Mol Cell Biochem (2012) 365:343–350
DOI 10.1007/s11010-012-1276-0

Accelerated aging as evidenced by increased telomere shortening and mitochondrial DNA depletion in patients with type 2 diabetes

Finny Monickaraj · Sankaramoorthy Aravind · Kuppan Gokulakrishnan · Chandrakumar Sathishkumar · Paramasivam Prabu · Durai Prabu · Viswanathan Mohan · Muthuswamy Balasubramanyam

Received: 21 December 2011 / Accepted: 24 February 2012 / Published online: 13 March 2012

Abstract Although shortened telomeres were shown associated with several risk factors of diabetes, there is lack of data on their relationship with mitochondrial dysfunction. Therefore, we compared the relationship between telomere length and mitochondrial DNA (mtDNA) content in patients with type 2 diabetes mellitus (T2DM; $n = 145$) and in subjects with normal glucose tolerance (NGT; $n = 145$). Subjects were randomly recruited from the Chennai Urban Rural Epidemiology Study. mtDNA content and telomere length were assessed by Real-Time PCR. Malonodialdehyde, a marker of lipid peroxidation was measured by thiobarbituric acid reactive substances (TBARS) using fluorescence methodology. Adiponectin levels were measured by radioimmunoassay. Oxidative stress as determined by lipid peroxidation (TBARS) was significantly ($p < 0.001$) higher in patients with T2DM compared to NGT subjects. In contrast, the mean telomere length, adiponectin and mtDNA content were significantly ($p < 0.001$) lower in patients with T2DM compared to NGT subjects. Telomere length was positively correlated with adiponectin, HDL, mtDNA content and good glycemic/lipid control and negatively correlated with adiposity and insulin resistance. On regression analysis, shortened telomeres showed significant association with T2DM even after adjusting for waist circumference, insulin resistance, triglyceride, HDL, adiponectin, mtDNA & TBARS. mtDNA depletion showed significant association with T2DM after adjusting for waist circumference and adiponectin but lost its significance when further adjusted for telomere length, TBARS and insulin resistance. Our study emphasizes the clustering of accelerated aging features viz., shortened telomeres, decreased mtDNA content, hypoadiponectinemia, low HDL, and increased oxidative stress in Asian Indian type 2 diabetes patients.

Keywords Telomere shortening · mtDNA depletion · Oxidative stress · Type 2 diabetes

Introduction

There is an upsurge in the prevalence of age-related diseases such as type 2 diabetes mellitus (T2DM) globally and more importantly in developing countries like India. Recent studies imply that regulation of aging and energy homeostasis share similar molecular pathways [1]. Many of the genes recently discovered that can be manipulated to slow the aging process belong to pathways involved in the control of metabolism. Energy homeostasis dysregulation occurs during the aging process and this appears to occur at an accelerated way in metabolic diseases like type 2 diabetes. Diabetes mellitus has recently been recognized as a cause of accelerated aging [2]. As the understanding of the metabolic syndrome has evolved, it has been recognized that the interaction of a panoply of factors in the presence of insulin resistance results in accelerated aging. The theory of aging [3] claims that the main place of production of free radicals (oxidative stress) is in mitochondria and this leads to mitochondrial DNA (mtDNA) damage and

F. Monickaraj · S. Aravind · K. Gokulakrishnan · C. Sathishkumar · P. Prabu · D. Prabu · V. Mohan · M. Balasubramanyam (✉)
Department of Cell and Molecular Biology, Madras Diabetes Research Foundation and Dr. Mohan's Diabetes Specialities Centre, WHO Collaborating Centre for Non-Communicable Diseases Prevention and Control, IDF Centre of Education, Gopalapuram, Chennai 600 086, Tamilnadu, India
e-mail: balusignal@gmail.com; drbalu@mdrf.in

mutations in mtDNA which further leads to progressive dysfunction in respiratory chain activity and mitochondrial dysfunction. Free radicals in excess, damage the DNA and in particular the guanine sites of the DNA are more vulnerable to this oxidative attack [4]. Telomere ends of the DNA which protects the genomic integrity are enriched with guanine sites and they are most susceptible to free radical attack leading to shortening of telomeres [5].

Accumulating evidence suggests that increased oxidative stress is considered as a unifying mechanism for the genesis of diabetes and its progression to vascular complications [6]. On the other hand, shortened telomeres have been shown to be associated with several disease states including cardiovascular diseases [7] and type 1 diabetes [8]. Our group was the first to report shortening of telomeres in patients with T2DM [9] and this has been later confirmed by several others [10–14]. Recently, it has been suggested that combining multiple biomarkers and conventional risk factors might substantially enhance the understanding of the underlying causes of T2DM [15]. While telomere shortening has been shown to be associated with T2DM, there is lack of studies that comprehensively explored the relationship among telomere length, oxidative stress, mtDNA content, and adiponectin levels. We reasoned that the higher insulin resistance and susceptibility to develop type 2 diabetes and cardiovascular diseases in Asian Indians could be explained by studying these emerging biomarkers. Therefore, we made an attempt to study some of the markers of accelerated aging with special reference to telomere length and mitochondrial DNA (mtDNA) content in Asian Indian subjects with type 2 diabetes and normal glucose tolerance.

