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Effect of omega-3 fatty acid supplementation on telomere length and telomerase activity: A systematic review of clinical trials

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ABSTRACT

Evidence suggests antioxidant and anti-inflammatory properties of omega-3 polyunsaturated fatty acids (n-3 PUFA). However, the effect of supplementation of this fatty acid profile on the telomere length and the telomerase enzyme activity was not revised yet. The PubMed and Embase® databases were used to search for clinical trials. A total of six clinical trials were revised. Omega-3 PUFA supplementation did not statistically affect telomere length in three out of three studies but affected telomerase activity in two out of four studies. The supplementation increased telomerase enzyme activity in subjects with first-episode schizophrenia. Besides, it decreased telomerase enzyme activity without modulating the effects of Pro12Ala polymorphism on the PPARγ gene in type 2 diabetes subjects. The methodological differences between the studies and the limited number of studies on the theme suggest that further studies are needed to elucidate the effects of n-3 PUFA supplementation on telomere length and telomerase enzyme activity in humans.

Abbreviations

DHA	docosahexaenoic acids
EPA	eicosapentaenoic acids
n-3 PUFA	omega-3 polyunsaturated fatty acid
MUFA	monounsaturated fatty acid
Nrf2	factor-2 related erythroid nuclear transcription factor 2
NF-kB	nuclear factor kappa B
PBMC	peripheral blood mononuclear cells
PPARγ	peroxisome proliferator activated receptor gamma
SFA	saturated fatty acid
SPMs	specialized pro-family mediators
TRF1	telomeric repeat-binding factor 1

1. Introduction

aging-related diseases have been responsible for a crescent number of premature deaths worldwide [1,2]. Type 2 diabetes, cardiovascular diseases, chronic kidney disease, neurodegenerative diseases, mental disorders [3,4], and even central fat accumulation [5] are some examples of common morbidities in aging. Telomere shortening is directly related to the chronological age of individuals and is proposed as a programmed theory of the cellular aging process [6,7]. Furthermore, telomere attrition has been associated with all these diseases [8–10].

Telomeres are complex structures formed by proteins and repeated sequences of DNA (5′-TAGGG-3′) located at the end of chromosomes, responsible for avoiding chromosomes fusion, for protecting the DNA from oxidative damage suffered throughout life, thus keeping genomic stability [11,12]. In its turn, the telomerase enzyme is responsible for maintaining telomere length [12,13]. In addition to genetic factors and the own cell division, nongenetic factors such as environmental, lifestyle events, and behavior are associated with telomeres shortening, leading to senescence and consequent cell dysfunction [11]. Although telomere shortening is a natural process in the organism [14], studies have shown that it can be accelerated by oxidative stress and inflammation [15,16].

Considering the etiology of age-related diseases linked to oxidative stress and inflammation process, nutrition is one of the behavioral and modifiable risk factors that can interfere with these processes [17,18]. Thus, it becomes necessary to investigate the role of foods and nutrients

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Table 1
Characterization of six clinical trials that investigated the n-3 polyunsaturated fatty acid supplementation on telomere length and/or in telomerase enzyme activity.

