Association of Marine Omega-3 Fatty Acid Levels With Telomeric Aging in Patients With Coronary Heart Disease

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http://jama.ama-assn.org/cgi/content/full/303/3/250
Association of Marine Omega-3 Fatty Acid Levels With Telomeric Aging in Patients With Coronary Heart Disease

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Context Increased dietary intake of marine omega-3 fatty acids is associated with prolonged survival in patients with coronary heart disease. However, the mechanisms underlying this protective effect are poorly understood.

Objective To investigate the association of omega-3 fatty acid blood levels with temporal changes in telomere length, an emerging marker of biological age.

Design, Setting, and Participants Prospective cohort study of 608 ambulatory outpatients in California with stable coronary artery disease recruited from the Heart and Soul Study between September 2000 and December 2002 and followed up to January 2009 (median, 6.0 years; range, 5.0-8.1 years).

Main Outcome Measures We measured leukocyte telomere length at baseline and again after 5 years of follow-up. Multivariable linear and logistic regression models were used to investigate the association of baseline levels of omega-3 fatty acids (docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]) with subsequent change in telomere length.

Results Individuals in the lowest quartile of DHA/EPA experienced the fastest rate of telomere shortening (0.13 telomere-to-single-copy gene ratio [T/S] units over 5 years; 95% confidence interval [CI], 0.09-0.17), whereas those in the highest quartile experienced the slowest rate of telomere shortening (0.05 T/S units over 5 years; 95% CI, 0.02-0.08; P < .001 for linear trend across quartiles). Levels of DHA/EPA were associated with less telomere shortening before (unadjusted coefficient $10^{-3}$ = 0.06; 95% CI, 0.02-0.10) and after (adjusted coefficient $10^{-3}$ = 0.05; 95% CI, 0.01-0.08) sequential adjustment for established risk factors and potential confounders. Each 1-SD increase in DHA/EPA levels was associated with a 32% reduction in the odds of telomere shortening (adjusted odds ratio, 0.68; 95% CI, 0.47-0.98).

Conclusion Among this cohort of patients with coronary artery disease, there was an inverse relationship between baseline blood levels of marine omega-3 fatty acids and the rate of telomere shortening over 5 years.
mers and cardiovascular morbidity and mortality has been documented in several populations.\textsuperscript{10-12}

Little is known concerning the dynamic regulation of telomere length over time, although it has recently become apparent that telomeres may lengthen as well as shorten.\textsuperscript{13,14} Given the cardioprotective effects of omega-3 fatty acids, we sought to determine whether omega-3 fatty acid levels were associated with changes in leukocyte telomere length over 5 years in a prospective cohort study of outpatients with coronary artery disease.

**METHODS**

**Participants**

The Heart and Soul Study is a prospective cohort study investigating the influence of psychosocial factors on cardiovascular events in stable coronary artery disease. The enrollment process has been previously described.\textsuperscript{15} Eligible participants were recruited from outpatient clinics in the San Francisco Bay Area of California if they met at least 1 of the following inclusion criteria: (1) history of myocardial infarction; (2) angiographic evidence of at least 50% stenosis by area in at least 1 coronary artery; (3) evidence of exercise-induced ischemia on treadmill electrocardiogram or stress nuclear perfusion imaging; or (4) history of coronary revascularization. Individuals were excluded if they had a history of myocardial infarction in the past 6 months, deemed themselves unable to walk 1 block, or were planning to move out of the local area within 3 years.

The study protocol was approved by the University of California San Francisco Committee on Human Research, the Research and Development Committee at the San Francisco Veterans Administration (VA) Medical Center, the Medical Human Subjects Committee at Stanford University, the Human Subjects Committee at the VA Palo Alto Health Care System, and the Data Governance Board of the Community Health Network of San Francisco. All participants provided written informed consent. Between September 2000 and December 2002, a total of 1024 participants enrolled in the study and completed a baseline examination including a venous blood draw. Five years later, all surviving participants were invited to return for a repeat examination. Of the 1024 original enrollees, 195 had died before the 5-year examination. Between September 2005 and December 2007, 667 (80%) of the eligible 829 participants completed the 5-year follow-up examination. Of 667 participants who completed the 5-year examination, 29 were excluded because they did not have baseline omega-3 measurements, 23 were excluded because they did not have baseline telomere length measurements, and 7 were excluded because they did not have 5-year telomere length measurements, leaving 608 participants for this analysis. Compared with the 221 participants who were alive at 5 years but not included in the analysis, these 608 participants had similar ages ($P=.27$), baseline omega-3 fatty acid levels ($P=.14$), and baseline telomere length ($P=.24$).

