Molecular Psychiatry
https://doi.org/10.1038/s41380-021-01077-2

ARTICLE

Omega-3 supplementation and stress reactivity of cellular aging biomarkers: an ancillary substudy of a randomized, controlled trial in midlife adults

Annelise A. Madison[1,2] · Martha A. Belury[1,3] · Rebecca Andridge[1,4] · Megan E. Renna[1] · M. Rosie Shrout[1] · William B. Malarkey[1,5] · Jue Lin[6] · Elissa S. Epel[7] · Janice K. Kiecolt-Glaser[1,8]

Received: 11 August 2020 / Revised: 5 March 2021 / Accepted: 24 March 2021

Abstract

Higher levels of omega-3 track with longer telomeres, lower inflammation, and blunted sympathetic and cardiovascular stress reactivity. Whether omega-3 supplementation alters the stress responsivity of telomerase, cortisol, and inflammation is unknown. This randomized, controlled trial examined the impact of omega-3 supplementation on cellular aging-related biomarkers following a laboratory speech stressor. In total, 138 sedentary, overweight, middle-aged participants ($n = 93$ women, $n = 45$ men) received either 2.5 g/d of omega-3, 1.25 g/d of omega-3, or a placebo for 4 months. Before and after the trial, participants underwent the Trier Social Stress Test. Saliva and blood samples were collected once before and repeatedly after the stressor to measure salivary cortisol, telomerase in peripheral blood lymphocytes, and serum anti-inflammatory (interleukin-10; IL-10) and pro-inflammatory (interleukin-6; IL-6, interleukin-12, tumor necrosis factor-alpha) cytokines. Adjusting for pre-supplementation reactivity, age, sagittal abdominal diameter, and sex, omega-3 supplementation altered telomerase ($p = 0.05$) and IL-10 ($p = 0.05$) stress reactivity; both supplementation groups were protected from the placebo group's 24% and 26% post-stress declines in the geometric means of telomerase and IL-10, respectively. Omega-3 also reduced overall cortisol ($p = 0.03$) and IL-6 ($p = 0.03$) throughout the stressor; the 2.5 g/d group had 19% and 33% lower overall cortisol levels and IL-6 geometric mean levels, respectively, compared to the placebo group. By lowering overall inflammation and cortisol levels during stress and boosting repair mechanisms during recovery, omega-3 may slow accelerated aging and reduce depression risk. ClinicalTrials.gov identifier: NCT00385723.

Introduction

Omega-3 fatty acid consumption may lessen accelerated aging and early mortality. Men and women in the top quintile of omega-3 fatty acid intake had 15% and 18% lower cardiovascular disease mortality 16 years later, respectively, compared to those in the lowest quintile [1]. At a cellular level, higher blood levels of omega-3 track

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41380-021-01077-2.

✉ Janice K. Kiecolt-Glaser
Janice.Kiecolt-Glaser@osumc.edu

[1] Institute for Behavioral Medicine Research, The Ohio State University College of Medicine, Columbus, OH, USA

[2] Department of Psychology, The Ohio State University, Columbus, OH, USA

[3] Department of Human Sciences, College of Education and Human Ecology, The Ohio State University, Columbus, OH, USA

[4] College of Public Health, The Ohio State University, Columbus, OH, USA

[5] Department of Internal Medicine, The Ohio State University College of Medicine, Columbus, OH, USA

[6] Department of Biochemistry and Biophysics, University of California, San Francisco, CA, USA

[7] Department of Psychiatry, University of California, San Francisco, CA, USA

[8] Department of Psychiatry and Behavioral Health, The Ohio State University College of Medicine, Columbus, OH, USA

Published online: 20 April 2021

with longer telomeres [2, 3], which are DNA repeats at the end of chromosomes that help to maintain genomic integrity during cell division [4], thereby promoting healthy cellular aging. Results from genome-wide association studies suggest a causal role of short telomeres in age-related disease, especially cardiovascular disease [5, 6]. Thus, there is evidence that omega-3 promotes longevity at both the epidemiological and cellular level.

A dysregulated physiological stress response is a risk factor for many physical and mental diseases, including depression [7, 8], and omega-3 may reduce morbidity by regulating stress-responsive systems. University students with higher serum omega-3 levels were buffered from an uptick in stimulated pro-inflammatory cytokine release during a high-stress exam period [9]. Heightened inflammatory reactivity to acute stress may increase risk for depression [10], and therefore, omega-3's anti-inflammatory properties may help to break the link between stress exposure and depression. Intriguingly, omega-3 supplementation also reduces sympathetic and cardiovascular reactivity to an acute stressor [11–13].

Prior publications from this randomized, placebo-controlled trial (RCT) showed that 4 months of omega-3 supplementation lowered basal inflammation and oxidative stress [2, 14], but this preplanned ancillary substudy investigated whether it altered the stress responsivity of biomarkers relevant to telomere length and cellular aging (i.e., cortisol, inflammatory cytokines, and telomerase). Cortisol and pro-inflammatory cytokines naturally rise after acute stress, but exaggerated cortisol responses are associated with shorter telomeres both cross-sectionally and longitudinally [15, 16], and pro-inflammatory cytokines fuel oxidative stress [17, 18], which shortens telomeres [19]. We hypothesized that omega-3 supplementation would reduce cortisol and inflammatory stress reactivity. Telomerase is an enzyme that maintains and restores telomeres, and Epel et al. [20] found differences in response to an acute laboratory stressor—the Trier Social Stress Test (TSST). We predicted that telomerase levels would not change following an acute stressor among those taking omega-3. In accord with the post-stress trajectory observed in Epel et al.'s [20] low-stress, non-caregiving cohort, we expected that the placebo group's telomerase would first rise within 45 min, and then fall at 120 min post stress. Importantly, we investigated these questions in a sedentary, overweight sample of middle-aged adults—a high-risk group for accelerated aging [21].

