Effects of Day-On-Day-Off Fasting on Telomere Length

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Abstract

The topic of aging has been studied in scientific literature for a long time. According to research, telomeres, the repetitive nucleotide sequence at the ends of chromosomes, naturally shorten with age, exposing the genetic material contained within the chromosome to damage and leading to impaired tissue regeneration. This damage leads to a variety of negative consequences, such as cellular and tissue dysfunction. Additionally, research indicates that shorter telomeres increase the risk for many of the diseases associated with aging, such as cancer, cardiovascular disease, Type 2 diabetes, and dementia. Ideally, researchers want to identify behavioral changes that will increase telomere length (TL). Some studies have demonstrated that fasting may lengthen telomeres in addition to lowering pro-inflammatory cytokines, and increasing high-density lipoprotein (HDL - good cholesterol) levels. Given the link between fasting and these positive physiological changes, and the wide variation in fasting types and durations, research is needed to determine which fasting types and durations might increase TL. Research indicates that intermittent fasting (IF) in particular has been associated with positive effects on heart health, blood pressure, and weight loss.

This study investigated a 68-year-old man using a crossover design in which he served as both the control and test subject over 140 days. During the 70-day control phase of the study, he ate his normal diet with no IF. During the 70-day test phase, he ate during a 12-hour period (a day on), followed by a 36-hour fast (a night, a day off, and another night). He repeated this two-day IF cycle 35 times. Blood was drawn and tested before the control phase, between the two phases, and following the test phase using the 6-Panel Flow Cytometry and Fluorescence in Situ Hybridization (Flow-FISH) TL test.

Contrary to the study's hypothesis that prolonged fasting without other behavior interventions would lengthen telomeres, the results showed that during the test phase, the subject's TL declined across all six blood-cell types tested. However, during the control phase, the subject's telomeres lengthened in three of six blood-cell types tested, declined in two, and remained unchanged in one. Overall findings of this study suggest that a day-on-day-off IF over 70 days did not increase telomere length and resulted in a decline in TL, which contradicts the study's hypothesis.

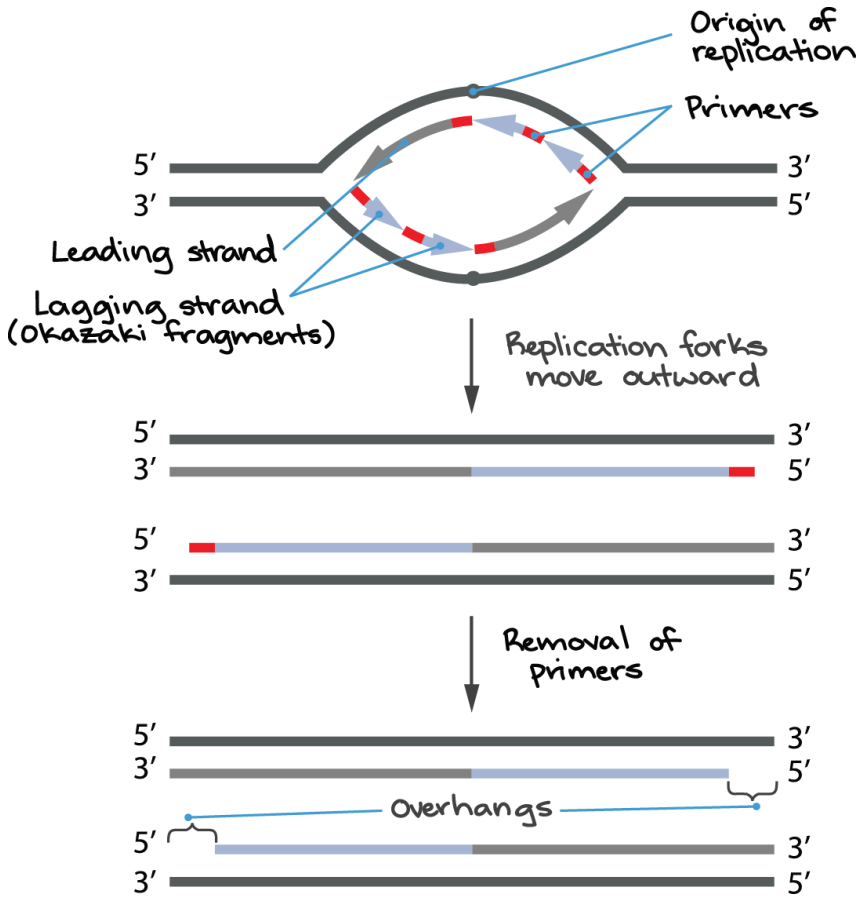
Keywords: telomeres, telomere length, intermittent fasting, aging

