Leukocyte Telomere Length Independently Predicts 3-Year Diabetes Risk in a Longitudinal Study of Chinese Population

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Cellular aging markers, including telomere length and mitochondrial function, as well as oxidative stress and inflammation markers influence each other and form a complex network, which is affected in diabetes. However, it remains unknown whether these markers could independently predict future diabetes after adjustment for their mutual effects. We conducted a 3-year longitudinal study in a Chinese cohort that comprised 108 nondiabetic individuals at baseline. The 2-hour 75 g oral glucose tolerance tests were performed at baseline and at 3-year follow-up. At baseline, leukocyte telomere length (LTL) and mitochondrial DNA copy number (mtDNAcn) in leukocytes were determined using the polymerase chain reaction method. Tumor necrosis factor (TNF-α), interleukin-6, 8-hydroxy-2-deoxyguanosine levels, and superoxide dismutase (SOD) activity were measured by the enzyme-linked immunosorbent assay. Participants who developed diabetes at the 3-year follow-up (n = 28) had shorter LTL and higher levels of TNF-α and SOD activity at baseline. Baseline LTL was found to be independently associated with the development of diabetes at the 3-year follow-up after the adjustment for mtDNAcn, markers of oxidative stress and inflammation, and conventional diabetes risk factors. Our findings suggest that LTL is an independent predictor for 3-year diabetes risk, which might inform timely prevention and treatment of diabetes. Telomere shortening might be involved in the pathogenesis of diabetes independently of conventional diabetes risk factors, mtDNAcn, or oxidative stress and inflammation pathways.

1. Introduction

Over the last several decades, numerous studies have revealed that telomere length, mitochondrial function, oxidative stress, and inflammation are cellular aging markers intertwined in the complex relationships that determine their roles in cellular senescence [1–3]. These cellular aging markers have also been reported to be associated with various age-related diseases [4, 5], including diabetes [6, 7]. The relationships between these cellular aging markers and diabetes are particularly complex. The potential associations between leukocyte telomere length (LTL), mitochondrial DNA copy number (mtDNAcn), markers of oxidative stress/antioxidants, and proinflammatory cytokines on the one hand and diabetes on the other hand have been extensively scrutinized. Unfortunately, the cross-sectional design of the majority of the previous population-based studies failed to uncover causal relationships between these cellular aging markers and diabetes, with many findings being inconsistent [8–14]. Prospective studies are scarce, with only three published reports [15–17] investigating the prospective association of LTL with the development of diabetes. Furthermore, the
results of those studies were discrepant and confusing. In a multiracial cohort of postmenopausal women, just a weak association was found between baseline LTL and future diabetes risk, which was attenuated after adjusting for conventional diabetes risk factors [15]. In contrast, in an American Indian cohort, baseline LTL was identified as a predictive marker independent of conventional diabetes risk factors [16]. Similar results were obtained in a European cohort [17]. The telomere length is characterized by strong heritability, and it varies substantially among different races [18], which could partly explain the discrepancy among these studies. Notably, none of these prospective studies included East Asian or, more specifically, Chinese subjects. Additionally, studies exploring the predictive role of mtDNAcn as well as markers of oxidative stress and inflammation in diabetes development are rare in general. Furthermore, considering the complex relationships between these biomarkers, it should be established whether they could affect each other’s predictive role in diabetes risk. Taking into account these considerations, we conducted a 3-year longitudinal study in a Chinese population to investigate the prospective associations of baseline LTL and mtDNAcn, as well as oxidative stress and inflammatory markers with the development of diabetes 3 years later.

2. Methods

2.1. Study Participants. The current study was conducted in a Chinese cohort from the ChangPing suburb of Beijing between March 2014 and May 2017. The study protocols and consent procedures had been approved by the Ethics Committee of the Peking Union Medical College Hospital. All participants have signed the informed consent forms. A total of 142 individuals who had completed data in 2014 and 2017 were recruited into this study. According to the World Health Organization criteria [19], participants with fasting plasma glucose (FPG) < 7.0 mmol/L and 2-hour postload plasma glucose (PG) < 11.1 mmol/L during the 75 g oral glucose tolerance test (OGTT) were identified as nondiabetic individuals, including those with normal glucose tolerance (NGT) and prediabetes (impaired fasting glucose (6.1 mmol/L ≤ FPG < 7.0 mmol/L and 2 hPG < 7.8 mmol/L) and/or impaired glucose tolerance (7.8 ≤ 2hPG < 11.1 mmol/L and FPG < 6.1 mmol/L)). There were 34 individuals who had diabetes at baseline and were thus excluded from the study. Hence, a total of 108 non-diabetic individuals at baseline were selected as the study population, including those with NGT (n = 55) and prediabetes (n = 53) at baseline. Demographic and anthropometric data were collected at baseline and at 3-year follow-up, including age, sex, height, weight, waist circumference (WC), and hip circumference (HC).

2.2. Biochemical Analysis. All the participants underwent a 75 g 2-hour OGTT, in which blood samples were drawn at four time points at baseline (0 min, 30 min, 60 min, and 120 min), whereas blood samples were obtained at two time points at the 3-year follow-up (0 min and 120 min). The levels of PG were determined by the glucose oxidase assay. Serum insulin (INS) and C-peptide (CP) were determined by the chemiluminescence immunoassay (Insulin IRI #02230141 (128434) and C-peptide #03649928 (129026), both from Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). Glycosylated hemoglobin (HbA1c) was measured by high-performance liquid chromatography, whereas total cholesterol (TC), total triglyceride (TG), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), uric acid (UA), creatinine (Cr), and urea were measured using an automatic analyzer.

