C-peptide is a predictor of telomere shortening: A five-year longitudinal study

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Aim: Relative telomere length (RTL) predicts the development of many age-related diseases. Yet, few studies have evaluated their longitudinal effect on RTL. We investigated longitudinally the association between cardiometabolic risk factors and RTL.

Methods: This was a longitudinal study with a 5-year follow-up period, based on data collected in 2014 and 2019. Of 478 participants in 2014, 198 consented to be followed-up in 2019. The associations between RTL and risk factors were analyzed using t-test, ANOVA or simple linear regression as applicable.

Results: RTL was significantly shortened after 5 years (P<0.001). Older age (P=0.018) and gender (P=0.05) were significantly associated with shorter RTL at follow-up. Higher baseline C-peptide correlated with shorter RTL (P=0.04) and shortening of RTL (P=0.03) after 5 years. Multivariate linear regression including both age and gender revealed a significant trend for C-peptide and change in RTL after 5 years (P=0.04). Interestingly, there was a trend of shorter RTL at follow-up with diabetes, though the findings were not statistically significant.

Conclusions: Higher C-peptide level contributes to telomere shortening over time, suggesting that metabolic dysregulation may play a role in early aging. Further understanding of this relationship and addressing high C-peptide levels can be important to prevent premature aging.

KEYWORDS
telomere length, C-peptide, aging, metabolic, insulin resistance, predictor, telomere shortening
Introduction

Telomeres are repetitive DNA sequences that shelter the linear ends of chromosomes in order to prevent DNA damage (1). They are added by the telomerase enzyme to prevent the loss of coding nucleotides, a process that normally occurs with each cell division due to incomplete replication of the 5′end of the DNA lagging strand (2). As a result, with each replicative cell division, telomeres will shorten, and this process has been correlated with cellular senescence (3). In addition to that, telomere shortening can be accelerated in the setting of disease conditions characterized by increased inflammation, which makes it a measurable marker of inflammation and oxidative stress (4).

Many studies have shown significant correlations between certain metabolic conditions associated with aging and relative telomere length (RTL) over time. As such, telomere length, as measured in leukocytes, is now widely recognized as a marker for cellular aging and as a clinical indicator for comorbidity and disease (2). For instance, a longitudinal cohort study that included 2848 adult men and women from the Netherlands recruited between 2004 and 2007, showed that a shorter baseline RTL was associated with worsening of all components of the metabolic syndrome at 2- and 6-years follow-up (5). Similarly, a study conducted on a cohort of 12792 Mexican American participants aged 20 to 85 years and followed annually, showed a doubling in the incidence of diabetes over time, between the lowest and highest quartiles of RTL at baseline (6). These findings support the role of RTL as a predictor of disease and morbidity, yet they only highlight a unidirectional relationship between shortening of RTL and the development of comorbidities years later. However, the relationship between RTL and comorbidities is complex and bidirectional. Baseline comorbidities such as obesity, hypertension, diabetes, and dyslipidemia could influence the change in RTL over time. Such a relationship would constitute a potential driver of a vicious metabolic cycle whereby a short RTL will lead to worsening metabolism, that in turn will accelerate RTL shortening even further (7).

Thus, it becomes important to better characterize the relationship between these cardiometabolic risk factors and their effect on RTL. There are few longitudinal studies that demonstrated the impact of such risk factors on RTL at follow-up, and on the rate of telomere shortening. For instance, a study on the same cohort from the Netherlands assessed baseline cardiometabolic risk factors and rate of RTL shortening over time in 1808 participants; it showed that a higher baseline waist circumference, lower HDL-cholesterol, and higher glucose levels all correlated with shorter telomere length at 6-year follow-up. This study also revealed that a larger increase in waist circumference over time was associated with significantly shorter RTL at follow-up (8). A separate longitudinal cohort study conducted a decade earlier in 1998 on 8074 participants ages 28-75 years from the Netherlands, and followed over 6.5 years, estimated the average loss in RTL to be 0.47 ± 0.16 annually. However, this rate was 3 times higher among active smokers, and was also accelerated in the presence of higher baseline waist, higher glucose levels and lower HDL-cholesterol (9).

Even though the above metabolic correlations allude to it, there are no studies reporting longitudinally on the presence of diabetes, a major cardiometabolic risk factor, and its effect on RTL. Moreover, there are few longitudinal reports on markers of dysglycemia, such as insulin and C-peptide levels. A longitudinal measure of both RTL and insulin resistance conducted over 12 years on 338 adult twin pairs found RTL to predict the development of insulin resistance, but not vice versa (10). The main driver of this relationship was insulin level and not glucose. Being strongly associated with aging, we were interested in determining longitudinally the effect of diabetes and other markers of dysglycemia on RTL over time (11). We have previously reported in a cross-sectional study in the Lebanese population an association between telomere length and risk factors such as a wider waist circumference and hypertension (12).

The purpose of the current study is to determine longitudinally whether the health profile or the presence of cardiometabolic risk factors influence the change in RTL over time in a sample of Lebanese individuals. This is with the aim of better characterizing the bidirectional relationship between RTL and these chronic conditions.