Authors, year, and country	Sample characteristics	Intervention characteristics	Method	Study design and follow-up	Markers	Main results
Holub et al., 2020 USA	**n:** 30 adults with T2DM **Age:** 56.6 (SD 8.9) y **BMI:** 34.6 (SD 7.5) kg/m^2	−81 mg/d of aspirin alone in capsules for 7 days −4 g/day of n-3 PUFA fish oil (EPA: 1600 mg + DHA: 800 mg) alone in capsules for 28 days -Combination of n-3 PUFA fish oil and aspirin in capsules for another 7 days.	TRAPeze telomerase detection kit; RT-PCR (sample: PBMC)	8-week sequential therapy unblinded clinical trial	Relative telomerase activity (copies)	No statistically significant change in telomerase enzyme copy number compared to baseline and between interventions
Pawełczyk et al., 2017 Poland	**n:** 71 subjects with a first episode of schizophrenia **Age:** G1: 23.2 (SD 4.8) y G2: 23.3 (SD 4.8) y	**G1:** 2.2 g/day of n-3 PUFA fish oil (EPA: 1320 mg + DHA: 880 mg) + 0.2% of vitamin E in capsules **G2:** olive oil (MUFA: 73.9% + PUFA: 9.8%) + 0.2% of vitamin E in capsules	TE ELISA kit (sample: PBMC)	Randomized placebo-controlled clinical trial −26 weeks	Telomerase concentration	**G1:** ↑ levels of telomerase *vs.* **G2**
Toupchian et al., 2016 Iran	**n:** 72 PPARy Pro12Ala polymorphism genotyped subjects with T2DM **Age:** **G1:** 55.9 (SD 7.8) y **G2:** 56 (SD 7) y	**G1:** 2.4 g/day of n-3 PUFA fish oil (EPA: 400 mg + DHA: 1450 mg) in capsules **G2:** 600 mg of paraffin in capsules	Telomeric repeat amplificaton protocol (TRAP); PCR-Elisa RT-PCR (sample: PBMC)	Double-blind randomized controlled clinical trial - 8 weeks	Telomerase activity	**G1:** ↓ telomerase activity *vs.* baseline and **G2** No statistically significant change in telomerase activity between genotypes
Barden et al., 2016 Australia	**n:** 74 subjects with chronic kidney disease **Age:** 56.5 (SEM 1.4) y **BMI:** 27.3 (SEM 0.5) kg/m^2	**G1:** 4 g/day of n-3 PUFA (EPA: 460 mg + DPA: 38 mg + DHA: 380 mg) in capsules **G2:** 200 mg/day of coenzyme Q10 in capsules **G3:** Combination of 4 g/day de n-3 PUFA + 200 mg/day of coenzyme Q10 in capsules **G4:** 4 g/day of olive oil in capsules	qPCR (sample: PBMC and neutrophils)	Double-blind randomized placebo-controlled clinical trial −8 weeks	Absolute telomere length (kb/diploide genome)	No statistically significant change in telomere length compared to baseline and between interventions n-3 PUFA supplementation: ↑ telomere length in neutrophils when results were adjusted by total neutrophil count *vs.* subjects who did not receive n-3
O' Callaghan et al., 2014 Australia	**n:** 33 older adults with mild cognitive impairment **G1:** **Age:** 74.8 (SD 5) y **BMI:** 28.1 (SD 4.1) kg/m^2 **G2:** Age: 74.2 (SD 7) y BMI: 26.8 (SD 2.6) kg/m^2 **G3:** Age: 73 (SD 3.9) y BMI: 28.1 (SD 5.3) kg/m^2	**G1:** fish oil (EPA: 1670 mg + DHA: 160 mg) in capsules **G2:** fish oil (EPA: 400 mg + DHA: 1550 mg) in capsules **G3:** 2.2 g/day of linoleic acid from safflower oil in capsules (total de 4 cápsulas diárias)	qPCR (sample: whole blood)	Double-blind, randomized placebo-controlled clinical trial −6 months (24 weeks)	Absolute telomere length (kb/diploide genome)	No statistically significant change in telomere length compared to baseline and between interventions
Kiecolt-glaser et al., 2013 EUA	**n:** 106 adults and older adults with overweight **G1:** **Age:** 50.6 (SD 6.5) y **BMI:** 30.7 (SD 3.8) kg/m^2 **G2:** **Age:** 50.3 (SD 7.8) y **BMI:** 31.7 (SD 4.5) kg/m^2 **G3:** **Age:** 51.2 (SD 8.9) y **BMI:** 31.1 (SD4.8) kg/m^2	**G1:** 2.5 g/day of n-3 PUFA fish oil (EPA: 2080 mg + DHA: 340 mg) + 1 UI vit. E in capsules **G2:** 1.25 g/day n-3 PUFA fish oil (EPA: 1040 mg + DHA: 170 mg) + 1 UI vit. E in capsules **G3:** SFA:MUFA:PUFA ratio= 37:42:21 + 1 UI vit. E in capsules	TRAPeze telomerase detection kit qPCR (sample: peripheral blood lymphocytes)	Double-blind, randomized placebo-controlled clinical trial −4 months (16 weeks)	Telomere length (base pairs) and telomerase activity	No statistically significant change in telomere length and telomerase activity between interventions ↓ n-6 PUFA:n-3 PUFA ratio = ↑ telomere length

Legend: G, group; BMI, body mass index; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; RT-PCR, reverse transcription polymerase chain reaction; qPCR, quantitative real-time PCR; EPA, eicosapentaenoic fatty acid; DHA, docosahexaenoic fatty acid; PBMC, peripheral blood mononuclear cell.

