

## Artículo de Revisión Sistemática / Systematic Review Article

### Dietary n-3 polyunsaturated fatty acids improve endothelial markers in metabolic syndrome: A systematic review

### Ácidos grasos dietarios poliinsaturados n-3 mejoran los marcadores endoteliales en el síndrome metabólico: Una revisión sistemática

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#### ABSTRACT

Metabolic syndrome (MS) is a global health problem. Dietary factors, especially fatty acids, may affect MS pathology. However, the associations between omega-3 polyunsaturated fatty acids (n-3 PUFAs) and MS risk demonstrate inconsistent results. To clarify the relationship between dietary n-3 PUFA and endothelial function on MS, we carried out a systematic review. An electronic literature search based on controlled clinical trials (CCTs) between 2004 and 2020 was conducted. A total of 28 articles were included in the systematic review. Studies were analyzed according intervention type: dietary interventions (12 CCTs), dietary supplementation interventions (9 CCTs) and mixed interventions (7 CCTs). Studies with dietary interventions characterized by n-3 PUFAs increased by food source, such as Mediterranean and Nordic-style diets, reported significant reduction in systolic and diastolic blood pressure, and also in inflammatory endothelial biomarkers. The same effect was also observed in mixed interventions and in CCTs with n-3 PUFAs supplementation. Dietary interventions with n-3 PUFAs contributes to improved endothelial and cardiovascular health in SM and associated risk factors.

Keywords: Controlled clinical trials; Endothelial function; Metabolic syndrome; n-3 Polyunsaturated fatty acids; Systematic review.

## RESUMEN

El síndrome metabólico (SM) es un problema sanitario global. Los factores dietéticos, especialmente los ácidos grasos, pueden afectar la patología del SM. Sin embargo, las asociaciones entre los ácidos grasos poliinsaturados omega-3 (AGPI n-3) y el riesgo de SM pueden ser inconsistentes. Para aclarar esta relación entre AGPI n-3 dietarios y la función endotelial en el SM, realizamos una revisión sistemática. Se realizó una búsqueda bibliográfica en fuentes electrónicas de ensayos clínicos controlados (ECC) entre 2004 y 2020. Se incluyeron un total de 28 artículos en la revisión. Los estudios fueron analizados según intervención realizada: intervención dietaria (12 ECC), intervención con suplementación dietética (9 ECC) e intervenciones mixtas (7 ECC). Los estudios que utilizaron intervenciones dietéticas con aumento de AGPI n-3 a través de alimentos, como las dietas mediterráneas y nórdicas, reportaron una reducción significativa de la presión arterial sistólica (PAS), diastólica (PAD) y de biomarcadores endoteliales inflamatorios. El mismo efecto se observó en intervenciones mixtas y ECC con suplementación de AGPI n-3. Las intervenciones dietéticas con AGPI n-3 contribuyen a mejorar la salud endotelial y cardiovascular y sus factores de riesgo asociados.

**Palabras clave:** Ácidos grasos poliinsaturados n-3; Ensayos clínicos controlados; Función endotelial; Revisión sistemática; Síndrome metabólico.

## INTRODUCTION

Due to the epidemiologic and nutritional transition, the metabolic syndrome (MS) is increasing throughout the world, with a prevalence of 31% in adults<sup>1</sup>. MS is a cluster of clinical and biochemical abnormalities characterized by central obesity, dyslipidemia (elevated triglycerides and LDL-cholesterol and reduced HDL-cholesterol), glucose intolerance, insulin resistance, endothelial dysfunction (ED), hypercoagulable state and elevated blood pressure, with increased risk of cardiovascular disease (CVD) and type 2 diabetes as well as all-cause mortality<sup>2</sup>.