Materials and methods

The current study builds on subjects, data and blood samples collected in 2014 with a follow up recruitment, data and blood samples collection 5 years later in 2019. The study was approved by the Institutional Review Board at the American University of Beirut with participants signing an informed consent form on both recruitment stages.

2014 baseline study

A detailed description of the study design has been reported elsewhere (12). Briefly, 501 adult Lebanese men and women, aged 18 years old and above, were recruited between February and June 2014. They were all residing in Greater Beirut. The objective was to determine Bisphenol A levels among residents of Greater Beirut (13).

Baseline data collection consisted of face-to-face interviews, anthropometric measurements, and blood withdrawal for laboratory tests. Data on demographic and socioeconomic characteristics namely age, gender, marital status, education,
Laboratory tests

Laboratory tests were performed with the following methodologies: fasting glucose by the enzymatic method (Cobas 6000, Roche), HbA1C by HPLC (Bio-Rad), insulin by radioimmunoassay (Cisbio), C-peptide by radioimmunoassay (Cisbio), and cortisol by radioimmunoassay (Cisbio). Levels of triglycerides, HDL, LDL, total cholesterol, and CRP were measured using Vitros 350 analyzer (Ortho Clinical Diagnostics, Johnson and Johnson).

2019 follow up study

Five years after the baseline study, and between February and May 2019, participants who consented to be contacted again were called by phone for the purpose of scheduling a follow-up visit for similar procedures performed in 2014. The sample that was available for follow-up consisted of 478 participants, out of which 198 agreed to be part of the study.

A detailed description of the follow up design has been recently reported elsewhere (21). Briefly, participants underwent face-to-face interview, anthropometric measurements, and laboratory studies. All previously (2014) collected demographics, socioeconomic information lifestyle factors, and concomitant diseases were similarly collected in the follow up study. The same applies for the anthropometric and blood pressure measurements and blood collection.

Total DNA was extracted from leucocytes of peripheral venous blood, and RTL was measured by amplifying telomere and single copy gene separately, using quantitative real-time PCR with the same analyses and quality control parameters as described for the 2014 baseline study in the same laboratory (12). The follow-up results were adjusted for systematic differences caused by the use of different reference samples.

Statistical analysis

Data were entered into SPSS and a P value <0.05 was used to indicate statistical significance. Variables on the presence of metabolic syndrome and the atherosclerotic vascular disease risk were computed as previously described (12). Results are presented as means ± standard deviation (SD) for continuous variables and as percentages for categorical variables.

The difference between mean RTL at baseline versus at follow up was compared using paired t-test. The associations of baseline demographics as well as lifestyle factors at initial recruitment (2014) with RTL at follow-up (2019) and change in RTL (defined as the difference between RTL at follow-up and RTL at recruitment) were first evaluated using Student t-test or One way ANOVA and simple linear regression using Pearson coefficient (r) as applicable. The same tests were conducted for the associations between baseline cardiometabolic factors factor, RTL and change in RTL at follow-up. Afterwards, morbidities that were found to be significantly associated with RTL or change in RTL at follow-up were assessed using a multivariate
linear regression model including both age and gender. Results of the multivariate analyses are reported as beta coefficient (β) with 95% confidence intervals (CI).

**Results**

DNA for RTL measurement was not available for two of the participants, hence the final sample size was 196. RTL was significantly shortened after 5 years follow up with a mean ± SD of 1.48± 0.85 in 2014 compared to 1.16 ± 1.00 in 2019 (P<0.001 with paired t-test). Furthermore, baseline creatinine (from the 2014 visit) of the 196 participants that were available for the 5-year follow-up was calculated as 0.75 ± 0.21, with only 5 participants with a GFR below 60 mL/min/1.73 m² (6.3%), none of whom had a GFR of below 30 mL/min/1.73 m².

**Baseline characteristics and RTL**

Table 1 depicts the association of baseline characteristics and lifestyle factors at initial recruitment (2014) with RTL at follow-up 5 years later, as well as the change in RTL in 2019 compared to 2014.

There was a significant association between gender and RTL at follow-up whereby males had a shorter RTL as compared to females (0.98±0.55 versus 1.27±1.17, \( P = 0.05 \)). Similarly, older age was associated with shorter RTL at follow-up (\( r = -0.168, P = 0.018 \)). RTL was otherwise not associated with socioeconomic indicators such as income, education or crowding index; nor with lifestyle factors such as coffee drinking, alcohol consumption, smoking, or physical activity. Finally, RTL did not correlate with any measures of obesity as reflected by BMI, waist circumference, or body fat mass.

**Baseline cardiometabolic risk factors and RTL**

Table 2 describes the association between baseline cardiometabolic risk factors (and related laboratory parameters) and RTL at follow-up 5 years later, as well as the change in RTL in 2019 as compared to 2014. A higher C-peptide level was significantly correlated with shorter RTL at follow-up (\( r = -0.14, P = 0.04 \)). As for the change in RTL (ΔRTL), the only significant result was observed with C-peptide. For instance, there was again a negative correlation whereby a higher C-peptide level was significantly associated with a negative change in RTL, meaning shortening of RTL at follow up (\( r = -0.15, P = 0.03 \)). There was an interesting trend between diabetes and RTL at follow-up, though it did not reach significance. For instance, participants with definite diabetes in 2014 had shorter RTL at follow up when compared to those who did not have diabetes (0.89±0.53 as compared to 1.22±1.06 respectively, with a \( P = 0.09 \)).

