Original Research

Leukocyte telomere length is inversely associated with post-load but not with fasting plasma glucose levels

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Impact statement

- Contradictory epidemiologic data have been obtained about the link between the leucocyte telomere length (LTL) and diabetes. Type 2 diabetes (T2D) is likely to be pathophysiologically heterogeneous, but comparison of the association of LTL separately with fasting plasma glucose (FPG) and 2-h post-load plasma glucose (2hPG) levels has not been done before. Thus, the study of LTL changes associated with different types of hyperglycaemia, that largely determine the heterogenity of T2D is important.
- In a population-based study of rural Ukrainians, we were the first to demonstrate that the increase of 2hPG (but not FPG) level increases the chances of revealing short telomeres.
- The obtained data can help to clarify the relationship between the LTL shortening and different conditions of the insulin resistance (mainly liver resistance in high FPG and mostly muscle and adipose tissue resistance in high 2hPG).

Abstract

Type 2 diabetes mellitus is characterized by shorter leukocyte telomere length, but the relationship between leukocyte telomere length and type 2 diabetes mellitus development is rather questioned. Fasting and post-load glycaemia associated with different types of insulin resistance and their relation with leukocyte telomere length remains unknown. We compared leukocyte telomere length and fasting or post-load glucose levels in persons who do not receive glucose lowering treatment. For 82 randomly selected rural residents of Ukraine, aged 45+, not previously diagnosed with type 2 diabetes mellitus, the WHO oral glucose tolerance test and anthropometric measurements were performed. Leukocyte telomere length was measured by standardized method of quantitative monochrome multiplex polymerase chain reaction in real time. Spearman's or Pearson's rank correlation was used for correlation analysis between fasting plasma glucose or 2-h post-load plasma glucose levels and leukocyte telomere length. Logistical regression models were used to evaluate risks of finding short or long telomeres associated with fasting plasma glucose or 2-h post-load plasma glucose levels. No association of fasting plasma glucose and leukocyte telomere length was revealed, whereas 2-h post-load plasma glucose levels demonstrated a negative correlation ($P < 0.01$) with leukocyte telomere length. Waist circumference and systolic blood pressure were negatively related ($P = 0.03$) with leukocyte telomere length in men. Oral glucose tolerance test result-based glycemic categories did not show differences between mean leukocyte telomere length in categories of normal fasting plasma glucose and 2-h post-load plasma glucose (NGT, $n = 33$); diabetes mellitus (DM), $n = 18$ and impaired fasting glucose/tolerance (IFG/IGT, $n = 31$) levels. A correlation relationship between leukocyte telomere length and 2-h post-load plasma glucose level in NGT; IFG/IGT and DM groups ($P = 0.027$; 0.029 and 0.049, respectively) was revealed; the association between leukocyte telomere length and fasting plasma glucose was confirmed in DM group only ($P = 0.009$). Increase of 2-h post-load plasma glucose (but not fasting plasma glucose) level improves the chances of revealing short telomeres: OR 1.52 (95% CI 1.04–2.22), $P = 0.03$. After the adjustment for age, gender, waist circumference, systolic blood pressure, and fasting plasma glucose, these phenomena remain significant. We conclude that 2-h post-load plasma glucose but not fasting plasma glucose is inversely associated with leukocyte telomere length.

Keywords: Type 2 diabetes, leukocyte telomere length, impaired fasting plasma glucose, impaired glucose tolerance

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Introduction

Telomeres are the repetitive nucleotide sequences that protect the ends of chromosomes and help to maintain the genomic integrity. Telomere length is known to play a key role in human health and longevity.[1] It was shown that telomeres shorten with age due to end replication problem. Some tissues, particularly reproductive, most stem cells and cancerous ones expressed enzyme called telomerase that can elongate telomere length using an RNA template as a matrix. With differentiation, the cells lose their telomerase potential and their telomeres shorten with each cell division.[2] If this reduction reaches a critical value, cellular arrest and apoptosis occur.[3] Thus, telomeres can be viewed as markers of aging and cellular dysfunction due to depletion of the proliferative potential.

