

The Association of Ramadan Fasting on Relative Leukocyte Telomere Length in Type 2 Diabetes Mellitus

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Abstract

Background. Leukocyte telomere length (LTL) is considered a biomarker of cellular senescence. Previous studies have reported the associations between shortened telomere length and type 2 diabetes mellitus (T2DM). However, the role of underlying factors remained unclear. While Ramadan fasting has consistently been shown to improve anthropometric and metabolic parameters in T2DM, its effect on cellular senescence has scarcely been reported.

Objective. We sought to determine whether Ramadan Fasting affects leukocyte telomere length in persons with T2DM.

Methodology. An interventional before-and-after study was conducted on 40 to 60-year-old subjects with T2DM consecutively recruited before Ramadan fasting (May 2018 and May 2019) from the Internal Medicine Outpatient Clinic at Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia. Relative LTL was compared between 48 subjects before Ramadan fasting and after at least 14 days of fasting and then adjusted with key clinical and biochemical parameters.

Results. The relative rLTL in subjects with T2DM was comparable between before and after at least 14 days of Ramadan fasting. (0.391 [0.021–1.515] vs 1.117 [0.528–1.741], $p = 0.112$).

Conclusion. No significant difference was found in relative leukocyte telomere length among subjects with type 2 diabetes who have undergone Ramadan fasting for at least 14 days. However, this study showed a tendency to have an increase in relative LTL.

Key words: Ramadan fasting, relative leukocyte telomere length, type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a degenerative disease with a growing prevalence each year. According to the International Diabetic Federation (IDF), the global prevalence of diabetes mellitus in 2019 reached 9.3% or approximately 463 million cases, with a mortality of 4.2 million.¹ Type 2 diabetes mellitus is related to the risk of age-related comorbidities through an acceleration of biological ageing, called cellular senescence. An increased turnover and chronic activation of inflammatory cells in T2DM contribute to the cellular oxidative stress that elicits the deletion of telomeres.^{2,3} Telomeres are the tandem repeats

of TTAGGG of the deoxyribonucleic acid (DNA) sequence required for DNA replication. During DNA replication, telomeres progressively get shorter, and once a critical limit is reached, cells undergo senescence and apoptosis. The G triplet of telomere is very susceptible to oxidative stress that potentially breaks the telomeric double-strands, resulting in shortened telomere length.^{4,5}

The relationship between shortened relative leukocyte telomere length (rLTL) and T2DM has been reported in previous studies. The first study to report this finding was by Jeanclos E et al.,⁶ in 1998, where they noted that T2DM is mediated via an immunologic process, hence patient's

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white blood cells (WBCs) profile might differ from those in the non-diabetic population. The accelerated telomere attrition rate is thought to play a significant role because it will result in premature senescence of leukocytes and an exhausted patient's immune system. A meta-analysis by Zhao et al.,⁷ which extracted telomere length from 9 population cohorts and pooled eligible studies resulted in a significant association between T2DM and telomere length (OR 1.291; 95% CI 1.112 - 1.498; $p < 0.001$), the risk is even more visible in high-quality studies (OR 1.452; 95% CI 1.204 - 1.753; $p = 0.005$). This risk was also adjusted for several factors, such as body mass index (BMI) and age. Interestingly, they also discovered that baseline rLTL was an independent predictor for the risk of cerebrovascular disease (CVD) in T2DM patients.

Inflammation and oxidative stress could accelerate telomere shortening. The relationship between telomere length and cardiometabolic factors has been reported in previous studies. A more recent cohort study by Cheng et al.,⁸ found that in 5,349 patients, rLTL was inversely correlated with age, diabetes duration, blood pressure, HbA1c, and urine albumin-to-creatinine ratio (uACR). The association of other clinical parameters, such as anthropometric measurements, calorie intake, physical activity, and lipid profile, with rLTL are points of research interest. Furthermore, any intervention addressing inflammatory improvement might benefit cellular senescence prevention.⁹⁻¹³ Particular emphasis was placed on calorie restriction, which has been hugely reported to improve inflammatory and metabolic parameters in T2DM.