Subjects and methods

Participants

Overall, 138 individuals (93 women, 45 men), ages 40–85, participated in this RCT. Campus and community print and web-based announcements were used for recruitment. The Ohio State University biomedical institutional review board approved this study. Each participant provided written informed consent. These ancillary hypotheses were pre-specified and are distinct from the primary results [2, 14].

Due to our desire to study sedentary, overweight individuals, only those who engaged in <2 h of vigorous physical activity per week and had a body mass index (BMI) between 22.5 and 50 were included. The parent study's exclusionary criteria, described elsewhere, yielded a sample that was free of metabolic, autoimmune, and inflammatory diseases and did not take medications that alter mood, cardiovascular, or immune function [2, 14].

Across groups, 63% of participants were women, 79% were white, and 16% were black. Participants' ages ranged from 40 to 85 with a mean of 51 years. Using the BMI cut point of 25 kg/m^2, 125 (91%) were overweight (Table 1).

Table 1 Baseline characteristics.

	Placebo ($n = 46$)	1.25 g/d ($n = 46$)	2.5 g/d ($n = 46$)
Age (years)[a]	51.1 (8.6)	51.1 (8.0)	51.0 (6.7)
Female	36 (78%)	28 (61%)	29 (63%)
Race			
White	33 (72%)	39 (85%)	37 (80%)
Black	9 (20%)	5 (11%)	8 (17%)
Asian	2 (4%)	1 (2%)	1 (2%)
Other	2 (4%)	1 (2%)	0 (0%)
Sagittal abdominal diameter (cm)[a]	22.8 (3.2)	23.9 (3.4)	22.9 (2.9)

[a]Data are mean (SD).

Randomization and blinding

At the baseline visit, participants were randomly assigned to a group using a permuted block randomization sequence and given their 1st month's supply. At every subsequent visit, participants returned unused supplements and received the next month's supply. Adherence was high and did not differ between groups, with 3.3%, 2.0%, and 2.6% percent of unused supplements returned in the placebo-, low-, and high-dose groups, respectively ($p = 0.31$). As previously described, participants and experimenters were adequately blinded [14].

Procedure

At the baseline and post-intervention visits, participants arrived at The Ohio State University's Clinical Research Center, a hospital research floor, at 07:45 and completed mood questionnaires, ate a standardized breakfast, had a baseline blood draw around 08:50 to assess pre-stressor telomerase and cytokine levels, and provided saliva for a baseline cortisol measure. Around 10:10, participants

completed a 20-min stressor, detailed below. Participants had their blood drawn to measure telomerase and cytokine levels 0.75 and 2 h post stress. Participants also provided saliva to measure cortisol immediately post stressor, as well as 0.75, 1.25, 1.75, and 2 h post stress. They also reported their state anxiety levels before and after the stressor.

Supplement and placebo

In this three-arm parallel group RCT, participants received either (a) 2.496 g/d omega-3 ($n = 46$), (b) 1.25 g/d omega-3, and placebo ($n = 46$), or (c) placebo ($n = 46$). All participants took six pills (3 g oil) daily. For the two supplement groups, each 500 mg gel capsule contained 347.5 mg eicosapentaenoic acid (EPA) and 58 mg docosahexaenoic acid (DHA). Thus, the high-dose group took 2085 g/d of EPA and 348 g/d of DHA and the low-dose group took 1042.5 g/d or EPA and 174 g/d of DHA. The placebo was a mixture of palm, olive, soy, canola, and coco butter oils that approximated the saturated:monounsaturated:polyunsaturated ratio consumed by US adults, 37:42:21 (USDA Continuing Survey of Food Intake by Individuals, 1994–1996). OmegaBrite (Waltham, MA) supplied both the omega-3 and the matching placebo. See Supplementary Table 1 for fatty acid analysis of supplement.

Psychosocial stressor

The TSST is a well-validated, widely used psychosocial laboratory acute stress paradigm [22]. After participants were given instructions, they had 10 min to prepare a speech about why they were the best job candidate. Without using notes or aides, participants then delivered a 5-min speech in front of a video camera and a panel of two judges wearing white lab coats who were told to maintain a neutral facial expression. If participants' speeches ended early, they were told to continue until the full 5 min had passed. After the speech, participants completed a 5-min serial-subtraction task out loud in front of the same panel. When participants made mistakes, they were told to restart from the beginning. The TSST reliably provokes strong neuroendocrine and inflammatory responses [23].

State Anxiety Index

Participants reported their state anxiety levels before and after the TSST on the widely used Spielberger et al. [24] 20-item State Anxiety Index. The measure asks participants to rate on a four-point scale how strongly they are experiencing each feeling (e.g., calmness, jitteriness) "right now, in this moment," ranging from "not at all" to "very much."

Cellular aging biomarkers

Salivary cortisol was assayed using the Cortisol Coat-A-Count Radioimmunoassay (Diagnostic Products Corporation). This plasma kit was modified to measure free cortisol in saliva per the manufacturers' directions. The assay was counted and calculated on the Packard Cobra II Gamma Counter (Packard Instrument Company). The sensitivity was 0.025 ul/dl and the inter-assay coefficient variation was 5.2%. This assay method for telomerase and these cytokines are described elsewhere [2, 14]. The inter-assay coefficients of variation were 6.8% for telomerase, 12.5% for interleukin-6 (IL-6), 12.1% for TNF-α, 6.4% for interleukin-10 (IL-10), and 10.5% for IL-12.