Furthermore, when adjusting for age and gender, the association between C-peptide and ΔRTL remained significantly negative with a \( P \) value of 0.04 (Table 3). A similar trend was observed for both diabetes and C-peptide levels in association with RTL at follow-up, though not statistically significant.

Of note that we did not perform a sub-analysis on the group with diabetes because of small sample size (N=33) and because 17 of them (51.5%) were getting treatment with hypoglycemic medications. The cohort was hence relatively controlled with a mean (± SD) fasting serum glucose of 154 ± 47 (mg/dL), Insulin of 34.7 ± 13.0 (µIU/mL), HbA1c of 7.7 ± 1.8%, and C peptide of 3.8 ± 1.70 ng/dL.

**Figure 1** illustrates the change in RTL with diabetes (A) and C-peptide (B) for the whole cohort. It shows that participants with diabetes had a shorter RTL than participants without diabetes at baseline. RTL shortening occurred in both groups over the 5-year follow-up but was steeper in the group with diabetes (Figure 1A). Similarly, higher C-peptide levels (above the mean) were associated with shorter RTL at baseline. There was RTL shortening among all individuals but the group with higher C-peptide had a steeper drop over time (Figure 1B).

**Discussion**

This study aimed to determine the relationship between baseline cardiometabolic risk factors, and telomere length over a 5-year period. Our results indicate that shorter RTL is significantly associated with higher C-peptide levels at baseline. A similar, though non-significant trend was observed for those who had diabetes at baseline. However, other risk factors such as hypertension and dyslipidemia did not show a statistically significant association with RTL at follow-up. To our knowledge, this is one of the very first studies to report on the long-term effect of metabolic derangement, and C-peptide in particular, on telomere length. This finding is of major clinical relevance as it may indicate how an individual’s health profile can influence RTL over the years, and therefore induce more rapid senescence. As a matter of fact, telomere loss is a widely described mechanism for cellular senescence, the irreversible loss of the cell’s replicative function, warranting its use as a marker for biological aging (22, 23).

Our results showed that older individuals had shorter telomere length at follow-up, a finding that is consistent with several studies (24–26). Furthermore, telomere length at follow-up was significantly associated with gender, with females having significantly longer RTL over the 5-year period. This is also consistent with the literature. A recent systematic review with meta-analysis concluded that females tend to have longer telomeres than males (27).
TABLE 1. Description and association of baseline characteristics and lifestyle at initial recruitment in 2014 with RTL at follow up in 2019 and ΔRTL.