Ramadan fasting (RF) is one calorie restriction model, particularly an intermittent or time-restricted feeding model. The benefit of RF on metabolic and anthropometric measures in T2DM has been reported in previous studies. However, the benefits of RF on cellular senescence in T2DM must be elaborated more. Therefore, this study aims to evaluate the effect of Ramadan fasting on rejuvenating cellular senescence in T2DM by examining the changes in the level of relative telomere length before and after at least 14 days of Ramadan fasting.

METHODOLOGY

Study design

This is an interventional before-and-after study without control (pre-and-post-study design) that analyzes the effect of Ramadan fasting on rLTL in persons with T2DM as a part of a larger study entitled, "Fasting in Type 2 Diabetes Mellitus Patients and Its Implications for Various Aspects: Glucose and Lipid Metabolism, Hemodynamic, Anthropometry and Body Composition, Nutrition, Cognitive System, Aging and Inflammation," conducted by the Division of Endocrinology and Metabolism, Internal Medicine Department, Faculty of Medicine, Universitas Indonesia (FMUI), Jakarta, Indonesia. Before Ramadan month in 2018 and 2019, we consecutively recruited persons with T2DM

aged 40 to 60 who planned to fast during Ramadan. The diagnosis of T2DM was based on a previous diagnosis of T2DM from a physician or the presence of A1c level of $\geq 6.5\%$. Those who had chronic kidney disease, severe liver disease, chronic gastrointestinal disease, cardiovascular disease, and autoimmune disease, who were pregnant or breastfeeding, and had a history of non-steroidal anti-inflammatory drug use, steroid consumption, or antibiotic consumption within the last month were excluded from this study. Patients considered to be at very high risk of fasting during Ramadan, according to International Diabetes Federation—Diabetes and Ramadan International Alliance (IDF-DAR) guidelines, were excluded. After the screening, eligible participants signed the study consent form. The visits were arranged 1-2 weeks before Ramadan (T0), at least 14 days during Ramadan (T1) of 1439 - 1440 Hijri, corresponding to about 16th May to 14th June 2018, and 5th May to 14th June 2019. For this current study, we selected subjects with complete data on age, body mass index, calorie intake, and blood parameters (fasting blood glucose, HbA1c, lipid profile). We compared rLTL data subjects with T2DM before and after fasting. This study was approved by the Ethical Health Research Committee of the Faculty of Medicine, Universitas Indonesia, Dr. Cipto Mangunkusumo National General Hospital, with ethical approval no. 0550/UN2.FI/ETIK/2018.

Telomeric length measurement

Whole blood samples of 25 mL were stored at a temperature of $-80\text{ }^{\circ}\text{C}$ in the Metabolic Disorder, Cardiovascular and Aging Cluster (MVA), Indonesia Medical Education and Research Institute (IMERI), FMUI, Jakarta, and then examined in October 2020. DNA was extracted from the peripheral blood leukocytes in MVA IMERI. The telomere length of the isolated DNA was measured using quantitative real-time PCR (RT-PCR) (Applied Biosystems 7900 HT) as a T/S ratio in the integrated laboratory of the FMUI. The T/S ratio compares telomeric DNA (T) with the level of single-copy (S) reference gene using quantitative real-time polymerase chain reaction (PCR). Cycling conditions for telomeres were 20-60 seconds at $95\text{ }^{\circ}\text{C}$, followed by at $95\text{ }^{\circ}\text{C}$ for 15 seconds and $60\text{ }^{\circ}\text{C}$ for 30-60 seconds. The P19-2-001, a single-copy gene, served as a reference gene in the conventional qPCR technique to measure telomeres.