Introduction

Deoxyribonucleic Acid (DNA) is the genetic code that serves as the building block of all living things (Genetic Science Learning Center, 2016). Long strands of DNA are packaged into chromosomes, and at the ends of each chromosome lie telomeres, a repetitive nucleotide sequence with specialized proteins that protect the DNA within the chromosome. As people age, their telomeres shorten naturally due to degradation, leaving the genetic material packaged within the chromosome exposed and leading to a myriad of adverse effects, including cell death, mutations, and potentially even cancer (Rossiello et al., 2022; Shay & Wright, 2005). Research indicates that shorter telomeres increase the risk of many diseases associated with aging, such as cancer, cardiovascular disease, Type 2 diabetes, and dementia (Zhu et al., 2011; Terry, D. F., 2008). Researchers have found that the rate of telomere shortening is a good indicator of a person's lifespan. Those with a faster rate of telomere shortening have higher mortality rates than those with a slower rate (Guzzardi et al., 2015).

To understand why telomeres shorten, it is essential to understand the replication process and its limitations (see Figure 1). In DNA replication, a ribonucleic acid (RNA) primer is used to initiate the process, which is carried out by DNA polymerase. Polymerase can only add nucleotides to the ends of the 3' (third of five prime carbon atoms on the deoxyribose sugar molecule). There are two strands of primers during DNA replication: the leading and the lagging. The leading 3' has enough room for RNA primers to attach, meaning polymerase can then fully repair that strand. However, the lagging 3' does not have enough space for the primers to attach to it, meaning polymerase can not fix this section, leaving about 50-100 base pairs unreplicated with each replication. Thus, the telomere shortens slightly with each replication.

Figure 1: *Mechanism of end truncation during linear DNA replication.*



Note: Diagram from "Telomere shortening," by Zlir'a, public domain.

However, the natural loss of base pairs during DNA replication is not the only process that affects the rate of telomere shortening (Greider, 1990; Shay & Wright, 2005). Aribonucleoprotein, which is known as telomerase, lengthens the telomere after DNA replication. Telomerase is found only in select cell types in the body, such as stem cells and specific white blood cells. Telomerase eventually loses its functionality as the person ages due to a variety of failures in other bodily functions throughout the aging person's system, and key genetic coding begins to be erased as the TL is eroded through repeated DNA replication (Greider, 1990; Shay & Wright, 2005).

A growing body of research suggests that lifestyle choices may not only slow the degradation in telomere length (TL), but even increase TL. These lifestyle choices are exercise (anaerobic and aerobic), diet (what is eaten - quantity and quality of food; how often food is eaten - fasting), sleep (quantity and quality), and stress management. Systematic reviews, such as the one by Perez et al. in 2017, find that diet has no effect on TL, but conclude that, due to the wide variety of fasting types, it cannot be confirmed that there is no effect. A 2024 study by Almuraikhy et al, “Joint Effects of Exercise and Ramadan Fasting on Telomere Length: Implications for Cellular Aging,” shows that a fasting regimen with an exercise training program increases telomere length (TL) by lowering TNF-α, a proinflammatory cytokine. A study group of 29 young (20 to 30-year-old), non-obese ($20 \leq$ body mass index (BMI) < 30), healthy women from Qatar University was divided into a control group and a test group. Both groups underwent a 4-week exercise training program. The test group underwent the 4-week exercise training program while fasting during Ramadan. The test group’s TL increased significantly during the 4-week test period, while the exercise-only control group’s TL showed no significant change. Not only did the test group observe increased TL, but they also observed a decrease in TNF-α

and an increase in high density lipoprotein (HDL - good cholesterol). The opposite of these relationships of shorter TL to higher TNF-α and lower HDL has been shown in other studies. A larger study done in 2020 found a relationship between shorter telomeres and higher TNF-α levels (Maekawa et al.). A 2015 study done on 1082 subjects from the Helsinki Birth Cohort confirmed a relationship between shorter telomeres and lower HDL levels; this was particularly true in men (Guzzardi et al., 2015).

