Association of omega-3 and omega-6 fatty acid intake with leukocyte telomere length in US males

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ABSTRACT

Background: Omega-3 (n–3) and omega-6 (n–6) fatty acids may contribute to oxidative stress and inflammation, which are related to telomere shortening. Evidence supporting an association between intake of n–3 or n–6 fatty acids and leukocyte telomere length (LTL) in males has been limited.

Objectives: We conducted a cross-sectional study to examine the associations of total or individual n–3 or total n–6 fatty acid intake with LTL in US males.

Methods: We included 2,494 US males with LTL measurement from 4 nested case–control studies within the Health Professionals Follow-Up Study. Individuals with previous histories of cancers, diabetes, and cardiovascular diseases at or before blood collection were excluded. Blood collection was performed between 1993 and 1995, and relevant information including n–3 and n–6 intake was collected in 1994 by questionnaire. The LTL was log-transformed and Z scores of the LTL were calculated for statistical analyses by standardizing the LTL in comparison with the mean within each selected nested case–control study.

Results: We found that consumption of DHA (22:6n–3) was positively associated with LTL. In the multivariable-adjusted model, compared with individuals who had the lowest intake of DHA (i.e., first quartile group), the percentage differences (95% CIs) of LTL were −3.7 (−13.7, 7.5), 7.0 (−4.3, 19.7), and 8.2 (−3.5, 21.3) for individuals in the second, third, and fourth quartiles of consumption, respectively (P-trend = 0.0498). We did not find significant associations between total n–3 or total n–6 fatty acid intakes and LTL. In addition, we found that males who consumed canned tuna had longer LTL than those who did not; in the multivariable-adjusted model, the percentage difference of LTL was 10.5 (95% CI: 1.3, 20.4) ($P = 0.02$).

Conclusions: Our results suggest that higher intakes of DHA and canned tuna consumption are associated with longer LTL. *Am J Clin Nutr* 2022;116:1759–1766.

Keywords: leukocyte telomere length, omega-3 fatty acid, docosahexaenoic acid, eicosapentaenoic acid, omega-6 fatty acid

Introduction

Telomeres are repetitive short DNA sequences (TTAGGG) located at the ends of eukaryotic chromosomes. In various somatic cells telomeres progressively shorten with each cycle of cellular replication (1). In particular, high levels of inflammation (2) and oxidative stress (3–5) accelerate telomere shortening, which in turn results in premature aging (6). However, it may be possible to promote telomere stability through improving dietary habits (7, 8).

Among nutrients, PUFAs from omega-3 (n–3) and omega-6 (n–6) families contribute to the inflammatory process. The main n–3 fatty acids consumed are alpha-linolenic acid (ALA, 18:3n–3), EPA (20:5n–3), docosapentaenoic acid (DPA, 22:5n–3), and DHA (22:6n–3), the latter 3 of which can also be biosynthesized

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Abbreviations used: AA, arachidonic acid; ALA, alpha-linolenic acid; DPA, docosapentaenoic acid; HPFS, Health Professionals Follow-Up Study; IRB, institutional review board; LA, linoleic acid; LTL, leukocyte telomere length; n–3, omega-3; n–6, omega-6.

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in our body from the former. The main n–6 fatty acids consumed are linoleic acid (LA, 18:2n–6) and arachidonic acid (AA, 20:4n–6); again, the latter can be biosynthesized from the former (9, 10). Most of these PUFAs, which are among the phospholipids in cell membranes, are involved in the inflammatory process (11). For example, AA can be converted into metabolites with proinflammatory or anti-inflammatory properties, whereas EPA, DPA, and DHA can be transformed into molecules capable of resolving inflammation (11). Thus, a balance in the dietary intake and metabolism of n–3 and n–6 PUFAs is necessary for an efficient inflammatory process. Intake of EPA + DHA has been inversely associated with soluble tumor necrosis factor receptor concentrations, a plasma inflammatory marker (10). That association was dependent on the intake of LA, e.g., the combination of the highest intake of EPA + DHA and LA was related to the lowest levels of inflammation, indicating that high concentrations of LA did not prevent the anti-inflammatory effect of EPA + DHA (10), which may beneficially affect telomere attrition. Also, compared with females, telomeres are shorter (12) and shortening (13) is faster in males, which might imply that males have a stronger interest to improve their eating habits than females. However, evidence to date on the association of total or individual n–3 or total n–6 PUFAs with leukocyte telomere length (LTL), as well as their joint effects on LTL, in US males is limited.