Meta-analysis of prospective, case–control, and cross-sectional studies (9 population cohorts; 5759 cases and 6518 controls) of Zhao *et al.*[4] provides evidence that the shortened leucocyte telomere length (LTL) is associated with type 2 diabetes mellitus (T2D) risk, which turned out to be quite moderate (OR: 1.117; 95% CI: 1.002, 1.246; $P = 0.045$) and reached statistical significance only after excluding three out of nine studies that were determined as ''the key contributors to the heterogeneity.'' The significant role of race, age, and gender in the association between LTL and T2D risk was also noted, and the question of causality of an association between LTL shortening and T2D development[4] remains unanswered. When studying US general population, Menke *et al.*[5] did not find any association between LTL and diabetes status, duration, or control after age, race–ethnicity, and sex adjustment, and these authors claim that their results suggest telomere attrition is not a cause or a consequence of diabetes.

Prior to this, in a large US multiethnic cohort of postmenopausal women (1675 incident diabetes case participants in six years of follow-up and 2382 control participants), a modest association between telomere length and diabetes risk according to the authors of this study could be explained by traditional diabetes risk factors.[6] This study was one of the three population cohorts excluded from the mentioned meta-analysis [4] Then again in order to build a full model, Nai-chieh *et al.*[6] used eight variables, not all of which seem reasonable. It is hard to imagine a relationship between biological and metabolic categories that could preserve statistical significance under such extensive adjusting. This study was one of the three population cohorts excluded from the mentioned meta-analysis.[4] At the same time, we should note that this study, similar to the study of Menke *et al.*,[5] had no oral glucose-tolerance testing (OGTT) to reveal diabetes categories and/or impaired glucose tolerance (IGT), i.e. the relationship between LTL and IGT was not studied.

Despite such ambiguous epidemiological data that sometimes challenge even the fact of independent association between LTL and T2D,[5] Japanese researchers seem to have no doubts that telomere shortening is a cause of diabetes and the inhibition of telomere attrition in various organs, including pancreatic β-cells, could be a new approach for preventing the progression of T2D and its complications.[7]

We believe that contradicting results of epidemiological studies still remain unexplained. This does not allow to directly use epidemiological data to prove pathophysiologic concepts, like the one recently proposed by Tamura *et al.*[7]

In the recent years, we have seen evidence of pathophysiological heterogeneity of pre-diabetic categories of hyperglycemia. Categories such as isolated impaired fasting glycaemia (i-IFG), isolated impaired glucose tolerance (i-IGT), and a combined category (IFG + IGT) were described. Clinicians acknowledge that T2D is multifactorial and has heterogeneous characteristics, but neither prevention nor treatment is systematically stratified.[8]

To address the question whether LTL is equally associated with different kinds of hyperglycemic conditions, we examined the fasting and post-load glucose levels in persons who did not receive glucose lowering treatment.

Methods

Study population

The study included 82 randomly selected residents of rural areas in Kyiv oblast, older than 44 years, not previously diagnosed with diabetes. All persons underwent standard (WHO) glucose tolerance test, survey, and anthropometric measurements.

The study was approved by the ethics committee of the Institute of Endocrinology and Metabolism (National Academy of Medical Sciences of Ukraine), and all participants gave written informed consent.

Blood sampling for further biochemical testing, DNA purification, and telomere length measurement was performed by medical staff of two local family medicine clinics.

Biochemical testing

Blood samples were taken on an empty stomach and in 2 h after taking a glucose solution (75 g of glucose/200 ml of water). Plasma was quickly separated with a centrifuge and stored in a cold environment for further tests during 24 h. The obtained biomaterial was transported in a thermal container with ice gel packs to the Institute's lab, which is authorized to perform glucose measurements (by enzymatic method) and zinc (Zn) and iron (Fe) by direct atomic absorption spectrophotometry (C-115 M PC, ''UKRROSPRIBOR,'' Ukraine). HbA1c levels were assessed using ClOVER A1c (Inforia Co., Ltd) system that uses boronate resin to bind HbA1c. HbA1c testing was performed only in persons with high plasma glucose, reaching diabetic levels, according to OGTT results.

