# Association Between Short Leukocyte Telomere Length, Endotoxemia, and Severe Periodontitis in People With Diabetes: A Cross-Sectional Survey

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## OBJECTIVE

**Shortened leukocyte telomere length (LTL) and diagnosis of periodontitis are associated with an increased risk of complications and mortality in diabetes. This study investigated the association between LTL, endotoxemia, and severity of periodontitis in a large cohort of people with diabetes.**

## RESEARCH DESIGN AND METHODS

**Six hundred thirty individuals (371 with type 2 and 259 with type 1 diabetes) were recruited from the University College Hospital in London, U.K. During a baseline visit, blood was collected for standard biochemical tests and DNA extraction, while a dental examination was performed to determine diagnosis and extent of periodontitis. LTL was measured by real-time PCR, and endotoxemia was assessed by the limulus amoebocyte lysate method.**

## RESULTS

**Two hundred fifty-five individuals were diagnosed with gingivitis, 327 with periodontitis (114 with moderate and 213 with severe disease), and 48 with edentulous. Diagnosis of periodontitis was associated with shorter LTL ($P = 0.04$). A negative association between LTL and endotoxemia was found in the severe periodontitis and type 2 diabetes groups ($P = 0.01$ for both). Shorter LTL was associated with increased extent of periodontitis ($P = 0.01$) and increased insulin resistance (homeostatic model assessment). Multiple adjustments for biochemical, anthropometric, and medication-use variables did not affect the results.**

## CONCLUSIONS

**LTL is associated with endotoxemia and diagnosis of periodontitis in people with diabetes. LTL shortening might represent a novel biological pathway accounting for previous epidemiological data that documented higher prevalence of diabetes and its complications in people with periodontitis and vice versa.**

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Diabetes care is complex and requires a multidisciplinary approach, as many issues and comorbidities may influence the quality of metabolic control and patient outcome (1). Further to this, premature cell senescence and increased oxidative stress may represent the most relevant mechanisms for the development of diabetes complications (2–4). One of the most common comorbidities observed in people with diabetes is periodontitis, an infectious inflammatory disease of the gingival tissue resulting in the progressive destruction of the bone supporting the tooth. Epidemiological data suggest susceptibility to periodontitis is increased by approximately threefold in people with diabetes (5). Additionally, the future risk of cardiorenal mortality and end-stage renal disease is believed to be two to three times higher in people with diabetes and concomitant severe periodontitis, compared with those with diabetes but good oral health (6–8). An exaggerated inflammatory response and chronic dysmetabolic state are currently considered the most plausible mechanisms linking these two common disorders (6). While consistent evidence suggests that improved glucose management in people with diabetes will reduce progression of periodontitis, there is still some debate as to whether treating periodontitis could also impact significantly the metabolic status of people with diabetes (9). A complete understanding of the potential pathways leading to an increased cardiorenal risk, mainly driven by vascular complications in individuals with diabetes and periodontitis, is yet to be achieved.

Telomeres are multiple repetitions of a standard nucleotide sequence that cap the ends of chromosomes and protect from genomic instability (10). In human somatic cells, telomeres shorten upon each cell division due to the end-replication problem associated with semiconservative DNA replication (11). In addition, oxidative stress exerts a major influence on the rate of telomere attrition over and above that of the end-replication problem, as the GGG triplets along the telomere sequence are highly sensitive to hydroxyl radical damage (12). Over the past few years, short telomere length, recorded in peripheral leukocytes, has been associated with increased morbidity and/or mortality due to a number of age-related diseases. Whether shortened telomeres are causally associated with such diseases or they are just a result of the increased inflammatory burden that characterizes the evolution of age-related disorders remains unclear.

Our group has previously reported for the first time that periodontitis is linked to shorter leukocyte telomere length (LTL) and that the severity of periodontal inflammation and oxidative stress burden was inversely associated with LTL (13). Our subsequent hypothesis was to test whether periodontal inflammation could be associated with shorter LTL in individuals with other comorbidities like diabetes. There are no reports on the effects of periodontal infections on LTL in people with diabetes. In this study, we therefore investigated the association between LTL and prevalence of periodontal inflammation in a cross-sectional survey of people with both type 1 and type 2 diabetes.