We hypothesized that habitual higher intake of total or individual n–3 PUFAs might be related to longer LTL in males and that this association might be influenced by dietary intake of total n–6 PUFAs. Therefore, we evaluated the associations between average consumption of total n–3, individual n–3, and total n–6 fatty acids derived from the FFQ closest to blood collection and LTL, as well as any potential joint effects on associations with LTL, in US males from the Health Professionals Follow-Up Study (HPFS) cohort with relevant data from middle age and older adulthood.

Methods

Study population

The HPFS is a US prospective cohort study that began in 1986. Participants consisted of 51,529 males aged 40–75 y in health professions including dentists, pharmacists, optometrists, osteopath physicians, podiatrists, and veterinarians, who were expected to be motivated and committed to participate in a long-term study at the beginning of the study. The participants filled out mailed surveys on diet at baseline and during follow-up every 4 y and on demographics and lifestyle at baseline and every 2 y. Between 1993 and 1995, a total of 18,018 participants provided blood samples.

In this study, we utilized available LTL data measured in previous nested case–control studies of colorectal (14), pancreatic (15), prostate (16), and skin cancers (17) within the HPFS. Participants with histories of cancer, diabetes, or cardiovascular disease at or before blood collection were excluded. Of 3029 participants, 2494 were included in this study (**Supplemental Figure 1**). This study protocol, titled Core C: Cohort Follow-Up and Database Management, was approved by the institutional review boards (IRBs) of the Brigham and Women's Hospital and the Harvard TH Chan School of Public Health (IRB number: 10162), and written informed consent was obtained from all study participants.

Measurement of LTL

We analyzed blood samples collected between 1993 and 1995 from the chosen participants. Detailed information on blood draw procedures, transportation, and plasma sample storage has been described previously (18). Coded DNA samples were examined by laboratory personnel blinded to participant characteristics including case–control status in each nested case–control study of the cohort (16, 19), and all assays were processed in triplicate by the same technician, under identical conditions (16). Briefly, genomic DNA was extracted from peripheral blood leukocytes using the QIAamp 96 DNA Blood Kit (Qiagen) and quantified using a PicoGreen reagent and a Molecular Devices 96-well spectrophotometer (Molecular Devices) (16, 20). Relative LTL was measured by a real-time qPCR-based telomere assay (15, 19, 21) run on an Applied Biosystems 7900HT Sequence Detection System (16). The average relative LTL was calculated as the ratio of telomere repeat copy number to a single gene copy number (T/S ratio) in the study subjects compared with that of a reference DNA sample (16, 19). The telomere and single-gene assay CVs for triplicates were $<1.2\%$.

Assessment of n–3 and n–6 fatty acid intakes and covariates

We utilized average nutrient intake data of total and individual n–3 fatty acids, total n–6 fatty acids, and other dietary factors, such as consumption of calories, fruit, vegetables, dietary fiber, and alcohol, derived from the 1994 FFQ whose cycle was closest to blood collection (1993–1995) or at the time of blood sampling. We estimated food composition values from 1992 USDA sources (22), supplemented with other data (10). Total n–3 fatty acids included ALA, EPA, DPA, and DHA; EPA and DHA were defined as individual types of n–3 fatty acids, and EPA + DHA as marine n–3 fatty acids. Total n–6 fatty acids included LA and AA. We also extracted average intakes of canned tuna (3–4 ounces/85.0–113.4 g) composed of white or light canned tuna in water (drained solids) and dark meat fish (3–5 ounces/85.0–141.7 g), including mackerel, salmon, sardines, bluefish, and swordfish, from the FFQ data. The information regarding consumption of canned tuna or dark meat fish was provided in response to the question, "How often on average you have used the amount specified during the past year?" with the following categories of responses: Never, 1–3/mo, 1/wk, 2–4/wk, 5–6/wk, 1/d, 2–3/d, 4–5/d, and $\geq$6/d. If the participants responded "Never," they were classified as "No" in consumption of canned tuna or dark meat fish; any answer besides "Never" was classified as "Yes." Validity and reproducibility of the FFQ were evaluated within the HPFS (23, 24). Specifically, the correlation between EPA measured by the FFQ and that calculated from 2 wk of diet recording was 0.60, with an average correlation of 0.65 among whole nutrients (24). Furthermore, significant correlations were observed between FFQ or dietary records and contents of subcutaneous adipose tissue biopsy specimens for EPA (0.47, diet record only) and PUFAs (0.60 or 0.50, respectively), indicating that the FFQ, diet records, and subcutaneous adipose tissue biopsy have similar validity in measuring PUFA intakes (23). Other covariate data