The Exercise and Ramadan Fasting study was conducted during 28 days of the 30-day Ramadan period, which in 2024 ran from 7 March to 6 April. The Ramadan fast begins at dawn and ends at sunset. Thus, the fasting period was getting a minute or two longer each day with the lengthening of the daylight time through the late winter and early spring. Additionally, the Ramadan fast is very strict. Ramadan fast practitioners put nothing in their bodies during the fast: no medicine, no liquids to include water, and of course no food. In 2024, the daily Ramadan fasting period began at just under 13 hours and gradually increased to just under 14 hours, four weeks later.

Fasting has been shown to increase TL, reduce proinflammatory cytokines, like TNF-α, and increase HDL levels. Due to the linkage of fasting to positive changes in both telomere length, HDL levels, and other health benefits, and given the vast variations in fasting types and periods, research is needed to determine which fasting types and periods might increase telomere length (TL). Research indicates that intermittent fasting (IF) in particular has been associated with positive health benefits. A systematic review of IF strongly associated it with benefits such as weight loss, although its long-term sustainability is questioned (Welton et al.). This study will look at a particular IF regimen as a possible means to increase TL independent

of the other lifestyle choices that may impact TL. The study's hypothesis is that adopting a day-on-day-off fasting regimen without other behavioral interventions will increase TL.

Methods

Ethics

An independent review board composed of administrators and teachers at the Augusta Preparatory Day School in Augusta, Georgia, USA, approved this study. The participant underwent a comprehensive physical on the day of his blood draw for the first telomere length measurement at the beginning of the control phase of the study, and reviewed the physical examination results with his primary care physician. The physical indicated the participant was in excellent health. The participant signed an informed consent form before the study beganand agreed to consult his primary care physician and to suspend participation if he experienced any health issues during the study.

Participant Description

The study subject is 68 years old. During the pre-study interview with the study subject, he indicated that he suffers from obstructive sleep apnea (OSA), which he mitigates with a continuous positive airway pressure (CPAP) device. He takes a statin drug, which keeps his cholesterol levels within normal range. He uses a topical steroidal cream occasionally to control eczema. He has no other chronic health conditions. He is 70 inches tall and weighs 195 pounds, yielding a BMI of 27.9, placing him near the upper end of the overweight BMI category (25-29.9). He walks his two dogs for 2 miles every morning and completes two 30-minute strength workouts at the gym each week. He indicated that he and his family would relocate from Georgia to Arizona, during the study's test phase. The study subject has routinely engaged in 8-hour-on-16-hour-off IF diets since age 50. When he was invited to participate in this study,

he engaged in a 48-day-long, day-on-day-off IF diet. This convinced him that he would have no difficulty sustaining a day-on, day-off IF diet for the 70 days required by the study.

Experimental Design

This study investigated a 68-year-old man using a crossover design, in which he served as both the control and test subject over 140 days. During the 70-day control phase, he ate his regular diet with no IF. During the 70-day test phase, which followed the control phase, he ate his regular diet for 12 hours (a day on), then fasted for 36 hours (a night, a day off, and another night). He repeated this two-day IF cycle 35 times.

Intermittent Fasting Protocol

While the study subject ate his regular diet during the control phase and during each day on of the test phase, he increased the portion size of the foods he would normally eat during the test phase to avoid excessive weight loss. During each fasting period, the study subject was encouraged to drink lots of water, allowed to drink up to two 8-ounce hot beverages, which could either be coffee or black tea with no additives, and required to drink two 8-ounce or larger servings of water each with 1 teaspoon of fresh lemon juice and a pinch of salt to help maintain a proper electrolyte balance during the long fasting period. The test subject was required to break every fast with an electrolyte-replacement beverage and a plate of fruits and vegetables to maintain proper electrolyte balance during the transition from a long fast to eating food again.