2.3. Calculations of Insulin Sensitivity and β-Cell Function Indices. Based on the results of the 75 g 2-hour OGTT, we evaluated the sensitivity to insulin and β-cell function of the participants by using the following equations [20–22]:

\[
\text{HOMA-IR} = \frac{\text{fasting INS (} \mu \text{U/L}) \times \text{FPG (mmol/L)}}{22.5},
\]

\[
\text{HOMA-β} = \frac{20 \times \text{fasting INS (} \mu \text{U/L})}{\text{FPG (mmol/L)} - 3.5}.\]

\[
\text{Matsuda index} = \frac{1000}{(\text{FPG (mg/dL)} \times \text{fasting INS (} \mu \text{U/mL}) \times \text{mean OGTT glucose concentration (mg/dL)} \times \text{mean OGTT insulin concentration (} \mu \text{U/mL)})}.
\]

\[
\text{Insulin secretion-sensitivity index} - 2(\text{ISSI-2}) = \frac{\text{Insulin AUC}}{\text{Glucose AUC}} \times \text{Matsuda index.}
\]

2.4. LTL Assays. The LTL assays were performed based on the blood samples collected at baseline. The details of LTL measurement have been described in our previous publication [23]. LTL was determined as the relative ratio of the telomere repeat copy number to the single copy number (T/S ratio) using the monochrome multiplex quantitative polymerase chain reaction protocol [24].

2.5. Measurement of mtDNAcn in Peripheral Blood. mtDNAcn was measured based on the blood samples...
collected at baseline. As described previously [25], genomic DNA was extracted from leukocytes using a QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) and subsequently purified, diluted, and quantified using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The relative mtDNAcn value was determined using the real-time polymerase chain reaction and adjusted by simultaneously measuring the amount of nuclear DNA.

2.6. Assessment of Oxidative Stress and Inflammatory Markers. The measurements of oxidative stress and inflammatory markers were performed based on the blood samples collected at baseline. The measurements of oxidative stress and inflammatory markers have also been described in detail previously [23]. Superoxide dismutase (SOD) activity, as well as the levels of 8-hydroxy-2-deoxyguanosine (8-OHdG), tumor necrosis factor (TNF-α), and interleukine-6 (IL-6) were determined according to the manufacturer’s instructions (Cloud-Clone Corp., Houston, USA). Absorbance kinetics were measured using an enzyme-linked immunosorbent assay reader.

2.7. Statistical Analysis. Statistical analysis was conducted using the SPSS 25.0 software package (IBM, R Studio (version 1.2.1335, https://www.r-project.org/), and GraphPad Prism 8.0 (https://www.graphpad.com). Continuous variables are presented as the mean ± standard error of the mean, whereas categorical variables are presented as percentages. To examine if the data were normally distributed, the Shapiro-Wilk and Kolmogorov-Smirnov tests were used. Normality transformations were performed on the variables that did not meet the normality assumptions using the appropriate formula when necessary. Comparisons of the continuous variables between groups were performed by Student’s t-test or nonparametric Mann-Whitney’s U-test, where appropriate. Pearson’s or Spearman’s correlation analysis was performed to determine the bivariate correlations, where appropriate. To explore whether mtDNAcn mediated the effect of TNF-α on 30-minute postload plasma glucose (PG30min), PROCESS macro Version 3.3 was used to generate simple mediation models with ordinary least squares. Mediation hypotheses were tested via a bias-corrected bootstrap method with 5,000 samples to calculate 95% confidence intervals (95% CI). If the 95% CI did not encompass zero, this represented statistical significance of the mediating effect. To identify the independent predictors for diabetes risk, multivariate logistic regression analysis was performed. In all comparisons, the statistical significance was set at P < 0.05.

3. Results

3.1. Relationships between LTL, TNF-α Level, and SOD Activity and the Risk of Diabetes Development. According to whether the participants developed diabetes at the 3-year follow-up, they were divided into progressors (n = 29) and non-progressors (n = 79). Tables 1 and 2 present the differences in characteristics between progressors and non-progressors at baseline and at the 3-year follow-up, respectively. The proportions of NGT subjects in progressors and non-progressors at baseline were similar (51.7% versus 50.6%, P = 0.920). At baseline, the progressors had a significantly higher level of PG30min (P = 0.001) and PG60min (P = 0.01) than non-progressors, with slightly higher HbA1c (P = 0.059). However, the differences of other conventional risk factors of diabetes at baseline, including age, BMI, WC, lipid profile, UA, insulin sensitivity indices, and insulin secretion indices between the two groups did not reach statistical significance (P > 0.05). Baseline age-adjusted LTL of progressors was significantly shorter than that of non-progressors (P = 0.005). TNF-α levels (P = 0.009) and SOD activity (P = 0.031) of progressors were higher than those of non-progressors, whereas we failed to observe statistically significant differences in the values of mtDNAcn (P = 0.221), IL-6 (P = 0.742), and 8-OHdG (P = 0.108) between groups. As expected, at the 3-year follow-up, HbA1c, FPG, PG120min, fasting insulin (FINS),