Power analysis

There were no prior data on omega-3 supplementation's effect on cortisol and telomerase stress reactivity, so we based our a priori power analysis on past reports of its association with inflammatory reactivity. In groups similar to our placebo group, stress boosted TNF-α by 0.6 standard deviations (effect size = 0.6) [9, 25]. However, among those with higher serum omega-3 levels, TNF-α increased only 0.1 standard deviations after stress, six times lower than those who have low serum levels of omega-3 [9]. Using these results and an estimated standard deviation of 0.79 pg/mL based on pilot data from our laboratory results in an estimated reduction in stress-related increase of 0.46 pg/mL in the lower dose group versus placebo. Thus, a sample size of 46 per group would allow us to detect this reduction (effect size 0.6) with 80% power.

Analytical plan

Zero-order correlations between variables of interest at the baseline visit were performed. All physiological outcomes were natural-log transformed due to their positive skew to ensure homoscedastic residuals. A time variable was created to index telomerase and cytokine reactivity time points (80 min before the stressor, 45 min after stress onset, and 120 min after stress onset). Because cortisol was assessed at six time points throughout the stressor, we calculated area under the curve with respect to ground (AUCg) as an index of total cortisol release as well as area under the curve with respect to increase (AUCi) as an index of cortisol reactivity to the stressor.

To test group differences in cortisol at the post-intervention visit, we used linear regression models with group predicting post-intervention cortisol reactivity (AUGi) and total cortisol (AUGg), adjusting for baseline values. To assess whether there were group differences in telomerase and cytokine stress reactivity and overall levels

at the post-intervention visit, we used hierarchical linear models with group, time, and a group by time interaction variable to predict telomerase, pro-inflammatory cytokine (i.e., IL-6, TNF-α, IL-12), and anti-inflammatory cytokine (i.e., IL-10) trajectories, adjusting for baseline values (at each reactivity time point). To account for sex-based differences in stress reactivity [26], we also tested sex as a moderator in all primary models. However, there were no sex-based differences in the effect of omega-3 supplementation on overall levels ($ps > 0.22$) or reactivity ($ps > 0.11$) of the outcomes of interest, so sex was not included as a moderator in final models. These hierarchical linear models used a subject-specific random intercept to account for the within-subject correlation of the repeated measurements within the visit, and the Kenward–Roger adjustment to the degrees of freedom. As in our study on basal telomere length and oxidative stress [2], all models were adjusted for age, sagittal abdominal diameter, and sex.

Our analytic strategy required the inclusion of participants' post-intervention outcome measurements in the models. We added telomerase and cortisol assessments after the trial began, so only 103 and 118 had baseline telomerase and cortisol data, respectively. Two participants were lost to follow-up ($n = 1$ in placebo, $n = 1$ in 1.25 g/d) and three ($n = 1$ in placebo, $n = 2$ in 2.5 g/d) discontinued the intervention. In addition, one participant did not have a sagittal abdominal diameter measurement and therefore was excluded from all analyses. Data from 97 participants were included in the telomerase model, 110 in the cortisol models, and 120–131 in the cytokine analyses (IL-6 $n = 131$, TNF $n = 131$, IL-10 $n = 127$, IL-12 $n = 120$). Supplementary Fig. 1 shows the participant flow through the trial.

Since the resulting analyses did not use all randomized subjects, we compared baseline outcome levels across the groups to ensure there were no differences. The same modeling strategy was used for these analyses as for the primary analyses (linear regression for cortisol AUCg and AUCi; hierarchical linear models for telomerase and cytokines; adjustment for age, sagittal abdominal diameter, and sex).

When a significant group by time interaction occurred, the following preplanned contrasts were performed to probe the interaction: (1) between-group mean differences at each time point (pre-stress, 45 min post stress, 120 min post stress) and (2) within-group changes between each time point. When a significant group main effect occurred (with nonsignificant interaction), we contrasted overall group means (pooled across time points). Two-tailed tests of significance were conducted and all alpha levels were set at $\alpha = 0.05$. Data were analyzed with SAS version 9.4 (SAS Institute, Cary, NC). Relevant data and statistical code will be made available upon written request.

Results

Manipulation check

At both visits, participants reported higher state anxiety levels immediately after the TSST than they did before the stressor (baseline visit: paired $t(133) = 8.7$, $p < 0.0001$; post-intervention visit: paired $t(122) = 6.8$, $p < 0.0001$), indicating that the manipulation was successful.

Omega-3 supplementation and telomerase reactivity

At the baseline visit, there were no group differences in overall levels ($ps > 0.17$) or reactivity ($ps > 0.08$) of the outcomes of interest. Estimated marginal means are shown in Supplementary Tables 2 and 3. However, omega-3 supplementation impacted post-intervention telomerase reactivity to the laboratory stressor ($p = 0.05$) (Fig. 1) but not overall telomerase levels ($p = 0.98$). Among those in the two supplement groups, telomerase did not change throughout the measurement period ($ps > 0.07$). In contrast, among those in the placebo group, telomerase did not change from pre-stress to 45 min post stress ($p = 0.90$), but sharply declined from 45 to 120 min post stress ($p = 0.001$). Preplanned contrasts showed that there were no between-group differences in telomerase before the stressor ($ps > 0.58$), 45 min ($ps > 0.26$), or 120 min ($ps > 0.13$) post stress onset.