Telomere Length and Measurement

The study subject's TL was tested before the control phase, between the two phases, and following the test phase. The RepeatDX laboratory in Vancouver, Canada tested the study subject's TL using the 6-panel flow cytometry and fluorescence in situ hybridization (Flow-FISH) TL test. The test reports the TL in kilobases (kb) per pair for six blood cell types: lymphocytes,

granulocytes, naive T cells, memory T cells, B cells, and NK cells. The 6-panel Flow-FISH test, developed in 2006, is currently the most accurate commercially available TL test with low variability and high reproducibility. Its accuracy makes it the best test for diagnosing TL disorders. While it is more costly than the most common, but much less accurate, quantitative polymerase chain reaction (qPCR) test, it made sense to use in this study for two reasons: 1) the small number of study subjects, and2) the likelihood of signaling the possibility that the study subject might have a telomere disorder. Although the study subject's TL was in the 1st-10th percentiles for age and sex across all six cell types tested, this along with the absence of any TL disorder symptoms, does not indicate a telomere disorder.

Assumptions and Limitations

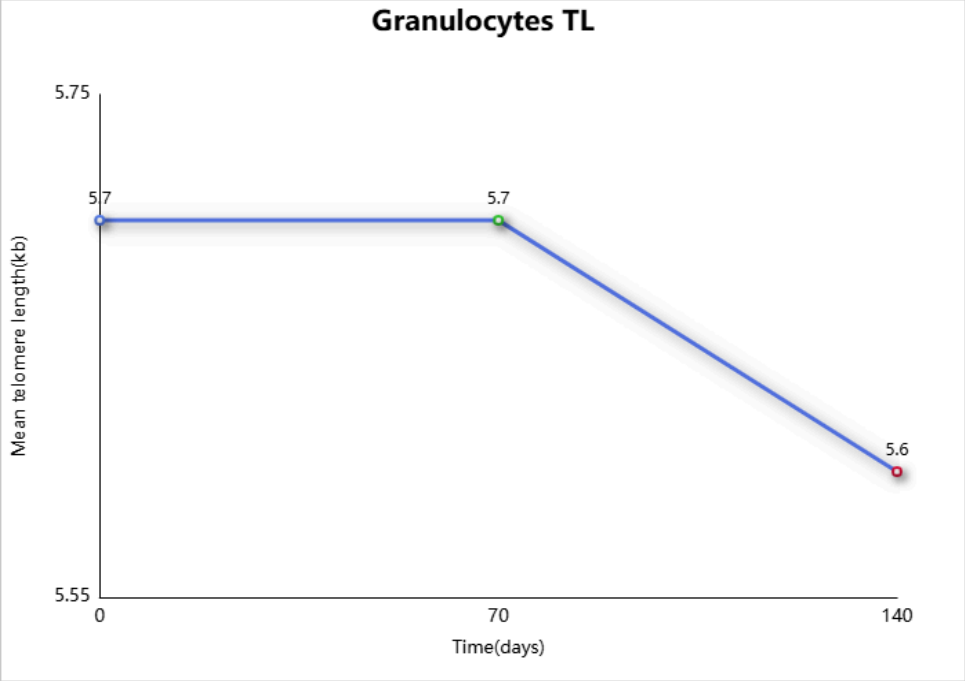
Assumptions: 1) other behavioral variables that may affect TL such as what is eaten, exercise, sleep, and stress remain nearly constant for the study subject across both phases of the study; 2) the subject followed the rules for the on and off periods during the test phase; 3) the test subject does not have a TL disorder, which was mitigated by using the most accurate telomere test for diagnosing TL disorders, and having the tests ordered and reviewed by his primary care physician. Limitations: 1) the study had a sample group of one for both the control and the test phases; 2) the same individual served as both the control and test groups; 3) the TL test was only performed at the beginning and end of each study phase with no tests during the phases; 4) no other behavioral variables that may affect TL were monitored during the control and test phases of the study.