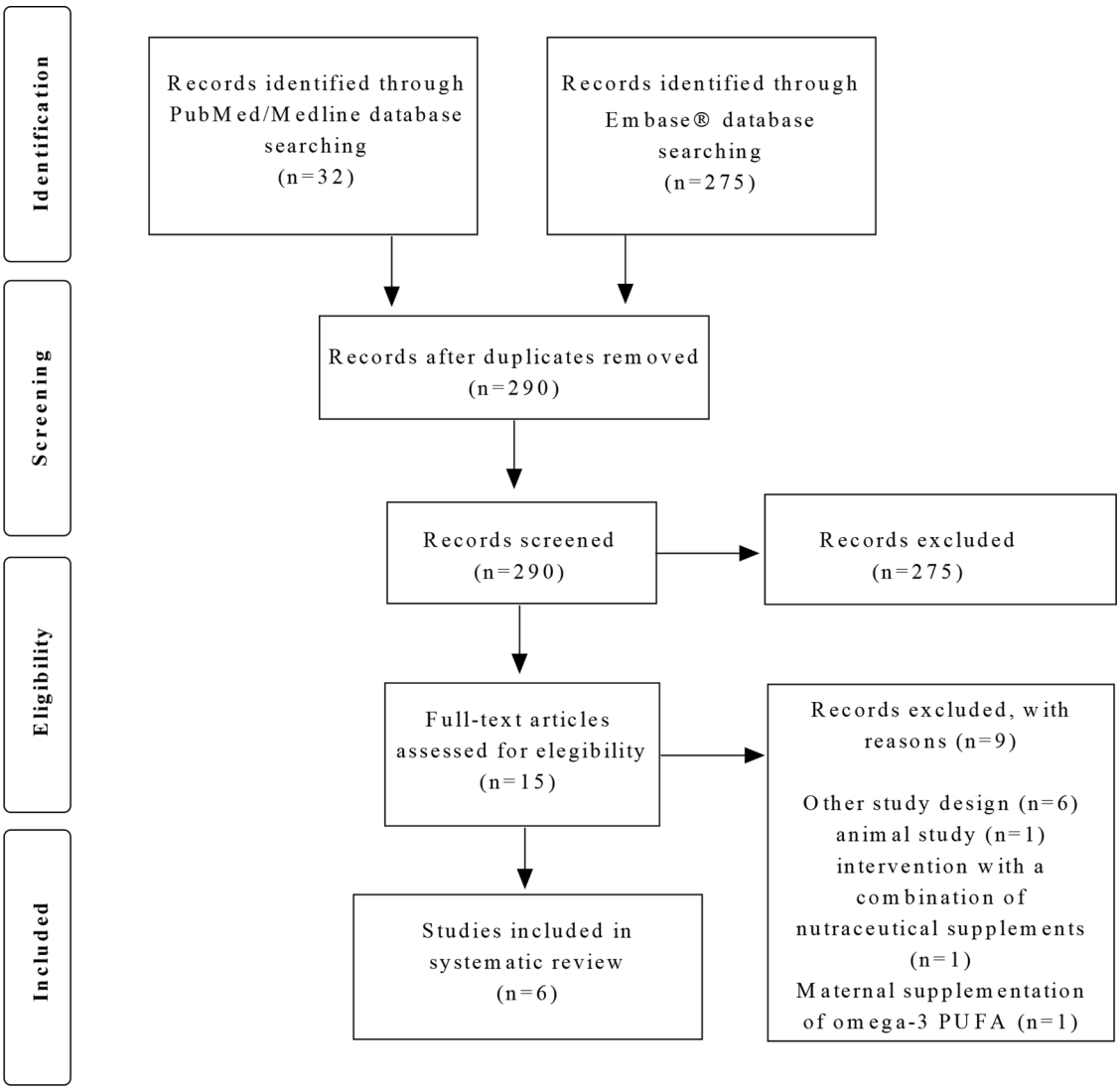

Fig. 1. Flowchart of studies included in the systematic review.

in developing, progressing, and protecting against diseases and the mechanism behind longevity and a healthy lifespan.