Research design and methods

Study subjects were recruited from the Chennai Urban Rural Epidemiology Study (CURES); the detailed study design are described elsewhere [16]. In Phase 3 of CURES, every tenth subject recruited in Phase 1 ($n = 2{,}600$) was invited to our centre for detailed anthropometric measurements and biochemical tests. Of these, 2,350 participated in the study (response rate: 90.4 %) and they underwent an oral glucose tolerance test using 75 g glucose load. Diagnosis of diabetes was based on WHO Consulting group criteria, i.e., 2 h post glucose load (plasma) (2 h PG) $\geq$ 11.1 mmol/l or 200 mg/dl and/or fasting plasma glucose (FPG) $\geq$ 126 mg/dl or self reported diabetic subjects on treatment by a physician. Normal glucose tolerance (NGT) was diagnosed if 2 h PG was $<$7.8 mmol/l or 140 mg/dl and/or FPG $\leq$100 mg/dl [17]. For the present study, we randomly selected (using computer-generated random numbers) 145 NGT subjects and 145 subjects with type 2 diabetes mellitus (T2DM). Among the patients with type 2 diabetes, 90 % were on oral-antidiabetic drugs and 10 % were on insulin along with oral-antidiabetic drugs. None of them were on statins. Ethical committee approval was obtained from the Madras Diabetes Research Foundation's institutional ethical committee and written informed consent was obtained from all study subjects.

Anthropometric measurements

Anthropometric measurements including weight, height, and waist circumference were obtained using standardized techniques as detailed elsewhere [16]. Height was noted with a tape measured to the nearest centimeter. Weight was measured with traditional spring balance that was kept on a firm horizontal surface. Body mass index (BMI) was calculated using the formula weight (kg)/height $(m)^2$. Waist circumference was measured using a non stretchable fiber measuring tape. Waist girth was measured as the smallest horizontal girth between the costal margins and the iliac crests at minimal respiration. Blood pressure was recorded from the right arm in a sitting position to the nearest 2 mm Hg with a mercury sphygmomanometer (Diamond Deluxe BP apparatus, Pune, India). Two readings were taken 5 min apart, and the mean of the two was taken as the blood pressure.

Biochemical parameters

Fasting plasma glucose (glucose oxidase–peroxidase method), serum cholesterol (cholesterol oxidase–peroxidase-amidopyrine method), serum triglycerides (glycerol phosphate oxidase–peroxidase-amidopyrine method), and HDL cholesterol (direct method-polyethylene glycol-pretreated enzymes) were measured using Hitachi-912 Autoanalyser (Hitachi, Mannheim, Germany). The intra- and inter-assay co-efficient of variation for the biochemical assays ranged between 3.1 and 7.6 %. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. Glycated hemoglobin (HbA1c) was estimated by high-pressure liquid chromatography using the Variant analyzer (Bio-Rad, Hercules, Calif., USA). The intra- and inter-assay co-efficient of variation of HbA1c was $<$10 %. Insulin resistance was calculated using the homeostasis assessment model (HOMA-IR) using the formula: fasting insulin (μU/ml)* fasting glucose (mmol/l)/22.5. Adiponectin levels were measured using radioimmunoassay method (Linco Research, St Charles, MO, USA). The intra-assay and the inter-assay co-efficient of variation were 3.8 and 7.4 %, respectively, and the lower detection limit was 1 ng/ml.

Measurement of telomere length

For the measurement of telomere length, DNA was isolated from whole blood by phenol–chloroform extraction and

ethanol precipitation [18]. Relative telomere length was determined by Real-time PCR approach as previously described by Cawthon [19] with a minor modification in the PCR temperature conditions. This method measures the factor by which the ratio of telomere repeat copy number to single—gene copy number differs between a sample and that of a reference DNA sample. PCR amplification was achieved using telomere (T) and single copy gene, 36B4 (encodes acidic ribosomal phosphoprotein) primers (S) which serves as a quantitative control. The mean telomere repeat gene sequence (T) to a reference single copy gene (S) is represented as T/S ratio which was calculated to determine the relative telomere length. In brief, PCR reactions were performed in triplicate in 20 µl reaction volumes (using 25 ng DNA sample per reaction) for all the samples studied. The PCR mixture contained 10 pmol of each of the primers, 100 µM of each dNTPs, and 0.3 × SYBR green dye and 0.5 Units of fast Taq DNA polymerase (Fermentas, USA). The PCR thermal conditions for relative telomere length assay using telomeric primers (T) and single copy gene primers (S) consisted of a initial denaturation of 5 min at 95 °C, followed by a total of 40 cycles at 95 °C for 5 s, 56 °C for 30 s, and 72 °C for 30 s and fluorescence acquisition. Crossing points (Cp) were determined using the ABI 7000. A standard curve derived from serially-diluted reference DNA was generated to check PCR efficiency between the plates. The average of telomere versus single copy gene (T/S) ratio was calculated which is proportional to telomere length of each individual. For quality control purposes, we have repeated many samples that were separately PCR amplified. All measurements were performed in a blinded fashion without knowledge of the clinical data.