| Variables in 2014                              | Total N=196 | RTL at follow-up | ΔRTL |
|------------------------------------------------|-------------|------------------|------|
|                                                 | Mean±SD     | Correlation (r)  | p-value | Mean±SD     | Correlation (r)  | p-value |
| Mean±SD                                         |             |                  |        |             |                  |        |
| Age (years)                                     | 46.88±13.34 | -0.168           | 0.018  | -0.060      | 0.407            |        |
| <40                                             | 58 (29.6)   | 1.42±1.56        | 0.07   | -0.31±1.56  | 0.559            |        |
| 40-60                                           | 113 (57.9)  | 1.07±0.61        |        | -0.26±0.80  | 0.419            |        |
| >60                                             | 25 (12.8)   | 1.02±0.61        |        | -0.53±1.14  | 0.371            |        |
| Gender                                          |             |                  |        |             |                  |        |
| Female                                          | 125 (63.8)  | 1.27±1.17        | 0.05   | -0.27±1.23  | 0.487            |        |
| Male                                            | 71 (36.2)   | 0.98±0.55        |        | -0.38±0.87  | 0.371            |        |
| Marital Status                                  |             |                  |        |             |                  |        |
| Married                                         | 146 (74.5)  | 1.15±1.08        | 0.54   | -0.34±1.13  | 0.724            |        |
| Single                                          | 21 (10.7)   | 1.38±0.73        |        | -0.24±1.51  | 0.487            |        |
| Other                                           | 29 (14.8)   | 1.07±0.70        |        | -0.17±0.65  | 0.724            |        |
| Income                                          |             |                  |        |             |                  |        |
| <600$                                           | 67 (34.5)   | 1.04±0.63        | 0.57   | -0.35±0.96  | 0.377            |        |
| 600-999.9$                                      | 79 (40.7)   | 1.21±1.34        |        | -0.31±1.39  | 0.75             |        |
| ≥1000$                                          | 37 (19.1)   | 1.21±0.76        |        | -0.40±0.81  | 0.371            |        |
| I don’t know/no answer                          | 11 (5.7)    | 1.43±0.74        |        | 0.24±0.61   | 0.724            |        |
| Education                                       |             |                  |        |             |                  |        |
| No schooling or primary school                  | 76 (39.0)   | 1.00±0.62        | 0.19   | -0.46±0.94  | 0.303            |        |
| Intermediate school                             | 60 (30.8)   | 1.37±1.52        |        | -0.26±1.38  | 0.724            |        |
| Secondary school or technical diploma           | 44 (22.6)   | 1.14±0.67        |        | -0.08±0.63  | 0.724            |        |
| University degree                               | 15 (7.7)    | 1.24±0.57        |        | -0.47±1.74  | 0.371            |        |
| Crowding Index                                  | 1.56±0.80   | -0.067           | 0.353  | 0.02        | 0.741            |        |
| Smoker                                          |             |                  |        |             |                  |        |
| Never                                           | 44 (22.4)   | 1.11±0.66        | 0.87   | -0.42±0.90  | 0.75             |        |
| Former                                          | 27 (13.8)   | 1.23±0.69        |        | -0.22±0.91  | 0.371            |        |
| Current                                         | 125 (63.8)  | 1.18±1.15        |        | -0.30±1.23  | 0.724            |        |
| Cigarette smoker                                |             |                  |        |             |                  |        |
| Never                                           | 97 (49.5)   | 1.23±1.28        | 0.68   | -0.32±1.29  | 0.66             |        |
|Former                                          | 22 (11.2)   | 1.12±0.48        |        | -0.11±0.50  | 0.724            |        |
| Current                                         | 77 (39.3)   | 1.10±0.65        |        | -0.36±1.01  | 0.724            |        |
| Narguileh (waterpipe) smoker                    |             |                  |        |             |                  |        |
| Never                                           | 111 (56.6)  | 1.14±0.67        | 0.88   | -0.28±0.81  | 0.80             |        |
| Former                                          | 29 (14.8)   | 1.19±0.68        |        | -0.28±0.92  | 0.724            |        |
| Current                                         | 56 (28.6)   | 1.22±1.56        |        | -0.40±1.63  | 0.371            |        |
| Current Alcohol Drinker                         |             |                  |        |             |                  |        |
| No                                               | 168 (85.7)  | 1.69±0.94        | 0.502  | -0.28±1.07  | 0.430            |        |
| Yes                                              | 28 (14.3)   | 1.60±1.04        |        | -0.46±1.35  | 0.371            |        |
| Coffee Drinker                                  |             |                  |        |             |                  |        |
| No                                               | 39 (19.9)   | 1.19±0.62        | 0.858  | -0.41±1.33  | 0.544            |        |
| Yes                                              | 157 (80.1)  | 1.16±1.07        |        | -0.28±1.06  | 0.724            |        |
| BMI (kg/m²)                                      | 29.99±5.87  | -0.048           | 0.508  | -0.059      | 0.412            |        |
| BMI (kg/m²): categorical                        |             |                  |        |             |                  |        |
| <30                                              | 100 (51.0)  | 1.16±1.22        | 0.987  | -0.32±1.22  | 0.904            |        |
| ≥30                                              | 96 (48.9)   | 1.17±0.70        |        | -0.30±1.01  | 0.724            |        |
| Waist Circumference (cm)                         | 97.85±17.23 | -0.026           | 0.714  | 0.009       | 0.901            |        |
| Within normal per gender*                       | 38 (19.4)   | 1.23±0.61        | 0.69   | -0.15±0.68  | 0.31             |        |
| Above normal per gender                         | 158 (80.6)  | 1.16±1.09        |        | -0.35±1.20  | 0.724            |        |
| Body Fat (Kg)                                   | 30.26±11.69 | 0.075            | 0.296  | 0.026       | 0.722            |        |
| Muscle Mass (Kg)                                 | 26.82±6.67  | -0.091           | 0.207  | -0.08±0.59  | 0.412            |        |
| Levels of Physical Activity                     |             |                  |        |             |                  |        |
| Low-intensity activity                          | 91 (46.4)   | 1.23±1.28        | 0.271  | -0.39±1.40  | 0.355            |        |
| Moderate-intensity activity                     | 72 (36.7)   | 1.02±0.50        |        | -0.31±1.73  | 0.544            |        |
| High-intensity activity                         | 33 (16.8)   | 1.31±0.89        |        | -0.06±0.87  | 0.724            |        |
| Physical Activity                               |             |                  |        |             |                  |        |
| None                                            | 28 (14.3)   | 0.98±0.52        | 0.293  | -0.40±0.76  | 0.647            |        |
| Any                                             | 168 (85.7)  | 1.20±1.05        |        | -0.29±1.16  | 0.724            |        |

(Continued)
Moreover, in a subset of the Offspring cohort of the Framingham Heart Study, insulin resistance and oxidative stress have been described to occur even before the development of pancreatic beta cells (50). Oxidative damage in the latter could potentially lead to their senescence and further contribute to the accelerated aging, as seen in diabetes.

Our main finding on C-peptide is supported by a large cross-sectional survey conducted on 1382 adult men and women, where a longer telomere was associated with lower C-peptide level (28). Very few studies report on this association, and none where a longer telomere was associated with lower C-peptide predicted RTL shortening may be through insulin resistance at the prediabetes and diabetes stage (31), with the fact that it is not metabolized by the liver (41), which provides more steady state levels for measurement (42), and more linear kinetics at the various glycemic stages. Alternatively, C-peptide may have effects independent of insulin (43), and is even hypothesized to have its own receptor (44).