were also collected through self-reported questionnaires at blood draw or at the questionnaire cycle closest to blood collection, including age at blood draw, smoking status and amount (pack-years), BMI calculated as weight/height squared (in kg/m^2), and physical activity indicated by metabolic equivalents per week (METs/wk). The validity of self-reported covariates has been reported in detail elsewhere (25).

Statistical analysis

Log-transformed relative telomere length was obtained. To adjust for potential batch effects, Z scores of log-transformed LTL were calculated by standardizing LTL in comparison with the mean within each batch.

We calculated age-adjusted demographic, dietary, and lifestyle characteristics of all participants across quartiles of relative LTL. Continuous variables were presented as mean ± SD and categoric variables were presented as *n* (%). We performed linear regression analyses to examine the associations of intakes of total n–3 and individual types of n–3 fatty acids (i.e., marine n–3, EPA, and DHA), as well as total n–6 fatty acids, with LTL. We built 3 models: model 1 was adjusted for age at blood collection; model 2 was adjusted for the covariate in model 1 + self-identified race (white and nonwhite), BMI, physical activity, alcohol consumption, total caloric intake, smoking status and pack-years (never, past smoker and pack-years < 20, past smoker and pack-years ≥ 20, current smoker and pack-years < 20, and current smoker and pack-years ≥ 20), and case–control status (i.e., whether a person was a case or control) in the previous nested case–control studies; and model 3 was adjusted for the covariates in model 2 + total n–6 in the analyses for total or individual n–3, and total n–3 in the analysis for total n–6. Percentage differences (95% CIs) in relative LTL were calculated by comparing LTLs in higher quartiles (i.e., quartiles 2, 3, or 4) with those in the lowest quartile (quartile 1) of n–3 or n–6 fatty acid intake using the following equation: [exp (β-coefficient) − 1] × 100% (26). In addition, we analyzed the association of intake of other individual n–3 or n–6 fatty acids (i.e., ALA, DPA, LA, and AA) with LTL using the same models described earlier. With regard to LA:ALA ratio, we also conducted analyses on its association with LTL using the foregoing models 1 and 2. Moreover, we examined the associations of canned tuna or dark meat fish consumption (Yes compared with No) with LTL using the aforementioned models.

In addition, to test the effect modification of total n–6 fatty acid intakes on the association between total or individual n–3 fatty acid intakes and LTL, we analyzed relative telomere length according to quartiles of n–3 fatty acid intakes in subgroups stratified by quartiles of total n–6 intake. Here we used linear regression models with adjustment for all covariates, and then examined multiplicative interaction. In the same way, we examined the role of total n–6 intake as an effect modifier on the association between canned tuna/dark meat fish intake and LTL. In addition, we conducted joint-group analyses with adjustment for all covariates to examine the joint effect of total or individual n–3 and n–6 fatty acid consumption on LTL. There were 2 types of analysis (analysis 1: high n–3 and low n–6 intake compared with low n–3 and high n–6 intake; analysis 2: high n–3 and low n–6 intake, low n–3 and high n–6 intake, and high intake in both compared with low intake in both), where high and low intakes corresponded to median intake or greater and less than median intake, respectively. These models were also used in the joint effect analyses of canned tuna/dark meat fish and total n–6 consumption on LTL, replacing high/low n–3 intake with consuming/not consuming canned tuna/dark meat fish. All 2-tailed *P* values < 0.05 were considered statistically significant. All analyses were conducted using SAS software (Unix 9.4; SAS Institute, Inc.).