Measurement of mtDNA content

DNA samples were divided into aliquots of 100 µl and were stored in −80 °C. The ABI PRISM 7000 Sequence Detection System (Applied Biosystems, ABI) was used to amplify the GAPDH (housekeeping) gene and the mitochondrial DNA encoded ATPase (MTATP) 8 gene. Primers used for detection of GAPDH and MTATP 8 gene sequences were: GAPDH (forward): 5′-CCC CAC ACA CAT GCA CTT ACC; (reverse): 5′-CCT AGT CCC AGG GCT TTG ATT; Mitochondrial DNA (forward): 5′-AAT ATT AAA CAC AAA CTA CCA CCT ACC; (reverse): 5′-TGG TTC TCA GGG TTT GTT ATA. The Real-time PCR was carried out in 25 µl of total reaction volume containing 5 µl (40 ng) of DNA, 12.5 µl of SYBR green master mix, an initial denaturation step at 95 °C for 10 min and 40 cycles of 1 min at 60 °C and 15 s at 95 °C. All samples were analyzed in triplicate. To determine the quantities of mtDNA and nuclear DNA (nDNA) present in blood samples, the average threshold cycle number (C_t) values of the nDNA and mtDNA were obtained from each case. The mtDNA content was calculated using the delta C_t (ΔC_t) of average C_t of mtDNA and nDNA ($\Delta C_t = C_{t,\ mtDNA} - C_{t,\ nDNA}$) in the same well as an exponent of 2 ($2\Delta C_t$).

TBARS measurement

Plasma levels of malonodialdehyde (MDA), a marker of lipid peroxidation was measured by thiobarbituric acid reactive substances (TBARS) using a fluorescence methodology [9, 20]. Absolute MDA levels were calculated by regression parameters using different concentrations of the standard, 1,1′,3,3′,-tetramethoxypropane. Inter- and intra-assay coefficients of variation of the above method were <5 and 10 %, respectively.

Statistical analysis

All data are represented as mean ± standard error mean (SEM). Age was adjusted in the data analysis related to mean and correlation determinations. Comparison between groups were performed using one-way ANOVA with $p < 0.05$ as the criterion for significance. Pearson correlation was done between variables and the risk factors. Multiple logistic regression analysis was carried out using disease-state as dependent variable and all the other factors (which reached significance in linear regression analysis) as independent variables. All analysis were done using windows based SPSS statistical package (version 12.0, Chicago, IL).

Results

Age-adjusted clinical and biochemical characteristics of the study subjects are presented in Table 1. Subjects with type 2 diabetes had higher BMI ($p < 0.001$), systolic and diastolic blood pressure ($p < 0.001$), HOMA-IR ($p < 0.001$), fasting plasma glucose ($p < 0.001$), HbA1c ($p < 0.001$), total cholesterol ($p < 0.001$), serum triglycerides ($p < 0.001$), LDL cholesterol ($p < 0.01$), and lower HDL cholesterol ($p = 0.04$) levels compared to control subjects.

Figure 1(a–d) presents the telomere length, mtDNA, adiponectin, and TBARS levels, respectively, in T2DM and control subjects. The mean telomere length (0.97 ± 0.011 vs 1.2 ± 0.013; $p < 0.001$) (Fig. 1a), mtDNA content (6.6 ± 0.17 vs 7.8 ± 0.24; $p < 0.001$) (Fig. 1b) and adiponectin (6.3 ± 0.31 ng/ml vs 7.9 ± 0.36 ng/ml; $p < 0.001$) (Fig. 1c) were significantly lower in T2DM patients compared to control subjects. Oxidative stress as

Table 1 Age-adjusted clinical and biochemical characteristics of the study subjects

Parameters	Control ($n = 145$)	Type 2 DM ($n = 145$)	p value
Age (years)	41.4 ± 4.9	43.6 ± 4.4	<0.001
BMI (kg/m^2)	24.5 ± 4.6	25.9 ± 4.1	<0.001
Waist circumference (cm)	86.3 ± 11.2	91.1 ± 9.9	<0.001
Systolic blood pressure (mm Hg)	119.5 ± 15.3	125.6 ± 18.1	<0.001
Diastolic blood pressure (mm Hg)	76.2 ± 10.4	79.3 ± 11.6	<0.001
HOMA-IR	1.90 ± 1.4	4.3 ± 2.7	<0.001
Fasting plasma glucose (mg/dl)	86.0 ± 8.5	163.6 ± 61.2	<0.001
HbA1c (%)	5.7 ± 0.51	8.8 ± 2.1	<0.001
Serum cholesterol (mg/dl)	184 ± 38	202 ± 43	<0.001
Serum triglycerides[a]	1.52	1.61	<0.001
LDL cholesterol (mg/dl)	116 ± 33.2	124 ± 39.1	<0.01
HDL cholesterol (mg/dl)	45 ± 10.6	43 ± 9.1	0.04

[a] Log transformed Geometric mean

determined by TBARS was significantly higher in T2DM compared to control subjects (7.8 ± 0.25 nM/ml vs 5.3 ± 0.34 nM/ml; $p < 0.001$) (Fig. 1d). Compared to males, there was a slight but significant increase in telomere length, mtDNA content, and adiponectin levels in females.