**Marine Omega-3 Fatty Acid Assay**

Levels of the marine omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were measured in fasting whole blood. Fatty acid methyl esters generated by treatment with boron trifluoride-methanol were analyzed by capillary gas chromatography (GC2010 [Shimadzu Corp, Columbia, Maryland] equipped with a 100-m SP2560 column [Supelco, Bellefonte, Pennsylvania]) and identified by comparison with a known standard (GLC-727; Nuchek Prep, Elysian, Minnesota). Blood levels of marine omega-3 fatty acids (EPA + DHA) were expressed as a percentage of total fatty acid methyl esters.\textsuperscript{16,17} The coefficient of variation for EPA + DHA was 5% to 6%.

**Telomere Length Assay**

Genomic DNA was isolated according to standard procedures from peripheral blood leukocytes collected at baseline and follow-up study visits and stored at −70°C. Purified DNA samples were diluted in 96-well microtiter source plates to a fixed concentration of 3 ng/μL. Relative mean telomere length was measured from DNA by a quantitative polymerase chain reaction (qPCR) assay that compares mean telomere repeat sequence copy number (T) to a reference single-copy gene copy number (S) in each sample as previously described and validated.\textsuperscript{18-20} The T/S ratio was determined from the mean quantity of reference DNA found to match with each experimental sample for the copy number of the targeted template (the number of telomere repeats for T and the number of beta-globin copies for S).

The primers for the telomere qPCR were tel1b (5’-CGGTTTGTTTGG-3’) and tel2b (5’-GGCTTGCTTAC5CCT-3’), each used at a final concentration of 900 nM. Human beta-globin qPCR primers were hbg1 (5’-GCTTCTGACAACACTGTG-3’) and hbg2 (5’-CACCAACTTCATCCAGTTCCAC-3’), used at a final concentration of 300 nM, and hbg2 (5’-CACCAACTTCATCCAGTTCCAC-3’), used at a final concentration of 700 nM. All PCRs were carried out on a Roche Lightcycler 480 real-time PCR machine (Roche Applied Science, Indianapolis, Indiana).

The T/S ratio at baseline and follow-up for each participant was measured in duplicate. When the duplicate T/S value and the initial value varied by more than 7%, the sample was run for a third time, and the 2 closest values were used to calculate the mean. Approximately 15% of samples required assay in triplicate. Using this method, the interassay coefficient of variability for telomere length measurement was 3.7%. The intra-assay coefficient of variability was 2.5%.

**Other Measurements**

Baseline demographics, age, sex, cardiovascular comorbidities, income, edu-
cation, and medical history were determined by questionnaire. Race/ethnicity has previously been shown to be associated with telomere length and was therefore ascertainment for inclusion in multivariable adjustment models. Participants self-identified their race/ethnicity by questionnaire in the following categories: Hispanic, Asian, black, white, and other.

Exercise capacity was determined at peak exertion during a symptom-limited exercise treadmill test as previously described. Medication use was determined by having participants bring bottles to the study appointment, during which study personnel recorded all medications. Medication use was categorized dichotomously using Epocrates Rx (San Mateo, California). Information regarding omega-3 fatty acid supplements was not collected. Waist and hip circumferences were measured with a flexible plastic measure to the nearest 0.1 cm.

Fasting venous blood samples were obtained at the baseline visit to measure serum biomarkers. Fasting high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and C-reactive protein (CRP) were measured in a clinical laboratory setting. C-reactive protein was measured using the Roche Integra high-sensitivity assay (Roche, Indianapolis) or (because of a change in the laboratory) the Beckman Extended Range high-sensitivity CRP assay (Beckman, Galway, Ireland). The R&D Systems (Minneapolis) Quantikine HS IL-6 Immunoassay was used to determine the concentration of interleukin 6 (IL-6). Blood samples were also obtained at the follow-up visit and stored as buffy coat, serum, and plasma.

All patients underwent complete resting 2-dimensional echocardiography and Doppler examination using an Acuson Sequoia ultrasound system (Siemens Medical Solutions, Mountain View, California) with a 3.5-MHz transducer. Left ventricular ejection fraction was calculated as (end diastolic volume – end systolic volume)/end diastolic volume.