Endothelial dysfunction may be described as a diminished production and/or availability of nitric oxide (NO), and an imbalance between the endothelium-derived vasodilators and vasoconstrictors. Oxidative stress and inflammation are the most representative and relevant mechanisms for ED pathogenesis. Besides, ED may be also induced by many factors such as dietary intake, drugs, and aging<sup>3</sup>. Evidence shows that the adherence of healthy dietary patterns, characterized by a relatively high fruits and vegetables intake (sources of vitamins, mineral, phytochemicals and dietary fiber), has a beneficial impact on endothelial function, reflected in decreased levels of circulating adhesion molecules, such as E-selectin (SELE), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1), as well as other inflammation markers released into the circulation during endothelial injury<sup>4,5</sup>. It has also been observed that a diet rich in fish and seafood, sources of omega-3 polyunsaturated fatty acids (n-3 PUFAs), is associated with a decreased risk of coronary heart disease and cardiac events<sup>6</sup>. Specifically, the most common dietary n-3 PUFAs include  $\alpha$ -linolenic acid (ALA; 18:3 n-3), eicosapentaenoic acid (EPA; 20:5 n-3), and docosahexaenoic acid (DHA; 22:6 n-3).

The n-3 PUFAs intake positively impact endothelial health through various mechanisms, such as decreased levels of triglycerides and LDL-cholesterol, increased concentrations of HDL-cholesterol, lower blood pressure values and platelet aggregation, as well as a minor arrhythmias incidence. These beneficial effects are mainly caused by the anti-inflammatory and antiaggregant effect of the derived eicosanoids<sup>7,8,9,10</sup>.

The studies about associations between n-3 PUFA and MS have demonstrated inconsistent results. The conclusions from epidemiological studies are controversial, suggested null, inverse and positive associations between n-3 PUFAs and MS risk<sup>11,12</sup>. To clarify the relationship between n-3 PUFAs and inflammatory markers, the aim of this study was to evaluate and synthesize findings from controlled clinical trials (CCTs) about the effects of n-3 PUFAs on endothelial function in MS, by performing a systematic review. We hypothesized that n-3 PUFAs would enhance endothelial function in MS.

## METHODS

**Search strategy.** We conducted a systematic search including data from 2004 to 2020 using the following electronic databases: Medline, Embase, Cochrane Library, Scielo, and Lilacs. In computer-based searches, we combined search terms related to the exposure: (e.g., n-3 OR omega 3 polyunsaturated fatty acids OR EPA OR eicosapentaenoic acid OR DHA OR docosahexaenoic acid OR ALA OR alpha-linolenic acid) and outcomes of interest (e.g., endothelial function OR blood pressure AND metabolic syndrome).

Study results were reported according to a protocol based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement<sup>13</sup>.

**Selection Criteria.** We included CCTs with dietary intervention and supplementation, carried on in adults  $\geq 18$  years of age, in the English language. To be included, CCTs had to report associations between n-3 PUFAs intake and blood pressure and endothelial markers values in MS patients.

To reduce the risk of bias, exclusion criteria included case-control studies, retrospective and prospective cohort studies, animal studies, non-original studies (meta-analyses, reviews, and commentaries) and intervention studies without a control group. Studies with participants with a diagnosis of type 2 diabetes and those that included populations younger than 18 years of age or with pregnant women were also excluded.

**Study selection, data collection and synthesis of the results.** Two independent reviewers used a predesigned database to extract relevant information from the selected

studies. First, they pre-screened all identified references, and selected potentially eligible studies based on title and abstract. Then, two reviewers independently evaluated full-text versions of all potentially eligible articles to evaluate whether inclusion criteria were met. Discrepancies were resolved through consensus. Extracted data included study authors, country, year of publication, study design (intervention), study size, participant age range, and main results.

## RESULTS

Through the mentioned search strategy, 341 studies were identified. After initial screening based on title and abstract, 52 articles remained for further evaluation. Of the full-text articles an additional 24 articles were excluded for various reasons. After applying all eligibility parameters, 29 articles were suitable and thus included for systematic review (Figure 1).

Studies were analyzed according to type of intervention, as detailed below: dietary interventions (dietary pattern or by n-3 source food), interventions with dietary supplementation and mixed interventions. Descriptive data of the included studies were summarized in tables 1, 2 and 3.

### Studies with dietary interventions

Using the indicated selection criteria, 12 CCTs with dietary interventions (1469 subjects in total) were

included (Table 1). Of these studies, 3 studies measured the impact of the Mediterranean diet<sup>14,15,16</sup>; two studies evaluated the effects of the Nordic diet<sup>17,18</sup>, 3 articles examined the benefits of increasing ALA intake mainly from rapeseed oil<sup>19,20,21</sup>, one study utilized a bread containing ground flaxseed<sup>22</sup>, two studies utilized increased fish consumption<sup>23,24</sup> and, finally, one study evaluated the effect of variation of n-3/n-6 ratio by the incorporation of flaxseed oil<sup>25</sup>.