Omega-3 supplementation and cortisol reactivity

Omega-3 supplementation did not impact cortisol reactivity to the laboratory stressor, as measured by the area under the curve increase ($p = 0.44$). However, omega-3 supplementation lowered total cortisol levels throughout the stressor in a dose-response manner, as indexed by AUCg ($p = 0.04$); those receiving the high dose had the lowest overall cortisol levels, and those in the placebo group had the highest (Fig. 2). Those taking the high dosage of omega-3 had significantly lower cortisol compared to the placebo group ($p = 0.01$); those taking the low dose did not differ from the placebo group ($p = 0.29$). Across all participants, the TSST caused a significant rise in cortisol at both visits (baseline visit: paired $t(119) = 5.6$, $p < 0.0001$; post-intervention visit: paired $t(112) = 4.1$, $p < 0.0001$).

Omega-3 supplementation and inflammatory reactivity

Omega-3 supplementation influenced IL-10 stress reactivity (interaction effect $F(4, 206) = 2.44$, $p = 0.05$) but not overall levels ($p = 0.30$). Although there were no group

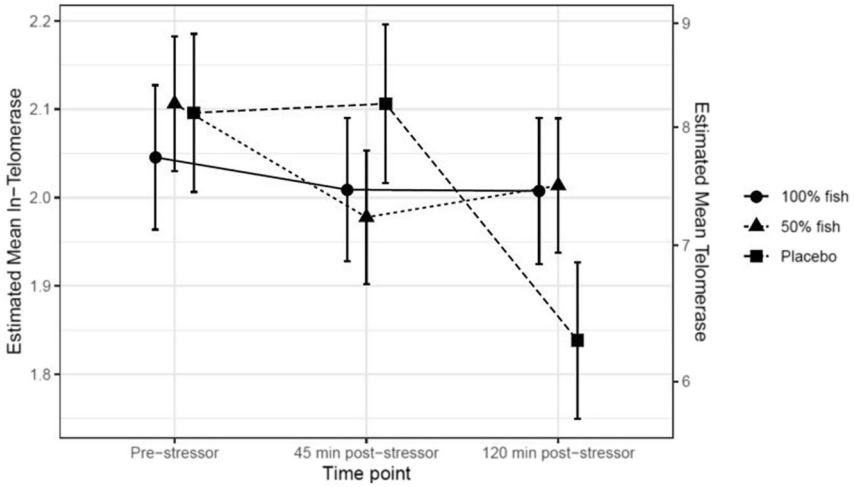

Fig. 1 Omega-3 supplementation impacted telomerase reactivity to an acute stressor ($p = 0.05$). Supplementation with either 2.5 or 1.25 g/d of omega-3 prevented changes in telomerase following an acute stressor ($ps > 0.07$). In contrast, those in the placebo group had a 24% decline in the geometric mean of telomerase from 45 to 120 min after the stressor ($p = 0.001$). Error bars are ±1 standard error.

differences before ($ps > 0.71$) or 45 min after the stressor ($ps > 0.14$), those in the omega-3 groups had higher levels of IL-10 120 min after the stressor ($ps < 0.05$) (Fig. 3). In the placebo group, IL-10 declined from pre-stress to 120 min post stress (change = −0.199, SE = 0.067, $t(206) = -2.95$, $p = 0.004$), but the omega-3 supplementation groups did not have this decline ($ps > 0.34$).

Omega-3 supplementation did not affect IL-6 stress reactivity (interaction effect $p = 0.11$), but it did lower overall IL-6 levels (main effect $F(2, 226) = 3.73$, $p = 0.03$). The high-dosage group had lower IL-6 levels than the placebo group ($B = 0.288$, SE = 0.106, $t(226) = 2.71$, $p = 0.007$) but there were no other group differences ($ps > 0.10$) (Fig. 4). Omega-3 supplementation did not impact TNF-α or IL-12 reactivity to the stressor (interaction effect $ps > 0.34$) or overall levels (main effect $ps > 0.59$). The TSST triggered an increase in IL-6 at both visits across all participants (baseline visit: paired $t(129) = 10.0$, $p < 0.0001$; post-intervention visit: paired $t(123) = 8.5$, $p < 0.0001$).

Discussion

In this RCT, omega-3 supplementation blocked stress-related decreases in telomerase and anti-inflammatory cellular signaling, while reducing overall cortisol and IL-6 levels among sedentary, overweight middle-aged adults. Specifically, the high dose (2.5 g/d) lowered overall cortisol and IL-6, while the 1.25 g/d dose was sufficient to ward off a post-stress drop in telomerase and IL-10 levels. These findings complement and extend our prior work, which showed that omega-3 supplementation reduced basal inflammation and oxidative stress [2, 14]. Our current findings were dose dependent, such that those receiving the high dose had the greatest differences compared to the placebo group—suggesting a causal relationship. Taken together, these results provide initial evidence that omega-3 may have a unique stress-buffering effect on biomarkers relevant to cellular aging and mental health among a sedentary, overweight middle-aged sample.

Omega-3 supplementation and telomerase reactivity

Among those in the supplement groups, telomerase levels did not change in response to an acute stressor. In contrast, the placebo group's geometric mean of telomerase dropped 24% between 45 and 120 min after the stressor. To our knowledge, this is the longest post-stress follow-up measurement of telomerase reported in the literature. Epel et al. [20] previously showed a slight numerical telomerase decline among non-caregiver control group between 50 and 90 min post stress. Data from our longer follow-up period suggest that without the buffering effects of omega-3, telomerase may ultimately drop below pre-stress levels 2 h post stressor. However, this finding is preliminary due to a paucity of research in this domain and replication is needed.

If telomerase does decline following stress, it could be intensified with repeated or chronic stressors. Indeed, chronically stressed caregivers had lower overall telomerase throughout an acute stressor, compared to non-caregivers [20]. Several samples experiencing adversity have shown a pattern of short telomeres with high telomerase activity [27, 28]—a compensatory profile of short but stable telomeres. However, some individuals under chronic or repeated stress may have the detrimental combination of short telomeres and low telomerase activity, leading to accelerated telomere shortening.