**Statistical Analyses**

In the absence of established cutoffs, we categorized participants a priori by quartiles of omega-3 fatty acid levels for descriptive purposes. Continuous variables with a skewed distribution were natural logarithm transformed (DHA + EPA, HDL-C, LDL-C, triglycerides, CRP, and IL-6). Differences in means and proportions of baseline characteristics were compared using 1-way analysis of variance and the chi² test, respectively. Based on a total of 608 participants (n = 152 in each quartile), we had more than 80% power to detect an interquartile difference in the rate of telomere shortening of 0.07 T/S units over 5 years, assuming an SD of 0.2. The significance level was a 2-tailed α of 0.05. Analysis of covariance was used to calculate the mean change in telomere length in each quartile, adjusted for age and baseline telomere length.

We used generalized linear regression models to determine the relationship between the natural logarithm of omega-3 fatty acid levels and change in telomere length (T/S ratio) as a continuous variable. Mixed models were not required because the difference between baseline and follow-up telomere length for each participant collapsed into a single observation rather than repeated measures. Multivariable adjustment was made for potential demographic, clinical, and biochemical confounders based on published associations and biological plausibility. Adjustment was also made for known downstream mediators of omega-3 fatty acid effects, including blood pressure, triglyceride levels, and biomarkers of systemic inflammation. Grouped covariates were added sequentially in multivariable models to assess the incremental attenuation attributable to each adjustment step. Within each adjustment step, all covariates were added simultaneously. The final adjustment model also included baseline telomere length, which has previously been identified as the most powerful predictor of subsequent change in telomere length.
sion models. Model adequacy was confirmed using the Hosmer-Lemeshow goodness-of-fit test. The presence of outlying and influential points was explored by visual inspection of standardized Pearson residuals.

To explore potential effect modifiers, we tested for interaction between omega-3 fatty acid levels and age, baseline telomere length, sex, race/ethnicity, smoking, income, education, and type 2 diabetes in the final multivariable-adjusted model. Stratified analysis was also performed for subgroups of age, baseline telomere length, sex, race/ethnicity, smoking, income, education, and type 2 diabetes mellitus. All subgroup analyses were prespecified. Statistical analysis was performed using Intercooled Stata, version 10.0 (Stata Corp, College Station, Texas).

RESULTS

All measurements except for follow-up telomere length were made at the baseline visit. The distribution of baseline omega-3 fatty acid levels was right-skewed (mean, 4.3%; median, 3.7%). In quartile analysis, higher levels of baseline omega-3 fatty acids were significantly associated with older age, white race/ethnicity, higher income, higher education level, and higher HDL-C and were inversely associated with history of myocardial infarction, type 2 diabetes mellitus, current smoking, waist-hip ratio, triglyceride levels, CRP, and IL-6 (Table 1). There was no significant association between omega-3 fatty acid levels and baseline telomere length (unadjusted P = .06; adjusted for age, P = .20).

Baseline omega-3 fatty acid levels were positively correlated with 5-year change in telomere length (r = 0.13; P = .001). The relationships between quartile of baseline omega-3 fatty acid levels and mean absolute and relative changes in telomere length, adjusted for age and baseline telomere length, are shown in the FIGURE. Individuals in the lowest quartile experienced a decrease in telomere length of 0.13 T/S units (95% confidence interval [CI],