Pearson correlation analysis of age-adjusted telomere length and mtDNA with metabolic risk factors in the total subjects is presented in Table 2. Telomere length was positively correlated with adiponectin ($p < 0.001$), mtDNA ($p < 0.001$), HDL cholesterol ($p = 0.003$) and negatively correlated with BMI ($p = 0.01$), waist circumference ($p = 0.008$), insulin resistance ($p < 0.001$), fasting plasma glucose ($p < 0.001$), glycated hemoglobin ($p < 0.001$), serum cholesterol ($p = 0.006$), serum triglycerides ($p < 0.001$), and LDL cholesterol ($p < 0.001$). Multiple logistic regression analysis was next done to determine the association of telomere length/mtDNA depletion with type 2 diabetes (Table 3). Telomere length showed significant negative association with T2DM even after adjusting for waist circumference, insulin resistance, triglyceride, HDL, adiponectin, mtDNA & TBARS levels (OR: 0.804, CI: 0.705–0.917; $p < 0.001$). mtDNA depletion showed significant association with T2DM after adjusting for waist circumference and adiponectin (OR: 0.875, CI: 0.778–0.983; $p = 0.024$) but lost its significance when further adjusted for telomere length, TBARS, and insulin resistance (OR: 1.03, CI: 0.886–1.199; $p = 0.698$).

Discussion

The important finding from this study is that there is an association among biomarkers of accelerated aging such as shortened telomeres, hypoadiponectinemia, low HDL, mtDNA depletion and increased oxidative stress in Asian Indian type 2 diabetes patients.

Patients with type 2 diabetes in our study were characterized with shortened telomeres and this feature showed positive association with decreased mtDNA content, hypoadiponectinemia, and increased oxidative stress. Telomere

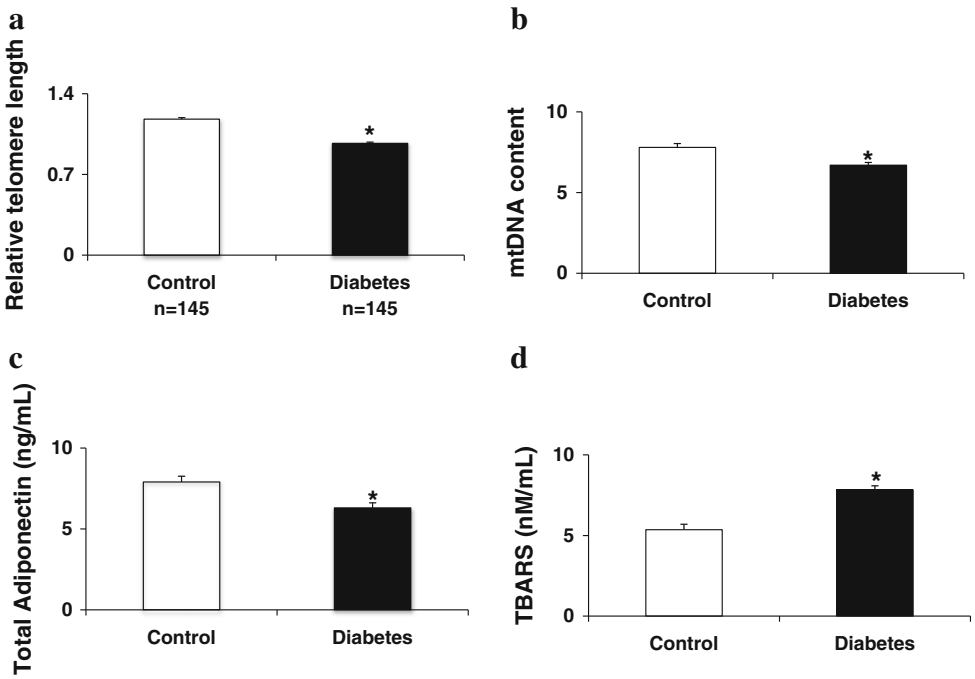

Fig. 1 Cumulative data [Mean (±SE)] of level of telomere length (**a**), mtDNA (**b**), adiponectin (**c**) and TBARS (**d**). * $p \leq 0.001$ compared to control

Table 2 Correlation of age-adjusted mtDNA, telomere length with metabolic risk factors

Parameters	Telomere length		mtDNA	
	r value	*p* value	*r* value	*p* value
Body mass index (BMI)	−0.151	0.01	−0.170	0.004
Waist circumference	−0.158	0.008	−0.203	0.001
Systolic blood pressure	−0.115	0.053	−0.105	0.080
Diastolic blood pressure	−0.094	0.113	−0.094	0.116
HOMA IR	−0.332	0.001	−0.224	0.001
Fasting plasma glucose	−0.308	0.001	−0.124	0.038
Glycated hemoglobin	−0.376	0.001	−0.138	0.022
Serum Cholesterol	−0.161	0.006	−0.057	0.345
Serum Triglycerides	−0.290	0.001	−0.108	0.072
LDL Cholesterol	−0.091	0.126	−0.035	0.556
HDL Cholesterol	0.174	0.003	0.107	0.075
Adiponectin	0.466	0.001	0.277	0.001
Telomere length	–	–	0.194	0.001
mtDNA	0.194	0.001	–	–
TBARS	−0.312	0.001	−0.187	0.002

Table 3 Multiple logistic regression analysis using type 2 diabetes as dependent variable