Intriguingly, in the control group, we did not replicate Epel et al.'s [20] finding of increased telomerase within 1 h after the stressor. Unlike Epel et al.'s sample, ours was overweight and rarely engaged in vigorous physical

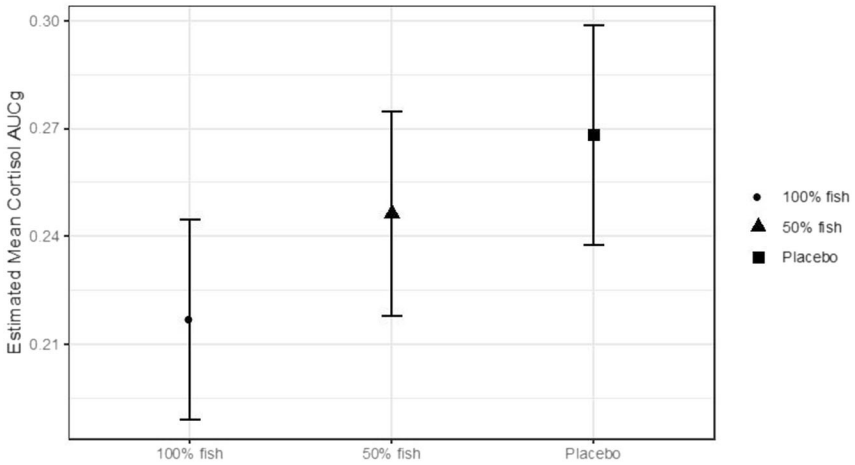

Fig. 2 Omega-3 supplementation lowered total salivary cortisol output throughout an acute stressor ($p = 0.04$). Specifically, supplementation with 2.5 g/d of omega-3 resulted in 19% lower total salivary cortisol throughout the stressor compared to the placebo group ($p = 0.01$), but supplementation with 1.25 g/d of omega-3 did not affect cortisol levels compared to placebo group ($p = 0.29$). Error bars are 95% confidence interval.

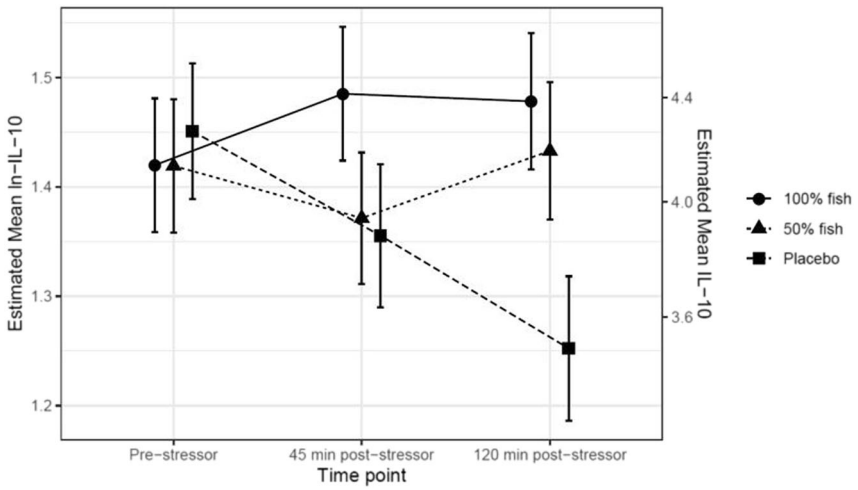

Fig. 3 Omega-3 supplementation influenced IL-10 stress reactivity ($p = 0.05$). Supplementation with either 2.5 or 1.25 g/d of omega-3 prevented changes in IL-10 following an acute stressor ($ps > 0.31$). In contrast, those in the placebo group had a 18% decline in the geometric mean of IL-10 from pre-stress to 120 min after the stressor ($p = 0.004$), such that their IL-10 geometric mean was 26% lower than the high-dose group ($p = 0.012$) and 20% lower than the low-dose group ($p = 0.047$) 120 min after the stressor. Error bars are ±1 standard error.

activity, two risk factors for accelerated aging [21, 29]; these factors may blunt the post-stress telomerase rise—a question for future exploration.

Omega-3 supplementation and cortisol

Omega-3 supplementation reduced total cortisol levels throughout the stressor. The high dose of omega-3 (2.5 g/d), but not the low dose (1.25 g/d), produced a significant (19%) reduction in total cortisol release compared to the placebo group. Importantly, across all participants, cortisol levels rose in response to the stressor, and therefore it is significant that those who received the omega-3 supplementation maintained lower cortisol levels throughout the stressor. These findings are especially notable in light of the growing literature implicating exaggerated cortisol stress reactivity in many clinically important outcomes, including hypertension [30], coronary artery calcification [31], and depression [32]. In fact, heightened cortisol reactivity uniquely predicted coronary artery calcification, whereas sympathetic nervous system reactivity did not [31]. This literature suggests that our finding is clinically meaningful; through lower stress-induced cortisol release, omega-3 supplementation may help to prevent common chronic diseases and depression.