| Table 1: Differences of baseline characteristics between progressors and non-progressors. |
|---------------------------------------------------------------|
| **Baseline characteristics**           | **Progressors** (n = 29) | **Non-progressors** (n = 79) | **P value** |
|-----------------------------------------|--------------------------|-------------------------------|------------|
| NGT (n (%))                             | 15 (51.7%)               | 40 (50.6%)                    | 0.920      |
| Sex (male/female)                       | 8/21                     | 57/22                         | 0.979      |
| Age (years)                             | 55.5 ± 1.8               | 54.1 ± 1.1                    | 0.519      |
| BMI (kg/m²)                             | 26.12 ± 0.55             | 26.05 ± 0.47                  | 0.930      |
| WC (cm)                                 | 86.8 ± 2.0               | 86.4 ± 1.1                    | 0.989      |
| WHR                                     | 0.95 ± 0.00              | 0.95 ± 0.00                   | 0.110      |
| HbA1c (%)                               | 5.8 ± 0.1                | 5.5 ± 0.1                     | 0.059      |
| FPG (mmol/L)                            | 5.84 ± 0.12              | 5.85 ± 0.06                   | 0.975      |
| PG30min (mmol/L)                        | 11.42 ± 0.43             | 9.69 ± 0.25                   | 0.001      |
| PG60min (mmol/L)                        | 10.58 ± 0.84             | 9.14 ± 0.28                   | 0.010      |
| PG120min (mmol/L)                       | 7.23 ± 0.42              | 6.89 ± 0.18                   | 0.465      |
| TC (mmol/L)                             | 5.29 ± 0.24              | 5.48 ± 0.12                   | 0.430      |
| TG (mmol/L)                             | 1.56 ± 0.15              | 1.60 ± 0.12                   | 0.774      |
| HDL-C (mmol/L)                          | 1.29 ± 0.05              | 1.31 ± 0.03                   | 0.734      |
| LDL-C (mmol/L)                          | 2.71 ± 0.17              | 2.84 ± 0.08                   | 0.470      |
| UA (µmol/L)                             | 274.2 ± 13.6             | 284.6 ± 9.4                   | 0.605      |
| Cr (µmol/L)                             | 73.1 ± 3.6               | 69.9 ± 2.2                    | 0.465      |
| Urea (µmol/L)                           | 5.15 ± 0.23              | 5.00 ± 0.14                   | 0.606      |
| HOMA-IR                                 | 2.97 ± 0.39              | 2.76 ± 0.20                   | 0.796      |
| Matsuda index                           | 4.43 ± 0.69              | 4.86 ± 0.39                   | 0.374      |
| HOMA-β                                  | 99.77 ± 12.36            | 91.23 ± 5.90                  | 0.813      |
| ISS1-2                                  | 449.44 ± 37.81           | 517.01 ± 24.43                | 0.074      |
| Age-adjusted LTL                        | 28.21 ± 0.17             | 28.80 ± 0.11                  | 0.005      |
| mtDNAcn                                 | 96.69 ± 7.73             | 109.15 ± 5.44                 | 0.221      |
| TNF-α (fmol/mL)                         | 29.88 ± 1.44             | 24.63 ± 1.31                  | 0.009      |
| IL-6 (pg/mL)                            | 4.31 ± 0.63              | 4.23 ± 0.37                   | 0.742      |
| 8-OHdG (pg/mL)                          | 33.15 ± 3.83             | 43.14 ± 3.45                  | 0.108      |
| SOD (U/mL)                              | 68.48 ± 2.96             | 62.36 ± 1.75                  | 0.031      |
3.2. Correlation of Cellular Aging Markers with Clinical Characteristics at Baseline. As indicated in Table 3, we found a modest positive correlation between baseline LTL and the Matsuda index ($r = 0.208$, $P = 0.039$), which proved to be a favorable OGTT-stimulated insulin sensitivity index. Nevertheless, we did not find an association between baseline LTL and any other conventional diabetes risk factor at baseline. mtDNAcn negatively correlated with PG30min, TG, and UA. The level of TNF-α positively correlated with HbA1c, PG30min, and UA, and negatively correlated with the Matsuda index. 8-OHdG level correlated with age, HDL-C, HOMA-IR, and HOMA-β. As for the correlations among the cellular aging markers per se, both LTL and mtDNAcn correlated with TNF-α level and SOD activity. To explore whether mtDNAcn mediated the effect of TNF-α on PG30min, mediation model analysis was performed. As shown in Figure 1, mtDNAcn fully mediated the effect of TNF-α on PG30min (direct effect $B = 0.0156$; 95% CI -0.0260–0.0571; indirect effect $B = 0.0308$; 95% CI 0.0077–0.0635).

3.3. Prospective Correlations of Baseline LTL, TNF-α, 8-OHdG, and SOD Activity with PG120min at the 3-Year Follow-Up. Subsequently, we performed Spearman’s correlation analysis to explore the associations between parameters of cellular aging markers at baseline and clinical characteristics at the 3-year follow-up. As indicated in Table 4, baseline LTL negatively correlated whereas TNF-α level and SOD activity positively correlated with PG120min at the 3-year follow-up ($r = -0.307$, 0.324, and 0.272, respectively, $P < 0.01$). Baseline 8-OHdG level inversely correlated with PG120min and HOMA-β at the 3-year follow-up ($r = -0.203$, $P < 0.05$, and $r = -0.257$, $P < 0.01$, respectively). Additionally, both baseline LTL and TNF-α correlated with TC at the 3-year follow-up ($r = -0.208$ and 0.203, respectively, $P < 0.05$).

3.4. Independent and Prospective Association between LTL and Future Diabetes Risk. Multivariate logistic regression analysis was performed to explore the independent predictors for the 3-year diabetes risk. As shown in Table 5, age and sex-adjusted LTL at baseline were significantly associated with the development of diabetes at the 3-year follow-up (OR = 0.423, 95% CI 0.231–0.776, adjusted $P = 0.005$, Model 1). Following further and progressive adjustments for conventional diabetes risk factors (Model 2), as well as oxidative stress and inflammatory markers at baseline (Model 3), the association remained significant (Model 2: OR = 0.414, 95% CI 0.187–0.919, adjusted $P = 0.030$; Model 3: OR = 0.341, 95% CI 0.139–0.839, adjusted $P = 0.019$). As shown in Table S1, multivariate logistic regression analysis according to LTL quintiles demonstrated that individuals with LTL values in the second quintile were more than twofold likely to develop diabetes as those with the longest LTL values in the highest quintile ($OR = 12.410$, 95% CI 1.240–124.252, adjusted $P = 0.032$). However, the elevation of diabetes risk in individuals with LTL values in the first ($OR = 8.626$, 95% CI 0.646–115.257, adjusted $P = 0.103$), third ($OR = 1.606$, 95% CI 0.155–16.684, adjusted $P = 0.691$), and fourth quintiles ($OR = 3.242$, 95% CI 0.315–33.411, adjusted $P = 0.323$) compared to those in individuals with the longest LTL values in the highest quintile did not reach statistical significance, suggesting a nonlinear association between LTL at baseline and diabetes risk at the 3-year follow-up.