Parameter	Odds ratio (OR)	95 % Confidence interval (CI)	*p* value
Independent variable: Telomere length			
Unadjusted	0.852	0.819–0.887	<0.001
Adjusted for waist circumference, Insulin resistance, triglyceride, HDL, adiponectin, mtDNA & TBARS	0.804	0.705–0.917	0.001
Independent variable: mtDNA			
Unadjusted	0.830	0.744–0.926	0.001
Adjusted for waist circumference and adiponectin	0.875	0.778–0.983	0.024
Adjusted for waist circumference, adiponectin, telomere length, TBARS & insulin resistance	1.03	0.886–1.199	0.698

shortening has been shown in patients with type 2 diabetes in several populations [9–14]. Reactive oxygen species were shown specifically to target the GGG triplets in telomeric DNA and result in increased telomere attrition [21]. Moreover, adiponectin levels were shown positively correlated to telomere length (13) implying protective effects of this hormone from accelerated aging. Recently, it has been suggested that shortening of telomeres could be the missing link between aging and the type 2 Diabetes epidemic [22]. Telomerase deficiency and hence short telomeres has also been shown to impair replicative capacity of pancreatic beta cells resulting in defective insulin secretion and glucose intolerance [23]. In a genetically defined mouse model (C57BL/6 mTR$^{-/-}$), Guo et al. [24] have recently shown that short telomeres are sufficient to impair glucose homeostasis. Recent studies emphasize several genetic mechanisms for the telomere length maintenance [25–27]. On the other hand telomere length had association with diet and lifestyle determinants [28]. Therefore, it appears that short telomere could be a potential gene-environment risk factor for diabetes contributing to both its onset and pathogenesis.

It is unlikely that the antidiabetic drug regimen used by the patients with type 2 diabetes had any influence on our results and interpretation. If at all any subtle effect indirectly by their anti-inflammatory or antioxidant actions, drugs might have only improved the telomere length, mtDNA content, and adiponectin levels. Despite this, we observed shortened telomeres, lowered mtDNA and adiponectin levels and increased oxidative stress in patients with type 2 diabetes. In our study, HDL levels were positively correlated to telomere length. Increased HDL cholesterol and telomere length were linked to increased longevity in animal models [29]. HDL levels were also positively correlated to mtDNA content in patients with type 2 diabetes in a Chinese population [30]. However, the correlation between HDL levels and mtDNA content was weak in our study. In addition to reduced levels, it appears that HDL particles themselves are dysfunctional in insulin-resistant states as evidenced by a recent study wherein anti-inflammatory and antioxidant properties of HDLs were shown impaired in type 2 diabetes [31]. Further studies should delineate whether dysfunctional HDL is linked to accelerated aging. Consistent with the observations of Harte et al. [32], triglyceride levels were seen inversely correlated to telomere length in our study. While oxidative stress is one of the underlying causes for the shortening of telomeres, it has been shown that increased trigylceride content in nonadipose tissue together with increased serum levels of trigylcerides may play an important role in augmentation of oxidative stress [33]. Therefore, the increased predisposition of South Asians to age-related chronic diseases such as type 2 diabetes can also be explained by lipid alterations and their influence on telomere length.

Mitochondria are central players in cellular energy metabolism and, consequently, defects in their function result in many characterized metabolic diseases. Critical for their function is mitochondrial DNA (mtDNA), which encodes subunits of the oxidative phosphorylation (OXPHOS) complexes essential for cellular respiration and ATP production. The mitochondrial genome is extremely susceptible to damages from constant exposure to ROS produced endogenously from oxidative phosphorylation.

mtDNA is also vulnerable to damage by ROS, because it is not protected by histone proteins and is located closed to inner mitochondrial membrane, where ROS are generated [34]. A role for mitochondrial dysfunction has been implicated in insulin resistance and type 2 diabetes [35, 36]. In our study, mtDNA content (which is a surrogate marker of mitochondrial function) was significantly decreased in patients with type 2 diabetes compared to control subjects. This is consistent with previous studies which demonstrated lower mitochondrial density and decreased mtDNA content in patients with type 2 diabetes [37–41]. The decreased mtDNA content in peripheral leukocytes was also shown to precede the development of type 2 diabetes [42]. Higher mtDNA copy number was shown associated with the lower prevalence of microalbuminuria in a Korean study [43]. Depletion of mtDNA in an insulin-sensitive cell line expressing an extracellular *myc* epitope–tagged glucose transporter 4 (L6 GLUT4myc) was directly correlated with a reduction in basal glucose utilization and resistance to insulin stimulation [44].

We found that adiponectin was positively associated with telomere length and mtDNA content and negatively associated with oxidative stress. Hypoadiponectinemia is a good marker of insulin resistance and an independent risk factor for type 2 diabetes and coronary artery disease [45–47]. As an adipocytokine with insulin-sensitizing and anti-inflammatory properties, this hormone has protective effects against metabolic abnormalities that accelerate aging. The negative association observed between adiponectin levels and oxidative stress in our study deserves attention. Apart from its insulin-sensitizing, antiatherogenic, and anti-inflammatory actions, adiponectin also appears to offer antioxidant function [48–52]. Detopoulou et al. [53] have recently proposed an adiponectin-mediated route through which antioxidant-rich foods exert beneficial effects in humans. While adiponectin expression was shown induced by Vitamin E via a peroxisome proliferator-activated receptor γ-dependent mechanism [54], adiponectin also prevented diabetic premature senescence in endothelial progenitor cells via suppression of ROS [55]. Apart from its role in metabolism, adiponectin also has a protective effect against metabolic abnormalities that accelerate aging [13]. These studies imply that strategies to upregulate adiponectin expression or to potentiate adiponectin signaling may favorably modulate cellular redox state and therefore reduce metabolic disease risk.