Anthropometry measurement

The body weight (BW) and body composition measurements were conducted using Tanita MC780MA bioelectrical impedance analysis (BIA), while a portable stadiometer (GEA Medical, SH-2A High Meter 2 M) was used to measure height. Waist circumference was measured using an ergonomic circumference measuring tape based on WHO standard protocol as the middle point between the last palpable costae and the top of the iliac crest. The blood pressure measurement was done in a sitting position after resting for 10-15 minutes using GEA Medical® type SH-2A High Meter 2 M.

Nutritional intake measurement

The nutritional intake data were attained using a 3-day non-consecutive food record, of which all subjects were asked to write their food and drink consumption for two days during the weekday and one day during the weekend. The food record data were then verified by a certified nutritionist when the continuous glucose monitors (CGMs) sensor was disconnected. Nutritional analysis was then performed using the Nutrisurvey® program. The final nutritional data was obtained after calculating each average parameter value, and these data were displayed on the table.

Physical activity measurement

The physical activity data were assessed using different questionnaires, where, in 2018, the Global Physical Activity Questionnaire (GPAQ) was used, and in 2019, Bouchard was used, and the examinations were performed at home. With the GPAQ questionnaire, subjects were asked to answer 16 questions. After converting the data into total physical activity Metabolic Equivalents (METs) in minutes/week, it was categorized as light, moderate or vigorous activity. The Bouchard questionnaire captured the activity of each subject every 15 minutes for 24 hours, resulting in 96 periods, and was also performed for two days during the weekday and one day during the weekend for each visit. For each 15 minutes, the subjects were instructed to fill it up with a number ranging from 1–9 according to the intensity of the predominant activity during that period. The questionnaire results were quantified to yield energy expenditure, depicted by METs in kcal/kg. The final energy expenditure data were obtained by counting the average of METs every 3 hours during the 3-day courses. The data were further transferred into a graph representing 24-hour METs during and after Ramadan fasting, which was then also stratified as light, moderate, or vigorous activity.

Data analysis

Normally distributed data were presented as the mean with standard deviation, whereas non-normally distributed data were displayed as the median with interquartile range. A normality test was conducted using the Shapiro-Wilk test. The paired T-test or Wilcoxon test was used to compare differences before and after fasting.

The sample size of 48 subjects with T2DM who had RF was calculated based on a hypothesis test on the difference between the means of two dependent groups at 80% desired power, a two-tailed alpha level of 0.05 and effect size or expected mean of the difference and standard deviation of the change in outcome of 0.27 and 0.49, respectively.¹⁴ Those null hypotheses were rejected at the 0.05 alpha level of significance. Data analysis was carried out using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

This study compared rLTL before and after at least 14 days of RF in 48 subjects with T2DM, with a mean age of 50 ± 5 years, 66.7% were females, whereas the mean BMI was 27.59 ± 4.69 kg/m². For the glycemic profiles, the median FBG was 147.00 (115.00 – 204.00) mg/dl; meanwhile, the median HbA1c profile was 8.00 (7.00 – 10.50)%. Baseline characteristics are shown in Table 1.

In individuals with T2DM, the median rLTL measured before fasting (T0) was 0.391 (0.024 – 1.515), while after fasting (T1), it increased to 1.117 (0.540 – 1.729). Figure 1 displays the boxplots comparing rLTL at T0 and T1. Nevertheless, the Wilcoxon test results show that there is no significant statistical difference in rLTL levels before and after fasting ($p = 0.112$).

Table 1. Baseline characteristics of subjects with T2DM who have undergone at least 14 days of Ramadan fasting