Results

Table 1 shows the age-standardized basic characteristics at blood collection according to quartiles of LTL in 2,494 males (2,351 whites and 143 nonwhites) of our study. Older males had shorter LTL (*P*-trend < 0.0001). Intakes of total n–3, marine n–3, EPA, DHA, and total n–6 fatty acids, LA:ALA ratio, as well as consumption of canned tuna and dark meat fish all had similar distributions across the LTL quartiles at large. Total n–3 fatty acids consisted of ALA (78.3%), EPA (7.3%), DHA (12.8%), and DPA (1.7%), and total n–6 fatty acids comprised LA (98.9%) and AA (1.1%). The overall average LA:ALA ratio was 10.48 ± 2.95:1. Other dietary factors, including total calories, total fruit, total vegetables, dietary fiber, and alcohol consumption, did not show any distinctive distribution in levels of LTL. Smoking status was similarly distributed across the quartiles of LTL, whereas pack-years among ever-smokers decreased from the lowest to highest quartiles (27.3 ± 17.9 and 22.7 ± 16.3, respectively; *P*-trend = 0.03). BMI and physical activity were similar across the levels of LTL.

We examined the associations between intake of total or individual types of n–3 or total n–6 fatty acids and LTL (**Table 2**). While we did not find significant associations between intakes of total n–3 or total n–6 and LTL, we observed a modest but statistically significant positive association of DHA intake and a marginally positive trend for marine n–3 fatty acids or EPA intake with LTL. In the multivariable-adjusted models, compared with the first quartile, percentage changes (95% CIs) in LTL were −3.7 (−13.7, 7.5), 7.0 (−4.3, 19.7), and 8.2 (−3.5, 21.3) among individuals in the second to fourth quartiles of DHA intake (*P*-trend = 0.0498); −2.6 (−12.7, 8.6), 7.9 (−3.4, 20.5), and 7.1 (−4.2, 19.6) among individuals in the second to fourth quartiles of marine n–3 intake (*P*-trend = 0.09); and 1.1 (−9.7, 13.1), 3.2 (−7.3, 15.0), and 9.9 (−1.8, 23.0) among individuals in the second to fourth quartiles of EPA intake (*P*-trend = 0.08). Among other individual n–3 or n–6 fatty acids, only LA showed a marginally positive trend with LTL (*P*-trend = 0.05) (**Supplemental Table 1**). Also, LA:ALA ratio was not associated with LTL (**Supplemental Table 2**).

In addition, we examined the associations between canned tuna or dark meat fish consumption and LTL and found a significant positive association with canned tuna consumption (**Table 3**). Compared with individuals not consuming canned tuna, those consuming canned tuna had 10.5% (95% CI: 1.3%, 20.4%) longer LTL in the multivariable-adjusted model (*P* = 0.02). Dark meat fish intake was not significantly associated with LTL.

In the analysis for potential effects of interactions between intakes of total or individual n–3, tuna or dark meat fish, and intake of total n–6 on LTL, we did not find any significant

TABLE 1 Age-standardized characteristics of 2,494 participants by quartiles of relative telomere length in the Health Professionals Follow-Up Study[1]