Well-described reviews of epidemiological studies [19,20] and also randomized clinical trials [20] showed that high adherence to the Mediterranean diet [19], as well as the consumption of some antioxidants, fruits, and vegetables, are associated with longer telomeres length [20]. On the other hand, several studies have investigated the role of n-3 polyunsaturated fatty acid (n-3 PUFA) supplementation as a potential modulators of telomere length and telomerase activity [21–25]. Two review studies also briefly cited the results of some original studies on the effect of n-3 PUFA supplementation on telomere length and/or telomerase activity [20,26]. Despite this, no review has focused on critically evaluating these studies, particularly reviewing omega-3 supplementation alone rather than in combination with other compounds.

N-3 PUFA is a long-chain fatty acid present in significant amounts in plant and animal foods. Health beneficial effects of n-3 PUFA supplementation primarily attributed to its antioxidant and anti-inflammatory properties have been suggested [17,27,28]. Eicosapentaenoic acids (EPA) and docosahexaenoic acids (DHA) present mainly in fish oil have been associated with cardiovascular risk reduction [29]. However, it has not been answered if the supplementation of n-3 PUFA affects the modulation of the senescence process through telomere length and telomerase enzyme activity. Thus, this systematic review aims to review the effect of n-3 PUFA supplementation on telomere length and telomerase enzyme activity in humans.

2. Materials and methods

2.1. Protocol and registration

This systematic review was carried out according to the guideline "Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)" [30] and was registered in PROSPERO (https://www.crd.york.ac.uk/prospero/), registration number CRD42020186349.

2.2. Literature search

Studies were identified by searching the following electronic databases: MEDLINE / PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Embase® (https://www-embase.ez35.periodicos.capes.gov.br/a/#/picoSearch). The keywords chosen for the search were based on MeSH terms and a list of synonyms suggested by Embase®. The intervention and outcome fields of the PICOS (population/intervention/comparator/outcome/study design) search strategy were used for the research. Then, filters were used to select studies in humans and clinical trials. Table 1 lists the key terms used in the search. The last search was carried out in June 2021. The search was performed independently by two authors (AS and BKSS). First, a selection was made by titles and abstracts. Then, the

articles were read in full and eligible studies were selected. Any disagreement between the authors was resolved by consensus. Finally, a backward search was performed to identify possible relevant articles to be included in the review. Duplicate articles were removed manually.

2.3. Eligibility criteria

The following criteria were applied for the inclusion of studies: (1) original clinical trials, randomized or not, controlled or not; (2) studies that evaluated the effect of consuming any dose of n-3 PUFA at any time; (3) studies that evaluated telomere length or the expression of telomerase enzyme activity. If data were duplicated in more than 1 study, the most complete and detailed study was included. The following exclusion criteria were applied: (1) studies with observational design, reviews, congress abstracts, letters, protocol articles, notes; (2) no investigation of telomere length or telomere-related markers; (3) interventions in which fatty acids were consumed along with mineral/vitamin supplements, other nutritional supplements; and (4) interventions that included behavioral modifications, such as physical activity.

2.4. Selection of studies and data extraction

The studies were selected by analyzing titles, abstracts, and full texts by two independent authors (AS and BKSS), and differences were resolved by consensus. In the absence of the whole article or when additional information was needed to compile the results, an email was sent to the corresponding author requesting the article or information.

Of the studies eligible for the review, two independent authors (AS and BKSS) extracted the following data from the studies: i) name of the first author, year of publication, and country of study, ii) sample characteristics (number of participants, presence of diseases, age, and body mass index), iii) characteristics of the intervention (description of each intervention group, as well as the doses of n-3 PUFA used), iv) study design and duration, v) analysis technique used to measure the telomeres length and telomerase and cell type used for the analyses, vi) markers evaluated in the study (telomere length and/or telomerase, and vii) main results.

2.5. Risk of bias assessment

The risk of bias was assessed independently by two authors (AS and BKSS) following the Joanna Briggs Institute Reviewer's Manual (JBI). The purpose of this appraisal is to assess the methodological quality of a study and to determine the extent to which a study has addressed the possibility of bias in its design, conduct, and analysis. Thirteen questions for randomized clinical trials and nine questions for non-randomized clinical trials were answered for each study included in the systematic review. Finally, the answers to these questions were classified as Yes, No, Unclear, or not applicable [31].