Characteristics	T2DM subjects (n = 48)
Age, year (mean [SD])	50 (5)
41 – 50 years old, n (%)	22 (45.83)
51 – 60 years old, n (%)	26 (54.17)
Gender	
Male, n (%)	16 (33.30)
Female, n (%)	32 (66.70)
Duration of DM, year (median [IQR])	5 (5 – 8)
History of DM medication	
Metformin, n (%)	33 (68.80)
Sulfonylurea, n (%)	23 (47.90)
Pioglitazone, n (%)	1 (2.10)
Acarbose, n (%)	4 (8.30)
Insulin, n (%)	5 (10.40)
Physical activity	
Light, n (%)	18 (37.50)
Moderate, n (%)	18 (37.50)
Vigorous, n (%)	1 (2.10)
Calorie intake, kcal (median [IQR])	1263.55 (1057.08–1556.03)
Carbohydrate, g (median [IQR])	170.00 (41.89)
Protein, g (median [IQR])	45.88 (38.67–76.18)
Protein g/kg BW (mean [SD])	0.50 (0.242)
Fat, g (median [IQR])	49.90 (34.77–59.93)
Smoking	
Never smoke, n (%)	34 (70.80)
Former smokers, n (%)	5 (10.40)
Current smokers, n (%)	9 (18.80)
Waist circumference, cm (mean [SD])	91.19 (11.69)
Female	89.44 (9.97)
Male	94.66 (14.27)
Body mass index, kg/m² (mean [SD])	27.59 (4.69)
Systolic blood pressure, mmHg (mean [SD])	130.55 (18.03)
Diastolic blood pressure, mmHg (median [IQR])	80 (70–80)
Fasting blood glucose, mg/dl (median [IQR])	147.00 (115.00–204.00)
HbA1c, % (median [IQR])	8.00 (7.00–10.50)
Total cholesterol, mg/dl (median [IQR])	199.00 (177.50–229.00)
HDL, mg/dl (median [IQR])	50.00 (43.755–57.75)
LDL, mg/dl (mean [SD])	129.05 (43.66)
Triglyceride, mg/dl (median [IQR])	172.50 (123.00–264.75)

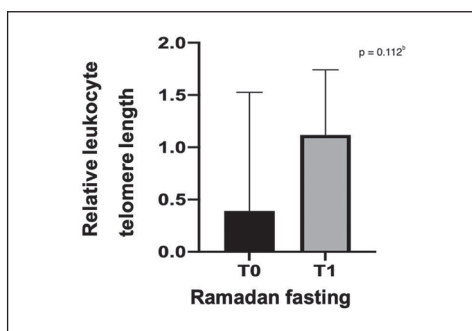


Figure 1. Boxplot of rLTL in T2DM subjects before and after Ramadan fasting.
 Note: ^b Wilcoxon test, significant *p*-value <0.05

Furthermore, this study analysed the effects of fasting on total calorie intake, metabolic profile, and anthropometric parameters in subjects with T2DM. Ramadan fasting significantly lowered total calorie intake, carbohydrate, protein in grams, protein in grams per body weight, and fat consumption (*p* <0.001, *p* = 0.002, *p* = 0.002, *p* = 0.001 and *p* = 0.002, respectively) (Figure 3). Fasting also significantly decreased anthropometric parameters, including body weight, BMI, and waist circumference. (Figure 2).

Systolic blood pressure, fasting blood glucose, HbA1c profile, and lipid profile, including total cholesterol, HDL, LDL, and triglyceride levels, were also significantly reduced after at least 14 days of Ramadan fasting (*p* <0.001, *p* = 0.001, *p* = 0.010, *p* = 0.015, *p* = 0.049, *p* = 0.002, and *p* <0.001 respectively) (Figure 3).

DISCUSSION

This study aims to evaluate the association of rLTL before and after at least 14 days of RF among subjects with T2DM. Our study showed that longer telomeres were found in subjects with T2DM who had undergone fasting for at least 14 days (T0 0.391 (0.024 – 1.515) vs T1 1.117 (0.540 – 1.729), *p* = 0.112), despite not being statistically significant. A study conducted by Asghar et al.,¹⁵ also showed a similar pattern in post-acute malarial infection. It is hypothesized that in post-infection patients, there is a decline in expression of CDKN2A which is responsible for suppressing telomerase enzyme activity during the infection process. Two distinct mechanisms regulate telomere length. Telomere shortening occurs with each round of DNA replication. When telomere length reaches its critical limit, cells undergo senescence.^{16,17} In contrast, telomere elongation is modulated by the telomerase enzyme by adding a third telomeric G-rich strand, which results in telomere elongation and cell viability and rejuvenation.^{18,19} Currently, there are no studies evaluating the effect of time-restricted feeding such as Ramadan fasting in telomerase enzyme activity and whether dynamic telomere elongation in our study results from telomerase activity or other unknown factors.