	Q1	Q2	Q3	Q4
Participants, *n*	632	615	632	615
Age at blood draw,[2] y	66.3 ± 7.4	64.2 ± 8.1	62.5 ± 8.1	62.7 ± 8.2
Race (white), %[3]	95.5	93.3	93.6	94.1
Telomere length, Z score	−1.2 ± 0.4	−0.3 ± 0.2	0.4 ± 0.2	1.3 ± 0.4
Total n–3 fatty acids,[4] g/d	1.54 ± 0.60	1.45 ± 0.57	1.50 ± 0.66	1.51 ± 0.57
ALA	1.20 ± 0.46	1.16 ± 0.46	1.15 ± 0.45	1.19 ± 0.49
Marine n–3 fatty acids[5]	0.31 ± 0.35	0.27 ± 0.25	0.32 ± 0.40	0.29 ± 0.24
EPA	0.12 ± 0.21	0.10 ± 0.13	0.12 ± 0.23	0.10 ± 0.12
DHA	0.20 ± 0.16	0.18 ± 0.13	0.20 ± 0.18	0.19 ± 0.13
DPA	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.02
Total n–6 fatty acids,[6] g/d	12.46 ± 4.98	12.05 ± 5.00	11.78 ± 4.72	12.28 ± 5.16
LA	12.32 ± 4.96	11.91 ± 4.97	11.64 ± 4.69	12.14 ± 5.13
AA	0.14 ± 0.06	0.14 ± 0.06	0.14 ± 0.06	0.14 ± 0.05
LA:ALA ratio[7]	10.46 ± 2.56:1	10.58 ± 2.79:1	10.44 ± 2.80:1	10.42 ± 2.61:1
Canned tuna,[8] servings/wk	0.79 ± 0.99	0.79 ± 0.87	0.85 ± 1.06	0.84 ± 0.91
Dark meat fish,[9] servings/wk	0.46 ± 0.66	0.43 ± 0.56	0.49 ± 0.71	0.44 ± 0.59
Other dietary factors				
Total calories, kcal/d	2,098 ± 570	2009 ± 553	2045 ± 579	2070 ± 561
Total fruit, servings/d	3.0 ± 1.7	2.8 ± 1.6	2.9 ± 1.5	2.8 ± 1.4
Whole fruit, servings/d	2.1 ± 1.3	2.0 ± 1.3	2.0 ± 1.2	2.0 ± 1.2
Fruit juices, servings/d	0.9 ± 0.8	0.8 ± 0.7	0.8 ± 0.7	0.8 ± 0.7
Total vegetables, servings/d	3.6 ± 1.8	3.3 ± 1.5	3.5 ± 1.7	3.5 ± 1.7
Dietary fiber, g/d	23.2 ± 6.2	22.6 ± 6.1	23.6 ± 6.5	22.8 ± 6.4
Alcohol consumption, g/d	12.3 ± 14.2	12.3 ± 14.9	12.2 ± 14.7	12.8 ± 15.2
Cigarette smoking				
Never smoker, %	47.6	49.5	50.3	44.2
Past smoker, %	47.5	45.7	44.8	52.7
Current smoker, %	4.9	4.8	4.9	3.1
Pack-years among ever smokers[10]	27.3 ± 17.9	25.9 ± 16.3	25.3 ± 16.8	22.7 ± 16.3
BMI at blood draw, kg/m^2	25.8 ± 3.0	25.6 ± 2.6	25.7 ± 3.1	25.5 ± 2.8
Physical activity, METs/wk	33.9 ± 27.4	32.0 ± 26.4	32.0 ± 32.7	33.8 ± 27.6

[1]Information presented was collected at the time of blood collection or at the questionnaire cycle closest to blood collection (HPFS: 1993–1995). Values are means ± SDs for continuous variables, percentages for categoric variables, and are standardized to the age distribution of the study population except for baseline age. AA, arachidonic acid; ALA, α-linolenic acid; DPA, docosapentaenoic acid; HPFS, Health Professionals Follow-Up Study; LA, linoleic acid; MET, metabolic equivalent; n–3, omega-3; n–6 omega-6; Q, quartile.

[2]*P*-trend < 0.0001 by simple linear regression.

[3]Race was self-identified and self-reported.

[4]Total n–3 fatty acids consisted of ALA (78.3%), EPA (7.3%), DHA (12.8%), and DPA (1.7%).

[5]EPA + DHA.

[6]Total n–6 fatty acids consisted of LA (98.9%) and AA (1.1%).

[7]Mean ± SD of the overall LA:ALA ratio was 10.48 ± 2.95:1.

[8]White or light canned tuna fish in water (drained solids).

[9]Dark meat fish includes mackerel, salmon, sardines, bluefish, and swordfish.

[10]*P*-trend = 0.03 by age-adjusted linear regression.

interactions (*P*- interactions > 0.05) (**Supplemental Tables 3** and **4**). In further joint-association analyses (**Supplemental Table 5**), only 1 significant association was observed: 17.0% (95% CI: 4.0%, 31.7%) longer LTL in the group consuming canned tuna and with low intake of total n–6 than in the group not consuming canned tuna and with low intake of total n–6 fatty acids ($P = 0.01$).