Regarding the 9 studies with systolic (SBP) and diastolic blood pressure (DBP) as an outcome, 6 reported a statistically significant reduction ( $p < 0.05$ ) in both parameters<sup>14,15,19,20,21,25</sup>, one article in SBP reduction<sup>16</sup> and another article showed effects only in reducing DBP<sup>24</sup>.

Regarding the 3 CCTs based on the Mediterranean diet recommendations, Esposito et al.<sup>14</sup> (2004) showed significant changes in endothelial dysfunction markers such as interleukins (IL-6, IL-7, IL-18), whereas Merino et al.<sup>16</sup> (2014) reported a reduction in levels of SELE, sICAM-1, sVCAM-1, tumor necrosis factor-alpha (TNF- $\alpha$ ) and oxidized LDL (LDL-ox). Both showed a significant reduction of high-sensitivity C-reactive protein (hs-CRP) with the dietary intervention. De Mello et al.<sup>23</sup> (2011), with increased fish intake as the food intervention, showed decreased levels of plasmatic hs-CRP and SELE ( $p < 0.05$ ).

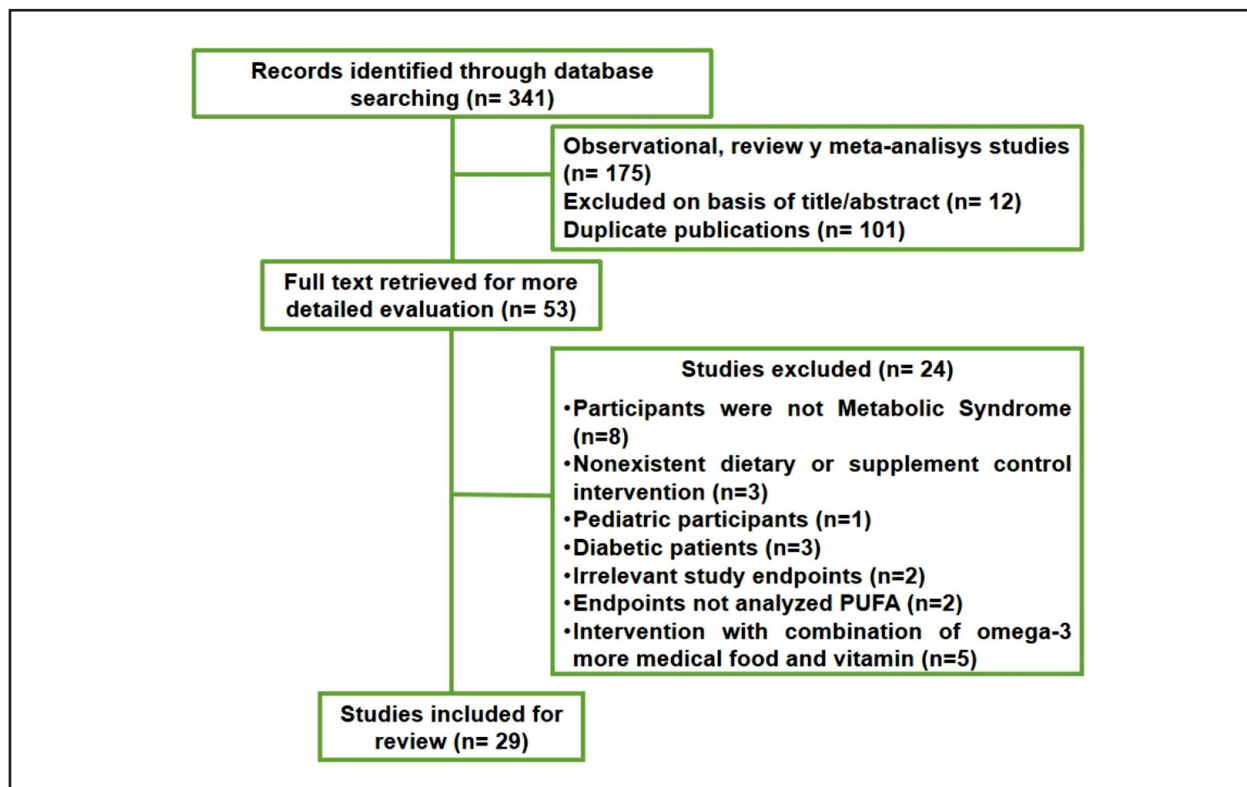


Figure 1: Flow diagram of literature search and selection of studies.