Despite not reaching significance in our study, the link between diabetes and shorter RTL is better characterized in the literature than the one with C-peptide, and was shown across different populations, settings, and risk factors, in support of the trend found in our study (11, 45). In vitro studies show that hyperglycemia may have a direct effect on RTL shortening and subsequent cell senescence (46). This finding was demonstrated by an experiment where skin fibroblasts were cultured in vitro in a medium with high glucose concentration. As compared to a normoglycemic milieu, these fibroblasts reached the threshold for apoptosis within a shorter life cycle as measured by population doubling times. More importantly, the effect of hyperglycemia was circumvented by telomerase overexpression (47). One mechanism through which hyperglycemia may promote RTL shortening is through oxidative stress (47). The generated oxygen radicals affect telomere ends which are rich in guanine residues, the latter fact rendering them vulnerable to oxidative stress (11, 48, 49). The relation between diabetes, oxidative stress and telomere shortening has been described in various human cells such as fibroblasts (47), leukocytes (2), and more recently pancreatic beta cells (50). Oxidative damage in the latter could potentially lead to their senescence and further contribute to the decrease in insulin release and worsening of hyperglycemia (50), a relationship which may explain the bidirectional findings in studies on RTL and metabolic dysfunction (7).

In the current study, our main finding is the longitudinal association whereby a higher C-peptide level predicted a shorter RTL and further shortening at 5-year follow-up. One other interesting, even though nonsignificant finding, is that the presence of diabetes at baseline was associated with a steeper drop in RTL over time. This sharper decrease in RTL (twice that of those who did not have diabetes at baseline) may imply accelerated aging, as seen in diabetes.
| Cardiometabolic risk factors and laboratory values in 2014 | All sample N=196 | RTL at follow-up | ΔRTL |
|--------------------------------------------------------|------------------|----------------|-------|
|                                                        | Correlation (r)  | p-value        | Correlation (r)  | p-value |
|                                                        | mean±SD          |                | mean±SD          |        |
| Diabetes                                               |                  |                |                  |        |
| Definite Diabetes                                      |                  |                |                  |        |
| No                                                     | 163 (83.2)       | 1.22±1.06      | -0.27±1.12       | 0.30   |
| Yes                                                    | 33 (16.8)        | 0.89±0.53      | -0.49±1.08       |        |
| Self-reported diabetes or hyperglycemia diagnosis       |                  |                |                  |        |
| No                                                     | 169 (86.2)       | 1.21±1.05      | -0.30±1.15       | 0.16   |
| Yes                                                    | 27 (13.8)        | 0.93±0.54      | -0.33±0.94       | 0.88   |
| Diabetes treatment                                     |                  |                |                  |        |
| No                                                     | 169 (86.2)       | 1.20±1.05      | -0.31±1.14       | 0.24   |
| Yes                                                    | 27 (13.8)        | 0.95±0.53      | -0.27±0.95       | 0.84   |
| Fasting serum glucose (mg/dL)                          |                  |                |                  |        |
| <100 – normal                                          | 90 (45.9)        | 1.10±0.55      | -0.44±0.96       | 0.37   |
| ≥100 – abnormal                                        | 106 (54.1)       | 1.22±1.26      | -0.20±1.13       |        |
| Insulin (μIU/mL), Mean (±SD)                           | 28.95±11.62      | -0.063         | 0.415            | 0.138  |
| HbA1c (%) Mean (±SD)                                   | 5.9±1.2          | -0.098         | 0.170            | 0.558  |
| C peptide (ng/dL), Mean (±SD)                          | 3.18±1.37        | -0.146         | 0.044            | 0.768  |
| Hypertension (HTN)                                     |                  |                |                  |        |
| Definite HTN                                           |                  |                |                  |        |
| No                                                     | 127 (64.8)       | 1.20±1.12      | -0.36±1.20       | 0.26   |
| Yes                                                    | 69 (35.2)        | 1.10±0.71      | -0.22±0.95       | 0.94   |
| Self-reported HTN diagnosis                            |                  |                |                  |        |
| No                                                     | 159 (81.1)       | 1.20±1.07      | -0.31±1.20       | 0.17   |
| Yes                                                    | 37 (18.9)        | 1.00±0.57      | -0.30±1.26       | 0.79   |
| HTN treatment                                          |                  |                |                  |        |
| No                                                     | 161 (82.1)       | 1.21±1.07      | -0.30±1.14       |        |
| Yes                                                    | 35 (17.9)        | 0.96±0.53      | -0.35±1.02       |        |
| Systolic Blood Pressure (mm/Hg), Mean (±SD)            | 121.97±19.05     | -0.120         | 0.093            |        |
| Diastolic Blood Pressure (mm/Hg), Mean (±SD)           | 75.73±10.28      | 0.008          | 0.908            |        |
| Dyslipidemia                                           |                  |                |                  |        |
| Self-reported dyslipidemia diagnosis                   |                  |                |                  | 0.83   |
| No                                                     | 146 (74.5)       | 1.17±1.09      | -0.36±1.20       |        |
| Yes                                                    | 50 (25.5)        | 1.14±0.65      | -0.16±0.80       |        |
| Dyslipidemia treatment                                 |                  |                |                  |        |
| No                                                     | 156 (79.6)       | 1.22±1.09      | -0.30±1.18       | 0.11   |
| Yes                                                    | 40 (20.4)        | 0.94±0.08      | -0.33±0.85       | 0.90   |
| HDL (mg/dL), Mean (±SD)                                | 49.38±13.80      | -0.052         | 0.465            |        |
| LDL (mg/dL), Mean (±SD)                                | 111.64±36.89     | 0.040          | 0.374            |        |
| Triglycerides (mg/dL), Mean (±SD)                      | 142.18±72.41     | 0.022          | 0.763            |        |
| Metabolic syndrome                                     |                  |                |                  |        |
| No                                                     | 86 (43.9)        | 1.13±0.60      | -0.39±1.04       | 0.11   |
| Yes                                                    | 110 (56.1)       | 1.20±1.23      | -0.24±1.17       | 0.90   |
| Atherosclerotic vascular disease (ASCVD) risk          |                  |                |                  |        |
| ASCVD 10 yrs risk (%), Mean (±SD)                      | 7.79±7.45        | -0.133         | 0.154            |        |
| Other                                                  |                  |                |                  |        |
| CRP (mg/L), Mean (±SD)                                 | 11.77±7.27       | -0.006         | 0.934            |        |
| Cortisol (μg/dL), Mean (±SD)                           | 18.47±12.27      | -0.052         | 0.484            |        |