## RESEARCH DESIGN AND METHODS

### Population

A total of 630 consecutive individuals referred to the Endocrinology Department of the University College of London Hospital were recruited for this study between May 2007 and September 2009 if willing to receive a periodontal screening and blood sample (371 with type 2 and 259 with type 1 diabetes mellitus based on the recent World Health Organization [WHO] definition). A physician collected participants' clinical (diagnosis, disease duration, complications) and smoking (current, former, or never) history. Medical records were screened by a separate investigator to obtain information on current medications. Anthropometric measurements and blood pressure were assessed using standard techniques. After consenting, all participants underwent blood collection first, and then an oral examination performed by two expert periodontists (N.G. and F.D.). Ethical approval was received from the National Research Ethics Service Committee London–Bentham (06/Q0502/97), and written informed consent was obtained from all participants.

### Oral Examination

Oral examination consisted of a single recording of periodontal health based on the Basic Periodontal Examination (BPE) index derived from the WHO Community Periodontal Index of Treatment Needs score (14,15). This screening tool was performed in each sextant of the whole dentition (scores ranging from 0 to 4), and the highest score of the whole mouth was entered as representative of the current gingival inflammatory status for each participant. Radiographic assessment of current bone levels and more detailed whole mouth gingival assessments could not be obtained. Scores 3 and 4 corresponded to the presence of clinical signs of periodontitis (indicating probing pocket depths of 4 to 5 mm for score 3 and of 6 mm or more for score 4) compared with healthy gingival tissue (score 0) or with simple gingivitis (score 1 and 2; reversible marginal gingival inflammation). Based on the fact that no individuals presented with score 0, we categorized individuals as presenting with prevalent gingivitis (BPE = 1 and 2), moderate periodontal pockets (BPE = 3), and severe periodontal pockets (BPE = 4) and individuals with no teeth (edentulous) using the highest score in each of the sextant examined. In addition, the sum of all sextants (BPE cumulative score) was created to define a continuous measure of extent of the disease. Both examiners were previously calibrated on a convenient sample of 10 individuals, and k scores were calculated ($>$0.90).

### Biochemical Tests

Blood samples were collected from all individuals after an overnight fast and processed for biochemical tests and telomere length assay. Lipid profile, glucose, $HbA_{1c}$, creatinine, and C-reactive protein (CRP) levels were assessed on an automated analyzer (Cobas Integra analyzer, Roche Diagnostics). Estimated glomerular filtration rate (eGFR) was calculated with the abbreviated MDRD (Modification of Diet in Renal Disease study) equation (16) and expressed in

milliliters per minute per 1.73 meters squared.

Serum levels of insulin were quantified with high-sensitivity ELISA (Mercodia, Sweden). Insulin resistance index was calculated as previously described (17). Serum endotoxin activity was determined by the limulus amoebocyte lysate test kit with a chromogenic substrate (Lonza, Walkersville, MD) on diluted (1:5, vol/vol in endotoxin-free water) samples. Intracoefficient and intercoefficient of variations for all assays were <5%.

### LTL Assay

An aliquot of whole blood was collected in an EDTA tube and immediately stored at −80°C for future DNA extraction and telomere length analysis. DNA was isolated using a modified salting-out method (18), optimized to yield high-molecular-weight DNA through DNA spooling at the last step of the extraction. A previously validated quantitative real-time PCR assay was used to measure telomere lengths in a blind and standardized fashion (19). Briefly, in each sample, the number of telomere repeats and single-copy gene (SCG) copies were determined in comparison with a reference sample in a telomere and a SCG quantitative PCR, respectively. Relative LTL was calculated from the ratio between telomere repeats to SCG copies and was expressed as T/S ratio. All PCRs were performed in duplicate on a Rotor-Gene 6000 machine (Corbett Research Ltd., Cambridge, U.K.), and the raw data were analyzed using comparative quantification analysis (Rotor-Gene 6000 software, Corbett Research Ltd.). The specificity of all amplifications was assessed by melting curve analysis. To test the reproducibility of our quantitative real-time PCR technique, a subset of 10 randomly selected DNA samples was rerun on a different day. Linear regression analysis of these measurements showed $r^2 = 0.79$ ($P = 0.001$), and the Spearman's nonparametric test resulted in a coefficient of 0.82 ($P = 0.004$). The coefficient of variation of the T/S ratios in the repeated measurements of the same sample was 5.6 (19).