Discussion

In our cross-sectional study in middle- to older-aged US males, DHA intake (medians of the first and fourth quartiles: 0.05 and 0.32 g/d, respectively) was modestly positively associated with LTL. Also, canned tuna consumption was significantly positively associated with LTL. In addition, there was neither any association between total n–6 fatty acid intake and LTL nor significant effect modification of total n–6 fatty acids on the association between total or individual n–3 fatty acids and LTL.

A similar association between DHA intake (1.55 g/d) and LTL was also observed in a previous randomized controlled pilot study, which included 33 mildly cognitively impaired patients aged >65 (DHA group, $n = 12$; EPA group, $n = 12$; and LA group, $n = 9$) (27). Individuals with the largest increase in concentrations of erythrocyte DHA showed the smallest decrease in telomere length over the intervention period of 6 mo among the DHA group, unlike other treatment groups with EPA or LA (27). In addition, a 5-y longitudinal follow-up study in 608 ambulatory outpatients (82.1% males, 59.5% whites) found that each 1-SD increase in blood concentrations of marine n–3 was associated with a 32% reduction in the odds

TABLE 2 Percentage differences (95% CIs) in relative telomere length according to quartiles of total or individual n–3 fatty acid and total n–6 fatty acid intakes[1]

	Q1 (ref.)	Q2	Q3	Q4	*P*-trend
Total n–3 fatty acids[2]					
Participants, *n*	622	629	620	623	
Median, g/d	0.87	1.21	1.56	2.16	
Model 1	0	−3.1 (−13.0, 7.9)	− 1.8 (−11.8, 9.5)	− 0.5 (−10.7, 10.9)	0.95
Model 2	0	− 3.6 (−13.8, 7.7)	− 3.0 (−13.9, 9.3)	− 1.7 (−14.1, 12.5)	0.92
Model 3	0	− 3.8 (−14.4, 8.2)	− 1.8 (−13.8, 11.7)	2.4 (−11.5, 18.5)	0.57
Marine n–3 fatty acids[3]					
Participants, *n*	603	637	624	630	
Median, g/d	0.07	0.16	0.28	0.49	
Model 1	0	− 2.4 (−12.4, 8.8)	7.9 (−3.2, 20.3)	8.2 (−2.9, 20.6)	0.056
Model 2	0	− 2.4 (−12.5, 8.8)	8.3 (−3.0, 20.9)	7.8 (−3.5, 20.4)	0.07
Model 3	0	− 2.6 (−12.7, 8.6)	7.9 (−3.4, 20.5)	7.1 (−4.2, 19.6)	0.09
EPA					
Participants, *n*	583	584	711	616	
Median, g/d	0.01	0.04	0.09	0.16	
Model 1	0	1.0 (−9.6, 12.9)	3.5 (−6.9, 15.1)	11.0 (−0.6, 23.9)	0.045
Model 2	0	1.5 (−9.3, 13.5)	3.8 (−6.8, 15.6)	10.8 (−0.9, 23.9)	0.054
Model 3	0	1.1 (−9.7, 13.1)	3.2 (−7.3, 15.0)	9.9 (−1.8, 23.0)	0.08
DHA					
Participants, *n*	559	674	642	619	
Median, g/d	0.05	0.12	0.19	0.32	
Model 1	0	− 3.3 (−13.3, 7.9)	7.5 (−3.7, 20.0)	9.2 (−2.3, 22.0)	0.03
Model 2	0	− 3.5 (−13.5, 7.7)	7.4 (−3.9, 20.2)	8.9 (−2.8, 22.1)	0.037
Model 3	0	− 3.7 (−13.7, 7.5)	7.0 (−4.3, 19.7)	8.2 (−3.5, 21.3)	0.0498
Total n–6 fatty acids[4]					
Participants, *n*	623	623	628	620	
Median, g/d	6.75	9.63	12.63	18.34	
Model 1	0	− 0.1 (−10.3, 11.3)	0.8 (−9.5, 12.3)	− 4.9 (−14.6, 6.0)	0.34
Model 2	0	− 2.0 (−12.4, 9.7)	− 2.7 (−13.9, 10.0)	− 11.1 (−23.7, 3.5)	0.12
Model 3[5]	0	− 1.0 (−12.0, 11.2)	− 2.0 (−14.3, 11.9)	− 12.2 (−25.7, 3.6)	0.10