### Table 1. Baseline Characteristics of Study Population by Quartile of Marine Omega-3 Fatty Acid Levels

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1 (n = 152)</th>
<th>2 (n = 152)</th>
<th>3 (n = 152)</th>
<th>4 (n = 152)</th>
<th>P Value 1-Way ANOVA or ( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine omega-3 fatty acid level</td>
<td>2.3 (1.1-2.8)</td>
<td>3.3 (2.9-3.7)</td>
<td>4.3 (3.8-5.1)</td>
<td>7.3 (5.2-18.4)</td>
<td></td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>64 (11)</td>
<td>66 (9)</td>
<td>67 (10)</td>
<td>67 (10)</td>
<td>.005</td>
</tr>
<tr>
<td>Male, No. (%)</td>
<td>128 (84)</td>
<td>123 (81)</td>
<td>120 (79)</td>
<td>128 (84)</td>
<td>.55</td>
</tr>
<tr>
<td>White, No. (%)</td>
<td>84 (55)</td>
<td>84 (55)</td>
<td>96 (63)</td>
<td>98 (64)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Income &gt;=50,000, No. (%)</td>
<td>19 (13)</td>
<td>25 (16)</td>
<td>34 (23)</td>
<td>52 (35)</td>
<td>.001</td>
</tr>
<tr>
<td>Education beyond high school, No. (%)</td>
<td>106 (69)</td>
<td>96 (63)</td>
<td>112 (74)</td>
<td>130 (86)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Exercise capacity, mean (SD), METs</td>
<td>7.5 (2.9)</td>
<td>7.3 (3.0)</td>
<td>8.0 (3.1)</td>
<td>9.1 (3.6)</td>
<td>.06</td>
</tr>
<tr>
<td>Prior myocardial infarction, No. (%)</td>
<td>94 (62)</td>
<td>82 (55)</td>
<td>69 (45)</td>
<td>72 (47)</td>
<td>.02</td>
</tr>
<tr>
<td>Prior congestive heart failure, No. (%)</td>
<td>21 (13)</td>
<td>23 (15)</td>
<td>21 (14)</td>
<td>25 (17)</td>
<td>.90</td>
</tr>
<tr>
<td>Prior stroke, No. (%)</td>
<td>22 (14)</td>
<td>21 (14)</td>
<td>13 (9)</td>
<td>19 (13)</td>
<td>.40</td>
</tr>
<tr>
<td>Type 2 diabetes, No. (%)</td>
<td>47 (31)</td>
<td>45 (30)</td>
<td>27 (18)</td>
<td>27 (18)</td>
<td>.004</td>
</tr>
<tr>
<td>Smoking, No. (%)</td>
<td>41 (27)</td>
<td>31 (20)</td>
<td>20 (13)</td>
<td>9 (6)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Waist-hip ratio, mean (SD)</td>
<td>0.96 (0.08)</td>
<td>0.96 (0.08)</td>
<td>0.95 (0.08)</td>
<td>0.94 (0.07)</td>
<td>.04</td>
</tr>
<tr>
<td>Blood pressure, mean (SD), mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>134 (20)</td>
<td>134 (21)</td>
<td>134 (21)</td>
<td>129 (19)</td>
<td>.13</td>
</tr>
<tr>
<td>Diastolic</td>
<td>76 (11)</td>
<td>75 (12)</td>
<td>74 (11)</td>
<td>74 (11)</td>
<td>.33</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, mean (SD), %</td>
<td>62 (9)</td>
<td>62 (8)</td>
<td>63 (8)</td>
<td>63 (10)</td>
<td>.22</td>
</tr>
<tr>
<td>Statin use, No. (%)</td>
<td>95 (63)</td>
<td>113 (74)</td>
<td>102 (67)</td>
<td>113 (74)</td>
<td>.06</td>
</tr>
<tr>
<td>β-Blocker use, No. (%)</td>
<td>100 (66)</td>
<td>89 (59)</td>
<td>85 (56)</td>
<td>77 (51)</td>
<td>.06</td>
</tr>
<tr>
<td>ACE inhibitor/ARB use, No. (%)</td>
<td>74 (49)</td>
<td>83 (55)</td>
<td>73 (48)</td>
<td>79 (52)</td>
<td>.60</td>
</tr>
<tr>
<td>Aspirin use, No. (%)</td>
<td>118 (78)</td>
<td>124 (82)</td>
<td>119 (78)</td>
<td>114 (75)</td>
<td>.58</td>
</tr>
<tr>
<td>Vitamin use, No. (%)</td>
<td>25 (16)</td>
<td>33 (22)</td>
<td>38 (25)</td>
<td>32 (21)</td>
<td>.33</td>
</tr>
<tr>
<td>HDL-C, mean (SD), mg/dL</td>
<td>45 (14)</td>
<td>44 (13)</td>
<td>47 (15)</td>
<td>48 (14)</td>
<td>.03 a</td>
</tr>
<tr>
<td>LDL-C, mean (SD), mg/dL</td>
<td>111 (39)</td>
<td>102 (29)</td>
<td>103 (33)</td>
<td>100 (32)</td>
<td>.04 a</td>
</tr>
<tr>
<td>Triglycerides, mean (SD), mg/dL</td>
<td>208 (239)</td>
<td>137 (88)</td>
<td>121 (79)</td>
<td>99 (50)</td>
<td>&lt;.001 a</td>
</tr>
<tr>
<td>C-reactive protein, mean (SD), mg/L</td>
<td>4.7 (6.5)</td>
<td>4.3 (6.6)</td>
<td>3.6 (6.5)</td>
<td>3.0 (7.9)</td>
<td>&lt;.001 a</td>
</tr>
<tr>
<td>Interleukin 6, mean (SD), (mg/L)</td>
<td>3.6 (2.7)</td>
<td>3.0 (2.2)</td>
<td>2.8 (2.1)</td>
<td>2.5 (2.1)</td>
<td>&lt;.001 a</td>
</tr>
<tr>
<td>Baseline telomere length, mean (SD), T/S units</td>
<td>0.96 (0.25)</td>
<td>0.93 (0.22)</td>
<td>0.89 (0.19)</td>
<td>0.91 (0.20)</td>
<td>.06</td>
</tr>
<tr>
<td>Year 5 telomere length, mean (SD), T/S units</td>
<td>0.83 (0.15)</td>
<td>0.83 (0.13)</td>
<td>0.82 (0.15)</td>
<td>0.86 (0.15)</td>
<td>.16</td>
</tr>
</tbody>
</table>