### Statistics

All data are presented as mean and SDs unless differently specified. All biochemical variables were log transformed if normality assumptions were not met. Descriptive analyses were performed on all survey participants comparing a number of confounders/variables across different levels of periodontal exposure (gingivitis, moderate and severe periodontal pockets, and edentulous) using a test for trend (Jonckheere trend test) for continuous and $\chi^2$ test for categorical variables (20). Nonparametric correlation analyses between LTL and age, body composition, and all biomarkers assayed were performed using Spearman rank testing. Linear regression models were used to investigate the univariate association between LTL and all common confounders. All those factors found to be statistically significant at the 0.10 level in the univariate analyses were then included in generalized linear models. Periodontal inflammation was defined both in a categorical (highest BPE score) fashion as to assess severity and in a continuous fashion (BPE cumulative score). Adjustment was made for age, gender, ethnicity, diabetes type, smoking, and waist circumference (model 1) and in addition for endotoxin, medication use, insulin, and CRP (model 2). Fully adjusted models were used to compare slopes of LTL by age across groups of prevalent periodontal inflammation. Despite the limited sample size of the group of edentulous individuals, all analyses were performed including and excluding this group in all multivariate models in order to confirm the results (data are presented with this group included).

In sensitivity analyses, we restricted our analysis sample by excluding those using medication, and we also compared the differences in LTL according to each medication category. The $\alpha$-value for statistical significance for association was set at 0.05. The IBM-SPSS statistical program (version 20) and Stata (version 12) were used for all analyses.

## RESULTS

A total of 255 participants were diagnosed with gingivitis and 237 with deeper periodontal pockets (114 with moderate and with 213 severe pockets), and 48 individuals were edentulous (Table 1). All groups with increased signs of periodontal inflammation were generally older, with higher prevalence of males, Caucasians, and type 2 diabetes diagnosis but lower number of current smokers, when compared with the gingivitis group. A linear trend of greater values of systolic and diastolic blood pressure as well as adiposity (both BMI and waist circumference) was observed in individuals with more severe gingival inflammation, and the difference was greatest in the edentulous group. There were no statistically significant differences in $HbA_{1c}$ and plasma glucose levels between groups, while levels of insulin, insulin resistance (calculated based on the homeostatic model assessment of insulin resistance [HOMA-IR] index), CRP, endotoxin, triglycerides, and creatinine were higher in individuals with increasing levels of periodontal inflammation. An inverse relationship was observed between serum levels of CRP and periodontal inflammation based on diabetes diagnosis. Indeed, while in patients with type 1 diabetes, severe periodontal inflammation and edentulousness were associated with lower levels of inflammation, the opposite was observed in type 2 diabetes patients (CRP levels in cases with severe periodontal inflammation and edentulous were higher than those with milder gingival inflammation [$P < 0.01$]). No association was found between LTL and CRP levels (data not shown).

All study participants with a diagnosis of deeper periodontal pockets (including moderate and severe) had shorter LTL when compared with healthier gingival scores ($P = 0.04$ when adjusted for age), and this difference remained statistically significant after multiple adjustments (Fig. 1*A*). When results were analyzed by severity of periodontal inflammation, individuals suffering from severe periodontal pockets exhibited shorter LTL when compared with the gingivitis group, independent of other confounders (Table 2). Edentulous individuals, albeit being older, did not present shorter LTL when compared with individuals with gingivitis in the fully adjusted model. The BPE cumulative score ($\beta = -0.003$;