4. Discussion

Our longitudinal findings indicate that short LTL at baseline may be predictive of increased future diabetes risk. This relationship was independent of the effects of conventional diabetes risk factors, mtDNAcn, or oxidative stress and inflammatory markers. Therefore, telomere shortening is possibly involved in the pathogenesis of diabetes via the pathways other than those mediating the effects of conventional
diabetes risk factors, mitochondrial dysfunction, oxidative stress, and inflammation.

Our observation that LTL predicts diabetes risk independently of conventional diabetes risk factors (e.g., age, BMI, WC, FPG, and lipid profile) was consistent with findings of previous prospective studies [16, 17]. We considerably extended the conclusions of those reports by showing that the correlation between LTL for diabetes risk is also independent of mtDNAcn and markers of oxidative stress and inflammation. Thus far, the role of the latter biomarkers in

Table 3: Baseline correlates of clinical characteristics and cellular aging markers among the 108 participants.

| Variables          | LTL  | mtDNAcn | TNF-α | Log2(IL-6) | 8-OHdG | SOD  |
|--------------------|------|---------|-------|------------|--------|------|
| Sex                | —    | —       | —     | —          | —      | —    |
| Age                | 0.007| -0.156  | 0.081 | -0.053     | 0.230* | 0.193*|
| BMI                | -0.058| -0.126 | 0.114 | -0.231*    | -0.111| -0.025|
| 1/(WC)             | -0.005| 0.192  | -0.140| 0.164      | 0.042  | -0.152|
| HbA1c              | -0.169| -0.086 | 0.278**| 0.181      | -0.012 | 0.234*|
| FPG                | -0.071| -0.124 | -0.101| 0.000      | 0.088  | 0.014 |
| PG30min            | -0.187| -0.420**| 0.200*| 0.027      | 0.030  | 0.130 |
| PG60min            | -0.160| -0.191 | 0.056 | -0.030     | -0.017 | 0.153 |
| PG120min           | -0.114| -0.057 | 0.088 | 0.258**    | 0.014  | 0.034 |
| NGT or prediabetes | —    | —       | —     | —          | —      | —    |
| TC                 | -0.047| -0.104 | 0.068 | -0.048     | 0.174  | -0.038|
| 1/sqrt(TG)         | 0.069 | 0.223* | -0.146| 0.061      | 0.031  | 0.072 |
| HDL-C              | -0.131| -0.129 | -0.007| 0.100      | 0.197* | 0.079 |
| LDL-C              | -0.075| -0.083 | 0.112 | -0.042     | 0.105  | -0.074|
| UA                 | -0.196| -0.310**| 0.216*| -0.005     | -0.113 | 0.001 |
| Log2(HOMA-IR)      | -0.149| -0.057 | 0.155 | -0.094     | -0.204*| -0.046|
| Ig(Matsuda index)  | 0.208*| 0.124  | -0.197*| 0.079      | 0.174  | 0.013 |
| Log2(HOMA-β)       | -0.102| 0.030  | 0.162 | -0.125     | -0.299**| -0.088|
| 1/sqrt(ISSI-2)     | -0.133| -0.178 | 0.060 | 0.089      | -0.076 | 0.091 |
| LTL                | 1.000 | 0.808  | -0.304**| -0.195     | -0.027 | -0.268**|
| mtDNAcn            | 0.080 | 1.000  | -0.359**| -0.022     | -0.005 | -0.213*|
| TNF-α              | -0.304**| -0.359**| 1.000 | 0.125      | -0.150 | 0.369**|
| Log2(IL-6)         | -0.195| -0.022 | 0.125 | 1.000      | 0.036  | 0.137 |
| 8-OHdG             | -0.027| -0.005 | -0.150| 0.036      | 1.000  | 0.022 |
| SOD activity       | -0.268**| -0.213* | 0.369**| 0.137 | 0.022  | 1.000 |

*P < 0.05. **P < 0.01. Abbreviations: BMI—body mass index; WC—waist circumference; HbA1c—glycosylated hemoglobin A1c; FPG—fasting plasma glucose; PG—plasma glucose after glucose load in OGTT; NGT—normal glucose tolerance; TC—total cholesterol; TG—total triglyceride; HDL-C—high-density lipoprotein cholesterol; LDL-C—low-density lipoprotein cholesterol; UA—uric acid; ISSI-2—insulin sensitivity index-2; LTL—leukocyte telomere length; mtDNAcn—mitochondrial DNA copy number; TNF-α—tumor necrosis factor-α; IL-6—interleukine-6; 8-OHdG—8-hydroxyl-2-deoxyguanosine; SOD—superoxide dismutase.

Figure 1: Mediation model of the association between TNF-α, mtDNAcn, and PG30min. The P values for regressions a and b are 0.0002 and 0.0001, respectively. The P value for the direct effect of TNF-α on PG30min is 0.4597. The absence of “0” in the 95% CI represents statistical significance of the indirect effect.
the development of diabetes remained confusing. Previous data indicated that telomere shortening and mitochondrial dysfunction likely affect each other and thus form a self-amplifying cycle [26]. The impaired mitochondria produce excessive reactive oxygen species that subsequently cause oxidative stress and inflammation [27]. In turn, oxidative stress and inflammation have also been demonstrated to damage telomere [28–30] and mtDNA [31, 32], which results in a vicious cycle. These changes may contribute to the development of diabetes via their possible involvement in insulin resistance [31, 33, 34] and β-cell dysfunction [35, 36]. Our finding of the positive correlation between LTL and the insulin sensitivity index, the Matsuda index, at baseline indicates an association between telomere shortening and resistance to insulin. The prospective inverse correlation between baseline 8-OHdG and HOMA-IR sensitivity index, the Matsuda index, at baseline indicates fi

of diabetes via their possible involvement in insulin resistance [31, 33, 34] and potential pathways might mediate the effects of telomere shortening on the predisposition to diabetes. Further investigations are necessary to uncover mechanisms and pathways underlying this association.