[1]Model 1 was adjusted for age at blood collection (continuous). Model 2 included the covariate in model 1 and was in addition controlled for race (white, nonwhite), BMI (continuous), physical activity (continuous), alcohol consumption (continuous), total caloric intake (continuous), smoking status and pack-years (never, past smoker and pack-years < 20, past smoker and pack-years ≥ 20, current smoker and pack-years < 20, current smoker and pack-years ≥ 20), and case–control status in previous nested case–control studies. Model 3 was in addition adjusted for total n–6 fatty acids. All models reflect linear regression. n–3, omega-3; n–6, omega-6; Q, quartile.

[2]Total n–3 fatty acids consisted of ALA (78.3%), EPA (7.3%), DHA (12.8%), and DPA (1.7%).

[3]EPA + DHA.

[4]Total n–6 fatty acids consisted of LA (98.9%) and AA (1.1%).

[5]Adjustment for total n–3 fatty acids in addition to the covariates used in model 2 for total n–6 fatty acids.

of telomere shortening (28). It is plausible that the different strengths of the associations may be partially attributable to our use of the data on DHA dietary intake, instead of blood DHA concentrations denoting its dietary bioavailability, biosynthesis from ALA, and potential anti-inflammatory biological status.

We also observed a marginally positive trend for intake of marine n–3 fatty acids with LTL. The potential beneficial effects of EPA + DHA on LTL might be explained by the following metabolic mechanisms: marine n–3 fatty acids attenuate nuclear factor kappa B activation, activate peroxisome proliferator-activated receptors, and play roles as substrates for the synthesis of resolvins, protectins, and maresins (29), which reduce production of inflammatory cytokines (30, 31) and active termination of inflammatory reactions (32, 33). Such lower inflammation and oxidative stress may retard telomere attrition (34, 35). Through a similar mechanism, antioxidants (e.g., carotenoids and n-acetylcysteine) and anti-inflammatory agents (e.g., resveratrol, aspirin, and statins) also demonstrated a positive role in slowing the pace of telomere shortening (36). In addition, EPA and DHA intake may help increase the life span of normal cells through stabilizing membranes (e.g., increase of membrane fluidity) (37), decreasing polymorphonuclear chemotaxis and infiltration, increasing macrophage phagocytosis and efferocytosis and leukocyte egress, and clearing bacteria and apoptotic cells (38), reducing telomere attrition.

Meanwhile, intake of total n–6 PUFAs including 98.9% of LA showed no association with LTL in our study; this was also the case in a Israel-based study in young males and females (39). Furthermore, previous findings among US males and females showed no relation between LA intake and any inflammatory markers (10). In addition, there are no data supporting the harmful side effects of n–6 fatty acids (40) or associations between total n–6 PUFAs or LA and risk of chronic diseases (41–43). These data together may help explain our findings.

TABLE 3 Percentage differences (95% CIs) in relative telomere length according to canned tuna/dark meat fish intake[1]

	No (ref.)	Yes[2]	*P* value
Canned tuna intake[3]			
Participants, *n*	667	1,827	
Median, servings/wk	0	0.56	
Model 1	0	11.1 (2.0, 21.1)	0.02
Model 2	0	10.6 (1.4, 20.6)	0.02
Model 3	0	10.5 (1.3, 20.4)	0.02
Dark meat fish intake[4]			
Participants, *n*	1,204	1,290	
Median, servings/wk	0	0.56	
Model 1	0	4.9 (−2.8, 13.2)	0.22
Model 2	0	4.6 (−3.2, 13.0)	0.25
Model 3	0	4.1 (−3.6, 12.5)	0.30

[1]Model 1 was adjusted for age at blood collection (continuous). Model 2 included the covariate in model 1 and was in addition controlled for race (white, nonwhite), BMI (continuous), physical activity (continuous), alcohol consumption (continuous), total caloric intake (continuous), smoking status and pack-years (never, past smoker and pack-years $<$ 20, past smoker and pack-years $\geq$ 20, current smoker and pack-years $<$ 20, current smoker and pack-years $\geq$ 20), and case–control status in previous nested case–control studies. Model 3 was in addition adjusted for total n–6 fatty acids. All models reflect linear regression.