**Table 1—Demographic, anthropometric, and biochemical characteristics of 630 patients with diabetes**

| Variables | Whole sample | Periodontal pockets | | | P for trend |
|---|---|---|---|---|---|
| | | Gingivitis (*n* = 255) | Moderate (*n* = 114) | Severe (*n* = 213) | Edentulous (*n* = 48) | |
| Age, years | 54.2 ± 16.0 | 50.0 ± 17.3 | 58.3 ± 15.4 | 61.7 ± 11.5 | 71.6 ± 8.2 | <0.001 |
| Gender, male | 375 (59.5) | 131 (51.4) | 79 (69.3) | 138 (65.1) | 26 (54.2) | 0.002 |
| Smoking, current | 96 (15.3) | 33 (12.9) | 26 (22.8) | 30 (14.1) | 7 (15.2) | 0.022 |
| Ethnicity, Caucasian | 433 (68.7) | 203 (79.6) | 81 (71.7) | 119 (55.9) | 29 (60.4) | <0.001 |
| Diabetes, type 2 | 371 (58.9) | 104 (40.8) | 69 (60.5) | 154 (72.3) | 44 (91.7) | <0.001 |
| BMI, kg/m$^2$ | 29.1 ± 7.6 | 27.5 ± 6.6 | 28.8 ± 7.0 | 28.7 ± 9.2 | 30.0 ± 5.1 | 0.004 |
| Waist circumference, cm | 103.2 ± 14.0 | 100.1 ± 13.1 | 103.4 ± 12.1 | 103.1 ± 16.1 | 108.1 ± 11.0 | <0.001 |
| Systolic blood pressure, mmHg | 132.1 ± 19.3 | 129.0 ± 19.7 | 131.4 ± 21.0 | 131.2 ± 18.2 | 136.1 ± 16.7 | 0.029 |
| Diastolic blood pressure, mmHg | 76.1 ± 11.5 | 74.4 ± 11.3 | 77.2 ± 11.0 | 75.2 ± 12.0 | 74.4 ± 11.5 | 0.570 |
| $HbA_{1c}$ | | | | | | 0.811 |
| mmol/mol | 65 ± 17.5 | 64 ± 17.5 | 63 ± 16.4 | 65 ± 18.6 | 63 ± 19.7 | |
| % | 8.1 ± 1.6 | 8.0 ± 1.6 | 7.9 ± 1.5 | 8.1 ± 1.7 | 7.9 ± 1.8 | |
| Glucose, mmol/L | 9.6 ± 4.8 | 8.4 ± 4.8 | 8.0 ± 4.9 | 8.5 ± 4.9 | 8.6 ± 4.0 | 0.834 |
| Insulin, pmol/L | 93.5 ± 133.1 | 79.5 ± 115.5 | 120.8 ± 187.9 | 118.2 ± 148.3 | 212.6 ± 262.8 | <0.001 |
| HOMA-IR index | 2.8 ± 10.1 | 0.1 ± 7.4 | 1.1 ± 11.9 | 1.3 ± 12.5 | 2.1 ± 4.5 | <0.001 |
| Endotoxin, endotoxin units/mL | 12.5 ± 8.4 | 10.9 ± 8.0 | 10.4 ± 10.6 | 9.8 ± 7.0 | 12.2 ± 10.0 | 0.04 |
| CRP, mg/L | 3.3 ± 5.6 | 1.3 ± 4.0 | 1.6 ± 7.3 | 1.5 ± 5.3 | 2.9 ± 8.5 | 0.002 |
| LDL, mmol/L | 2.4 ± 0.9 | 2.3 ± 0.8 | 2.3 ± 0.9 | 2.1 ± 0.9 | 2.0 ± 0.9 | 0.001 |
| HDL, mmol/L | 1.2 ± 0.5 | 1.3 ± 0.5 | 1.1 ± 0.5 | 1.1 ± 0.4 | 0.9 ± 0.3 | <0.001 |
| Total cholesterol, mmol/L | 4.2 ± 1.0 | 4.4 ± 0.9 | 4.3 ± 1.1 | 4.1 ± 1.0 | 4.0 ± 1.0 | 0.002 |
| Triglycerides, mmol/L | 1.4 ± 0.9 | 1.1 ± 0.8 | 1.2 ± 1.1 | 1.2 ± 0.08 | 1.5 ± 0.9 | <0.001 |
| Creatinine, mg/L | 88.4 ± 52.2 | 78.5 ± 36.9 | 80.5 ± 30.1 | 82.0 ± 56.4 | 105.0 ± 104.3 | <0.001 |
| eGFR, mL/min/1.73 m$^2$ | 88.2 ± 28.7 | 86.6 ± 27.9 | 86.3 ± 29.2 | 82.3 ± 27.0 | 57.3 ± 30.8 | <0.001 |

Data are mean ± SD or *n* (%). *P* values are calculated from test for trend. Categorical variables are compared with $\chi^2$ test.

95% CI −0.005 to −0.001; *P* = 0.013) and circulating levels of endotoxins (β = −0.027; 95% CI −0.052 to −0.027; *P* = 0.034) were independently associated with LTL when adjusting for all confounders. Indeed there was a negative linear association between LTL and increased BPE scores and circulating endotoxin levels (Fig. 2*A*). When the association between LTL and endotoxin levels was analyzed by subgroup of prevalent periodontal inflammation, a stronger negative correlation was found in patients with deeper periodontal pockets (*r* = −0.14; *P* = 0.04) than in those with simple gingival gingivitis (Fig. 2*B*). Patients with severe periodontal pockets as well as edentulous presented with higher levels of endotoxemia when compared with cases with simple gingivitis in the group of type 2 diabetes (13.2 ± 1.6 endotoxin units/mL severe periodontal inflammation and 14.5 ± 1.9 endotoxin units/mL edentulous versus 10.5 ± 1.2 endotoxin units/mL in the gingivitis group; *P* = 0.025 and *P* = 0.026, respectively). An association of opposite direction (lower endotoxin levels with higher level of periodontal inflammation) was observed in type 1 diabetes (*P* < 0.001; data not shown).