**Table 1.** Studies assessing effects of n-3 PUFAs utilizing intervention dietary.

| Author, year                            | Country | Age, mean/range   | Duration | N      | Intervention   | Main results   |
|---|---------|-------------------|----------|--------|--|--|
| Esposito et al. <sup>14</sup> (2004)    | Italy   | 43.9/NR years     | 2 years  | n= 180 | Intervention group: Mediterranean diet (carbohydrates 50-60%, proteins 15-20%, total fat <30%, saturated fat <10%, cholesterol <300 mg/day)<br>Control group: prudent diet (carbohydrates 50-60%; proteins, 15-20% and total fat <30%) | Blood pressure SBP (p= 0.01) y DBP (p= 0.03) reduced in Mediterranean diet<br>Dysfunction endothelial biomarkers<br>IL-6 (p=0.04), IL-7 (p= 0.04), IL-18 (p= 0.03), and hs-CRP (p= 0.01) reduced in intervention group                   |
| Palkowska et al. <sup>25</sup> (2012)   | Poland  | 44/22–65 years    | 12 weeks | n= 23  | Intervention group: PUFAs n-3/n-6 ratio 1:5, flaxseed oil<br>Control group: PUFAs n-3/n-6 ratio 1:10, sunflower oil  | Blood pressure<br>Decreased SBP, 11.5±2.2 mmHg (p= 0.0003)<br>Reduced DBP, 7.1±0.9 mmHg (p= 0.0008) in intervention group  |
| Baxheinrich et al. <sup>19</sup> (2012) | Germany | 51.3/NR years     | 6 months | n= 81  | RO group: rapeseed oil and margarine (3.5 g/d ALA)<br>OO group: olive oil and margarine (0.78 g/d ALA)   | Blood pressure<br>Reduced SBP (-10.0 mmHg, p<0.05)<br>Reduced DBP (-8.4 mmHg, p<0.05)<br>More pronounced for RO group compared to OO group (p<0.026).<br>Dysfunction endothelial biomarkers<br>Leptin reduction (p<0.05) in RO           |
| Rallidis et al. <sup>15</sup> (2009)    | Greece  | 50.4/<70 years    | 2 months | n= 82  | Intervention group: Mediterranean-style diet and ≥1 portion of fish weekly with close supervision by a dietitian.<br>Control group: Mediterranean diet, meeting with the dietitian only at the beginning and end of the study          | Blood pressure<br>Reduced DBP (p= 0.041) in intervention group   |
| Merino et al. <sup>16</sup> (2014)      | Spain   | 55.66/30-70 years | 1 year   | n= 108 | Intensive intervention: Mediterranean dietary pattern with at least two servings per week of fatty fish<br>Standard of care control group  | Blood pressure<br>Reduced SBP (p<0.05) in subjects in second tertile of n-3 PUFAs intake.<br>Dysfunction endothelial biomarkers<br>Subjects in top tertile (increased n-3 PUFAs intake) showed a significant increase of serum ApoA1 and |

...continuation table 1.