Glycemic categories

All individuals were separated into three groups according to glucose tolerance testing results: normal fasting glucose and normal glucose tolerance (NGT) – fasting plasma glucose (FPG) level less than 6.1 and 2-h post-load level less than 7.8 mmol/l; impaired fasting glucose and/or impaired glucose tolerance (IFG/IGT) – FPG level 6.1 and over, but less than 7.0 and 2-h post-load level 7.8 mmol/l and over,

but less than 11.1 mmol/l; screen-detected diabetes mellitus (DM) – FPG level 7.0 and over and/or 2-h post-load level 11.1 mmol/l and over.

Anthropometric categories

Central (abdominal) obesity was defined using waist circumference (WC) cut-offs according to current IDF definitions for Caucasians: male $\geq$ 94 cm; female $\geq$ 80 cm. To create categories of arterial hypertension, IDF definitions were used as well: systolic BP $\geq$ 130 or diastolic BP $\geq$ 85 mm Hg or treatment of previously diagnosed hypertension.[9]

Other methodological aspects of a larger epidemiologic investigation, which this study is a part of are described elsewhere.[10]

LTL measurement

To determine the average relative telomere length (RTL), standardized method of quantitative monochrome multiplex polymerase chain reaction in real time has been used, which was proposed by Cawthon.[11] DNA was extracted from the whole blood by phenol–chloroform purification method.[12] For PCR amplification were used strips of eight tubes that are compatible with Bio-Rad Chromo4, in which was added the previously prepared reaction mixture for PCR in volume 8 mkl and 2 mkl DNA. For obtaining calibration curve, PCR was carried out at four concentrations of the reference DNA in duplicates which cover a range of 27-fold dilutions, prepared by serial dilution. All experimental DNA samples were analyzed in triplicate.

PCR reaction mix was prepared on the basis of ''Kit for RT-PCR in the presence of an intercalating dye SYBR Green I'' (''Syntol'' RF) as recommended by the manufacturer. For the quantitative monochrome multiplex, PCR pair of primers telg and telc (final concentration of each – 450 nm) were mixed with albumin primers albu and albd (final concentration of 250 nM each) and added to the master mix.

Thermal cycling profiles were taken from the original method without any changes.

When PCR was done, two standard curves were generated by program Opticon Monitor 3, one for the telomeric signal and another for the signal from the single copy gene When signal for telomere product overcomes the threshold – Ct value was obtained and used with calibration curve to determine telomeric DNA amount (T) relative to reference DNA, data for albumin DNA(S) amount were obtained in a similar way. Relative telomere length was determined by division T to S.

Statistical analysis

Shapiro–Wilk test was used to check for the normality of value distribution of the T/S indicator. Despite all the T/S values distribution being normal, many biochemical and anthropometric data distributions were not; therefore, we employed nonparametric statistical methods and Spearman's or parametric Pearson's rank correlation for correlation analysis.

Student *t*-test and W-Wilcoxon test were used to compare normally distributed data and non-normally distributed data, respectively

When comparing the values of more than two groups, ANOVA and *post hoc* test (Scheffé's method) for pairwise comparison of subgroups were used. For the analysis of non-normally distributed parameter, the nonparametric Kruskal–Wallis test and *post hoc* (Dunn's test) pairwise comparison of subgroups were used. Logistical regression models were also used to evaluate the risks of finding short (1st quartile) or long (4th quartile) telomeres, associated with fasting or post-load plasma glucose levels.

Results

The investigated biochemical indicators in blood, FPG and plasma glucose after 2 h of standard loading levels, levels of Fe an Zn, as well as telomere length (relative telomere length, T/S ratio) in 62 women and 20 men did not differ. Median age was also virtually the same (61.5 and 62 years in men and women accordingly), $P = 0.875$, see Table 1(a).