In addition to these disease outcomes, a greater cortisol response to acute stress dovetails with shorter telomeres [7, 15, 16]. Therefore, telomerase may rise in tandem with cortisol to protect telomeres. Indeed, Epel et al. [20] reported that those with greater cortisol reactivity had higher total telomerase levels throughout the stressor. In this sample, the placebo group had higher cortisol levels

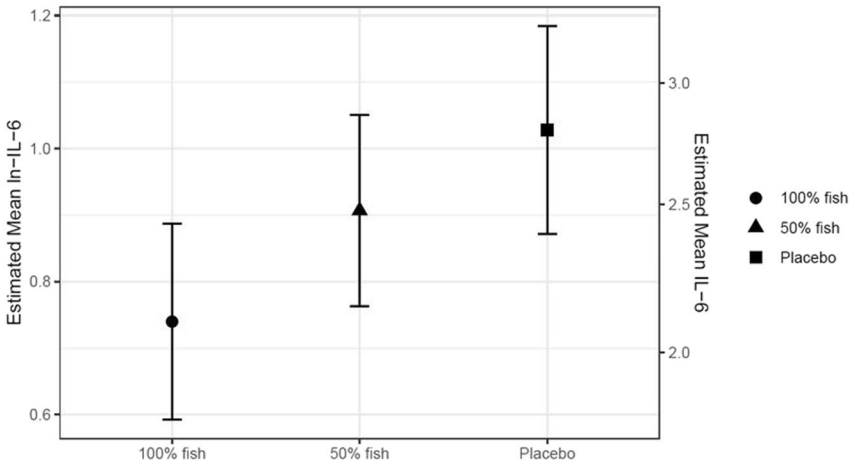

Fig. 4 Omega-3 supplementation lowered overall IL-6 release throughout an acute stressor ($p = 0.03$). Specifically, supplementation with 2.5 g/d of omega-3 resulted in a 33% lower geometric mean of IL-6, compared to the placebo group ($p = 0.007$), but supplementation with 1.25 g/d of omega-3 did not affect IL-6 levels, compared to placebo ($p = 0.26$). Error bars are 95% confidence interval.

compared to the omega-3 groups throughout the stressor, but we observed a telomerase drop 120 min post stress. This finding aligns with the observation that cortisol suppresses lymphocyte telomerase activity in vitro [33]. Without a compensatory telomerase rise, telomeres may ultimately shorten at a faster rate overtime. In contrast, those in the omega-3 supplementation groups had lower total cortisol release and maintained stable levels of telomerase throughout the stressor, a combination that could help to maintain telomere length across time.

Omega-3 supplementation and inflammation

The high 2.5 g/d dose of omega-3 lowered the overall pro-inflammatory IL-6 geometric mean throughout the stressor by 33%. The stressor triggered an increase in IL-6 across all participants, but even so, omega-3 supplementation decreased overall IL-6 levels during this stressful period. Although the 1.25 g/d dose did not lower overall IL-6 levels compared to the placebo, it was sufficient to prevent a 18% post-stress drop in the anti-inflammatory cytokine IL-10 geometric mean. In contrast, neither omega-3 dosage impacted TNF-α or IL-12 reactivity or overall levels. These RCT findings extend evidence from an observational study in which those with higher serum omega-3 levels had lower pro-inflammatory responses during high-stress periods [9]; our results suggest that omega-3 may directly modulate the inflammatory stress response.

Meta-analytic evidence suggests that IL-6 robustly increases following acute stress with a moderate to large effect size, while TNF-α shows a smaller increase [34], which may explain our pattern of results. There were too few studies to assess IL-12 reactivity in the meta-analysis [34]. However, contrary to our placebo group's trajectory, IL-10 usually increases following acute stress, but publication bias is a concern [34]. Our sample characteristics (i.e., overweight, sedentary) may be responsible for the placebo group's post-stress decline in IL-10. If this is the case, omega-3 supplementation appears to reverse this effect, which could help to weaken the link between obesity and accelerated aging.

According to the concept of inflammaging, people with heightened inflammatory reactivity and an imbalance of pro- and anti-inflammatory cellular signaling may age too quickly on a cellular level [35]. Pro-inflammatory cytokines provoke the release of reactive oxygen species [17], contributing to oxidative stress, inefficient cellular functioning, and more inflammation. However, the anti-inflammatory cytokine IL-10 has anti-oxidant properties [36]. By balancing pro- and anti-inflammatory cytokine release throughout an acute stressor, omega-3 promotes healthy cellular aging.

Omega-3 may reduce the risk of developing depression via lowered stress-induced inflammation. Meta analyses indicate that omega-3 supplementation can lower depressive symptoms [37, 38]. Several studies suggest that depressed individuals have a heightened inflammatory stress response [39–41], and, even more intriguingly, one study found that such elevated reactivity predicted increased depressive symptoms 1 year later [10]. Therefore, our finding that omega-3 supplementation blunted the inflammatory stress response may help to explain omega-3 supplementation's antidepressant effect.

Clinical implications

Most US adults' dietary intake of omega-3 is well below recommended values. The Academy of Nutrition and Dietetics recommends that the general population consume 500 mg/day of EPA and DHA [42], but data from a nationally representative sample revealed that the median intake from food and dietary supplements was 18 and 15 mg/day, respectively [43]. For those with preexisting conditions (e.g., mood disorders, cardiovascular disease), the recommendations are even higher [44, 45]. Although it can be

difficult for those at high risk for heart disease to implement and sustain dietary changes [46], our high adherence rate indicates that daily omega-3 supplementation is feasible. Moreover, these findings suggest that apart from other dietary changes, daily omega-3 supplementation alone may help protect cells from the toll of acute stressors, thereby facilitating a healthy biological aging process.

Strengths and limitations

This RCT's multiple strengths include the 4-month supplementation period, the three-arm design with a placebo control, the baseline and post-intervention administration of a well-validated laboratory speech stressor, and the repeated biological measurements for 3.5 h throughout the stressor. Additionally, telomerase measurement extended beyond the previously reported 90-min post stress, which facilitated the discovery of a post-stress telomerase dip in the placebo group that was prevented by omega-3 supplementation. The three-arm design supports causal claims; the biological outcomes were dose dependent, in that the high-dose and placebo groups had the greatest observed differences. Lastly, because each participant completed the acute laboratory stressor before and after the intervention, we could adjust for baseline reactivity to account for potential interindividual variability.