Multiple biological processes driven by diverse molecular factors conspire progressively to diminish organ function with advancing age. It is in this context the findings in our study assumes significance as we show an association between shortened telomeres, decreased mtDNA content and hypoadiponectinemia in patients with type 2 diabetes. Increased ROS and oxidative stress pathway could be the root-cause for cellular and molecular alterations that leads to accelerated aging. Mitochondrial DNA (mtDNA) damage is closely interrelated with mitochondrial ROS production, and this might also play a causal role for cellular senescence. Improvement of mitochondrial function results in less telomeric damage and slower telomere shortening, while telomere-dependent growth arrest is associated with increased mitochondrial dysfunction [56]. Adiponectin treatment of human myotubes in primary culture induced mitochondrial biogenesis, palmitate oxidation, and citrate synthase activity, and reduced the production of reactive oxygen species [57]. Decreased level of adiponectin has been demonstrated to have a causal role in mitochondrial dysfunction and insulin resistance via alterations in PGC1α [58]. Moreover, an essential role of mitochondrial function has been implicated in adiponectin synthesis in adipocytes [59], and impaired mitochondrial function in adipose tissue may explain decreased plasma adiponectin levels in diabetes. In our study, the negative association of mtDNA content with type 2 diabetes was lost when adjusted for telomere length or oxidative stress. This implies that there might be a connection between telomere length maintenance and appropriate mitochondrial function. In support of this, Sahin et al. [60] have demonstrated that telomere-dysfunction-induced repression of the PGC (PPARγ coactivator) network is associated with mitochondrial dysfunction as evidenced by compromised OXPHOS and respiration, decreased ATP generation capacity, and increased oxidative stress. Adopting a mouse model with impaired telomere maintenance caused by a targeted deletion of the enzyme telomerase, Sahin et al. [60] have demonstrated that telomere-dysfunction activates p53 which in turn binds and represses PGC-1α and PGC-1β promoters, thereby forging a direct link between telomere and mitochondrial biology. It is also plausible that mtDNA depletion in type 2 diabetes might be originated from an imbalance of mitochondrial fusion and fission dynamics as a consequence of PGC-1 alterations [61, 62]. As suggested by Kelly [63], the mitochondrial derangements driven by telomere-dysfunction and the subsequent loss of PGC-1 activity may lower the threshold for the generation of ROS (oxidative stress), which damage mitochondrial DNA, thus setting up a vicious cycle of further mitochondrial dysfunction. Our findings in a clinical setting support this potentially unifying mechanism for cellular aging.

To conclude, there is an association of shortened telomeres with decreased mtDNA, hypoadiponectinemia and increased oxidative stress pointing toward the existence of a molecular connection between the nuclear and mitochondrial aging processes in patients with type 2 diabetes. It appears that maintenance of appropriate mitochondrial function and telomere length either by pharmacological

means or lifestyle modification will have promising therapeutic potential for type 2 diabetes and associated disorders. While our findings expand the existing knowledge on the molecular pathogenesis of type 2 diabetes, prospective studies are needed to prove whether lifestyle modifications could counteract accelerated aging and thereby prevent diabetes and its associated complications.

Acknowledgments Authors thank the Department of Biotechnology (DBT), New Delhi for the financial assistance. A senior research fellowship from the Council of Scientific and Industrial Research (CSIR), New Delhi is also thankfully acknowledged. This is the 123rd publication of CURES (CURES—123).

Conflict of interest None.