Age of the investigated individuals demonstrates a negative correlation with FPG levels and levels of Fe and Zn, whereas FPG levels positively correlate with the levels of these metals in plasma. Glycaemia measured in 2 h after standard glucose loading demonstrates a negative correlation ($P < 0.01$) with relative telomere length.

In case of assessing these same correlations for women only, a negative association ($P = 0.001$) of telomere length and fasting glycaemia is added to the above-mentioned ones, whereas in men, there is no association of fasting glycaemia with age and plasma Zn levels (see Table 1(b)).

Comparing anthropometric features revealed a lower body mass index (BMI) and neck circumference in men. According to the survey data, over half of the women (53%) received medications to control arterial hypertension, whereas among men, this indicator was at 20%, see Table 2(a). Only 2 out of 20 men were smokers at the moment of the investigation. There were no smokers among women.

Age of men and women was positively associated with systolic blood pressure. Other anthropometric features (BMI, waist, and neck circumference) demonstrate a high degree of mutual positive associations. Waist and neck circumference were negatively related with telomere length in men (Table 2(b)).

LTL, FPG, and 2hPG correlations according to glycemic categories of the study subjects

Highlighting OGTT result-based traditional glycemic categories did not show differences between mean LTL T/S ratio in groups with normal fasting glycaemia and glucose tolerance (NGT, $n = 33$), screen-detected diabetes mellitus (DM, $n = 18$), and pre-diabetes group (IFG/IGT, $n = 31$), Table 3. Correlation analysis performed in these groups revealed a statistically significant relation between LTL T/S ratio and 2hPG level in NGT, IFG/IGT, and DM groups ($P = 0.027$; 0.029 and 0.049, respectively). The association between LTL and FPG was confirmed in DM group only ($P = 0.009$), Table 3.

Table 1 Age, biochemical and relative telomere length characteristics of the study subjects (a) and their Indicators of Spearman's rank correlation (b)

a. Age, biochemical and relative telomere length characteristics of the study subjects

Parameters	All, $n = 82$	Women, $n = 62$	Men, $n = 20$	*P* value (women vs. men)
Age, yrs;	61.5 (56–69)	62.0 (56–68)	61.0 (55–68)	0.875
Fasting plasma glucose, mmol/l;	6.08 (5.69–6.75)	6.16 (5.73–6.77)	6.01 (5.08–6.6)	0.290
Post-load plasma glucose, mmol/l;	6.12 (5.12–7.16)	6.21 (5.12–7.18)	6.08 (5.07–6.48)	0.560
Fe plasma level, mcg/l;	1178.9 (906.2–1500.2)	1200.9 (906.2–1496.4)	1144.9 (802.7–1566.3)	0.765
Zn plasma level, mcg/l;	1070.1 (910.7–1258.2)	1077.6 (933.9–1232.2)	1069.3 (882.4–1290.0)	0.682
Relative telomere length, T/S ratio;	0.58465 (0.4854–0.6948)	0.58725 (0.4957–0.6948)	0.5778 (0.43515–0.67365)	0.311

b. Indicators of Spearman's rank correlation between some biochemical variables and T/S ratio

Variables	Age	Fasting plasma glucose	Post-load plasma glucose	Fe plasma level	Zn plasma level	T/S ratio
All						
Age	–	−0.314	–	−0.409	−0.389	–
Fasting plasma glucose	−0.314	–	–	0.372	0.279	–
Post-load plasma glucose	–	–	–	–	–	**−0.353**
Fe plasma level	0.409	0.372	–	–	0.261	–
Zn plasma level	0.389	0.279	–	0.261	–	–
T/S ratio	–	–	**−0.353**	–	–	–
Women						
Age	–	−0.275	–	−0.34	−0.287	–
Fasting plasma glucose	−0.275	–	–	0.322	0.291	**−0.309**
Post-load plasma glucose	–	–	–	–	–	**−0.271**
Fe plasma level	−0.34	0.322	–	–	–	–
Zn plasma level	−0.287	0.291	–	–	–	–
T/S ratio	–	**−0.309**	**−0.271**	–	–	–
Men						
Age	–	–	–	−0.608	−0.614	–
Fasting plasma glucose	–	–	–	0.514	–	–
Post-load plasma glucose	–	–	–	–	–	**−0.537**
Fe plasma level	−0.608	0.514	–	–	–	–
Zn plasma level	−0.614	–	–	–	–	–
T/S ratio	–	–	**−0.537**	–	–	–