Results

The six charts in Figures 2-7 show telomere lengths for each of the six blood-cell types, measured using the 6-panel Flow-FISH TL tests. The three length points on each chart

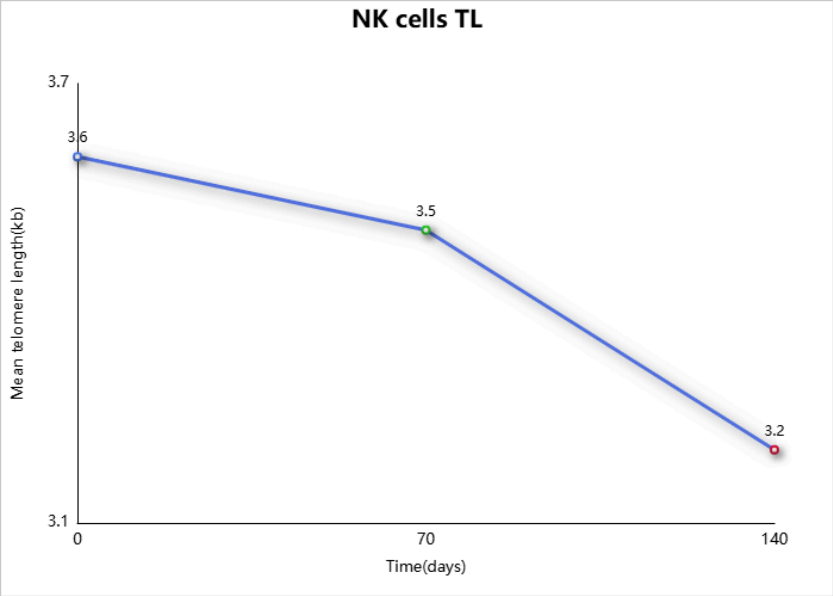
represent the results of the three TL tests conducted during the study. The first blood draw was on February 10, 2025, at the beginning of Phase 1, the second blood draw at the end of Phase 1 and beginning of Phase 2 took place on April 21, 2025, and the final blood draw at the end of Phase 2 took place on June 30, 2025.

Figure 2: *Telomere length of granulocyte blood cells*



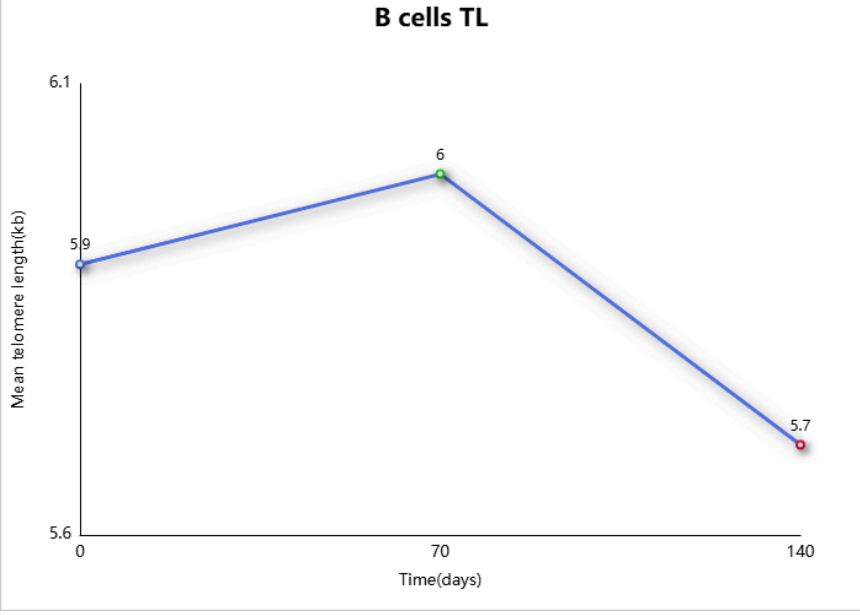
Note. The graph shows no change in granulocyte TL during the control phase of the study, but a 1 kb decrease during the test phase. Thus, the total change in length over the duration of the study was minus .1 kb.

Figure 3: *Telomere length of NK blood cells*



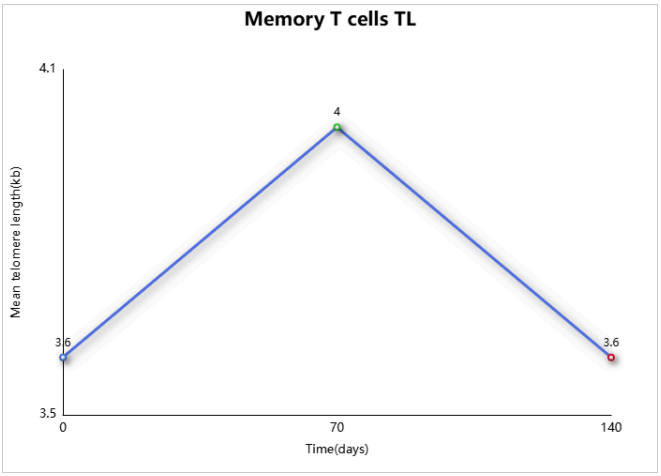
Note. The graph shows that the NK cell TL decreased by 0.1 kb during the control phase and by an additional 0.3 kb during the test fasting phase. This resulted in a total decrease in length of .4 kb.