There is another explanation regarding inhibition of telomere shortening after fasting. In our study, there was a significant decrease in total calorie intake after fasting, which may affect increasing rLTL. As discussed previously, chronic inflammation is often seen in adipose tissue, exerting senescence-associated secretory phenotype (SASP) in patients with T2DM. SASP triggers age-related degenerative

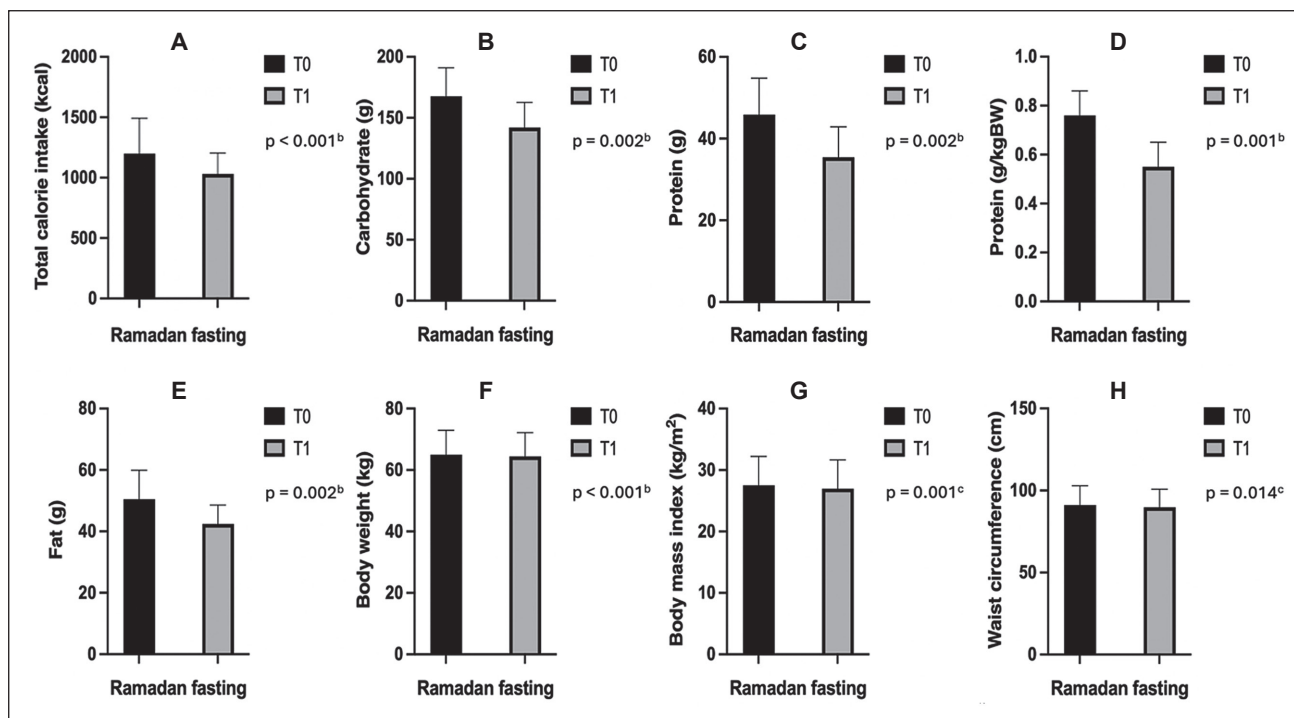


Figure 2. Changes in calorie intake and anthropometric measures after fasting; **(A)** total calorie intake, kcal (median [IQR]), **(B)** carbohydrate, g (median [IQR]), **(C)** protein, g (median [IQR]), **(D)** protein, g/kgBW (median [IQR]), **(E)** fat, g (median [IQR]), **(F)** body weight, kg (median [IQR]), **(G)** body mass index, kg/m² (mean [SD]), **(H)** waist circumference, cm (mean [SD]).
 Note: ^b Wilcoxon test, ^c Paired t-test, significant *p*-value <0.05

