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Shorter telomeres in adults with Type 1 diabetes correlate with diabetes duration, but only weakly with vascular function and risk factors

Andrzej S. Januszewski [a,b,1], Surya S. Sutanto [c,1], Susan McLennan [c,d], David N. O'Neal [b], Anthony C. Keech [a], Stephen M. Twigg [c,d,2], Alicia J. Jenkins [a,b,2,*]

[a] NHMRC Clinical Trials Centre, The University of Sydney, Camperdown, Sydney, NSW, Australia
[b] University of Melbourne, Department of Medicine, St Vincent's Hospital, Fitzroy, Melbourne, VIC, Australia
[c] Greg Brown Diabetes and Endocrine Laboratory, Sydney Medical School, Charles Perkins Centre, The University of Sydney, Camperdown, Sydney, NSW, Australia
[d] Department of Endocrinology, Royal Prince Alfred Hospital, Camperdown, Sydney, NSW, Australia

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ABSTRACT

Objective: To determine if white blood cell (WBC) telomeres are shorter in Type 1 diabetes (T1D) than in subjects without diabetes (non-DB), and shorter in T1D subjects with vs. without vascular complications; and to determine associations with vascular biomarkers.
Research design and methods: WBC relative telomere length (RTL) was determined by quantitative PCR in a cross-sectional study of 140 non-DB and 199 T1D adults, including 128 subjects without vascular complications (T1DNoCx) and 71 subjects with vascular complications (T1DCx). Relationships of RTL with age, T1D duration, arterial elasticity, pulse pressure and vascular risk factors were determined.
Results: RTL did not differ by gender within T1D and non-DB groups. Age-adjusted RTL was shorter in T1D vs. non-DB subjects (1.48 ± 0.03 AU vs. 1.64 ± 0.04 AU, $p = 0.002$), but did not differ by T1D complication status (T1DNoCX 1.50 ± 0.04 vs. T1DCX 1.46 ± 0.05, $p = 0.50$), nor correlate with arterial elasticity. Univariate analysis in T1D showed RTL correlated (inversely) with age $r = -0.27$, $p = 0.0001$, T1D duration $r = -0.16$, $p = 0.03$, and pulse pressure ($r = -0.15$, $p = 0.04$), but not with HbA1c, BP, renal function (serum creatinine, ACR, eGFR), lipids, insulin sensitivity, inflammation (CRP, CAMs) or oxidative stress (OxLDL, OxLDL/LDL-C, MPO, PON-1). Multiple regression analysis showed independent determinants of RTL were age and T1D presence ($r = 0.29$, $p < 0.0001$).
Conclusions: In this cross-sectional study telomeres were shorter in T1D. RTL correlated inversely with T1D duration, but did not differ by complication status and weakly correlated with pulse pressure and vascular risk factors. Only age and T1D were independent determinants of RTL. Longitudinal studies are merited.

* *Corresponding author at:* NHMRC Clinical Trials Centre, The University of Sydney, 92-94 Parramatta Rd, Camperdown, 2050 Sydney, NSW, Australia. Tel.: +61 2 95625000; fax: +61 2 95651863.
E-mail address: alicia.jenkins@ctc.usyd.edu.au (A.J. Jenkins).
[1] Equal contribution.
[2] Equal senior authors.
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1. Introduction

Diabetes is a condition of accelerated ageing, and may be associated with reduced life-span relative to subjects without diabetes, particularly with co-existent renal or cardiovascular disease (CVD) [1]. Chronic complications of Type 1 diabetes (T1D) are promoted by hyperglycaemia, hypertension, dyslipidaemia, smoking, adiposity, insulin resistance and inflammation, each of which may also shorten telomeres [2,3].

Telomeres are repetitive specialised DNA protein loop structures at chromosomal ends that stabilise chromosomes. Telomeres shorten (≈20–30 nucleotides) with each cell cycle, and once they reach a critical length cell cycle arrest or apoptosis is activated [2]. Shorter telomeres may be both a marker and mediator of ageing and cardiometabolic disorders [2,4].

There are many telomere studies in the general population and in Type 2 diabetes (T2D) [5], but relatively few and only small studies in T1D [6–9]. We conducted a cross-sectional study of white blood cell (WBC) telomere length in well-characterised T1D patients with and without vascular complications and in subjects without diabetes (non-DB). We hypothesised that: firstly telomeres are shorter in T1D vs. non-DB subjects, secondly, telomeres are shorter in T1D subjects with vs. without vascular complications and vascular dysfunction, and thirdly, that adverse vascular risk factors are associated with shorter telomeres.