To the best of our knowledge, this is the first study to explore an independent effect of mtDNAcn on the risk of future diabetes. We did not detect an independent effect of this parameter on the risk of diabetes at the 3-year follow-up. Another prospective study observed that adding mtDNAcn as an adjunctive marker augmented the predictive power of HbA1c and OGTT for future diabetes, but its independent role was not investigated [41]. Considering the small sample size of our present study, larger population studies are needed to validate our conclusion.

Several previous cross-sectional and prospective studies reported associations between LTL and the fasting insulin sensitivity index HOMA-IR [33, 42, 43]. In our correlation analysis, we found that LTL positively correlated with the Matsuda index at baseline, but not with HOMA-IR either at baseline or at the 3-year follow-up, which conflicts with the previous reports. Although the small sample size of the present study might partially explain this discrepancy, it is worth noting that these two indices provide different information: the Matsuda index reflects whole-body insulin sensitivity, whereas HOMA-IR has been recognized as an index of peripheral or hepatic insulin sensitivities based on the assumption that they are equivalent [21]. In addition, the Matsuda index has been reported to highly correlate with the gold standard whole-body insulin sensitivity index derived from euglycemic insulin clamp, suggesting that the Matsuda index provides a reasonable approximation of the

Table 4: Spearman’s correlation between baseline cellular aging markers and clinical characteristics at the 3-year follow-up.

| Clinical characteristics at 3-year follow-up | LTL    | mtDNAcn | Aging markers at baseline | 8-OHdG | SOD activity |
|---------------------------------------------|--------|---------|--------------------------|--------|-------------|
| HbA1c                                       | -0.086 | 0.065   | 0.023                    | -0.098 | 0.092       | -0.010     |
| FPG                                         | -0.008 | -0.148  | -0.108                   | -0.138 | 0.105       | -0.073     |
| PG120min                                    | -0.307**| -0.113  | 0.324**                   | 0.030  | -0.203*     | 0.272**    |
| FINS                                        | -0.097 | 0.013   | 0.118                    | -0.164 | -0.185      | -0.125     |
| INS120min                                   | -0.133 | -0.020  | 0.158                    | -0.120 | -0.180      | -0.075     |
| FCP                                         | -0.062 | -0.037  | 0.121                    | -0.124 | -0.206*     | -0.155     |
| CP120min                                    | -0.273**| -0.126  | 0.251*                   | -0.038 | -0.227*     | 0.019      |
| HOMA-IR                                     | -0.066 | 0.015   | 0.048                    | -0.118 | -0.129      | -0.097     |
| HOMA-IR-β                                   | -0.090 | 0.080   | 0.123                    | -0.032 | -0.257**    | -0.075     |
| TC                                          | -0.208*| 0.017   | 0.203*                   | -0.031 | 0.109       | 0.020      |
| TG                                          | -0.084 | -0.097  | 0.029                    | 0.049  | 0.076       | -0.104     |
| HDL-C                                       | -0.079 | -0.119  | 0.163                    | -0.039 | 0.104       | 0.197*     |
| LDL-C                                       | -0.138 | 0.050   | 0.240*                   | 0.003  | 0.095       | 0.091      |
| UA                                          | -0.138 | -0.181  | 0.114                    | -0.004 | -0.182      | -0.007     |

*P < 0.05. **P < 0.01. Abbreviations: HbA1c—glycosylated hemoglobin A1c; FPG—fasting plasma glucose; PG—plasma glucose; INS—insulin; CP—C-peptide; TG—total triglyceride; HDL-C—high-density lipoprotein cholesterol; LDL-C—low-density lipoprotein cholesterol; UA—uric acid; LTL—leukocyte telomere length; mtDNAcn—mitochondrial DNA copy number; TNF-α—tumor necrosis factor-α; IL-6—interleukine-6; 8-OHdG—8-hydroxy-2-deoxyguanosine; SOD—superoxide dismutase.
whole-body insulin sensitivity [21]. Unfortunately, during the OGTT at the 3-year follow-up, PG30min and PG60min were not obtained; therefore, the value of the Matsuda index could not be calculated. The prospective association of LTL and the Matsuda index needs to be explored in future studies.

Table 5: Multivariate logistic regression models to evaluate the effect of covariates on the association between the baseline LTL and 3-year diabetes risk.

