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Omega-3 fatty acids, oxidative stress, and leukocyte telomere length: A randomized controlled trial ☆

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ABSTRACT

Shorter telomeres have been associated with poor health behaviors, age-related diseases, and early mortality. Telomere length is regulated by the enzyme telomerase, and is linked to exposure to proinflammatory cytokines and oxidative stress. In our recent randomized controlled trial, omega-3 (*n*-3) polyunsaturated fatty acid (PUFA) supplementation lowered the concentration of serum proinflammatory cytokines. This study assessed whether *n*-3 PUFA supplementation also affected leukocyte telomere length, telomerase, and oxidative stress. In addition to testing for group differences, changes in the continuous *n*-6:*n*-3 PUFA ratio were assessed to account for individual differences in adherence, absorption, and metabolism. The double-blind four-month trial included 106 healthy sedentary overweight middle-aged and older adults who received (1) 2.5 g/day *n*-3 PUFAs, (2) l.25 g/day *n*-3 PUFAs, or (3) placebo capsules that mirrored the proportions of fatty acids in the typical American diet. Supplementation significantly lowered oxidative stress as measured by F2-isoprostanes ($p = 0.02$). The estimated geometric mean log-F2-isoprostanes values were 15% lower in the two supplemented groups compared to placebo. Although group differences for telomerase and telomere length were nonsignificant, changes in the *n*-6:*n*-3 PUFA plasma ratios helped clarify the intervention's impact: telomere length increased with decreasing *n*-6:*n*-3 ratios, $p = 0.02$. The data suggest that lower *n*-6:*n*-3 PUFA ratios can impact cell aging. The triad of inflammation, oxidative stress, and immune cell aging represents important pre-disease mechanisms that may be ameliorated through nutritional interventions. This translational research broadens our understanding of the potential impact of the *n*-6:*n*-3 PUFA balance. ClinicalTrials.gov identifier: NCT00385723.

1. Introduction

1.1. Telomeres, telomerase, inflammation, and oxidative stress

Telomeres, the caps found at the ends of chromosomes, are essential for chromosomal stability and replication; the enzyme telomerase is important for telomere formation, maintenance, and restoration (Blackburn, 2005; Epel et al., 2004). A growing literature has linked shorter telomeres with health behaviors, age-related diseases, and earlier mortality (Brouilette et al., 2003; Epel et al., 2009; Kimura et al., 2008; Valdes et al., 2005).

Telomeres can be maintained or lengthened by telomerase, an intra-cellular enzyme that adds telomeric DNA to shortened telomeres (Chan and Blackburn, 2003). Telomere length is also linked to, and likely regulated by, exposure to proinflammatory cytokines and oxidative stress (Aviv, 2006; Carrero et al., 2008; Damjanovic et al., 2007). Inflammation triggers T-cell proliferation, one known cause of telomere shortening (Aviv, 2004; Carrero et al., 2008; Gardner et al., 2005). Oxidative stress promotes telomere erosion during cellular replication *in vitro* and also stimulates the synthesis of proinflammatory cytokines (Aviv, 2006; Lipcsey et al., 2008).

1.2. Telomeres, telomerase, and omega-3 PUFAs

Although telomeres typically shorten with aging, shortening is not inevitable, and telomeres can also lengthen (Aviv et al., 2009; Ehrlenbach et al., 2009; Epel et al., 2009; Farzaneh-Far et al.,

☆ Please see Brief Commentary by Sophie Layé found on page 14 of this issue.
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Table 1
Baseline characteristics of study population, by intervention group.

	Placebo (n = 31)	1.25 g/day n-3 (n = 40)	2.5 g/day n-3 (n = 35)
Age, mean (SD), years	51.2 (8.9)	50.3 (7.8)	50.6 (6.5)
Female, No., %	23 (74%)	24 (60%)	22 (63%)
Race, No., %			
White	23 (74%)	33 (83%)	27 (77%)
Black	4 (13%)	5 (13%)	7 (20%)
Asian	2 (6%)	1 (3%)	1 (3%)
Other	2 (6%)	1 (3%)	0 (0%)
Body mass index, mean (SD), kg/m^2	31.1 (4.8)	31.7 (4.5)	30.7 (3.8)
Sagittal abdominal diameter, mean (SD), cm	23.1 (3.1)	23.4 (3.1)	22.9 (2.4)
Telomere length at baseline, base pairs			
N	31	40	35
Mean (SD)	5866 (430)	5809 (374)	5916 (432)
Median (IQR)	5810 (5588–6257)	5782 (5566–6092)	5838 (5681-6280)
Range	4981–6531	4963–6612	5042–6764
Telomerase at baseline, activity/10,000 cells			
N	27	36	31
Mean (SD)	8.5 (4.2)	8.2 (4.4)	7.9 (3.3)
Median (IQR)	7.0 (5.8–10.9)	7.1 (5.8–9.8)	7.4 (5.2–10.5)
Range	2.0–20.3	2.1–25.5	2.6–16.6
F2-isoprostanes at baseline, ng/ml			
N	27	38	32
Mean (SD)	0.036 (0.017)	0.037 (0.015)	0.031 (0.012)
Median (IQR)	0.032 (0.025–0.044)	0.033 (0.026–0.045)	0.032 (0.024–0.036)
Range	0.017–0.087	0.01–0.069	0.011–0.081