Note: Data are medians (1,3 Qs) or Indicators of Spearman's rank correlation (Ro). The shown values of the coefficients other than 0 ($P < 0.05$); For Ro = **−0.353; −0.309; −0.271; −0.537** $P < 0{,}01$; $P = 0.01$; $P = 0.03$ and $P = 0.01$ respectively. Post-load plasma glucose – 120 min after 75 g glucose per os.

In the DM group, median (1,3Q) value of the HbA1c level was 6.3 (5.6–6.87) %. Hba1c levels were positively and with a statistical significance associated with age and FPG. A similar association with LTL T/S ratio was absent, data not shown.

Characteristics of participants by quartile of leukocyte telomere length

Median value of FPG levels, median age, NC, and WC in the short telomere group and in long telomere group had no statistically significant differences. This difference only occurred when plasma glucose was compared after standard loading ($P = 0.030$), Table 4.

Short LTL logistic regression risk's assessment (1st vs. 4th quartiles) of the study subjects (n = 82) depending of their fasting and post-load plasma glucose levels

Increase of plasma glucose levels after glucose loading (continued variable) increases the chances of revealing short telomeres, and this pattern remains after binary or multi-adjusting according to all the potential confounders that could influence this risk according to our assessment of

Table 2 Anthropometric data of the study subjects depending of gender (a) and their Indicators of Spearman's rank correlation (b)

a.

Parameters	Women ($n = 62$)	Men ($n = 20$)	*P* value (women vs. men)
BMI, kg/m^2	31.83 (29.0–38.01)	28.08 (25.57–31.39)	<0.001
Waist circumference, cm	103.5 (91–116)	99.0 (91.0–108.5)	0.176
Neck circumference, cm	36 (35–38)	40.0 (37.75– 42.5)	<0.001
Systolic BP, mm Hg	140 (125–160)	126 (125–148.5)	0.206
Diastolic BP, mmHg	80 (75–95)	80 (80–85)	0.704
High BP, *n* (%)	35 (56.5)	8 (40)	0.404
High BP treatment, *n* (%)	33 (53.2)	4 (20.0)	0.016

b.

Variables	Age	BMI	Waist circumference	Neck circumference	Sysolic BP	Diastolic BP	T/S ratio
Women							
Age	–	–	–	–	0.391	–	–
BMI	–	–	0.892	0.732	0.255	–	–
Waist circumference	–	0.892	–	0.764	0.283	–	–
Neck circumference	–	0.732	0.764	–	0.313	–	–
Sysolic BP	0.391	0.255	0.283	0.313	–	0.69	–
Diastolic BP	–	–	–	–	0.69	–	–
T/S ratio	–	–	–	–	–	–	–
Men							
Age	–	–	–	–	0.533	–	–
BMI	–	–	0.842	0.837	–	–	–
Waist circumference	–	0.842	–	0.842	–	–	**−0.494**
Neck circumference	–	0.837	0.842	–	–	–	–
Systolic BP	0.533	–	–	–	–	–	**–0.478**
Diastolic BP	–	–	–	–	–	–	–
T/S ratio	–	–	−0.494	–	−0.478	–	–

Note: Data are medians (1,3 Qs) or *n* (%) or Indicators of Spearman's rank correlation (Ro). The shown values are the coefficients other than 0 ($P < 0.05$); For Ro = **−0.494** or **−0.478;** $P = 0.03$.
BMI: body mass index; BP: blood pressure.

the association between telomere length and some biochemical (FPG levels in women) and anthropometric parameters (systolic blood pressure and WC in men), accounting for gender and age. All similar assessments of FPG did not reach statistical significance (Table 5).