| Models  | Variables     | Adjusted OR | 95% CI      | P values |
|---------|---------------|-------------|-------------|----------|
| Model 1 | Age           | 1.010       | 0.962-1.060 | 0.699    |
|         | Male sex      | 1.080       | 0.389-2.996 | 0.883    |
|         | LTL           | 0.423       | 0.231-0.776 | 0.005    |
| Model 2 | Age           | 0.981       | 0.924-1.042 | 0.537    |
|         | Male sex      | 2.199       | 0.387-12.481| 0.374    |
|         | BMI           | 0.999       | 0.801-1.247 | 0.993    |
|         | WC            | 1.009       | 0.937-1.087 | 0.807    |
|         | HbA1c         | 2.513       | 0.825-7.652 | 0.105    |
|         | FPG           | 0.555       | 0.175-1.765 | 0.319    |
|         | PG30min       | 1.517       | 0.984-2.339 | 0.059    |
|         | PG60min       | 1.157       | 0.779-1.718 | 0.470    |
|         | PG120min      | 0.869       | 0.568-1.330 | 0.518    |
|         | TG            | 0.691       | 0.325-1.469 | 0.337    |
|         | HDL-C         | 0.163       | 0.007-3.586 | 0.250    |
|         | LDL-C         | 0.898       | 0.328-2.455 | 0.833    |
|         | UA            | 0.994       | 0.985-1.004 | 0.239    |
|         | Matsuda index | 1.050       | 0.852-1.293 | 0.648    |
|         | ISSI-2        | 0.756       | 0.174-3.274 | 0.708    |
|         | LTL           | 0.414       | 0.187-0.919 | 0.030    |
| Model 3 | Age           | 0.996       | 0.931-1.066 | 0.902    |
|         | Male sex      | 3.008       | 0.410-22.079| 0.279    |
|         | BMI           | 0.944       | 0.746-1.196 | 0.636    |
|         | WC            | 1.003       | 0.931-1.081 | 0.939    |
|         | HbA1c         | 2.356       | 0.685-8.105 | 0.174    |
|         | FPG           | 0.608       | 0.169-2.184 | 0.445    |
|         | PG30min       | 1.501       | 0.930-2.422 | 0.097    |
|         | PG60min       | 1.094       | 0.705-1.697 | 0.689    |
|         | PG120min      | 1.004       | 0.628-1.604 | 0.988    |
|         | TG            | 0.656       | 0.283-1.520 | 0.326    |
|         | HDL-C         | 0.140       | 0.004-4.392 | 0.263    |
|         | LDL-C         | 0.904       | 0.305-2.675 | 0.855    |
|         | UA            | 0.994       | 0.984-1.003 | 0.191    |
|         | Matsuda index | 1.129       | 0.902-1.413 | 0.289    |
|         | ISSI-2        | 0.635       | 0.143-2.817 | 0.550    |
|         | mtDNAcn       | 0.994       | 0.979-1.010 | 0.471    |
|         | TNF-α         | 1.007       | 0.933-1.086 | 0.865    |
|         | IL-6          | 0.874       | 0.721-1.059 | 0.169    |
|         | 8-OHdG        | 0.977       | 0.950-1.005 | 0.108    |
|         | SOD activity  | 1.003       | 0.955-1.052 | 0.917    |
|         | LTL           | 0.341       | 0.139-0.839 | 0.019    |

Model 1: adjusted for age and sex. Model 2: adjusted for Model 1+conventional diabetes risk factors (BMI, WC, HbA1c, FPG, PG30min, PG60min, PG120min, TG, HDL-C, LDL-C, UA, Matsuda index, and ISSI-2). Model 3: adjusted for Model 2+mtDNAcn, as well as biomarkers related to oxidative stress and inflammation (mtDNAcn, TNF-α, IL-6, 8-OHdG, and SOD). Abbreviations: BMI—body mass index; WC—waist circumference; HbA1c—glycosylated hemoglobin A1c; FPG—fasting plasma glucose; PG—plasma glucose; TG—total triglyceride; HDL-C—high-density lipoprotein cholesterol; LDL-C—low-density lipoprotein cholesterol; UA—uric acid; ISSI-2—insulin sensitivity index-2; LTL—leukocyte telomere length; mtDNAcn—mitochondrial DNA copy number; TNF-α—tumor necrosis factor-α; IL-6—interleukine-6; 8-OHdG—8-hydroxy-2-deoxyguanosine; SOD—superoxide dismutase.
Interestingly, we found that both mtDNAcn and TNF-α correlated with PG30min. The mediation model analysis revealed that TNF-α indirectly contributed to the elevation of PG30min via mitochondrial dysfunction rather than other pathways, but this remains to be confirmed. Notably, except for PG30min, parameters such as FPG, PG60min, and PG120min did not correlate with mtDNAcn and TNF-α. After glucose loading, the first-phase insulin secretion occurs within 30 min, suppressing glucose production in the liver [44]. Impaired first-phase insulin secretion leads to the elevation of PG30min. Therefore, our result suggests that mtDNAcn and TNF-α are possibly associated with impaired first-phase insulin secretion.

Overall, we made several important observations in the present study. First, to the best of our knowledge, our analysis for the first time demonstrated that LTL independently predicted 3-year diabetes risk after adjusting for the confounding effects of mtDNAcn, oxidative stress, and inflammation marker levels, as well as of the conventional diabetes risk factors. Second, we investigated the associations between the cellular aging markers on the one hand and PG30min and PG60min values on the other hand, which were rarely determined in previous studies. Intriguingly, this is the first study that reports correlations of mtDNAcn and TNF-α level with PG30min, although the exact mechanisms underlying these relationships need to be clarified in future studies.

However, several limitations also need to be mentioned. The relatively small sample size limited statistical power and thereby likely precluded confirmation of the associations between some parameters that could be revealed in a larger cohort. This circumstance partially explains the absence of correlations among the indicators. On the other hand, the follow-up time was relatively short; thus, the earlier predictive value of these cellular aging markers was not explored in the current study.

5. Conclusion
Collectively, our observations suggest that LTL is an independent predictor for the 3-year diabetes risk. Its predictive role, which was independent of mtDNAcn and markers of oxidative stress and inflammation, as well as of conventional diabetes risk factors, might provide novel insights into the potential pathways that mediate the effect of telomere shortening on diabetes development. These specific pathways will need to be identified in future studies. Importantly, identification of telomere length as an additional risk modifier could assist early prediction for future diabetes and its timely prevention. However, large population-based studies are needed to validate the clinical value of our present findings.