Indeed prevalence of combined moderate and severe inflammation was associated with shorter LTL in individuals with type 2 (*P* = 0.039) rather than type 1 diabetes (Fig. 1*B*). A progressive reduction of LTL was noted with increasing severity of periodontal inflammation only in cases with type 2 diabetes. Those individuals with prevalent severe periodontal pockets showed shorter LTL when compared with the individuals with gingivitis (*P* = 0.004) (Fig. 1*B*).

Among all metabolic parameters, a strong and direct association was only found between LTL and insulin resistance (HOMA-IR index) (β = 0.001; 95% CI 0.001–0.002; *P* = 0.002) in the fully adjusted model (model 2). Lastly, LTL and eGFR were linearly associated (*r* = 0.164; *P* < 0.001), and adjustment for diabetes type, smoking, waist circumference and blood pressure did not affect the association (β = 0.001; 95% CI 0.001–0.002; *P* = 0.002).

Medication use differed greatly among individuals grouped based on prevalent periodontal inflammation, but these differences were statistically significant only in the subgroup of people with type 1 diabetes (Supplementary Table 1). There was, however, no association between LTL and class of medications in the whole cohort as well as in the group of people with type 2 diabetes (Supplementary Table 2).

## CONCLUSIONS

In this study, we reported for the first time an association between LTL and severity of periodontal inflammation in people with diabetes. The main finding was that LTL ratio was lower when severity of periodontal inflammation was greater, and that this association was independent of age, diabetes type, adiposity, and circulating levels of inflammatory biomarkers. We also reported that circulating endotoxin