One limitation is that our sample was predominately white, female, and highly educated. Even so, one noteworthy feature of our middle-aged sample is that they were overweight and not physically active, and therefore at increased risk for accelerated aging [21, 29]; these sample characteristics allowed us to examine whether omega-3 supplementation could lessen the divergence between chronological and biological aging. Another limitation is that the analyses did not use all randomized subjects due to the decision to assess telomerase and cortisol reactivity after the trial had already begun. However, we ensured that those included in these models did not differ in their baseline reactivity. Lastly, we did not connect stress-related telomerase fluctuations to telomere length, which is the biomarker associated with disease risk.

Conclusion

Four months of omega-3 supplementation led to a profile of stress resilience—lower overall levels of cortisol and inflammation during stress, and higher levels of telomerase and anti-inflammatory activity during recovery. This has direct relevance to aging biology and psychiatry. These findings are preliminary, but if replicated, they suggest that omega-3 supplementation may limit the impact of repeated stress on cellular aging and depression risk.

Acknowledgements This study was supported in part by NIH grants AG029562, AG038621, UL1RR025755, TL1TR002735, and CA16058. OmegaBrite (Waltham, MA) supplied the omega-3 PUFA supplement and placebo without charge and without restrictions; OmegaBrite did not influence the design, funding, implementation, interpretation, or publication of the data.

Compliance with ethical standards

Conflict of interest ESE and JL are co-founders of Telome Health, Inc., a telomere measurement company. All other authors report no competing interests.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Zhang Y, Zhuang P, He W, Chen J, Wang W, Freedman N, et al. Association of fish and long-chain omega-3 fatty acids intakes with total and cause-specific mortality: prospective analysis of 421 309 individuals. J Intern Med. 2018;284:399–417.
2. Kiecolt-Glaser JK, Epel ES, Belury MA, Andridge R, Lin J, Glaser R, et al. Omega-3 fatty acids, oxidative stress, and leukocyte telomere length: a randomized controlled trial. Brain Behav Immun. 2013;28:16–24.
3. Farzaneh-Far R, Lin J, Epel ES, Harris WS, Blackburn EH, Whooley MA. Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease. JAMA. 2010;303:250–7.
4. Blackburn EH, Epel ES, Lin J. Human telomere biology: a contributory and interactive factor in aging, disease risks, and protection. Science. 2015;350:1193–8.
5. Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, et al. Identification of seven loci affecting mean telomere length and their association with disease. Nat Genet. 2013;45:422–7.
6. Haycock PC, Burgess S, Nounu A, Zheng J, Okoli GN, Bowden J, et al. Association between telomere length and risk of cancer and non-neoplastic diseases: a Mendelian randomization study. JAMA Oncol. 2017;3:636–51.
7. Turner AI, Smyth N, Hall SJ, Torres SJ, Hussein M, Jayasinghe SU, et al. Psychological stress reactivity and future health and disease outcomes: a systematic review of prospective evidence. Psychoneuroendocrinology. 2020;114:104599.
8. Kiecolt-Glaser JK, Renna ME, Shrout MR, Madison AA. Stress reactivity: what pushes us higher, faster, and longer—and why it matters. Curr Dir Psychol Sci. 2020;29:492–8.
9. Maes M, Christophe A, Bosmans E, Lin A, Neels H. In humans, serum polyunsaturated fatty acid levels predict the response of proinflammatory cytokines to psychologic stress. Biol Psychiatry. 2000;47:910–20.
10. Aschbacher K, Epel E, Wolkowitz O, Prather A, Puterman E, Dhabhar F. Maintenance of a positive outlook during acute stress protects against pro-inflammatory reactivity and future depressive symptoms. Brain Behav Immun. 2012;26:346–52.
11. Ginty AT, Conklin SM. Preliminary evidence that acute long-chain omega-3 supplementation reduces cardiovascular reactivity to mental stress: a randomized and placebo controlled trial. Biol Psychol. 2012;89:269–72.
12. Monahan KD, Wilson TE, Ray CA. Omega-3 fatty acid supplementation augments sympathetic nerve activity responses to physiological stressors in humans. Hypertension. 2004;44:732–8.
13. Rousseau D, Moreau D, Raederstorff D, Sergiel JP, Rupp H, Müggli R, et al. Is a dietary n-3 fatty acid supplement able to