Data Availability
The SPSS Statistics data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials
Table S1: multivariate-adjusted OR (95% CI) for diabetes risk and oxidative stress and inflammation according to baseline LTL quintiles. (Supplementary Materials)

References
[1] Y. Zhu, X. Liu, X. Ding, F. Wang, and X. Geng, “Telomere and its role in the aging pathways: telomere shortening, cell senescence and mitochondria dysfunction,” Biogerontology, vol. 20, no. 1, pp. 1–16, 2019.
[2] B. Liu, T. Maekawa, K. Yoshida et al., “Telomere shortening by transgenerational transmission of TNF-α-induced TERRA via ATF7,” Nucleic Acids Research, vol. 47, no. 1, pp. 283–298, 2019.
[3] E. Zole and R. Ranka, “Mitochondria, its DNA and telomeres in ageing and human population,” Biogerontology, vol. 19, no. 3–4, pp. 189–208, 2018.
[4] M. Herrmann, I. Pusceddu, W. März, and W. Herrmann, “Telomere biology and age-related diseases,” Clinical Chemistry and Laboratory Medicine, vol. 56, no. 8, pp. 1210–1222, 2018.
[5] A. L. Fitzpatrick, R. A. Kronmal, J. P. Gardner et al., “Leukocyte telomere length and cardiovascular Risk Scores for Prediction of cardiovascular Mortality,” American Journal of Epidemiology, vol. 28, no. 2, pp. e13–e15, 2007.
[6] M. J. D’Mello, S. A. Ross, M. Briel, S. S. Anand, H. Gerstein, and G. Paré, “Association between shortened leukocyte telomere length and cardiometabolic outcomes: systematic review and meta-analysis,” Circulation Cardiovascular Genetics, vol. 8, no. 1, pp. 82–90, 2015.
[7] K. D. Salpea, P. J. Talmud, J. A. Cooper et al., “Association of telomere length with type 2 diabetes, oxidative stress and UCP2 gene variation,” Atherosclerosis, vol. 209, no. 1, pp. 42–50, 2010.
[8] Q. Shen, X. Zhao, L. Yu et al., “Association of leukocyte telomere length with type 2 diabetes in mainland Chinese populations,” The Journal of Clinical Endocrinology & Metabolism, vol. 97, no. 4, pp. 1571–1574, 2012.
[9] F. Monickaraj, S. Aravind, K. Gokulakrishnan et al., “Accelerated aging as evidenced by increased telomere shortening and mitochondrial DNA depletion in patients with type 2 diabetes,” Molecular and Cellular Biochemistry, vol. 365, pp. 343–350, 2012.
[10] M. J. Sampson, M. S. Winterbone, J. C. Hughes, N. Dozio, and D. A. Hughes, “Monocyte telomere shortening and oxidative
DNA damage in type 2 diabetes,” *Diabetes Care*, vol. 29, no. 2, pp. 283–289, 2006.

[11] F. X. Xu, X. Zhou, F. Shen, R. Pang, and S. M. Liu, “Decreased peripheral blood mitochondrial DNA content is related to HbA1c, fasting plasma glucose level and age of onset in type 2 diabetes mellitus,” *Diabetic Medicine*, vol. 29, no. 7, pp. e47–e54, 2012.

[12] E. C. C. C. Rosa, R. R. C. dos Santos, L. F. A. Fernandes, F. de Assis Rocha Neves, M. S. Coelho, and A. A. Amato, “Leukocyte telomere length correlates with glucose control in adults with recently diagnosed type 2 diabetes,” *Diabetes Research and Clinical Practice*, vol. 135, pp. 30–36, 2018.

[13] E. Jeanclós, A. Krolewski, J. Skurnick et al., “Shortened telomere length in white blood cells of patients with IDDM,” *Diabetes*, vol. 47, no. 3, pp. 482–486, 1998.

[14] A. L. Fitzpatrick, R. A. Kronmal, J. P. Gardner et al., “Leukocyte telomere length and cardiovascular disease in the cardiovascular health study,” *American Journal of Epidemiology*, vol. 165, no. 1, pp. 14–21, 2007.

[15] N. C. You, B. H. Chen, Y. Song et al., “A prospective study of leukocyte telomere length and risk of type 2 diabetes in post-menopausal women,” *Diabetes*, vol. 61, no. 11, pp. 2998–3004, 2012.

[16] J. Zhao, Y. Zhu, J. Lin et al., “Short leukocyte telomere length predicts risk of diabetes in American Indians: the Strong Heart Family Study,” *Diabetes*, vol. 63, no. 1, pp. 354–362, 2014.

[17] P. Willeit, J. Raschenberger, E. E. Heydon et al., “Leucocyte telomere length and risk of type 2 diabetes mellitus: new prospective cohort study and literature-based meta-analysis,” *PLoS One*, vol. 9, no. 11, article e12483, 2014.

[18] A. V. Diez Roux, N. Ranjit, N. S. Jenny et al., “Race/ethnicity and telomere length in the multi-ethnic study of atherosclerosis,” *Aging Cell*, vol. 8, no. 3, pp. 251–257, 2009.

[19] The Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus, Y. Seino, K. Nanjo et al., “Report of the committee on the classification and diagnostic criteria of diabetes mellitus,” *Journal of Diabetes Investigation*, vol. 1, no. 5, pp. 212–228, 2010.

[20] D. R. Matthews, J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner, “Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man,” *Diabetologia*, vol. 28, no. 7, pp. 412–419, 1985.

[21] M. Matsuda and R. A. DeFronzo, “Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp,” *Diabetes Care*, vol. 22, no. 9, pp. 1462–1470, 1999.

[22] R. Retnakaran, Y. Qi, M. I. Goran, and J. K. Hamilton, “Evaluation of proposed oral disposition index measures in relation to the actual disposition index,” *Diabetic Medicine*, vol. 26, no. 12, pp. 1198–1203, 2009.

[23] M. Zhou, L. Zhu, X. Cui et al., “Influence of diet on leukocyte telomere length, markers of inflammation and oxidative stress in individuals with varied glucose tolerance: a Chinese population study,” *Nutrition Journal*, vol. 15, p. 39, 2016.