2. Methods

2.1. Subjects and samples

The study was approved by the St. Vincent's Hospital Ethics Committee and each subject gave a written informed consent. Adult patients were recruited from the hospital's Diabetes Clinics and controls were recruited from the community. All were volunteers and no payment was made, other than an offer of travel/parking reimbursement, which >95% declined. Exclusion criteria (for all participants) were: cancer, end-stage renal failure, inflammatory conditions, anti-oxidant vitamin supplement intake, recent (<3 months) surgery, infection, myocardial infarction, stroke, cardiac arrhythmia, diabetic ketoacidosis or retinal laser therapy. Additional exclusion criteria for non-diabetic subjects were: Type 1 or Type 2 diabetes or known pre-diabetes. A history and examination was performed and complication status verified by treating clinicians. Diabetes complications were defined as current or previous proliferative or pre-proliferative diabetic retinopathy, increased albuminuria (in ⩾2 of three timed 12 or 24 h urine collections in the absence of urinary infection or ketoacidosis) or clinically evident CVD. Subjects were evaluated after an overnight fast pre-medication. Pulse-wave analysis (PWA), including large and small artery elasticity (LAE and SAE), which correlates with pulse-wave velocity and brachial artery flow mediated dilation respectively [10], was performed on rested supine subjects (Pulse Wave™ CR-2000, Hypertension Diagnostics Inc., Eagan MN) as a previous study [10]. Inter-measurement CVs for LAE and SAE were 7% and 5% respectively.

Serum renal and liver function, lipids, HbA_{1c}, full blood count and erythrocyte sedimentation rate (ESR), and mid-stream urine for cell count, albumin/creatinine ratio and culture to exclude infection were performed. Glomerular Filtration Rate (eGFR) was calculated by the Cockcroft–Gault equation and estimated glucose disposal rate (eGDR), a measure of insulin sensitivity, was calculated: eGDR $[mg \times kg^{-1} \times min^{-1}] = 24.31 - 12.22 \times WHR - 3.29 \times HT - 0.57 \times HbA_{1c}$, with WHR being waist/hip ratio and HT representing history of hypertension [11]. Blood was centrifuged (3000 rpm, 4 °C, 10 min,) and plasma stored (−80 °C) until analysis. EDTA plasma for F2-isoprostanes also contained BHT and reduced glutathione. All non-diabetic subjects had normal fasting glucose and HbA1c levels performed in the same accredited Clinical Chemistry Laboratory as the T1D subjects.

2.2. Inflammation & oxidative stress

Complementing white cell count (WCC) and ESR, C-reactive protein (hsCRP) was measured by high-sensitivity immunonephelometry (Dade Behring, Marburg, Germany). *Soluble Cell Adhesion Molecules* (CAMs): vascular cell adhesion molecule-1 (sVCAM-1), intercellular adhesion molecule-1 (sICAM-1) and endothelial leucocyte adhesion molecule-1 (sE-Selectin) were measured by ELISA (R&D Systems, MN). *Oxidised* (*Ox*) *LDL* and *myeloperoxidase* (MPO) were measured by ELISA (Mercodia, Uppsala, Sweden) with OxLDL results expressed as absolute values and OxLDL/LDL-C (CVs < 10%). Serum *paraoxonase-1 activity* (PON1) was measured by rates of hydrolysis of paraoxon and of phenylacetate [12]. All CVs < 9%. *F2-isoprostanes* were by gas chromatography/mass spectroscopy in one run (CV < 8%). Low Molecular Weight (LMW-) AGEs were quantified by fluorescence spectroscopy with intra- and inter-assay CVs of 2.2% and 13% respectively [13].

2.3. Telomere length

Genomic DNA (gDNA) was extracted from whole blood (QIAamp DNA Blood Mini kit, Qiagen, Australia) and its concentration and quality determined (NanoDrop ND-1000, ThermoFisher Scientific, Australia) with acceptable gDNA A260:A280 ratios of 1.60–1.80. gDNA were stored (−80 °C) until analysis.

Relative telomere length (RTL) was measured in quadruplicates by qPCR using a modification of published methods [14,15] as described in supplementary material, and the mean was used in data analyses. A representative gel of the qPCR products is shown in the supplementary materials (Fig. 1).

3. Statistics

STATISTICA version 12 (StatSoft Inc. Tulsa OK) was used. Non-normally distributed variables (Triglycerides, hsCRP, eGFR, eGDR, WCC, ESR, MPO, ACR) were log-transformed or analysed using non-parametric tests. Descriptive statistics were performed and frequency data compared using 2-tail Chi-square test. Univariate correlations used Pearson's or partial