3. Results

3.1. Studies selection

We identified three hundred and seven articles in our search and removed nineteen duplicate articles. During the screening of titles and abstracts, two hundred and seventy-five articles were excluded for not meeting the eligibility criteria. Fifteen articles remained for full-text evaluation, and then nine articles were excluded. Other study designs (observational studies), animal studies, and interventions with a combination of nutraceutical supplements were the reasons for excluding the studies. As a result, six articles met the eligibility criteria and were included in this systematic review [21–25,32] (**Fig. 1**). The characteristics of these studies are presented in Table 1.

3.2. Studies characteristics

The six eligible studies were published between 2013 and 2020. Five studies were randomized [21–23,25,33], only one was a sequential intervention [32], and four were double-blinded [21–23,25]. The studies were conducted in Australia [22,23], US [21,32], Iran [25], and Poland [24]. A total of 386 subjects were evaluated, being adolescents and adults with first episodes of schizophrenia [24], adults with chronic kidney disease [23], older adults with mild cognitive impairment [22], and adults with overweight [21]. In addition, three studies assessed telomerase activity [24,25,32], another two studies assessed telomere length [22,23] and one study evaluated both markers [21]. Of the studies that evaluated telomerase activity, the intervention period ranged from 28 days to 6 months, with doses of EPA from 1040 mg to 2080 mg/day and DHA between 170 mg to 880 mg/day. On the other hand, of the studies that evaluated telomere length, the intervention period ranged from 8 weeks to 6 months, with doses of EPA from 400 mg to 2080 mg/day and DHA between 160 mg to 1550 mg/day.

Controls used in the studies were olive oil [23,24], aspirin [32], n-6 PUFA [22], paraffin [25], and a mixture of oils - SFA:MUFA:PUFA ratio of 37:42:21 [21]. Furthermore, in all studies, n-3 PUFA were taken in capsules and taken in the context of a habitual diet. Peripheral blood mononuclear cells (PBMC) were used in most studies.

3.3. Results of individual studies

In summary, omega-3 PUFA supplementation did not statistically affect telomere length in three out of three studies but affected telomerase activity in two out of four studies. In patients with chronic kidney disease, the supplementation of 4 g of n-3 PUFA (EPA: 460 mg + DHA: 380 mg) or combined with the supplementation of coenzyme Q10 did not change telomere length in eight weeks compared to the supplementation of coenzyme Q10 alone and, also with olive oil. However, when analyzed together, those who received n-3 PUFA had increased neutrophil telomere length adjusted by total neutrophil count compared to patients who did not receive n-3 PUFA. This result was independent of age, sex, and body mass index [23]. In another study, the supplementation of different doses of EPA and DHA (EPA: 1.670 mg + DHA: 160 0.16 mg and EPA: 400 mg + DHA: 1550 mg) did not affect telomere length in older adults with mild cognitive impairment compared to baseline and the supplementation of 2.2 g/day of safflower oil in six months [22].

In line with these findings, one study found that the supplementation of different doses of n-3 PUFA (EPA: 1040 mg + DHA: 170 mg and EPA: 2080 mg + DHA: 340 mg, respectively) did not impact telomere length and telomerase enzyme activity of overweight older adults over four months. Such results are of comparison between doses and with the placebo group. To further explore the data obtained, the authors unified the groups that received both doses of n-3 PUFA and observed that the decrease in the n-6 PUFA:n-3 PUFA ratio was associated with an increase in telomere length [21]. In subjects with type 2 diabetes mellitus, the supplementation of 4 g of n-3 PUFA (EPA: 1600 mg + DHA: 800 mg) for twenty-eight days also did not affect the copy number of the telomerase enzyme activity compared to baseline and aspirin consumption [32].

On the other hand, supplementation of 2.4 g of n-3 PUFA (EPA: 400 mg + DHA: 1450 mg) for eight weeks decreased the telomerase enzyme activity of subjects with type 2 diabetes without modulating effects of PPARγ Pro12Ala polymorphism compared to the placebo group [25]. In another study, supplementation of 4 g of n-3 PUFA (EPA: 1320 mg + DHA: 880 mg) for twenty-six weeks more pronouncedly increased telomerase enzyme levels in subjects diagnosed with first-episode schizophrenia compared to the placebo group who received olive oil. However, after eight weeks of intervention, this effect was not observed [24].