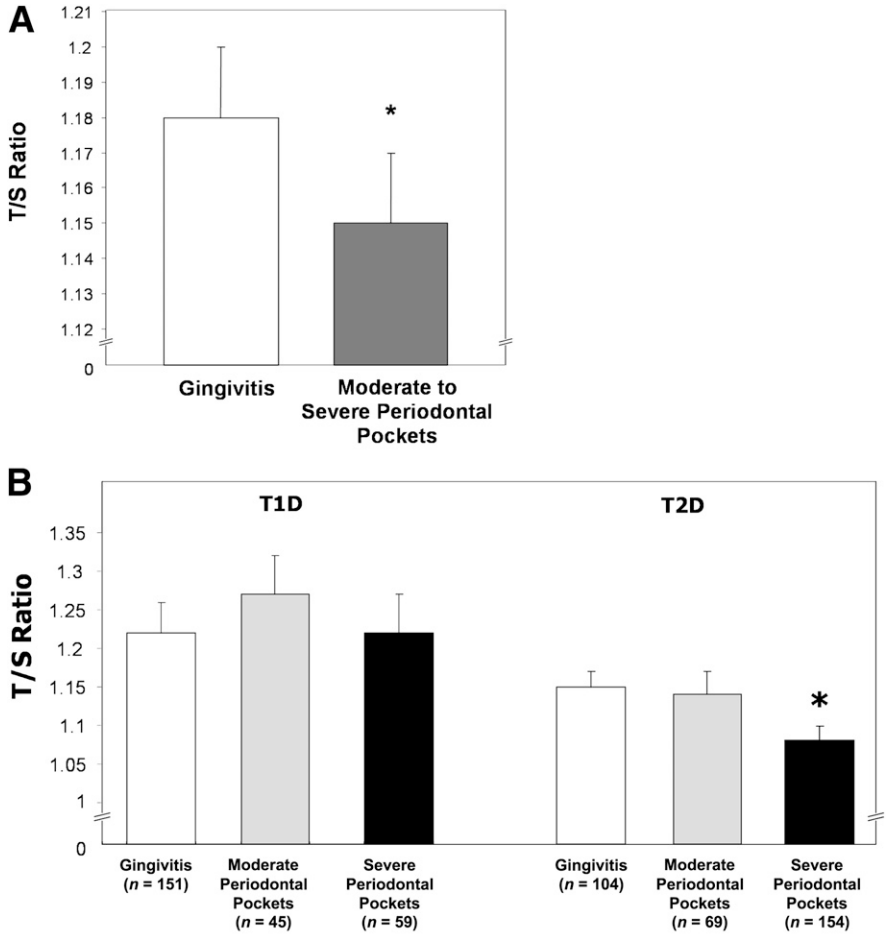


**Figure 1—**Difference in LTL (T/S ratio) of 630 individuals sorted by (*A*) prevalent periodontal inflammation, including gingivitis (*n* = 280) and moderate to severe periodontal pockets (*n* = 350), and (*B*) diabetes type and severity of periodontal inflammation. Data are reported as mean ± SE, and analysis is adjusted (according to model 2) for age, gender, ethnicity, diabetes type, smoking, waist circumference, and circulating levels of endotoxin, insulin, and CRP. *$P < 0.01$ versus gingivitis. T1D, type 1 diabetes; T2D, type 2 diabetes.

levels, insulin resistance (HOMA-IR), and eGFR were associated with LTL in people with diabetes.

Our group previously reported shorter LTL in individuals with periodontitis when compared with healthy controls, suggesting that increased systemic inflammation and oxidative stress could account for this association. We reported increased levels of systemic inflammation/oxidative stress to be associated with both diagnosis of periodontitis and shorter LTL (13). In this study, we documented that these associations are present also in individuals suffering from diabetes, a disorder characterized by a chronic state of increased inflammation and oxidative stress, and we suggested the bacterial burden as a possible factor accounting for this association.

Current understanding of LTL dynamics and its determinants in humans are in line with the hypothesis that chronic exposure to inflammatory stimuli determines faster LTL attrition rate (21). Indeed, the continuous recruitment and differentiation of inflammatory cells are thought to be the primary mechanisms causing a higher rate of LTL shortening in individuals with chronic infectious and/or inflammatory diseases like periodontitis. On the other hand, shorter LTL could represent a proxy of increased susceptibility to acute and chronic infections (22). Indeed, in this

**Table 2—Mean (± SE) telomere length (T/S ratio) of 630 individuals with diabetes by severity of periodontal inflammation**

| | | Periodontal pockets | | | |
|---|---|---|---|---|---|
| | Gingivitis (*n* = 255) | Moderate (*n* = 114) | Severe (*n* = 213) | Edentulous (*n* = 48) | *P* value* |
| Age adjusted | 1.13 ± 0.01 | 1.13 ± 0.02 | 1.10 ± 0.01† | 1.19 ± 0.03 | 0.04 |
| Model 1 | 1.16 ± 0.02 | 1.17 ± 0.03 | 1.12 ± 0.02† | 1.22 ± 0.04 | 0.01 |
| Model 2 | 1.16 ± 0.02 | 1.17 ± 0.03 | 1.12 ± 0.02† | 1.23 ± 0.04 | 0.002 |

Model 1 is adjusted for age, gender, ethnicity, diabetes type, smoking, and waist circumference. Model 2 is model 1 + circulating levels of endotoxin, medication use, insulin, and CRP. **P* value for difference in severity of periodontal inflammation categories unordered. †$P < 0.05$ compared with gingivitis.

