

## ABSTRACT

**CROSS-SECTIONAL ANALYSIS OF TELOMERE LENGTH IN PEOPLE 33-80 YEARS OF AGE: EFFECTS OF DIETARY SUPPLEMENTATION.** Calvin B. Harley, PhD<sup>1</sup>; Joanne Chan, BS<sup>1</sup>; Marsha Blauwkamp, PhD<sup>1</sup>; Francis C. Lau, PhD, FACN<sup>2</sup>; Jamie F. McManus, MD, FAAFP<sup>2</sup>; Drew Watson, PhD<sup>1</sup>; Evangelos Hytopoulos, PhD<sup>1</sup>; and Bruce P. Daggy, PhD, FACN<sup>2</sup>. <sup>1</sup>Telomere Diagnostics Inc., Menlo Park, CA; <sup>2</sup>Shaklee Research Center, Pleasanton, CA.

Telomere length has been associated with aging, age-related diseases, adverse conditions, and mortality. Moreover, studies in humans suggest a causal role of short telomeres or accelerated telomere shortening in disease and mortality risk. A previous cross-sectional study has shown that supplement usage significantly improved various health parameters and nutritional status. The objective of the current cross-sectional study was to explore the effect of dietary supplementation on telomere length.

The normal range of telomere lengths was determined from saliva samples in a population of healthy, non-smoking subjects aged 33-80 from the San Francisco Bay Area (control group; n=324; 147 males and 177 females) who took no more than 3 supplements daily. The telomere lengths of heavy supplement users (supplement group; n=80; 21 males and 59 females), the majority of whom took more than 12 supplements at least 4 days per week, were compared to the age-matched control group. Disease and smoking status were not exclusion criteria for the supplement group. Telomere length was measured by quantitative PCR to determine the telomere-to-single copy gene (T/S) ratio. Change in T/S ratio over time was fitted to a linear regression. Blood biomarkers were also assessed.

Overall, women had longer telomeres than men in the control group, but this trend was reversed in the supplement group. T/S ratio of the supplement group was 11.2% greater than that of the control group ( $p < 0.0001$ ). Supplementation resulted in a greater treatment effect in men vs. women ( $p < 0.005$ ). By linear regression, the rate of change in T/S ratio was reduced by 40% in the supplement group vs control. Blood biomarkers in both groups were comparable and were within the normal physiological ranges.

The results of this cross-sectional study suggest that heavy dietary supplementation significantly attenuated telomere shortening in subjects compared to a healthy control group. Longitudinal studies are warranted to further explore the link between nutritional supplementation and healthy aging in the context of reduced rate of telomere shortening.

## BACKGROUND AND OBJECTIVES

Telomere length is a biomarker of overall health status. It appears to be an "integrator" of a broad range of current and lifelong factors that impact health, including genetics, diet, fitness, toxins, and chronic stress. Telomere length is thought to reflect physiological age (as opposed to chronological age) as well as health status based on studies demonstrating that short telomeres accelerate age-related decline and disease in the body. In addition, telomeres are the "changeable" part of the genome, and studies have suggested that improved lifestyle choices can increase telomere length and promote individual wellness. A previous cross-sectional study has shown that supplement usage significantly improved various health parameters and nutritional status [1]. It is unclear whether dietary supplementation influences telomere length. The goal of this study was to investigate the effect of dietary supplementation on telomere length in healthy non-supplement users and heavy supplement users between the ages of 30-80.

[1]. Usage patterns, health, and nutritional status of long-term multiple dietary supplement users: a cross-sectional study. Block G, Jensen CD, Norkus EP, Dalvi TB, Wong LG, McManus JF, Hudes ML. Nutr J. 2007 Oct 24;6:30.

# Cross-Sectional Analysis of Telomere Length in People 33-80 Years of Age: Effects of Dietary Supplementation

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## METHODS

**Study design:** cross-sectional study

**Population:** 1) Control group – San Francisco Bay Area healthy male and female non-smokers aged 30-80 who took no more than 3 supplements daily. 2) Supplement group – male and female supplement users aged 30-80 who took at least 5 supplements 4-6 times weekly for at least 5 years regardless of their health and/or smoking status. Participants in this group were from all regions of the country.

**Recruitment:** 1) Control group – recruited through agreements with local companies who agreed to advertise the study and allow their employees to participate at the workplace by sending email flyers from HR department. Other residents in the Bay area were recruited through, newspaper and radio advertisements, fliers and craigslist ads. 2) Supplement group – recruited at the Shaklee 2012 Leadership Conference held in San Francisco.

**Inclusion criteria:** 1) Control group – healthy men and women aged 30-80 residing in SF Bay Area who were English-speaking and willing to sign the informed consent form (ICF) and keep healthy behaviors stable for one year. 2) Supplement group: men and women aged 30-80 residing in mainland USA who used at least 5 supplements 4-6 times weekly for at least 5 years and were willing to sign the ICF.

**Exclusion criteria:** 1) Control group – those with health conditions and taking medications that would affect telomere length, all smokers (cigarettes or recreational drugs), BMI>35, people taking more than 3 daily supplements. 2) Supplement group – no exclusion if inclusion criteria were satisfied.