3.4. Risk of bias

Supplementary Figure 1 summarizes the results of the risk of bias assessment. Most of the studies (76%) did not address the possibility of bias in its design, conduct, and analysis. For example, the intention-to-treat analysis was mentioned in just one article, which justifies the prevalence of increased risk of bias on the item "Patients analyzed in the groups to which they were randomized". In addition, most studies did not mention the number and training of raters and their intra and inter reliability, which confer some unclear answers.

4. Discussion and conclusions

This is the first systematic review of clinical trials focusing on supplementation of n-3 PUFA as a potential modulator of telomere length and telomerase enzyme activity in humans. Despite being a natural phenomenon of cell fate [12], telomere shortening has been associated with behavioral characteristics such as food consumption [19,20]. In this sense, behavioral changes focused on improving dietary constituents have shown promising results in maintaining telomere length or decelerating telomere attrite [34] and in greater telomerase enzyme activity [24].

To date, six clinical trials investigated the effects of n-3 PUFA supplementation on telomere length (EPA: 400 mg to 2080 mg + DHA: 160 mg to 1550 mg) and/or telomerase enzyme activity (EPA: 1040 mg to 2080 mg + DHA: 170 mg to 880 mg), with unanimity regarding the absence of effect on telomere length. Omega-3 PUFA supplementation did not statistically affect telomere length in three out of three studies but affected telomerase activity in two out of four studies. Similar results were observed when n-3 PUFA was supplemented with other compounds. The supplementation of NucleVital®Q10 complex, which contains n-3 PUFA (1350 mg), ubiquinone (300 mg), astaxanthin (15 mg), lycopene (45 mg), lutein palmitate (30 mg), zeaxanthine palmitate (6 mg), L-selenomethionine (330 mg), cholecalciferol (30 μg), and α-tocopherol (45 mg) for twelve weeks unchanged telomere length, but increased telomerase levels by more than 25% in healthy subjects [33]. Interestingly, linseed-oil-supplemented pigs showed lower levels of the shelterin telomeric repeat-binding factor 1 (TRF1) protein compared to the control group [35]. TRF1 increased is associated with oxidative stress and is involved in the negative regulation of the telomere length by inhibiting the telomerase activity [36–38] suggesting a possible protective role of n-3 PUFA from linseed oil on telomere attrition.

Despite the lack of statistical significance, a trend towards lesser shortening of telomeres after n-3 PUFA supplementation could be observed [21–23]. Thus, this result may be relevant from a clinical point of view. Considering that telomere attrition is associated with the triggering of diseases [38], slowing down this process, can be advantageous, even if it is minimal.

The anti-inflammatory and antioxidant bioactivity of n-3 PUFA is recognized in the literature either from vegetables [39] or from animal sources such as fish oil [40]. The increase in reactive oxygen species in the organism parallel to the state of imbalance redox and inflammation seem to contribute to telomeric attrition and, consequently, exposure of genetic material to oxidation. In most studies included in this review, the subjects evaluated had some morbidity, such as type 2 diabetes, chronic kidney disease, overweight, and schizophrenia. Both inflammation and oxidative stress are a consequence and are also involved in the progression of these morbidities [41,42]. Thus, it would be precisely through these two mechanisms of action that we expected to observe the beneficial effects of n-3 PUFA supplementation on telomere length and telomerase activity [7].

Studies suggest that peroxidation of n-3 PUFA increases the concentration of 4-hydroxyhexenal in the cytosol, which activates factor-2 related erythroid nuclear transcription factor 2 (Nrf2). Thus, antioxidant enzymes are produced, giving antioxidant characteristics to this fatty acid profile [43,44]. In this sense, EPA and DHA could attenuate oxidative stress in vascular endothelial cells through upregulation of Nrf2 [18].