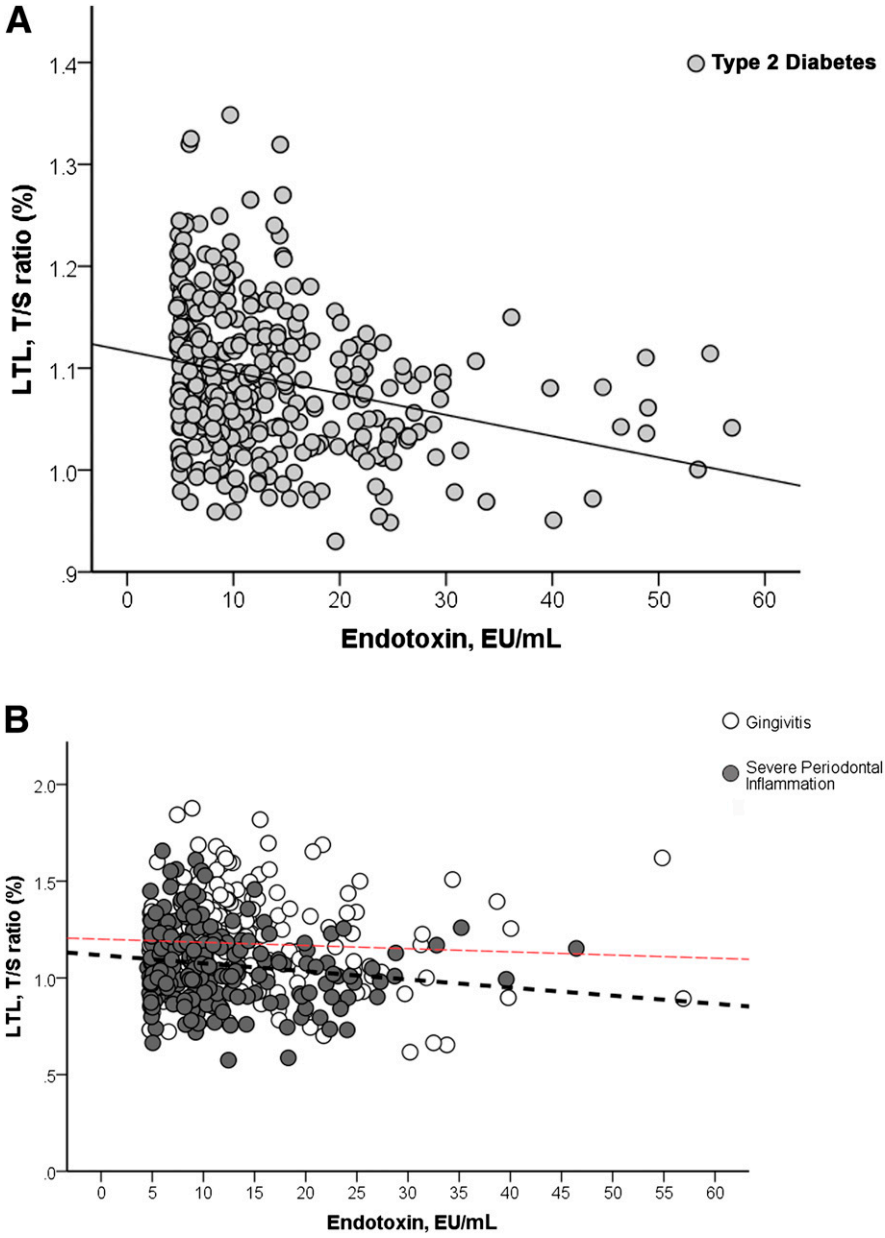


**Figure 2—***A*: Scatter plot of predicted T/S ratios against circulating endotoxin level in people with type 2 diabetes; adjusted model for age, gender, ethnicity, diabetes type, smoking, waist circumference, and circulating levels of endotoxin, insulin, and CRP ($r = -0.29$; $P < 0.001$). *B*: Scatter plot of predicted T/S ratios by endotoxin values (log transformed) and adjusted for age, gender, ethnicity, smoking, waist circumference, and diabetes type in individuals with gingivitis (open circles) and severe periodontal inflammation (closed circles). *P* value of statistical difference in slope between gingivitis (slope = 0.023; red dotted line) and severe periodontal inflammation (slope = −0.051; black dotted line) is 0.001. EU, endotoxin units.

study, endotoxin levels increased with greater extent of periodontitis but were inversely associated with a single measure of LTL. Epidemiological evidence suggests that endotoxemia predicts incident diabetes (23), higher concentrations are found in people with diabetes, and it is associated with the development of diabetic nephropathy (24).

In this study, we did not observe a cross-sectional association between circulating levels of inflammatory markers and LTL. In individuals with concomitant diabetes and either gingivitis or severe gingival inflammation, however, inflammatory burden is likely to be affected by the activity of the underlying diseases (25). Therefore, a single measure of inflammatory markers is more likely to reflect the metabolic status at the time of blood collection, rather than informing on the individual's cumulative inflammatory exposure. Conversely, LTL represents a record of the cumulative burden of inflammation and oxidative stress over individual's life span, and its dynamics are minimally affected by acute inflammatory changes (21). However, we did observe consistent and of opposite direction associations between LTL and prevalent periodontal inflammation and CRP

levels in type 1 versus type 2 diabetes cases.

A robust and independent association was found between LTL and both insulin resistance and eGFR. These findings confirm previous evidence suggesting LTL could act as a reliable marker of future diabetes complications, especially with regard to nephropathy (26,27). Interestingly, diagnosis of periodontitis has been linked to future increased risk of cardiorenal mortality of individuals with diabetes (6). Our study, therefore, provides a possible mechanistic explanation of this association. Further research is needed to confirm the exact pathway linking local inflammation, systemic exposure to endotoxin, and reduced LTL.