Abbreviations: ACE, angiotensin-converting enzyme; ANOVA, analysis of variance; ARB, angiotensin II receptor blocker; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MET, metabolic equivalent task; T/S, telomere-to-single-copy gene ratio.

SI conversions: To convert HDL-C and LDL-C to mmol/L, multiply by 0.0259; to convert triglycerides to mmol/L, multiply by 0.0113.

aSignificance test by 1-way ANOVA after natural log transformation.

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(Reprinted) JAMA, January 20, 2010—Vol 303, No. 3 253
0.09-0.17), whereas those in the highest quartile experienced a decrease of 0.05 T/S units (95% CI, 0.02-0.08; P<.001 for trend by linear contrasts).

In the unadjusted linear regression model, higher baseline log omega-3 fatty acid levels were associated with increases in absolute telomere length (unadjusted β coefficient=0.06; 95% CI 0.02-0.10). After sequential adjustment for demographics, comorbidities, blood pressure, serum lipids, inflammatory biomarkers, medications, and baseline telomere length (TABLE 2), the association of baseline log omega-3 fatty acid levels with lengthening of telomeres over time persisted (adjusted β coefficient=0.05; 95% CI, 0.01-0.08). The greatest attenuation in the β coefficient occurred after adjustment for baseline telomere length.

### Table 2. Association of Log Omega-3 Fatty Acid Levels (as a Continuous Variable) With Absolute Increase in Leukocyte Telomere Length Over 5 Years