influence the cardiac effect of the psychological stress? Mol Cell Biochem. 1998;178:353–66.
14. Kiecolt-Glaser JK, Belury MA, Andridge R, Malarkey WB, Hwang BS, Glaser R. Omega-3 supplementation lowers inflammation in healthy middle-aged and older adults: a randomized controlled trial. Brain Behav Immun. 2012;26:988–95.
15. Steptoe A, Hamer M, Lin J, Blackburn EH, Erusalimsky JD. The longitudinal relationship between cortisol responses to mental stress and leukocyte telomere attrition. J Clin Endocrinol Metab. 2017;102:962–9.
16. Tomiyama AJ, O'Donovan A, Lin J, Puterman E, Lazaro A, Chan J, et al. Does cellular aging relate to patterns of allostasis? An examination of basal and stress reactive HPA axis activity and telomere length. Physiol Behav. 2012;106:40–5.
17. De Biase L, Pignatelli P, Lenti L, Tocci G, Piccioni F, Riondino S, et al. Enhanced TNFα and oxidative stress in patients with heart failure: effect of TNFα on platelet O2-production. Thromb Haemost. 2003;90:317–25.
18. Gidron Y, Russ K, Tissarchondou H, Warner J. The relation between psychological factors and DNA-damage: a critical review. Biol Psychol. 2006;72:291–304.
19. Kurz DJ, Decary S, Hong Y, Trivier E, Akhmedov A, Erusalimsky JD. Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. J Cell Sci. 2004;117:2417–26.
20. Epel ES, Lin J, Dhabhar FS, Wolkowitz OM, Puterman E, Karan L, et al. Dynamics of telomerase activity in response to acute psychological stress. Brain Behav Immun. 2010;24:531–9.
21. Horvath S, Erhart W, Brosch M, Ammerpohl O, von Schönfels W, Ahrens M, et al. Obesity accelerates epigenetic aging of human liver. Proc Natl Acad Sci. 2014;111:15538–43.
22. Kirschbaum C, Pirke K-M, Hellhammer DH. The 'Trier Social Stress Test'—a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology. 1993;28:76–81.
23. Allen AP, Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Biological and psychological markers of stress in humans: focus on the Trier Social Stress Test. Neurosci Biobehav Rev. 2014;38:94–124.
24. Spielberger C, Gorsuch R, Lushene R, Vagg P, Jacobs G. Manual for the state-trait anxiety inventory. Palo Alto, California: Consulting Psychologists Press; 1983.
25. Altemus M, Rao B, Dhabhar FS, Ding W, Granstein RD. Stress-induced changes in skin barrier function in healthy women. J Investig Dermatol. 2001;117:309–17.
26. Bekhbat M, Neigh GN. Sex differences in the neuro-immune consequences of stress: focus on depression and anxiety. Brain Behav Immun. 2018;67:1–12.
27. Zalli A, Carvalho LA, Lin J, Hamer M, Erusalimsky JD, Blackburn EH, et al. Shorter telomeres with high telomerase activity are associated with raised allostatic load and impoverished psychosocial resources. Proc Natl Acad Sci. 2014;111:4519–24.
28. Chen SH, Epel ES, Mellon SH, Lin J, Reus VI, Rosser R, et al. Adverse childhood experiences and leukocyte telomere maintenance in depressed and healthy adults. J Affect Disord. 2014;169:86–90.
29. Du M, Prescott J, Kraft P, Han J, Giovannucci E, Hankinson SE, et al. Physical activity, sedentary behavior, and leukocyte telomere length in women. Am J Epidemiol. 2012;175:414–22.
30. Hamer M, Steptoe A. Cortisol responses to mental stress and incident hypertension in healthy men and women. J Clin Endocrinol Metab. 2012;97:E29–34.
31. Hamer M, Endrighi R, Venuraju SM, Lahiri A, Steptoe A. Cortisol responses to mental stress and the progression of coronary artery calcification in healthy men and women. PLoS ONE. 2012;7:e31356.
32. Burke HM, Davis MC, Otte C, Mohr DC. Depression and cortisol responses to psychological stress: a meta-analysis. Psychoneuroendocrinology. 2005;30:846–56.
33. Choi J, Fauce SR, Effros RB. Reduced telomerase activity in human T lymphocytes exposed to cortisol. Brain Behav Immun. 2008;22:600–5.
34. Marsland AL, Walsh C, Lockwood K, John-Henderson NA. The effects of acute psychological stress on circulating and stimulated inflammatory markers: a systematic review and meta-analysis. Brain Behav Immun. 2017;64:208–19.
35. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging: an evolutionary perspective on immunosenescence. Ann N Y Acad Sci. 2000;908:244–54.
36. Haddad JJ, Fahlman CS. Redox-and oxidant-mediated regulation of interleukin-10: an anti-inflammatory, antioxidant cytokine? Biochem Biophys Res Commun. 2002;297:163–76.
37. Liao Y, Xie B, Zhang H, He Q, Guo L, Subramaniapillai M, et al. Efficacy of omega-3 PUFAs in depression: a meta-analysis. Transl Psychiatry. 2019;9:1–9.
38. Mocking R, Harmsen I, Assies J, Koeter M, Ruhé H, Schene A. Meta-analysis and meta-regression of omega-3 polyunsaturated fatty acid supplementation for major depressive disorder. Transl Psychiatry. 2016;6:e756.
39. Miller GE, Rohleder N, Stetler C, Kirschbaum C. Clinical depression and regulation of the inflammatory response during acute stress. Psychosom Med. 2005;67:679–87.
40. Fagundes CP, Glaser R, Hwang BS, Malarkey WB, Kiecolt-Glaser JK. Depressive symptoms enhance stress-induced inflammatory responses. Brain Behav Immun. 2013;31:172–6.
41. Pace TW, Mletzko TC, Alagbe O, Musselman DL, Nemeroff CB, Miller AH, et al. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. Am J Psychiatry. 2006;163:1630–3.
42. Vannice G, Rasmussen H. Position of the academy of nutrition and dietetics: dietary fatty acids for healthy adults. J Acad Nutr Diet. 2014;114:136–53.
43. Papanikolaou Y, Brooks J, Reider C, Fulgoni VL. US adults are not meeting recommended levels for fish and omega-3 fatty acid intake: results of an analysis using observational data from NHANES 2003–2008. Nutr J. 2014;13:31.
44. Freeman MP, Hibbeln JR, Wisner KL, Davis JM, Mischoulon D, Peet M, et al. Omega-3 fatty acids: evidence basis for treatment and future research in psychiatry. J Clin Psychiatry. 2006;67:1954.
45. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation. 2002;106:2747–57.
46. Dansinger ML, Gleason JA, Griffith JL, Selker HP, Schaefer EJ. Comparison of the atkins, ornish, weight watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. JAMA. 2005;293:43–53.