Bolded P-values are statistically significant as they are less or equal to 0.05.
In brief, a higher C-peptide and the presence of diabetes, are both associated with telomere shortening. Taken together, these risk factors are on a continuum of glucose metabolism, starting with insulin resistance, prediabetes, and diabetes. Our findings and those of the aforementioned studies indicate that biological aging could be influenced by these metabolic abnormalities. Given the prediabetes and diabetes epidemics, and the world increase in aging, and more specifically, premature aging, it becomes important and relevant to better characterize the link found in our study between elevated C-peptide and telomere shortening.

Our study suffers from some limitations. First, the small sample size for those who have definite diabetes (n=33) does not permit further exploration of diabetes control, duration of diabetes, and medication intake in relation to telomere shortening. In addition, the sample size may have been insufficient to show a statistically significant association between diabetes status and RTL at follow-up as well as between characteristic laboratory abnormalities in type 2 diabetes patients (HbA1c and fasting blood glucose) with change in RTL over time. In fact, even with regards to the significant association between C-peptide and RTL, the calculated power was only 55%. Furthermore, we looked at other cardiovascular risk factors such as obesity and smoking to evaluate whether there is a similar association as the one observed with diabetes, but the latter did not reach statistical significance, potentially due to the small sample size. Another limitation is that the participants were only recruited from the Greater Beirut area, which impedes its representativeness and warrants the need for further studies to explore the influence of diabetes on telomere length. Finally, telomere length was measured using quantitative PCR, a method described in 2009 by Cawthon (19). This method has some shortcomings including a low reliability for measurements at the bottom and top percentiles of RTL, as compared to the Southern blot technique, which is the gold standard method for estimating telomere length (51). However, the qPCR method is still widely used in most epidemiological studies (51).

Nonetheless, despite the limitations, the comprehensive evaluation of general risk factors is one of this study’s strengths. In fact, this reduces the risk of having any confounding bias that could affect the association between metabolic abnormalities and telomere length. Moreover, the follow-up period (5-year) was sufficient to observe the changes in telomere length over time. The consistency in measure and the fact that it is a prospective design are further unique strengths of this study.

**Data availability statement**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.
et al. Short telomere length is associated with aging, central obesity, poor sleep and disease. *Cite Rev Clin Lab Sci* (2018) 55(7):443–65. doi: 10.1080/10408536.2018.1504274

3. Vincenzi S, Passos JF. Telomeres and cell senescence - size matters not. *Ebiomedi* (2017) 21:14–20. doi: 10.1016/j.ebiomedi.2017.03.027

4. Zhang J, Rane G, Dai X, Shannugam MK, Ariusof F, Samy RP, et al. Aging and the telomere connection: An intimate relationship with inflammation. *Aging Res Rev* (2016) 25:55–69. doi: 10.1016/j.arr.2015.11.006

5. Rèvez D, Milaneschi Y, Verhoeven JE, Penninx BW. Telomere length as a marker of cellular aging is associated with prevalence and progression of metabolic syndrome. *J Clin Endocrinol Metab* (2014) 99(12):4607–15. doi: 10.1210/jc.2014-1851

6. Zhao H, Han L, Chang D, Ye Y, Shen J, Daniel CR, et al. Social-demographics, health behaviors, and telomere length in the Mexican American mano a mano cohort. *Oncotarget* (2017) 8(157):96553–67. doi: 10.18632/oncotarget.19903

7. Ellis CE, Scott RA. The long and short of telomere length and diabetes. *Diabetes* (2014) 63(1):65–7. doi: 10.2337/db13-1469

8. Rèvez D, Milaneschi Y, Verhoeven JE, Lin J, Penninx BW. Longitudinal associations between metabolic syndrome components and telomere shortening. *J Clin Endocrinol Metab* (2015) 100(8):3050–9. doi: 10.1210/jc.2015-1995

9. Huzen J, Weng LS, van Veldhuisen DJ, Samani NJ, Zwierdenman AH, Codd V, et al. Telomere length loss due to smoking and metabolic traits. *J Intern Med* (2014) 275(2):155–63. doi: 10.1111/j.1017-0309.2014.001491.x