In addition to antioxidant effects, n-3 PUFA has anti-inflammatory properties. N-3 PUFA's are involved in the synthesis of specialized pro-family mediators (SPMs), such as resolvins, maresins, and protectins, which are involved in the resolution of inflammation [45]. Besides, dietary fats have a key role in modulating cell membranes, and thus inflammatory mediators such as leukotrienes, prostaglandins, among others, can be produced depending on the lipid composition of the membranes. In this sense, a balanced diet between the proportions of n-6 PUFA: n-3 PUFA can favor the balance between arachidonic fatty acid and EPA in cell membranes. When removed by the phospholipase A2 enzyme into cells, the EPA fatty acids will be bioconverted by the lipoxygenase and cyclooxygenase 1 enzymes into unique leukotrienes, prostaglandins, and thromboxanes. These substances have anti-inflammatory characteristics, unlike the leukotrienes produced by arachidonic acid. The anti-inflammatory capacity of n-3 PUFA is also related to the disarticulation of the complete TAK-1-TAB-1/2-ubiquitin. EPA and DHA fatty acids bind to the GPR120 receptor on cell membranes, recruiting the β-arrestin protein. When activated by GPR120, β-arrestin rescues the binding protein 1/2 (TAB-1/2) to TAK-1 from the inflammatory protein complex initiated by inflammatory mediators such as TNF or by activation of toll-like receptors, preventing dephosphorylation of the kappa B inhibitor kinase (IKK). Thus, nuclear factor kappa B (NF-kB) does not target the cell nucleus to stimulate the production of cytokines and other inflammatory mediators. Furthermore, n-3 PUFA increases the expression of the peroxisome proliferator-activated receptor γ (PPARγ) and consequently negatively impacts the activity of the transcription factor NF-kB through the mechanism of transrepression [28,46]. In addition to these effects, n-3 PUFA also modulates the expression of sterol regulatory element-binding protein (SREBP), decreasing the liver's synthesis of fatty acids and cholesterol [47].

In one of the revised studies, the decrease in the n-6:n-3 PUFA ratio was associated with longer telomere length [21]. This association may indicate that the imbalance between these two types of fat consumption is related to inflammatory processes and oxidative stress, which are the main links in the cellular senescence process. The imbalance between the consumption of n-6:n-3 fatty acids, typical of the Western dietary pattern, may favor the production of serial pair pro-inflammatory mediators such as leukotrienes, prostaglandins, and thromboxanes [28]. In patients with stable coronary artery disease, there was an inverse relationship between baseline blood levels of marine n-3 PUFA and the rate of telomere shortening over five years [48]. In another study, higher n-6: n-3 PUFAs ratio and lower EPA and DHA were associated with shorter telomere length in the Chinese population [49]. Furthermore, in the Nurse's Health Study, the n-6 PUFA linoleic acid intake was inversely associated with telomere length [50].

In subjects with type 2 diabetes, supplementation of n-3 PUFA for 28 days numerically increased telomerase activity, but without statistical significance in relation to aspirin consumption and the same amount of EPA and DHA combined with the consumption of aspirin [32]. In contrast, supplementation of a lower dose of EPA and higher dose of DHA compared to the study above [32] decreased telomerase levels in type 2 diabetes subjects with PPARy Pro12ALA polymorphism within eight weeks [25]. In the first study [32], the short intervention time may be insufficient to detect a statistically significant increase in telomerase activity. Studies have shown that subjects with the rs1801282 variant may have decreased type 2 diabetes susceptibility and may have positive, beneficial effects on insulin sensitivity and body mass index [51, 52]. Despite this, DHA-enriched fish oil upregulated cyclin-dependent kinase inhibitor 2A expression, a marker of cell senescence, which is related to inhibition of the telomerase activity [25].

Subjects with first-episode schizophrenia seem to benefit from n-3 PUFA supplementation, as they had increased telomerase enzyme activity after six months of supplementation compared to supplementation