| Author, year                            | Country                          | Age, mean/range  | Duration       | N      | Intervention  | Main results   |
|---|----------------------------------|------------------|----------------|--------|---|--|
| Egert et al. <sup>20</sup><br>(2014)    | Germany                          | 51.3/18-70 years | 6 months       | n= 81  | Energy-restricted diet enriched with ALA (approximately 3.4 g ALA/d). Sources: refined rapeseed oil and commercial margarine<br>Energy-restricted control diet (approximately 0.9 g ALA/d). Sources: refined olive oil and commercial margarine | reduction of serum hs-CRP, TNF- $\alpha$ , sICAM-1, sVCAM-1 and oxLDL values ( $p= 0.028$ )<br><br>Blood pressure<br>Reduced SBP and DBP ( $p<0.001$ )<br>Dysfunction endothelial biomarkers<br>Decreased serum concentrations of CRP, TNF-a, IL-6, sICAM-1, SELE and asymmetric dimethylarginine ( $p<0.001$ ) in diet with ALA |
| De Mello et al. <sup>23</sup><br>(2011) | Finland                          | 59/40-70 years   | 12 weeks       | n= 104 | Healthy Diet: participants consumed (100–150 g fish per meal) three times per week.<br>Whole-grain-enriched diet (WGED): no change to current fish consumption. Control diet: fatty fish once a week  | Dysfunction endothelial biomarkers<br>Decreased Plasma SELE, hs-CRP only in the Healthy Diet group ( $p<0.05$ )  |
| Vázquez et al. <sup>24</sup><br>(2014)  | Spain                            | 57.3/NR years    | 8 weeks        | n= 257 | Patients were randomized to<br>a) Advice on a healthy diet supplemented with 100 g/day of white fish (Namibia hake)<br>b) Advice on a healthy diet avoiding fish or seafood   | Blood pressure<br>Decreased DBP in the intervention with fish ( $p= 0.014$ )   |
| Marklund et al. <sup>1</sup><br>(2014)  | (Finland-Sweden-Denmark-Iceland) | 54.9/30-65 years | 18 or 24 weeks | n= 154 | Healthy Nordic diet: whole-grain products, berries, fruits and vegetables, rapeseed oil, three fish meals per week and low-fat dairy products.<br>Control diet: low-fiber cereal products and dairy fat-based spread (butter)                   | Blood pressure<br>SBP and DBP tended to decrease ( $p= 0.07$ and $p= 0.08$ , respectively) in Nordic diet  |
| Uusitupa et al. <sup>18</sup><br>(2013) | (Finland-Sweden-Denmark-Iceland) | 54.4/30-65 years | 18 or 24 weeks | n= 166 | Healthy Nordic diet: whole-grain products, berries, fruits and vegetables, rapeseed oil, three fish meals per week and low-fat dairy products<br>Control diet: received low-fiber cereal products and dairy fat-based spread (butter)           | Dysfunction endothelial biomarkers<br>IL-1 receptor antagonistic (Ra) a was markedly and consistently elevated during the control diet ( $p= 0.00053$ )  |

...continuation table 1.

| Author, year                          | Country | Age, mean/range  | Duration | N      | Intervention  | Main results  |
|---------------------------------------|---------|------------------|----------|--------|---|---|
| Akrami et al. <sup>21</sup><br>(2017) | Iran    | 48.5/30-60 years | 7 weeks  | n= 60  | Intervention group: 25 mL/d of flaxseed oil (FO)<br>Control group: 25 mL/d of sunflower seed oil (SO)   | Blood pressure<br>Significant reductions in SBP and DBP in the FO group related to SO group                     |
| Zong et al. <sup>22</sup><br>(2013)   | China   | 48,8/35-65 years | 12 weeks | n= 173 | Intervention group: lifestyle counseling group received two portions of bread containing 30g/day of ground whole flaxseed, providing 7 g ALA per day<br>Lifestyle counseling group: two 100 g isocaloric breads | Dysfunction endothelial biomarkers<br>Decreased hs-CRP, IL-18, SELE, sICAM-1, and PAI-1 (p<0.05) in both groups |

NR: not reported; SBP: systolic blood pressure; DBP: diastolic blood pressure; IL-6: interleukin 6; IL-7: interleukin 7; IL-18: interleukin 18; hs-CRP: high-sensitivity C-reactive protein; PUFAs: polyunsaturated fatty acids; g/d: gram/day; ALA: alpha-linolenic acid; ApoA1: Apolipoprotein A 1; TNF- $\alpha$ : tumor necrosis factor-alpha; sICAM-1: soluble intercellular adhesion molecule-1; sVCAM-1: soluble vascular cell adhesion molecule-1; oxLDL: oxidized LDL; SELE: E-selectin; CRP: C-reactive protein; IL-1: interleukin 1; mL/d: milliliter/day; PAI-1: plasminogen activator inhibitor-1.