Figure 4: *Telomere length of B blood cells*



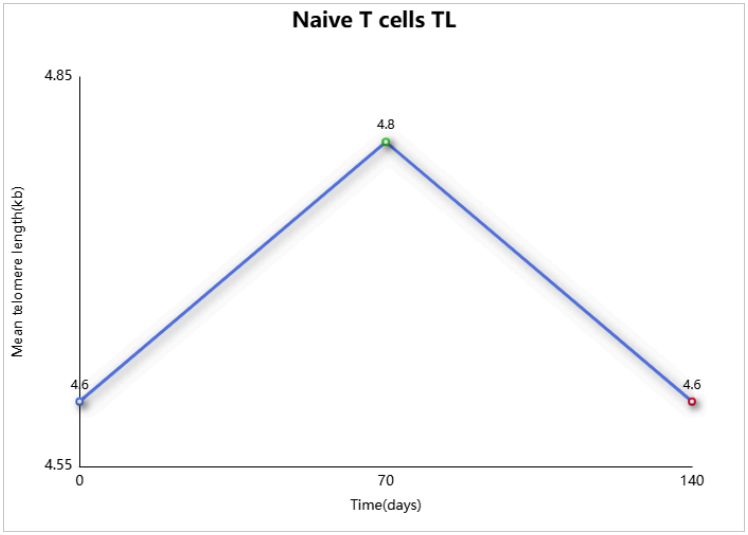
Note. The graph shows that B-Cell TL increased by 0.1 kb during the control phase and decreased by 0.3 kb during fasting. Thus, the total reduction in length during the study was .2 kb.

Figure 5: *Telomere length of memory T blood cells*



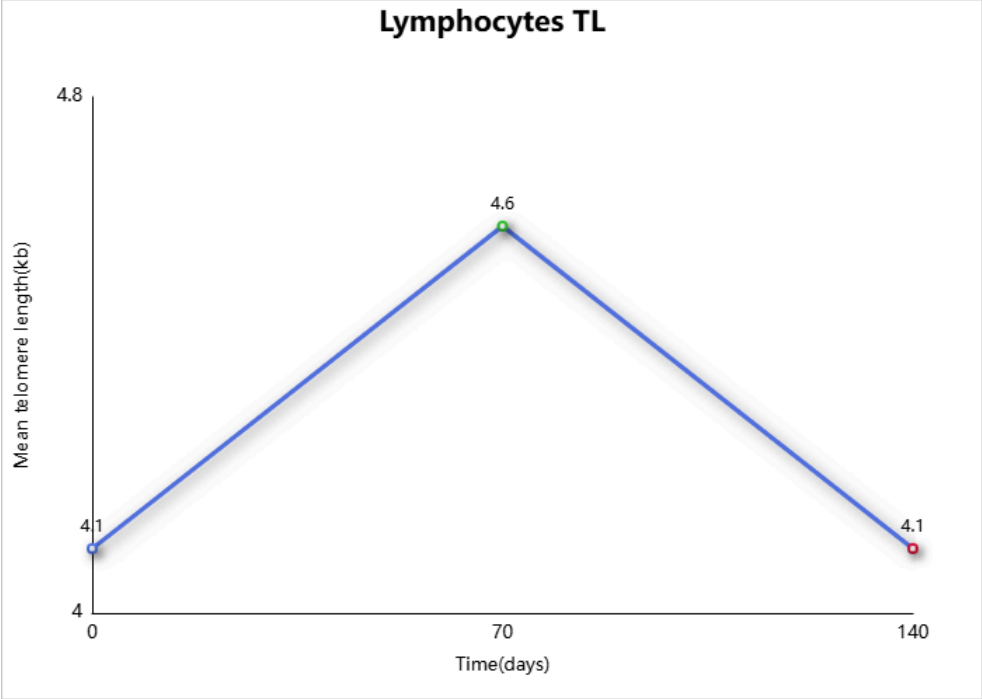
Note. The graph shows that memory T-cell TL increased by 0.4 kb during the control phase and decreased by 0.4 kb during the test phase. Thus, there was no change in length during the study.