Comparing LTL in categories selected according to WC or presence of arterial hypertension did not reveal any significant differences (Supplement Table 1).

Discussion

In the studied population of rural residents of central Ukraine, LTL (T/S ratio) correlates with both fasting and 2hPG levels in women, but only with 2hPG levels in men (Tables 2(b) and 3(b)). When evaluating the association between LTL and plasma glucose levels without regard to gender (for both genders simultaneously), the correlational dependence was present only in 2hPG levels but not in FPG (Tables 1(b) and 4). We can assume that the revealed correlation between FPG and LTL in women is in fact secondary as regards to the association between fasting and 2hPG levels. Indeed, mutual adjusting of telomere shortening chances depending on fasting glycaemia levels and/or after glucose loading revealed a preservation of the association for 2hPG levels (Table 5).

Content of metals in blood plasma (Fe, Zn) that are known not only as risk factor of developing chronic hyperglycemia[13,14] but also as factors that may affect telomere length by influencing telomerase activity[15] and can reduce telomere length[16] did not correlate with LTL in our study.

Our data indicate that the telomere length is negatively related to impaired glucose tolerance but not to increased

Table 3 Leucocyte telomere length, fasting and post-load plasma glucose levels and their Indicators of Pearson's rank correlation according to categories of the study subjects

Glycemic categories	*n*	LTL T/S ratio	FPG, mmol/l			2hPG, mmol/l;		
				Correlation with LTL			Correlation with LTL	
		Mean (SD)	Median (1,3 qs)	R	P value	Median (1,3 qs)	R	P value
NGT	33	0.6076 (0.1059)	5.69 (4.93–5.86)	0.085	0.642	5.46 (4.99–6.41)	–0.384	0.027
IFG/IGT	31	0.5745 (0.1473)	6.28 (6.1–6.61)	0	0.827	6.21 (4.85–8.15)	–0.393	0.029
DM	18	0.554 (0.1273)	7.37 (7.25–7.92)	–0.593	0.009	7.06 (6.06–12.98)	–0.471	0.049

Note: Data are means (SD), medians (1,3 Qs) or Indicators of Pearson's rank correlation (R); mean LTL T/s ratios of NGT, IFG/IGT and DM groups did not differ with any statistical significance ($P = 0.322$).

Table 4 Characteristics of participants by quartile of leukocyte telomere length

Characteristics	1Q, $n = 20$	2Q, $n = 22$	3Q, $n = 20$	4Q, $n = 20$	*P* value
FPG, mmol/l	6.44 (5.99–6.84)	6.17 (5.64–7.33)	5.79 (5.61–6.17)	6.09 (5.54–6.67)	0.065
2hPG, mmol/l	7.01 (6.16–9.86)	6.15 (5.34–7.1)	5.78 (5.30–6.74)	5.32 (4.83–6.22)	0.030
Age, yrs	61.5 (58.5–68.0)	58.5 (51.0–78.0)	62.5 (58.0–68.5)	62.0 (57.0–67.0)	0.898
WC in men, cm	108.0 (102.0–111.0) $n = 7$	100.0 (91.5–105.0) $n = 4$	93.0 (91.0–96.5) $n = 4$	90.0 (89.0–105.8) $n = 5$	0.111
SBP in men, mm Hg	147.0 (125.0–162.0) $n = 7$	125.0 (123.5–126.0) $n = 4$	133.0 (125.5–144.5) $n = 4$	125.0 (116.5–133.8) $n = 5$	0.141
WC in women, cm	105.0 (94.0–116.0) $n = 13$	105.0 (96.0–117.0) $n = 18$	101.5 (90.5–106.5) $n = 16$	103.0 (90.3–120.5) $n = 15$	0.767
SBP in women, mm Hg	140.0 (127.5–152.5) $n = 13$	143.5 (125.0–162.0) $n = 18$	139.5 (128.5–155.0) $n = 16$	140.0 (120.5–163.8) $n = 15$	0.997

Note: Data are medians (25–75 percentile). Significance level assessed by Kruskal–Wallis test.

fasting glycaemia. The revealed pattern remained after adjusting by age, gender, and some anthropometric characteristics of studied population (Table 5).