[2]Yes or no refers to reported consumption of canned tuna or dark meat fish.

[3]White or light canned tuna fish in water (drained solids).

[4]Dark meat fish includes mackerel, salmon, sardines, bluefish, and swordfish.

Our study did not find a significant association of total n–3 fatty acid intake with LTL, which may be due mainly to the lack of association between ALA (~78% of total n–3) and LTL. While ALA is a major n–3 fatty acid in seeds, nuts, and vegetables, EPA and DHA are the most abundant n–3 fatty acids in fish, which indicates their biological and functional differences (44). In addition, dietary ALA is rarely present in the body because it is used for production of ATP (45) and biosynthesis of long-chain n–3 PUFAs, partitioned towards β-oxidation, or stored in adipose tissue as triglycerides (46), whereas dietary DHA is rapidly incorporated primarily into phospholipids of plasma membranes (47) and thus has a good correlation with DHA concentrations in membranes (48).

In our study, while intake of white or light canned tuna in water (drained solids) showed a positive relation with LTL, dark meat fish intake showed no association with LTL, which can be explained by different methods of preparation for eating between canned tuna and other fishes. In general, canned tuna is used directly in its original form, whereas other fishes may be prepared differently by consumers (e.g., deep-frying, grilling, boiling, or baking). The way in which canned tuna is usually consumed may optimize the bioavailability of DHA. On the other hand, pan-frying and baking may elevate the concentrations of oxidized products, especially derived from DHA, suggesting a significant change in EPA and DHA concentrations (49). Furthermore, although high mercury content is a long-standing concern related to canned tuna, no adverse effect of methylmercury exposure on telomere length was demonstrated in the Seychelles Child Development Study in mothers and their children at 5 y of age (50). In another study conducted in Lebanon, canned tuna was not associated with any life-threatening risk related to heavy metals (51). In addition, selenium, which may mitigate the negative effects of mercury, is also highly concentrated in tuna (52). These lines of evidence could help explain the positive association between canned tuna consumption and LTL that we observed.

Our study has several strengths, including a relatively large sample size (n = 2494). In addition, detailed collection of dietary, lifestyle, and medical information using well-designed and validated self-reported questionnaires allowed us to adjust for widely recognized potential confounders in the relation of interest. Moreover, we used high-quality habitual nutrient intake data from a validated FFQ, which is more valuable than data from short-term food records in measuring average long-term diet (25). Such data are especially beneficial to our investigation, because we sought to explore the relation between modifiable risk factors, i.e., habitual diet, and a long-latent biological change like LTL.

At the same time, our study has several limitations. First, residual and unmeasured confounding cannot be fully ruled out because of the nature of the observational data; yet, we adjusted many well-known confounding factors and excluded males with all prevalent cancers, diabetes, and cardiovascular diseases at or before blood draw. Second, we studied only compliant healthy professional males belonging to a specific social stratum, which may create a lack of external validity; nevertheless, the results could be applied to males in mid- to older life with similar dietary and life patterns. Third, because the dietary information on n–3 and n–6 fatty acid intakes was obtained through self-reported diet questionnaires, some misclassification is inherent; however, the questionnaire has been extensively validated in subsamples of this cohort, and any misclassification would likely be some nondifferential error to bias our results toward the null. Fourth, our study is based on dietary amounts of n–3 and n–6 PUFAs and not on their concentrations in biofluids (e.g., blood), which is a more relevant marker for studying the association with LTL. Future studies using their blood concentrations are warranted to confirm our findings. Lastly, antioxidants and anti-inflammatory agents that can affect LTL could not be adjusted in our analyses because of a lack of relevant data.

In conclusion, our cross-sectional findings from the HPFS suggest that higher intake of DHA or canned tuna consumption may be associated with longer LTL in middle-aged or older

US males. We acknowledge that the evidence we provided was suggestive and that the observed associations were not causal relations. Thus, we should remain cautious when interpreting the findings. Interventional studies with DHA supplementation are warranted to verify causality.

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Data Availability

Data described in the article, code book, and analytic code will be made available upon request pending application and approval.

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