>
<td>27 (67.5)</td>
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<td>Use of ACE-I and/or ARBs (yes) n (%)</td>
<td>15 (34.1)</td>
<td>23 (57.5)</td>
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<td>Use of statins (yes) n (%)</td>
<td>8 (18.2)</td>
<td>18 (45)</td>
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<td>Any retinopathy (yes) n (%)</td>
<td>7 (15.9)</td>
<td>8 (20.0)</td>
<td>0.62</td>
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<td>Albumin excretion (mg/24 hours)*</td>
<td>5.5 (0.0-13.5)</td>
<td>88.6 (44.0-150.0)</td>
<td>&lt;0.001</td>
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<td>Treatment for diabetes n (%)</td>
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<tr>
<td>Diet alone</td>
<td>7 (15.9)</td>
<td>3 (7.5)</td>
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<tr>
<td>Antidiabetic tablets</td>
<td>31 (70.5)</td>
<td>30 (75.0)</td>
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<td>Insulin</td>
<td>6 (13.6)</td>
<td>7 (17.5)</td>
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</table>
Legend to Figure 1.

Bars represent mean values ± SD of the telomere length (TRF) and pulse wave velocity (PWV) in the subjects according to the presence (MA+) or not of microalbuminuria (MA-). cr: carotid-radial segment; cf: carotid-femoral segment.