Table 2
Fatty acid composition of dietary oil supplements (% fatty acid).

Fatty acid	Name	Placebo	Supplement
C14:0	Myristic acid	6.4	0.1
C16:0	Palmitic acid	19.6	0.1
C16:1*n*7	Palmitoleic acid	0.4	0.1
C18:0	Stearic acid	3.1	0.5
C18:1*n*9	Oleic acid	43.5	0.7
C18:1*n*7	Vaccenic acid	1.4	0.4
C18:2*n*6	Linoleic acid	20.4	0.2
C18:3*n*6	Gamma linolenic acid	0.0	0.7
C18:3*n*3	Alpha linolenic acid	3.3	0.2
C18:4*n*3	Stearidonic acid	0.1	6.9
C20:1*n*9	Cetoleic acid	0.2	0.4
C20:2*n*6	Eicosadienoic acid	0.0	0.1
C20:3*n*6	Dihomo-gamma-linolenic acid	0.0	0.1
C20:4*n*6	Arachidonic acid	0.1	3.5
C20:4*n*3	Eicosatetraenoic acid	0.0	1.5
C20:5*n*3	Eicosapentaenoic acid	1.2	75.0
C22:4*n*6	Adrenic acid	0.0	0.0
C22:5*n*3	Docosapentaenoic acid	0.0	0.8
C22:6*n*3	Docosahexaenoic acid	0.2	8.9
Total reported		100	100
Saturated fatty acids[a]		29.1	0.7
Monounsaturated fatty acids[b]		45.3	1.2
Omega 3 fatty acids[c]		4.8	93.3
Omega 6 fatty acids[d]		20.5	4.6
n-6/*n*-3 ratio[e]		4.27	0.05

[a] C14:0, C16:0, C18:0.
[b] C16:1*n*7, C18:1*n*7, C18:1*n*9.
[c] Sum of (C18:3*n*3 + C18:4*n*3 + C20:4*n*3 + C20:5*n*3 + C22:5*n*3 + C22:6*n*3).
[d] Sum of (C18:2*n*6 + C18:3*n*6 + C20:2*n*6 + C20:3*n*6 + C20:4*n*6 + C22:4*n*6).
[e] Sum of (C18:2*n*6 + C18:3*n*6 + C20:2*n*6 + C20:3*n*6 + C20:4*n*6 + C22:4*n*6)/sum of (C18:3*n*3 + C18:4*n*3 + C20:4*n*3 + C20:5*n*3 + C22:5*n*3 + C22:6*n*3).

2010a; Nordfjall et al., 2009). It is important to identify malleable factors that might promote telomere stability over time. Based on theoretical and empirical reasons, it is possible that blood levels of polyunsaturated fatty acids (PUFAs) may be one of the factors that can prevent telomere shortening over time. The omega-3 (*n*-3) PUFAs can reduce inflammation and decrease oxidative stress (Calder, 2005; Kiecolt-Glaser et al., 2011; Mori et al., 1999; Nalsen et al., 2006), described below, and thus could buffer telomeres from their damaging effects.

In the Heart and Soul Study, which followed 608 people with stable coronary heart disease over 5 years, average telomere length increased in 23% of the individuals, shortened in 45%, and remained unchanged in 32% (Farzaneh-Far et al., 2010a). Slower telomere attrition was predicted by higher baseline levels of the two key *n*-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), which were the only significant predictors out of 16 clinical and behavioral factors examined (Farzaneh-Far et al., 2010b). Each standard deviation increase in the DHA + EPA total was associated with a 32% reduction in the odds of telomere attrition. In a different pilot study, an intensive three-month lifestyle change program that included *n*-3 PUFA supplementation significantly increased telomerase activity (Ornish et al., 2008).