[24] R. M. Cawthon, “Telomere length measurement by a novel monochrome multiplex quantitative PCR method,” *Nucleic Acids Research*, vol. 37, no. 3, article e21, 2009.

[25] M. C. Zhou, L. Zhu, X. Cui et al., “Reduced peripheral blood mtDNA content is associated with impaired glucose-stimulated islet β cell function in a Chinese population with different degrees of glucose tolerance,” *Diabetes/Metabolism Research and Reviews*, vol. 32, no. 7, pp. 768–774, 2016.

[26] J. F. Passos, G. Saretzki, and T. von Zglinicki, “DNA damage in telomeres and mitochondria during cellular senescence: is there a connection?,” *Nucleic Acids Research*, vol. 35, no. 22, pp. 7505–7513, 2007.

[27] A. Salminen, J. Ojala, K. Kaariranta, and A. Kauppinen, “Mitochondrial dysfunction and oxidative stress activate inflammasomes: impact on the aging process and age-related diseases,” *Cellular and Molecular Life Sciences*, vol. 69, no. 18, pp. 2999–3013, 2012.

[28] T. Maekawa, B. Liu, D. Nakai et al., “ATF7 mediates TNF-α-induced telomere shortening,” *Nucleic Acids Research*, vol. 46, no. 9, pp. 4487–4504, 2018.

[29] R. P. Barnes, E. Fouquerel, and P. L. Opresko, “The impact of oxidative DNA damage and stress on telomere homeostasis,” *Mechanisms of Ageing and Development*, vol. 177, pp. 37–45, 2019.

[30] V. Cattan, N. Mercier, J. P. Gardner et al., “Chronic oxidative stress induces a tissue-specific reduction in telomere length in CAST/Ei mice,” *Free Radical Biology and Medicine*, vol. 44, no. 8, pp. 1592–1598, 2008.

[31] J. M. Dos Santos, D. S. de Oliveira, M. L. Moreli, and S. A. Benite-Ribeiro, “The role of mitochondrial DNA damage at skeletal muscle oxidative stress on the development of type 2 diabetes,” *Molecular and Cellular Biochemistry*, vol. 449, no. 1-2, pp. 251–255, 2018.

[32] N. Suematsu, H. Tsutsui, J. Wen et al., “Oxidative stress mediates tumor necrosis factor-alpha-induced mitochondrial DNA damage and dysfunction in cardiac myocytes,” *Circulation*, vol. 107, no. 10, pp. 1418–1423, 2003.

[33] S. Verhulst, C. Dalgård, C. Labat et al., “A short leucocyte telomere length is associated with development of insulin resistance,” *Diabetologia*, vol. 59, no. 6, pp. 1258–1265, 2016.

[34] G. S. Hotamisligil, N. S. Sharpill, and B. M. Spiegelman, “Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance,” *Science*, vol. 259, no. 5091, pp. 87–91, 1993.

[35] D. Kuhlow, S. Florian, G. van Figura et al., “Targeted and persistent 8-oxoguanine base damage at telomeres promotes telomere loss in cardiac myocytes,” *Mitochondrial DNA: Preceding Research and Reviews*, vol. 32, no. 7, pp. 768–774, 2011.

[36] M. Çalan et al., “Elevated urinary levels of 8-oxo-2′-deoxyguanosine, (5′R)- and (5′S)-8,5′-cyano-2′-deoxyadenosines, and 8-iso-prostaglandin F2α as potential biomarkers of oxidative stress in patients with prediabetes,” *DNA Repair*, vol. 48, pp. 1–7, 2016.

[37] S. Lenzen, J. Drinkern, and M. Tiedge, “Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues,” *Free Radical Biology and Medicine*, vol. 20, no. 3, pp. 463–466, 1996.

[38] E. Fouquerel, R. P. Barnes, S. Uttam, S. C. Watkins, M. P. Bruçez, and P. L. Opresko, “Targeted and persistent 8-oxoguanine base damage at telomeres promotes telomere loss and crisis,” *Molecular Cell*, vol. 75, no. 1, pp. 117–130.e6, 2019.

[39] M. subdivision, M. A. Cakan, M. C. G cleans, “Determined urinary levels of 8-oxo-2′-deoxyguanosine, (5′R)- and (5′S)-8,5′-cyano-2′-deoxyadenosines, and 8-iso-prostaglandin F2α as potential biomarkers of oxidative stress in patients with prediabetes,” *DNA Repair*, vol. 48, pp. 1–7, 2016.

[40] L. Maschierow, K. Khalaf, H. A. al-Aubaidy, and H. F. Jelinek, “Inflammation, coagulation, endothelial dysfunction and...
oxidative stress in prediabetes — Biomarkers as a possible tool for early disease detection for rural screening,” *Clinical Biochemistry*, vol. 48, no. 9, pp. 581–585, 2015.

[41] S. B. Cho, I. S. Koh, H. Y. Nam, J. P. Jeon, H. K. Lee, and B. G. Han, “Mitochondrial DNA copy number augments performance of A1C and oral glucose tolerance testing in the prediction of type 2 diabetes,” *Scientific Reports*, vol. 7, no. 1, article 43203, 2017.

[42] A. Adaikalakoteswari, M. Balasubramanyam, and V. Mohan, “Telomere shortening occurs in Asian Indian Type 2 diabetic patients,” *Diabetic Medicine*, vol. 22, no. 9, pp. 1151–1156, 2005.

[43] S. Demissie, D. Levy, E. J. Benjamin et al., “Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study,” *Aging Cell*, vol. 5, no. 4, pp. 325–330, 2006.

[44] L. Luzi and R. A. DeFronzo, “Effect of loss of first-phase insulin secretion on hepatic glucose production and tissue glucose disposal in humans,” *American Journal of Physiology-Endocrinology and Metabolism*, vol. 257, no. 2, pp. E241–E246, 1989.