In our survey, only people with type 2 diabetes presented a linear reduction in LTL with increasing severity of periodontal inflammation. A number of factors could account for this finding. Firstly, a relatively low number of individuals with type 1 diabetes presented with greater severity of periodontal inflammation, reducing the power of our analyses. Furthermore, people generally develop type 1 diabetes early in life. They become more accustomed and compliant to diabetes treatment than people who develop type 2 diabetes in older age. This might account for the increased levels of cardiometabolic risk factors and inflammatory markers recorded in the type 2 rather than type 1 diabetes group, also explaining the higher prevalence of moderate to severe periodontal inflammation in participants with type 2 diabetes. Lastly, in our study cohort, people with type 1 diabetes were generally younger than those with type 2 diabetes. This might result in a shorter period of exposure to increased levels of periodontal inflammation. It is possible that the chronic immuno-inflammatory response related to the gingival disease did not have enough time to significantly impact on the relatively slow rate of LTL shortening in humans.

A number of limitations in our study should be highlighted. Firstly, we are reporting a cross-sectional association without information on the direction or causality of the association found. Short LTL has been associated with aberrant cytokine production and altered immune cell function (28). Similarly, etiology of periodontitis is currently thought to be dependent upon a dysregulated immuno-inflammatory response to the dental plaque biofilm, leading to a progressive destruction of the tooth-supporting tissues (29). Therefore, shorter LTL may be a marker of individuals with higher risk of developing severe infections (i.e., periodontitis) due to an age-related deficit or dysregulated activity of their immuno-inflammatory system rather than being its consequence. Secondly, our method of assessment of periodontal diagnosis was limited to the WHO/BPE index of prevalent gingival inflammation and treatment needs. This is considered not a clinical method of periodontitis diagnosis (as lacking of measures of gingival recession/attachment loss and radiographic assessment of alveolar bone levels) but rather a method of prevalent severity of gingival inflammation in population surveys. While in the past, Community Periodontal Index of Treatment Needs/BPE has been used as method of periodontal exposure, clear limitations of the index have been reported, especially as it could allow an underestimation of the periodontal tissue destruction. Thirdly, the presence of severe gingival inflammation in people with periodontitis might determine a change in the relative proportion of leukocyte subpopulation in peripheral blood. As the quantitative PCR-based assay used in this study provides only the average telomere length across all leukocytes, we cannot exclude that this factor could partially account for our results. However, it is now well established that the high interindividual variability in telomere length far exceeds the variations among cell types within the same individual (30,31). This results in a high synchronization of telomere length between different cells and tissues of both healthy and diseased subjects (32,33). Fourthly, the endotoxin assay used in our study measures the total amount of endotoxin activity in peripheral blood, not providing specific information on the possible origins of the infectious burden. As individuals with diabetes normally have a higher risk of infection, we cannot exclude that other sources of bacterial products (i.e., gut flora) could account for the high endotoxin levels. Lastly, the eradication of a chronic exposure to a continuous infectious/inflammatory stimulus (as that should be observed in edentulous individuals) could account for the lack of a statistically significant difference in LTL ratio observed in edentulous individuals who, albeit older individuals, did not present with shorter LTL than people with periodontitis. However, the limited sample size and greater age range present in our analyses preclude any reliable interpretation of the results.

In individuals with diabetes, prevalent moderate to severe periodontal inflammation was associated with shorter LTL and endotoxemia. This association can mark an age-related dysfunction of the immune-inflammatory system (potentially increasing the individual predisposition to chronic infections), or it can be a consequence of the higher levels of inflammatory and oxidative stress exposure related to periodontitis. Short LTL have been previously associated with higher risk of complications in people with diabetes. Therefore, regardless of its origins, the association between short LTL, endotoxemia, and periodontitis can provide a biological pathway explaining the increased incidence of diabetes as well as increased risk of complications (cardiorenal mortality) observed in people with periodontitis.

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**Author Contributions.** S.M. performed all LTL analyses and wrote the manuscript. N.G. collected clinical data, performed laboratory analyses, and contributed to discussion. K.L., K.D.S., and M.P. performed laboratory analyses and statistics and contributed to discussion. M.O., J.E.S., S.T., N.D., J.E.D., and S.H. contributed to discussion and the manuscript preparation. H.L.E. collected clinical periodontal data. K.P. and U.D. contributed to collecting data and discussion. S.E.H. initiated the project, contributed to discussion, and wrote the manuscript. F.D. supervised all research work, contributed to discussion, and wrote the manuscript. F.D. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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