References

1. Curtis R, Geesaman BJ, DiStefano PS (2005) Ageing and metabolism: drug discovery opportunities. Nat Rev Drug Discov 4:569–580
2. Morley JE (2008) Diabetes and aging: epidemiologic overview. Clin Geriatr Med 24:395–405
3. Wei YH, Lee HC (2002) Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. Exp Biol Med (Maywood) 227:671–682
4. Rhee DB, Ghosh A, Lu J et al (2011) Factors that influence telomeric oxidative base damage and repair by DNA glycosylase OGG1. DNA Repair (Amst) 10:34–44
5. Kanvah S, Schuster GB (2005) The sacrificial role of easily oxidizable sites in the protection of DNA from damage. Nucleic Acids Res 33:5133–5138
6. Ceriello A, Ihnat M (2010) Oxidative stress is, convincingly, the mediator of the dangerous effects of glucose variability. Diabet Med 27:968
7. Calvert PA, Liew TV, Gorenne I et al (2011) Leukocyte telomere length is associated with high-risk plaques on virtual histology intravascular ultrasound and increased proinflammatory activity. Arterioscler Thromb Vasc Biol 31:2157–2164
8. Jeanclos E, Krolewski A, Skurnick J et al (1998) Shortened telomere length in white blood cells of patients with IDDM. Diabetes 47:482–486
9. Adaikalakoteswari A, Balasubramanyam M, Mohan V (2005) Telomere shortening occurs in Asian Indian Type 2 diabetic patients. Diabet Med 22:1151–1156
10. Sampson MJ, Winterbone MS, Hughes JC et al (2006) Monocyte telomere shortening and oxidative DNA damage in type 2 diabetes. Diabetes Care 29:283–289
11. Adaikalakoteswari A, Balasubramanyam M, Ravikumar R et al (2007) Association of telomere shortening with impaired glucose tolerance and diabetic macroangiopathy. Atherosclerosis 195:83–89
12. Zee RY, Castonguay AJ, Barton NS et al (2010) Mean leukocyte telomere length shortening and type 2 diabetes mellitus: a case-control study. Transl Res 155:166–169
13. Al-Attas OS, Al-Daghri NM, Alokail MS et al (2010) Adiposity and insulin resistance correlate with telomere length in middle-aged Arabs: the influence of circulating adiponectin. Eur J Endocrinol 163:601–607
14. Salpea KD, Talmud PJ, Cooper JA et al (2010) Association of telomere length with type 2 diabetes, oxidative stress and UCP2 gene variation. Atherosclerosis 209:42–50
15. Wu H, Yu Z, Qi Q et al (2011) Joint analysis of multiple biomarkers for identifying type 2 diabetes in middle-aged and older Chinese: a cross-sectional study. BMJ Open 1:e000191
16. Deepa M, Pradeepa R, Rema M et al (2003) The Chennai Urban Rural Epidemiology Study (CURES)—Study design and Methodology (Urban Component) (CURES—1). J Assoc Physicians India 51:863–870
17. Alberti KG, Zimmet PZ (1998) Definition diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus, provisional report of a WHO Consultation. Diabet Med 15:539–553
18. Kochl S, Niederstatter H, Parson W (2005) DNA extraction and quantitation of forensic samples using the phenol-chloroform method and real-time PCR. Methods Mol Biol 297:13–30
19. Cawthon RM (2002) Telomere measurement by quantitative PCR. Nucleic Acids Res 30:e47
20. Yagi K (1976) A simple fluorometric assay for lipoperoxide in blood plasma. Biochem Med 15:212–216
21. Serra V, Grune T, Sitte N et al (2000) Telomere length as a marker of oxidative stress in primary human fibroblast cultures. Ann N Y Acad Sci 908:327–330
22. Mulder H (2010) Is shortening of telomeres the missing link between aging and the type 2 diabetes epidemic? Aging 2:634–636
23. Kuhlow D, Florian S, von Figura G et al (2010) Telomerase deficiency impairs glucose metabolism and insulin secretion. Aging 2:650–658
24. Guo N, Parry EM, Li LS et al (2011) Short telomeres compromise β-cell signaling and survival. PLoS ONE 6:e17858
25. Slagboom PE, Droog S, Boomsma DI (1994) Genetic determination of telomere size in humans: a twin study of three age groups. Am J Hum Genet 55:876–882
26. Atzmon G, Cho M, Cawthon RM et al (2010) Evolution in health and medicine Sackler colloquium: genetic variation in human telomerase is associated with telomere length in Ashkenazi centenarians. Proc Natl Acad Sci USA 26(Suppl 1):1710–1717
27. Shen Q, Zhang Z, Yu L et al (2011) Common variants near TERC are associated with leukocyte telomere length in the Chinese Han population. Eur J Hum Genet 19:721–723
28. Cassidy A, De Vivo I, Liu Y et al (2010) Associations between diet, lifestyle factors, and telomere length in women. Am J Clin Nutr 91:1273–1280
29. Joeng KS, Song EJ, Lee KJ et al (2004) Long lifespan in worms with long telomeric DNA. Nat Genet 36:607–611
30. Xu F, Zhou X, Shen F et al (2012) Decreased peripheral blood mitochondrial DNA content is related to HbA(1c), fasting plasma glucose level and age of onset in type 2 diabetes mellitus. Diabet Med (in press)
31. Morgantini C, Natali A, Boldrini B et al (2011) Anti-inflammatory and antioxidant properties of HDLs are impaired in type 2 diabetes. Diabetes 60:2617–2623
32. Harte AL, da Silva NF, Miller MA et al (2012) Telomere length attrition, a marker of biological senescence, is inversely correlated with triglycerides and cholesterol in south asian males with type 2 diabetes mellitus. Exp Diab Res (in press)
33. Bakker SJ, IJzerman RG, Teerlink T et al (2000) Cytosolic triglycerides and oxidative stress in central obesity: the missing link between excessive atherosclerosis, endothelial dysfunction, and beta-cell failure? Atherosclerosis 148:17–21
34. Croteau DL, Stierum RH, Bohr VA (1999) Mitochondrial DNA repair pathways. Mutat Res 434:137–148
35. Szendroedi J, Phielix E, Roden M (2011) The role of mitochondria in insulin resistance and type 2 diabetes mellitus. Nat Rev Endocrinol 8:92–103
36. Michel S, Wanet A, De Pauw A et al (2012) Crosstalk between mitochondrial (dys)function and mitochondrial abundance. J Cell Physiol 227:2297–2310