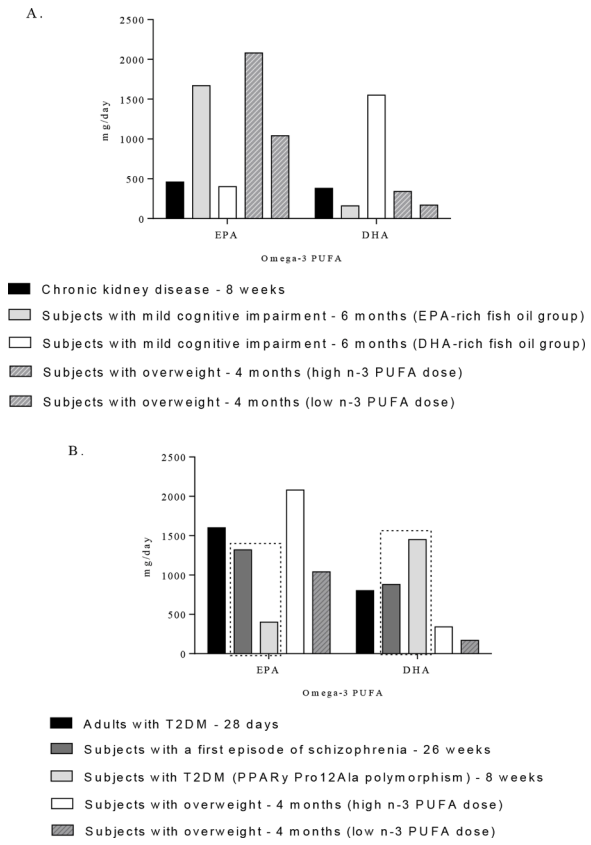

Fig. 2. Comparison of EPA and DHA doses used in studies that evaluated the effects of n-3 supplementation on: **A.** telomere length and **B.** telomerase enzyme activity. Data dashed are from studies that observed a statistically significant effect of n-3 PUFA supplementation on telomerase enzyme activity.

with olive oil [24]. In contrast, the supplementation with a higher dose of EPA but a lower dose of DHA compared to the prior study did not impact telomerase activity in adults and older adults with overweight compared to the placebo group after sixteen weeks. Evidence suggests that oxidative stress and inflammation are involved in the pathology of schizophrenia [53]. Due to the bond between schizophrenia pathophysiology and the anti-inflammatory and antioxidant properties of n-3 mentioned earlier, individuals with the disease may benefit from supplementation with EPA + DHA. A detail of all three studies that observed a trend toward less telomere shortening after supplementation with n-3 PUFA [21–23] is that the dose of EPA was higher than those of DHA. Furthermore, studies have shown improvement in lipid profile markers after EPA supplementation, whereas DHA supplementation appears to increase low-density lipoprotein cholesterol concentrations [54–56]. In another study, EPA, but not DHA, markedly activated the sirtuin 1 gene expression in THP1 cells [57]. In fact, molecular mechanisms link telomeres to sirtuin expression, which are positively related to telomere stabilization [58]. Despite this, the mechanisms of EPA and DHA in cell senescence markers still need to be further studied. It is noteworthy that the studies differed regarding the doses of EPA and DHA supplemented, intervention time, profile and number of evaluated subjects, and comparison group (**Fig. 2**). These observations may justify the different results regarding telomerase activity between studies and allow us to infer that more studies are necessary for the field.

In this systematic review, we critically analyzed the studies that assessed the effects of the n-3 PUFA supplementation on telomere length and telomerase enzyme activity. Heterogeneous studies were compared in this review. Differences between studies regarding the doses of EPA and DHA supplemented, intervention time, profile and number of evaluated subjects, and the comparison group were detected and critically assessed. It is necessary to conduct more studies with n-3 PUFA supplementation and other promising fatty acid sources. In addition, it would be interesting to assess markers related to telomere constituents such as proteins from the shelterin complex and others. Finally, we emphasize the lack of human studies on mechanisms behind the effects of n-3 PUFA on the senescence process through modulation of telomere length and telomerase enzyme activity.

Supplementation of n-3 PUFA had no statistically significant effect on telomere length, but showed a tendency to reduce telomere attrition in three of three studies that assessed telomere length. The supplementation increased telomerase enzyme activity in subjects with first-episode schizophrenia. Besides, it decreased telomerase enzyme activity without modulating the effects of Pro12Ala polymorphism on the PPARγ gene in type 2 diabetes subjects. The methodological differences between the studies and the limited number of studies on the theme allow us to infer that further studies are needed to elucidate the effects of n-3 PUFA supplementation on telomere length and telomerase enzyme activity in humans.

CRediT authorship contribution statement

Alessandra da Silva: Writing – original draft, Writing – review & editing, Visualization. **Brenda Kelly Souza Silveira:** Writing – original draft, Writing – review & editing, Visualization. **Helen Hermana Miranda Hermsdorff:** Writing – review & editing, Supervision. **Walmir da Silva:** Writing – review & editing, Supervision. **Josefina Bressan:** Writing – review & editing, Supervision.

Declarations of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.plefa.2022.102451.

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