**Study site:** sample collection and processing as well as telomere length assay and data collection were performed by Telomere Diagnostics, Menlo Park, CA.

**Telomere length test:** qPCR was used to measure average telomere length per genome (i.e. telomere-to-single copy gene (T/S) ratio) in saliva cellular DNA. Saliva was used because of its ease of collection and storage. Saliva samples were collected with Oragene DNA collection kit (Ont., Canada).

**Statistics:** Telomere length was reported as a T/S ratio (telomere signal normalized relative to a single copy gene signal). T/S ratios of the supplement group were compared to those in the age-matched control group. Student's *t*-test was used for comparisons between two endpoints. For comparison of multiple endpoints, ANOVA was used. *P*-values less than 0.05 were considered to be statistically significant.

## RESULTS

Table 1. Demographic characteristics

Parameter	Control (n=324)	Supplement (n=80)
Female [n (%)]	177(54.6)	59 (73.8)
Age (years)	56.4 ± 0.7	61.2 ± 1.3
Total Fe (µg/dL)	104.1 ± 1.8	119.9 ± 3.5*
TIBC (µg/dL)	340.6 ± 2.6	353.5 ± 4.4*
Glucose (mg/dL)	87.2 ± 0.6	91.3 ± 1.0*
Triglycerides (mg/dL)	102.2 ± 3.0	94.5 ± 4.7
Cholesterol ratio	3.30 ± 0.06	3.26 ± 0.12

Values expressed in Mean ± SEM where applicable; \* $P < 0.05$  vs. Control.

Table 2. Summary of Telomere Length (TL) Between Groups

	Control (n=324)	Supplement (n=80)	P-value	% Increase vs. Control
Overall	1.10 ± 0.01	1.23 ± 0.02	< 0.0001*	11.20
Male	1.09 ± 0.02	1.30 ± 0.06	< 0.0010*	19.61
Female	1.12 ± 0.01	1.20 ± 0.02	< 0.0025*	7.60

\* Significantly different from control group.

Figure 1. Effect of dietary supplements on TL

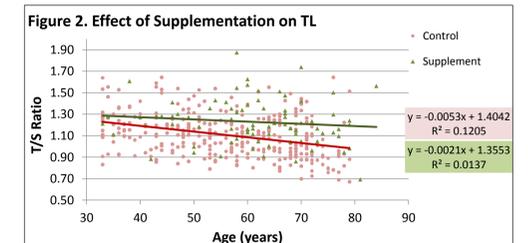
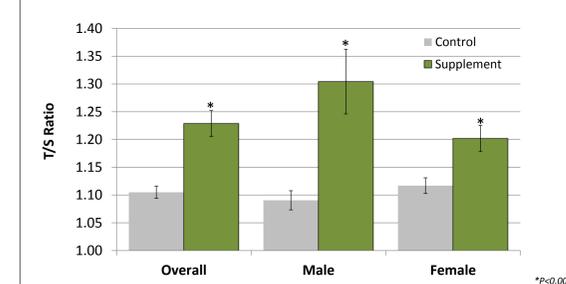


Figure 3. Effect of Supplementation on TL (Women)

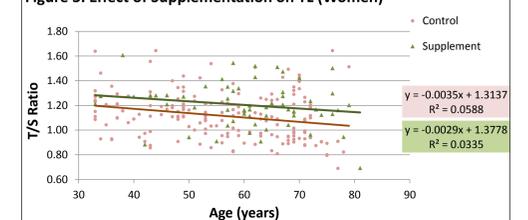
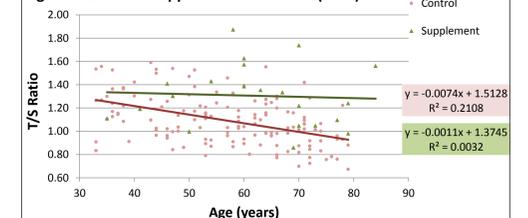


Figure 4. Effect of Supplementation on TL (Men)



## SUMMARY

- Demographic data showed that on average, the control group was younger than the supplement group even though the two groups were age-matched (Table 1).
- Participants in the supplement group were heavy supplement users who took more than 12 supplements at least 4 days per week for at least 10 years; much higher than the minimum requirements stipulated in the inclusion criteria.
- Supplement group had significantly higher total iron, total iron binding capacity and fasting glucose levels than those in the control group; however, these values were within normal range for both groups (Table 1).
- No significant difference in blood triglyceride concentration or cholesterol ratio was observed between the control and supplement group (Table 1).
- Supplement group had significantly greater T/S ratio compared to control group (Fig. 1).
- Women had longer telomeres than men in the control group, but this trend was reversed in the supplement group (Fig. 1).
- T/S ratio of the supplement group was 11.2% greater than that of the control group ( $p < 0.0001$ ). Supplementation resulted in a greater treatment effect in men vs. women ( $p < 0.005$ ) (Table 2).
- Linear regression indicated that the rate of change in T/S ratio was reduced by 40% in the supplement group vs control (Fig. 2).

## CONCLUSIONS

- The results of this cross-sectional study suggest that dietary supplementation significantly attenuated telomere shortening.
- Longitudinal studies are warranted to further explore the link between nutritional supplementation and healthy aging in the context of reduced rate of telomere shortening.