37. Song J, Oh JY, Sung YA et al (2001) Peripheral blood mitochondrial DNA content is related to insulin sensitivity in offspring of type 2 diabetic patients. Diabetes Care 24:865–869
38. el-Sharnooby JA, Ahmed LM (2003) Potential relationship between peripheral blood mitochondrial DNA content and insulin resistance and secretion in offspring of type 2 diabetic patients. Egypt J Immunol 10:57–66
39. Morino K, Petersen KF, Shulman GI (2006) Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. Diabetes 55:S9–S15
40. Wong J, McLennan SV, Molyneaux L et al (2009) Mitochondrial DNA content in peripheral blood monocytes: relationship with age of diabetes onset and diabetic complications. Diabetologia 52:1953–1961
41. Hsieh CJ, Weng SW, Liou CW et al (2011) Tissue-specific differences in mitochondrial DNA content in type 2 diabetes. Diabetes Res Clin Pract 92:106–110
42. Lee HK, Song JH, Shin CS et al (1998) Decreased mitochondrial DNA content in peripheral blood precedes the development of non-insulin-dependent diabetes mellitus. Diabetes Res Clin Pract 42:161–167
43. Lee JE, Park H, Ju YS et al (2009) Higher mitochondrial DNA copy number is associated with lower prevalence of microalbuminuria. Exp Mol Med 41:253–258
44. Park SY, Choi GH, Choi HI et al (2005) Depletion of mitochondrial DNA causes impaired glucose utilization and insulin resistance in L6 GLUT4myc myocytes. J Biol Chem 280: 9855–9864
45. Mohan V, Deepa R, Pradeepa R et al (2005) Association of low adiponectin levels with the metabolic syndrome—the Chennai Urban Rural Epidemiology Study (CURES-4). Metabolism 54:476–481
46. Wasim H, Al-Daghri NM, Chetty R et al (2006) Relationship of serum adiponectin and resistin to glucose intolerance and fat topography in South-Asians. Cardiovasc Diabetol 5:10
47. Ryu HK, Yu SY, Park JS et al (2010) Hypoadiponectinemia is strongly associated with metabolic syndrome in Korean type 2 diabetes patients. J Am Coll Nutr 29:171–178
48. Lam KS, Xu A (2005) Adiponectin: protection of the endothelium. Curr Diab Rep 5:254–259
49. Sandhya N, Gokulakrishnan K, Ravikumar R et al (2010) Association of hypoadiponectinemia with hypoglutathionemia in NAFLD subjects with and without type 2 diabetes. Dis Markers 29:213–221
50. Deng G, Long Y, Yu YR et al (2010) Adiponectin directly improves endothelial dysfunction in obese rats through the AMPK–eNOS pathway. Int J Obes 34:165–171
51. Essick EE, Ouchi N, Wilson RM et al (2011) Adiponectin mediates cardioprotection in oxidative stress-induced cardiac myocyte remodeling. Am J Physiol Heart Circ Physiol 301: H984–H993
52. Antonopoulos AS, Lee R, Margaritis M et al (2011) Adiponectin as a regulator of vascular redox state: therapeutic implications. Recent Pat Cardiovasc Drug Discov 6:78–88
53. Detopoulou P, Panagiotakos DB, Chrysohoou C et al (2010) Dietary antioxidant capacity and concentration of adiponectin in apparently healthy adults: the ATTICA study. Eur J Clin Nutr 64:161–168
54. Landrier JF, Gouranton E, El Yazidi C et al (2009) Adiponectin expression is induced by vitamin E via a peroxisome proliferator-activated receptor gamma-dependent mechanism. Endocrinology 150:5318–5325
55. Chang J, Li Y, Huang Y et al (2010) Adiponectin prevents diabetic premature senescence of endothelial progenitor cells and promotes endothelial repair by suppressing the p38 MAP kinase/p16INK4A signaling pathway. Diabetes 59:2949–2959
56. Passos JF, Saretzki G, von Zglinicki T (2007) DNA damage in telomeres and mitochondria during cellular senescence: is there a connection? Nucleic Acids Res 35:7505–7513
57. Civitarese AE, Ukropcova B, Carling S et al (2006) Role of adiponectin in human skeletal muscle bioenergetics. Cell Metab 4:75–87
58. Iwabu M, Yamauchi T, Okada-Iwabu M et al (2010) Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca(2^+) and AMPK/SIRT1. Nature 464:1313–1319
59. Koh EH, Park JY, Park HS et al (2007) Essential role of mitochondrial function in adiponectin synthesis in adipocytes. Diabetes 56:2973–2981
60. Sahin E, Colla S, Liesa M et al (2011) Telomere dysfunction induces metabolic and mitochondrial compromise. Nature 470: 359–365
61. Joseph A, Joanisse DR, Baillot RG et al (2012) Mitochondrial dysregulation in the pathogenesis of diabetes: potential for mitochondrial biogenesis-mediated interventions. Exp Diab Res (in press)
62. Jheng HF, Tsai PJ, Guo SM et al (2012) Mitochondrial fission contributes to mitochondrial dysfunction and insulin resistance in skeletal muscle. Mol Cell Biol 32:309–319
63. Kelly DP (2011) Ageing theories unified. Nature 470:342–343