It is interesting that in the studied population of rural residents, we were unable to find any gender differences of telomere length. We may assume that to reveal such associations, we would require a large number of studied persons and greater age range; however, other researchers, who studied two Swedish populations ($n = 476$, age range 48–68, median 62 years, and $n = 513$, age range 26–75, median 42 years), also revealed that ''the yearly telomere loss was not observed for men.''[17] At the same time, a greater age range possibly helped to reveal a reduction of LTL in the older age group. Our previous study of telomere length in a Ukrainian population of a greater age range demonstrated expected age differences.[18]

Swedish researchers found a significant association between obesity parameters (BMI, WC) and telomere length in women only. In men, a negative correlation to 2 h oral glucose-tolerance test (OGTT) was observed.[17] We revealed a negative association between LTL, WC, and systolic blood pressure in men only, and we also found an association between FPG levels and telomere length for women only and post-load plasma glucose for both women and men.

As for the absence of a correlation between post-load plasma glucose levels and LTL in women according to Swedish data, we must note that OGTT data were not available on all patients, and because of the low number of individuals examined (148 from 475 women) and the borderline significance, these results should be interpreted with caution.

In a longitudinal study from South Korea,[19] a significant inverse association between changes in WC and LTL was found in men but not in women, which corresponds to our data.

Table 5 Short telomeres length multi-adjusted logistic regression risk's assessment (1st vs. 4th quartiles) of the study subjects ($n = 82$) depending of their fasting and post-load plasma glucose levels

Models description	Models adjusting	OR	95% CI		P
Fasting plasma glucose, mmol/l	Absent	1.89	0.87	4.13	0.108
	Age	1.93	0.85	4.35	0.115
	Gender	1.89	0.86	4.18	0.115
	Waist circumference	1.84	0.81	4.15	0.114
	Systolic BP	1.85	0.85	4.04	0.112
	Systolic BP + waist circumference + gender	1.82	0.8	4.16	0.156
	Post-load plasma glucose	2.09	0.71	6.16	0.181
Post-load plasma glucose, mmol/l	Absent	1.52	1.04	2.22	0.03
	Age	1.6	1.05	2.43	0.029
	Gender	1.57	1.05	2.37	0.029
	Waist circumference	1.54	1.04	2.28	0.03
	Systolic BP	1.52	1.04	2.24	0.032
	Systolic BP + Waist circumference + Gender	1.61	1.05	2.46	0.028
	Fasting plasma glucose	1.58	1.05	2.38	0.029

In general, one should agree with an opinion[20] that biologic effects on LTL may differ under certain psychosocial and racial/ethnic circumstances and could impact future health disparity studies.

Besides, it is clear that when comparing the association between LTL and diabetes or impaired glucose regulation (pre-diabetes), we must account for the heterogeneity of the hyperglycemic categories. Thus, in a population-based study of the US general population, lower LTL was associated with higher prevalence of diabetes in unadjusted models. However, in models adjusted for age, race–ethnicity, and sex and in multivariable adjusted models, the association between LTL and diabetes was attenuated and no longer significant. In addition, LTL was not associated with diabetes duration or diabetes control in unadjusted or adjusted models.[5]

In this US study, the authors defined diabetes as a previous diagnosis of diabetes, A1c $\geq$ 6.5% (48 mmol/mol) or fasting glucose $\geq$ 126 mg/dl; yet, the fraction of diabetes cases diagnosed based only on the corresponding FPG levels in this study is not reported.