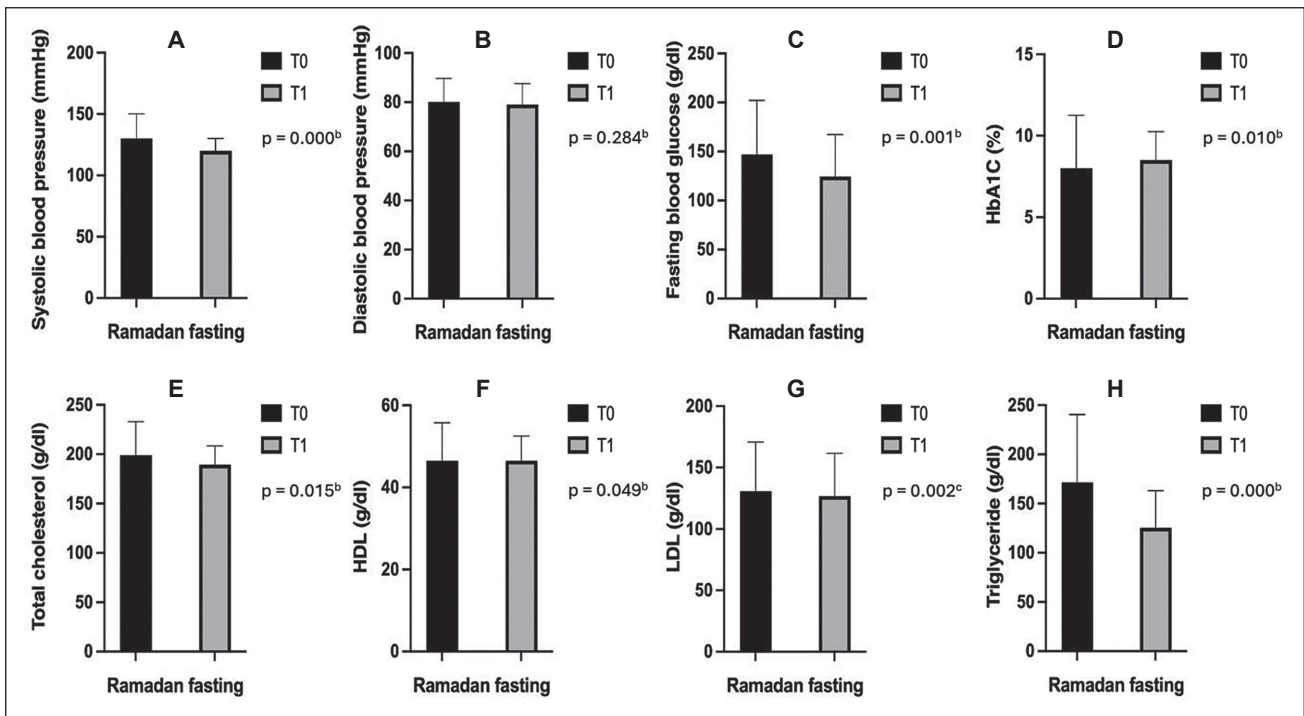


Figure 3. Changes in metabolic parameters after fasting; **(A)** systolic blood pressure, mmHg (median [IQR]), **(B)** diastolic blood pressure, mmHg (median [IQR]), **(C)** fasting blood glucose, g/dl (median [IQR]), **(D)** HbA1c, % (median [IQR]), **(E)** total cholesterol, g/dl (median [IQR]), **(F)** HDL, g/dl (median [IQR]), **(G)** LDL, g/dl (mean [SD]), **(H)** triglyceride, g/dl (median [IQR]). Note: ^bWilcoxon test, ^cPaired t-test, significant *p*-value <0.05