10. Verbalst S, Dalgaard C, Labat C, Kark JD, Kimura M, Christensen K, et al. A short leucocyte telomere length is associated with development of insulin resistance. *Diabetologia* (2016) 59(6):1258–65. doi: 10.1007/s00125-016-3915-6

11. Cheng F, Carroll J, Joglekar MV, Januszewski AS, Wong KK, Hazdik AA, et al. Diabetes, metabolic disease, and telomere length. *Lancet Diabetes Endocrinol* (2021) 9(2):117–26. doi: 10.1016/s2213-8587(20)30365-x

12. Zghib NK, Sleiman F, Nasreddine L, Nasrallah M, Nakhoul N, Isma’el H, et al. Bisphenol a urinary level, its correlates, and association with cardiometabolic risks in Lebanese urban adults. *Environ Monit Assess* (2017) 189(10):517. doi: 10.1007/s10661-017-6216-8

13. Moumenim Y, Nasrallah M, Khouryier Zghib N, Nasreddine L, Nakhoul N, Isma’el H, et al. Bisphenol a urinary level, its correlates, and association with cardiometabolic risks in Lebanese urban adults. *Environ Monit Assess* (2017) 189(10):517. doi: 10.1007/s10661-017-6216-8

14. Netzer NC, Stooobs RA, Netzer CM, Clark K, Strohl KP. Using the Berlin questionnaire to identify patients at risk for the sleep apnea syndrome. *Ann Intern Med* (1999) 131(7):485–91. doi: 10.7326/0003-4819-131-7-199910050-00002

15. Nasrallah MP, Nakhoul NF, Nasreddine L, Moumenim Y, Abiad MG, Isma’el H, et al. PREVALENCE OF DIABETES IN GREATER BEIRUT AREA: WORSENING OVER TIME. *Endocr Pract* (2017) 23(9):1991–100. doi: 10.4158/ep171876.0r

16. Armstrong C. JNC8 guidelines for the management of hypertension in adults. *Am Fam Physician* (2014) 90(7):303–4.

17. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome–a new worldwide definition. *Lancet* (2005) 366(9491):1059–62. doi: 10.1016/s0140-6736(05)67402-8

18. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res* (2002) 30(10):e47. doi: 10.1093/nar/gof047

19. Cawthon RM. Telomere length measurement by a novel monochromate multiplex quantitative PCR method. *Nucleic Acids Res* (2009) 37(3):e21. doi: 10.1093/nar/gkn1027

20. Pfaff MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* (2001) 29(9):e45. doi: 10.1093/nar/28.9.e45

21. Nasrallah MP, Elbejjani M, Nasreddine L, Chami H, Ismaeiel H, Fleifel M, et al. Incidence of diabetes and its predators in the greater Beirut area: a five-year longitudinal study. *Diabetol Metab Syndr* (2022) 14(1):67. doi: 10.1186/s13098-022-00833-w

22. Aubert G, Lansodpr PM. Telomeres and aging. *Physiol Rev* (2008) 88(2):557–79. doi: 10.1152/physrev.00026.2007

23. Bernadotte A, Mikkelson VM, Spivak IM. Markers of cellular senescence. Telomere shortening as a marker of cellular senescence. *Aging (Albany NY)* (2016) 8(1):3–11. doi: 10.18632/aging.100871

24. Ehrlenbach S, Willeit P, Kiechl S, Willeit J, Reindl M, Schanda K, et al. Telomere shortening and markers of inflammation in the elderly. *Am J Epidemiol* (2009) 169(2):1725–34. doi: 10.1093/aje/kwp273

25. Steenstrup T, Hjelmborg JV, Mortensen LH, Kimura M, Christensen K, Aviv A. Leukocyte telomere dynamics in the elderly. *Eur J Epidemiol* (2013) 28(2):181–7. doi: 10.1007/s10654-013-9780-4

26. Berglund K, Reynolds CA, Ploner A, Gerritsen L, Hovatta I, Pedersen NL, et al. Longitudinal decline of leukocyte telomere length in old age and the association with sex and genetic risk. *Aging (Albany NY)* (2016) 8(7):1398–415. doi: 10.18632/aging.100995

27. Gardner M, Bann D, Wiley L, Cooper R, Hardy R, Nitsch D, et al. Gender and telomere length: systematic review and meta-analysis. *Exp Gerontol* (2014) 51:15–27. doi: 10.1016/j.exger.2013.12.004
28. Yang M, Jiang P, Jin C, Wang J. Longer telomere length and its association with lower levels of c-peptide. Front Endocrinol (Lausanne) (2017) 8:244. doi: 10.3389/fendo.2017.00244

29. Mangge H, Herrmann M, Almer G, Zebler S, Moeller R, Horesjø R, et al. Telomere shortening associates with elevated insulin and nuchal fat accumulation. Sci Rep (2020) 10(1):6863. doi: 10.1038/s41598-020-63916-6

30. Khan HA, Sobki SH, Ekhaizmy A, Khan I, Almusawi MA. Biomarker potential of c-peptide for screening of insulin resistance in diabetic and non-diabetic individuals. Saude J Biol Sci (2018) 25(8):1729–32. doi: 10.1016/j.sjbs.2018.05.027