It seems significant that the above research did not look at the relation between LTL and post-load/postprandial glucose levels, which may be responsible for such a result. It should be also noted that this study joins cases based on screen-detected ''diabetic'' FPG level and previously known cases of diabetes.[5] In an Italian study, no significant difference in LTL was observed between control subjects and patients with Type 2 diabetes without complications. Patients with diabetes complications had significantly shorter leukocyte telomere length (LTL) than both patients without diabetes complications and healthy control subjects.[21]

In our study, telomere length was compared in groups and selected based on OGTT results. The absence of a statistically significant difference between NGT and DM groups may be probably explained by the absence of high fraction of diabetes complications in persons from the screen-detected DM group.

Another epidemiologic study from US has shown an association of LTL shortening with the history of T2D. Only subjects with normal FPG and 2 h OGTT levels were considered as a control in this study. It is interesting that this relatively large control group ($n = 424$) did not demonstrate an association between FPG and LTL, which does not contradict our data. A recent Russian study (50 patients with diabetes and 49 control group participants) that shows a positive association between LTL shortening and intima–media complex thickness did not reveal any relationship between FPG and LTL, which is consistent with our results. Unfortunately, these studies do not give results of a similar evaluation for 2 h OGTT levels.[22,23]

It is surprising that although the association between IGT and LTL shortening was revealed a long time ago, epidemiological studies of correlational association between LTL and distinctly FPG and 2 h OGTT levels such as ours seem to not have been previously performed. Greek researchers reported[24] that significant telomerase activity was detected in circulating peripheral blood mononuclear cells in persons with metabolic syndrome (a cluster of cardiovascular and pro-thrombotic risk factors, such as insulin resistance, impaired glucose tolerance, dyslipidemia, obesity, and elevated blood pressure), which may partially explain the absence of association between several indicators of insulin resistance (FPG and LTL) that we have revealed. The absence of telomere shortening in persons with screen-detected DM or complication-free diabetes can be explained by an elevation of telomerase activity as well.

Thus, the presence of an association between LTL and chronic hyperglycemia may be determined by a balance between telomerase activity level and the degree of oxidative stress, that have different influence on the telomere length, which lowers the chances of revealing this association. It seems that either an increase of FPG is more associated with an elevation of telomerase activity than IGT, or the last category is related to a more significant oxidative stress that leads to telomere shortening with the development of atherosclerosis in large vessels.[25]

Interestingly, the subjects with IGT and newly diagnosed diabetes, but not isolated IFG, exhibit a greater arterial stiffness.[26]

The hyperglycemic categories that we have studied are being currently looked at as patho-physiologically different conditions of insulin resistance (mainly liver resistance in i-IFG and mostly muscle and adipose tissue resistance in i-IGT) that require different approaches towards prevention of type 2 diabetes development and treatment.[8,27]

For the first time, we were possibly able to demonstrate an association between LTL shortening and glucose tolerance, as well as the absence of such association for LTL and FPG. Epidemiological nature of the study and the absence of drug treatment could have contributed to this result. This can be viewed as one of the strong points of our study. Quantitative deficit that prevented conducting a convincing comparison in isolated glycemic groups can be considered as one of this study's weak points.

In the studied population sample of Caucasians, median age was above 60 and women significantly dominated over men. All of these factors (race, female gender, and age over 60) have a negative influence on the strength of association between telomere length and T2D.[4]

Nevertheless, we believe that LTL demonstrates a negative correlational relationship with post-load 2hPG not just in the hyperglycemic categories (IFG/IGT) but in the NGT category as well. As far as we are aware, such results have not been seen before. It is possible that the negative relationship between LTL and glucose tolerance in fact decreases with the increase of glycaemia. This may partially explain the absence of telomere shortening, noted by some researchers in heterogeneous populations of diabetes patients.

Further studies are needed for a more detailed assessment of telomere shortening risk, associated with specific categories of hyperglycemia. They must also evaluate telomerase activity and the presence of diabetic complications. It is quite possible that these studies will help determine the causality of telomere shortening and development of diabetes.

Authors' contributions: MK: conception and design of the analysis. DK, IA, NO and AK were responsible of the data acquisition and/or measurements. VK performed the statistical analysis. MK, AV and VK intellectual content; and the final approval of the manuscript was done by all authors.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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