diseases mediated by chronic-low grade inflammation and oxidative stress.²⁰ A decrease in total calorie intake may affect the nutrient-sensing pathway by stimulating sirtuin 1 (SIRT1), AMP-activated protein kinase (AMPK), and inhibit mammalian target of rapamycin (mTOR), which plays a role in autophagy stimulation, suppressing chronic inflammation, and stimulating mitochondrial biogenesis. SIRT1 is also known to stimulate telomerase activity directly. After fasting, cells may exert an improved adaptive response to stress and DNA damage. Thus, we may infer that telomere shortening in patients with T2DM is inhibited by other factors that are suppressing chronic inflammatory processes in adipose tissue.^{21,22}

In our study, hypoglycemic episodes did not occur in all subjects. This may be due to the recruitment process that did not include patients who are high-risk for fasting based on the IDF-DAR stratification. A study conducted by Harbuwono et al.,²³ also assessed blood glucose variability using the mean amplitude of glycaemic excursion (MAGE) to evaluate glycaemic profile before and after Ramadan fasting in patients with T2DM on oral anti-diabetic agents. The study showed no significant difference before and after fasting.²³ We may conclude that Ramadan fasting is relatively safe if patients are educated to carefully monitor their blood glucose, most importantly before iftar, as hypoglycemic management before iftar may undermine their fasting on that day. Also, to reduce the risk of hypoglycemia, preliminary efforts such as hypoglycemia risk assessment and adjustment of drug dosage and food intake may be beneficial before Ramadan fasting.

To our knowledge, this was the first study discussing telomere length differences on subjects with T2DM in Indonesia. This study also pioneered evidence regarding the effects of Ramadan fasting on leukocyte telomere length in patients with T2DM. However, there were several limitations to our study. We did not include subjects with T2DM who did not undergo Ramadan fasting, because the focus of this study was to determine the association of rLTL on patients with T2DM who did RF. We also used secondary data retrospectively and encountered data availability issues, resulting in a reduced number of subjects being analyzed due to incomplete data on metabolic profile and anthropometric measures. Furthermore, data obtained from the questionnaire were relatively subjective, bringing about the issue of recall bias. However, to minimize this risk, patient data was adjusted with objective data obtained. Additionally, the minimum duration of Ramadan fasting was set to only 14 days to minimize lost-to-follow-up occurrences in the study due to subjects' unavailability during Eid-al-Fitr (end of Ramadan celebration); consequently, effects may not have reached the optimum. Lastly, this study did not consider the subjects' previous sunnah (optional) fasting habits, which may have a cumulative positive effect on telomere length.

CONCLUSION

This study showed that there was no significant difference in rLTL before and after at least 14 days of Ramadan fasting in subjects with T2DM; however, this study showed a tendency to have an increase in rLTL. Therefore, further

research is needed to evaluate the effects of time-restricted feeding, such as Ramadan fasting, on telomere length in patients with T2DM and correlate this with telomere and telomerase levels.

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Statement of Authorship

All authors certified fulfilment of ICMJE authorship criteria.

CRedit Author Statement

MDH: Formal Analysis, Writing – original draft preparation; **FK:** Methodology, Data Curation, Writing – review and editing; **PWL:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft preparation, Writing – review and editing, Supervision; **SW:** Methodology, Data curation, Writing – review and editing; **RMM:** Writing – review and editing; **AJB:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – review and editing; **DSH:** Conceptualization, Methodology, Data curation, Writing – original draft preparation, Writing – review and editing, Supervision, Funding acquisition; **DLT:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft preparation, Writing – review and editing, Supervision, Project administration, Funding acquisition.

Data Availability Statement

Datasets analyzed in the study are under license and not publicly available for sharing.

Author Disclosure

The authors declared no conflict of interest.

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