Figure 6: *Telomere length in Naive T blood cells*



Note. The graph shows naive T cells TL increased by 0.2 kb during the control phase of the study and decreased by 0.4 kb during the test phase. Thus, there was no change in length during the study.

Figure 7: *Telomere length of lymphocytes*



Note. The graph shows the lymphocyte TL increased by 0.5 kb during the control phase of the study and decreased by 0.5 kb during fasting. Thus, there was no change in TL during the study.

Discussion

The data from the study's results do not support the hypothesis that adopting a day-on-day-off fasting regimen for 70 days without other behavioral interventions will increase TL. In fact, during the test phase of the study, the TL of all six blood-cell types decreased by an average of 0.3 kb per blood-cell type. During the control phase, the subject's telomeres lengthened in four of six blood-cell types tested, declined in one, and remained the same length

in one, with an average increase of 0.2 kb per blood-cell type. During the entire study, the TL of three cell types was the same for the first and last TL tests, and three decreased by one to four kilobase pairs with an average decrease per blood-cell type of .1 kb. Additionally, the study subject's telomere lengths across all six blood-cell types and all three TL tests were in the low range for his age, between the 1st and 10th percentiles.

Previous research and meta-analyses have not shown that IF reduces TL. Thus, other factors may have contributed to the TL shortening observed in this study during the test phase. One potential confounding factor was the subject's relocation, which occurred during the test phase. The study participant reported missing his two-mile walks with his dogs and his gym workouts for approximately half of the test phase. He also indicated that he had to give up some sleep during the test phase to do all the moving-related activities in addition to all the study subject's usual daily activities. Finally, the study subject reported experiencing mild fatigue during the final 3-4 weeks of the IF intervention. The telomere shortening pressure from lack of sleep (Carrol, J. E. & Prather, A. A., 2021) and exercise (Sánchez-González, J. L., 2025) along with regular shortening due to aging may have overcome any telomere lengthening benefits from the IF intervention. Finally, the IF intervention may have been too severe, particularly with respect to the fasting period and the intervention duration. This may have led to physiological stress, which would explain the mild fatigue and, ultimately, greater telomere shortening pressure toward the latter part of the test phase.

Another potentially confounding factor in the study was the subject's 48-day self-assurance fast before the study's initiation. Interestingly, the study subject's average TL of all six blood cell types increased during the control phase. This may have been due to some residual effect of the study subject's 48-day self-assurance fast prior to the control phase. This

seems even more likely given the low length range of the study subject's telomeres. It is unlikely that his normal lifestyle, without intervention, would result in telomere lengthening over a 70-day period. Additionally, the shorter 48-day pre-study day-on-day-off IF period, which appears to have influenced telomere lengthening during the control phase of the study, strengthens the case for the 70-day study IF period creating telomere shortening pressure towards the end of the IF intervention period.

Conclusion

While the results of this study did not support the hypothesis, and it had some severe limitations, its results map a clear path to future research. First the results of this study would have been more meaningful, and quite likely more explainable, had additional TL tests been conducted periodically during the control and test phases of the study and had other behavioral variables been tracked during the control and test phases of the study. The results raise the possibility that even though an IF regimen may be safe for a particular individual, it may actually result in shorter TL. Thus, future research needs to focus on optimizing the IF regimen to provide maximum TL increases. Intermittent fasting without consideration of other lifestyle factors may not always increase TL. Intermittent fasting is one of many lifestyle factors that probably can be used to manage an individual's telomere length. Based on the results of this study, it could reasonably be assumed that additional research may be necessary to optimize TL increase in other behavioral interventions such as sleep and exercise. Thus, similar to the Exercise and Ramadan Fasting study (Almuraikhy, S., 2024), future research must look at how combinations of lifestyle changes affect TL and how to optimize combinations of lifestyle changes to maximize telomere lengthening. Finally, future research should look at the possibility

of tailoring interventions to each individual and their particular circumstances in any effort to restore TL lost to age.

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