<table>
<thead>
<tr>
<th>Model</th>
<th>β Coefficient ×10^{-3} (95% Confidence Interval)</th>
<th>P Value</th>
<th>Model R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>0.06 (0.02-0.10)</td>
<td>.001</td>
<td>0.02</td>
</tr>
<tr>
<td>Model 1</td>
<td>0.08 (0.04-0.13)</td>
<td>&lt;.001</td>
<td>0.08</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.09 (0.04-0.14)</td>
<td>&lt;.001</td>
<td>0.13</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.09 (0.04-0.14)</td>
<td>&lt;.001</td>
<td>0.13</td>
</tr>
<tr>
<td>Model 4</td>
<td>0.09 (0.04-0.14)</td>
<td>&lt;.001</td>
<td>0.14</td>
</tr>
<tr>
<td>Model 5</td>
<td>0.05 (0.01-0.08)</td>
<td>.008</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Note: All covariates were modeled continuously except the following categorical variables: sex (male, female); ethnicity (Hispanic, Asian, black, white, other); income (<$10,000, $10,000-$19,999, $20,000-$29,999, $30,000-$39,999, $40,000-$50,000, ≥$50,000); education (no formal schooling, 5th grade or less, 6th-8th grade, 9th-11th grade, high school graduate, some college/vocational school, college degree, graduate/professional degree); prior myocardial infarction; type 2 diabetes; current smoking status; vitamin use; statin use; β-blocker use. In the final adjusted model (Model 5), the linear regression equation is change in telomere length=0.101−0.008(income $20,000-$29,999)−0.001(income $30,000-$39,999)−0.005(income $40,000-$49,999)−0.003(income ≤$50,000)−0.004(income ≥$50,000)−0.001(log level)−0.003(age)−0.06(male)−0.03(Asian)−0.02(black)−0.03(white)−0.03(other)−0.006(income $10,000-$19,999)−0.006(income $20,000-$29,999)−0.001(income $30,000-$39,999)−0.005(income $40,000-$49,999)−0.003(income ≥$50,000)+0.04(5th grade)−0.08(6th-8th grade)−0.03(9th-11th grade)−0.08(12th grade or higher)−0.08(high school graduate)−0.11(college degree)+0.09(college degree)+0.08(bachelor degree)+0.06(exercise capacity)+0.01(exercise capacity−0.05(prior myocardial infarction)−0.02(type 2 diabetes)−0.02(smoking)−0.01(left ventricular ejection fraction)−0.12(waist−hip ratio)+0.003(systolic blood pressure)+0.007(diastolic blood pressure)+0.003(log triglycerides)+0.03(log low-density lipoprotein cholesterol)+0.005(log high-density lipoprotein cholesterol)−0.005(log C-reactive protein)−0.007(log interleukin 6)−0.02(multivitamin use)+0.0008(statin use)+0.025(β-blocker use)−0.77(baseline telomere length)+0.009(baseline telomere length−log omega-3 level). Model 1 was adjusted for age, sex, race/ethnicity, income, education, and exercise capacity. Model 2 = model 1 + prior myocardial infarction, type 2 diabetes, smoking, left ventricular ejection fraction, waist−hip ratio, systolic and diastolic blood pressure, log triglycerides, log low-density lipoprotein cholesterol, and log high-density lipoprotein cholesterol. Model 3 = model 2 + log C-reactive protein and log interleukin 6. Model 4 = model 3 + use of vitamins, statins, and β-blockers. Model 5 = model 4 + baseline telomere length and baseline telomere length−log omega-3 level. During the median 6-year (range, 5.0-8.1 years) follow-up period, 276 participants (45%) exhibited greater than 10% reduction in telomere length. Each 1-SD increase in baseline log omega-3 fatty acid levels was associated with a 19% decrease in the odds of telomere shortening (unadjusted odds ratio, 0.81; 95% CI, 0.69-0.95). After sequential adjustment for demographics, comorbidities, blood pressure, serum lipids, inflammatory biomarkers, medications, and baseline telomere length (TABLE 3), each 1-SD increase in baseline log omega-3 fatty acid levels was associated with a 32% decrease in the odds of telomere shortening (adjusted odds ratio, 0.68; 95% CI, 0.47-0.98).

In multivariable models, we found no evidence that the effect of baseline omega-3 fatty acid levels on telomeric aging was modified by age, sex, race/ethnicity, smoking, income, education, or type 2 diabetes (all P>.10). However, the effect of baseline omega-3 fatty acids on telomeric aging was stronger in participants with longer baseline telomere length (P=.04 for interaction).

## COMMENT

Leukocyte telomere length is an emerging marker of biological age that independently predicts morbidity and mortality in patients with cardiovascular diseases. In this longitudinal study, we observed that baseline levels of marine omega-3 fatty acids were associated with decelerated telomere attrition over 5 years. The association was linear and persisted after adjustment for potential confounders. These findings raise the possibility that omega-3 fatty acids may protect against cellular aging in patients with coronary heart disease.

Several studies have reported cross-sectional associations between longer telomeres and nutritional supplements, including multivitamins, vitamin C, vitamin D, vitamin E, and folic acid. The lack of longitudinal data on telomere trajectory limits the degree to which causal inferences can be
in patients with CHD

Table 3. Association of Log Omega-3 Fatty Acid Levels as a Continuous Variable per 1-SD Increase With Leukocyte Telomere Shortening, Defined as >10% Decrease in Telomere Length Over 5 Years

<table>
<thead>
<tr>
<th>Model</th>
<th>Odds Ratio per 1-SD Increase (95% Confidence Interval)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>0.81 (0.69-0.95)</td>
<td>.01</td>
</tr>
<tr>
<td>Model 1</td>
<td>0.72 (0.60-0.88)</td>
<td>.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.68 (0.54-0.85)</td>
<td>.001</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.68 (0.54-0.86)</td>
<td>.001</td>
</tr>
<tr>
<td>Model 4</td>
<td>0.68 (0.47-0.98)</td>
<td>.04</td>
</tr>
</tbody>
</table>