31. Gardner JP, Li S, Srinivasan SR, Chen W, Kimura M, Lu X, et al. Rise in insulin resistance is associated with escalated telomere attrition. Circulation (2005) 111(17):2171–7. doi: 10.1161/01.cir.0000163550.70487.0b

32. Demissie S, Levy D, Benjamin EJ, Cupples LA, Gardner JP, Herbert A, et al. Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the framingham heart study. Aging Cell (2006) 5(4):325–30. doi: 10.1111/j.1474-9726.2006.00224.x

33. Luc K, Schramm-Luc A, Güriz TJ, Mikołajczyk TP. Oxidative stress and inflammatory markers in prediabetes and diabetes. J Physiol Pharmacol (2019) 70(6):809–24. doi: 10.26402/jpp.2019.6.01

34. Evans JL, Geldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev (2002) 23(5):599–622. doi: 10.1210/er.2001-0039

35. Rani V, Deep G, Singh RK, Palle K, Yadav UC. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. J Life Sci (2016) 148:183–93. doi: 10.1007/s10166-016-0202

36. Carr ALJ, Oram RA, Marren SM, McDonald TJ, Narendran P, Andrews RC. Measurement of peak c-peptide at diagnosis informs glycemic control but not telomere length in type 1 diabetes. J Clin Lab Anal (2007) 21(2):85. doi: 10.1002/cji.20138

37. Ido Y. Diabetic complications within the context of aging: Nicotinamide adenine dinucleotide redox, insulin c-peptide, sirtuin 1-liver kinase B1-adenosine monophosphate-activated protein kinase positive feedback and forkhead box O3. J Diabetes Investig (2016) 7(4):448–58. doi: 10.1111/jdi.12485

38. Rokseth R, Kierner LM, Borst MH, Muller Kobold A, Koostro-Ron JE, Gloeirsch J, et al. Plasma c-peptide and risk of developing type 2 diabetes in the general population. J Clin Med (2020) 9(9):3001. doi: 10.3390/jcm9093001

39. Al Qarni AA, Joatar FE, Das N, Awad M, Eltayeb M, Al-Zubair AG, et al. The clinical utility of c-peptide measurement in the care of patients with diabetes. Diabetes Med (2013) 30(7):803–17. doi: 10.1111/dme.12159

40. Sokooti S, Kierner LM, Borst MH, Muller Kobold A, Koostro-Ron JE, Gloeirsch J, et al. Plasma c-peptide and risk of developing type 2 diabetes in the general population. J Clin Med (2020) 9(9):3001. doi: 10.3390/jcm9093001

41. Y. A Clinical utility of c-peptide measurement in the care of patients with diabetes. Diabetes Med (2013) 30(7):803–17. doi: 10.1111/dme.12159

42. Sokooti S, Kiener LM, Borst MH, Muller Kobold A, Koostro-Ron JE, Gloeirsch J, et al. Plasma c-peptide and risk of developing type 2 diabetes in the general population. J Clin Med (2020) 9(9):3001. doi: 10.3390/jcm9093001

43. Y. A Clinical utility of c-peptide measurement in the care of patients with diabetes. Diabetes Med (2013) 30(7):803–17. doi: 10.1111/dme.12159

44. Hills CE, Brunskill NJ. Intracellular signalling by c-peptide. Exp Diabetes Res (2008) 2008:635158. doi: 10.1155/2008/635158

45. Wang J, Dong X, Cao L, Sun Y, Qiu Y, Zhang Y, et al. Association between telomere length and diabetes mellitus: A meta-analysis. J Int Med Res (2016) 44(6):1156–73. doi: 10.1177/0300060516667132

46. Blazer S, Khankin E, Segev Y, Ofir R, Yalon-Hacothen M, Kra-Oz Z, et al. High glucose-induced replicative senescence: point of no return and effect of telomerase. Biochim Biophys Acta 2002 296(1):93–101. doi: 10.1016/s0006-291x(02)00815-5

47. Araki E, Nishikawa T. Oxidative stress: A cause and therapeutic target of diabetic complications. J Diabetes Investig (2010) 1(3):90–6. doi: 10.1111/j.2040-1124.2010.00013.x

48. Ma D, Zhu W, Hu S, Yu X, Yang Y. Association between oxidative stress and telomere length in type 1 and type 2 diabetic patients. J Endocrinol Invest (2013) 36(11):1032–7. doi: 10.3275/9036

49. Barnes RP, Fouquerel E, Oppreko PL. The impact of oxidative DNA damage and stress on telomere homeostasis. Mech Ageing Dev (2019) 177:37–45. doi: 10.1016/j.mad.2018.03.013

50. Tamura Y, Takubo K, Aida J, Araki A, Ito H. Telomere attrition and diabetes mellitus. Geriat Gerontol Int (2016) 16 Suppl 166–74. doi: 10.1111/ggi.12738

51. Wang Y, Savage SA, Alsaggar R, Aubert G, Dagnall CL, Spellman SR, et al. Telomere length calibration from qPCR measurement. Limitations of current method. Cells (2018) 7(11):183. doi: 10.3390/cells7110183