All covariates were modeled continuously except the following categorical variables: sex (male, female); ethnicity (Hispanic, Asian, black, white, other), income (<$30 000, $30 000-59 999, $60 000-89 999, $90 000-119 999, $120 000-149 999, $150 000-179 999), education (less than high school, high school, some college, college graduate), marital status (single, married, divorced), smoking (never, past, current), physical activity (low, moderate, high), number of children, family history of myocardial infarction, diabetes, hypertension, and cancer. Model 1 was adjusted for age, sex, race/ethnicity, income, education, and exercise capacity. Model 2 included the following additional variables: BMI, waist-to-hip ratio, systolic and diastolic blood pressure, log triglycerides, log high-density lipoprotein cholesterol, log C-reactive protein, and log interleukin-6. Model 3 included all variables in model 2 plus use of vitamin C supplements. Model 4 included all variables in model 3 plus use of vitamin D supplements. Model 5 included all variables in model 4 plus use of statins, atorvastatin, and other lipid-lowering medications. Model 6 included all variables in model 5 plus use of aspirin, beta-blockers, and antiplatelet medications. Model 7 included all variables in model 6 plus use of aspirin, beta-blockers, antiplatelet medications, and warfarin. Model 8 included all variables in model 7 plus use of revascularization procedures and coronary artery bypass graft surgery.
of physical activity, are believed to cumulatively determine telomere length throughout adult life. Our paired-sample longitudinal study was specifically designed to isolate the effect of omega-3 fatty acids levels on subsequent within-person changes in telomere length. Further studies are needed to understand the interindividual variability in the complex integration of bidirectional influences on telomere length.

Among the strengths of the present study is the detailed characterization of demographic, clinical, and biochemical covariates. In addition, the longitudinal study design improves on prior cross-sectional studies. However, several limitations should be considered in the interpretation of our results. First, the association reported in this study is observational, and therefore no definitive conclusions can be made regarding causality. Although we adjusted for multiple carefully measured potential confounding variables, the possibility of residual confounding by measured or unmeasured covariates cannot be excluded. For example, the absence of detailed dietary information may have contributed to residual confounding by other nutritional factors associated with telomere length, such as red meat intake. To definitively address the question of whether omega-3 fatty acids inhibit cellular aging, a double-blind, randomized, placebo-controlled trial would be necessary. Second, our measurements were restricted to telomere length in leukocytes and do not necessarily reflect telomere trajectory in other cell compartments such as myocardium, endothelium, or the atherosclerotic plaque. Third, due to long-term storage of blood samples, we could not measure biomarkers of oxidative stress or telomerase activity, which might elucidate the mechanisms of deceleration of telomeric aging by omega-3 fatty acids. Fourth, our study sample comprised mainly male outpatients with coronary artery disease, a population that might be particularly responsive to the effects of omega-3 fatty acids. The findings may not be generalizable to other patient populations, and further studies are required to validate our findings in other demographic groups.

In summary, among patients with stable coronary artery disease, there was an inverse relationship between baseline blood levels of marine omega-3 fatty acids and the rate of telomere shortening over 5 years.

Author Contributions: Dr Farzaneh-Far had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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Acquisition of data: Farzaneh-Far, Lin, Epel, Harris, Whooley.
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Critical revision of the manuscript for important intellectual content: Lin, Epel, Harris, Blackburn, Whooley.
Statistical analysis: Farzaneh-Far, Lin.
Obtained funding: Farzaneh-Far, Whooley.
Administrative, technical, or material support: Lin, Epel, Harris, Blackburn, Whooley.
Study supervision: Farzaneh-Far, Whooley.

Financial Disclosures: Dr Harris reports that he is an advisor to, a speaker for, and has received research grants from companies with interests in omega-3 fatty acids, including GlaxoSmithKline and Monsanto. In addition, he has recently founded a company (OmegaQuant Analytics) to offer blood omega-3 fatty acid testing. No other disclosures were reported.

Funding/Support: Dr Farzaneh-Far is supported by an American Heart Association Fellow-to-Faculty Transition Award (grant 0875014N). The Heart and Soul Study was supported by the Department of Veterans Affairs (Epidemiology Merit Review Program), the National Heart, Lung, and Blood Institute (grant R01 HL079235), the Robert Wood Johnson Foundation (Generalist Physician Faculty Scholars Program), the American Federation for Aging Research (Paul Besson Faculty Scholars in Aging Research Program), the Ischemia Research and Education Foundation, and the Nancy Kirkman Heart Research Fund. Dr Lin is supported by the Bernard and Barbro Foundation.

Role of the Sponsor: The funding organizations had no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript.

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