Regarding the studies that evaluated the effects of increasing rapeseed oil intake, Baxheirich et al.<sup>19</sup> (2012) reported a reduction in leptin concentrations, whereas Egert et al.<sup>20</sup> (2014) showed a decrease in hs-CRP, TNF- $\alpha$ , IL-6, sICAM-1, and SELE concentrations. In the same sense, Zong et al.<sup>22</sup> (2013) showed decreased levels of hs-CRP, IL-18, SELE, sICAM-1, and PAI-1 (plasminogen activator inhibitor-1) after an intervention with ground whole flaxseed, although the same effect was also evident in the control group.

The Nordic diet, consumed in countries such as Sweden, Denmark, Norway, Finland and Iceland, consists of foods of Nordic origin, mainly whole grains, rapeseed oil, berries, fruits, vegetables, fish, nuts, and low-fat dairy products<sup>26</sup>. One CCT by Marklund et al.<sup>17</sup> (2014) showed a significant tendency of decreased SBP and DBP ( $p=0.07$  and  $p=0.08$ , respectively) in the intervention group, whereas Uusitupa et al.<sup>18</sup> (2013) found elevated levels of IL-1 receptor antagonist (IL-1 Ra), an inflammatory cytokine, in the control diet.

### Studies with enriched supplementation

We identified 9 CCTs (562 subjects in total) utilizing n-3 PUFAs oral supplements (Table 2). The main source of

n-3 PUFAs was fish oil<sup>27,28,29,30,31,32</sup>. In two CCTs, purified EPA was employed<sup>33,34</sup> and one study utilized flaxseed oil and fish oil supplements<sup>35</sup>.

Regarding interventions with fish oil supplements, Augustine et al.<sup>31</sup> (2014) showed a significant and beneficial change in mean arterial pressure values. Tardivo et al.<sup>32</sup> (2015), with 900 mg/day of n-3 as supplementation, reported a significant reduction in both SBP and DBP in the intervention compared to control group. In the same sense, two CCTs found significant change, but only in SBP, in the intervention group compared to controls<sup>27,29</sup>. About endothelial dysfunction markers as an outcome of fish oil supplement intervention, two studies found a significant reduction in IL-6 values<sup>27,30</sup> and one study on hs-CRP concentrations<sup>29</sup>. Also, Tousoulis et al.<sup>30</sup> (2014) showed a significant increase of PAI-1.

Two studies with purified EPA as intervention, showed a significant reduction in sVCAM-1 and sICAM-1 plasmatic levels<sup>34</sup>. These studies reported decreased hs-CRP concentrations and increased adiponectin concentrations<sup>33</sup> in the intervention group compared to controls. Finally, mix supplements (flaxseed oil and fish oil), did not show significant differences in plasma inflammatory marker concentrations between groups<sup>35</sup>.

**Table 2.** Studies assessing effects of n-3 PUFA utilizing a supplement.

| Author, year  | Country | Age, mean/range   | Duration | N     | Intervention   | Main Results  |
|---|---------|-------------------|----------|-------|--|---|
| Soares de Oliveira Carvalho et al. <sup>27</sup> (2014) | Brazil  | 40,05/30-45 years | 3 months | n= 30 | Microencapsulated fish oil group: received enriched jam with 3 g/day of fish oil (ME FO)<br>Placebo group: Not enriched jam  | Blood pressure<br>Reduced SBP compared to the placebo group (p<0.05)  |
| Root et al. <sup>28</sup> (2013)                        | USA     | 20.9/18-30 years  | 4 weeks  | n= 41 | Intervention group: supplementation with 1.7 g/day fish oil omega 3.<br>Placebo group: 1.0 g/day of safflower oil  | Blood pressure<br>No affect to SBP (p= 0.26).<br>Dysfunction endothelial biomarkers<br>No effect on IL-6 (p= 0.51), hs-PCR (p= 0.53), TNF- $\alpha$ (p= 0.75)   |
| Satoh et al. <sup>33</sup> (2009)                       | Japan   | 51.7/NR years     | 3 months | n= 92 | EPA-treated group: administered 1.8 g/day EPA capsule<br>Control group: diet alone   | Blood pressure<br>No effect for SBP and DBP<br>Dysfunction endothelial biomarkers<br>EPA treatment increased the plasma adiponectin concentrations (p<0.01) and decreased the CRP concentrations (p<0.01) |
| Ebrahimi et al. <sup>29</sup> (2009)                    | Iran    | 52.9/40-70 years  | 6 months | n= 90 | Intervention group: 1 g/day of fish oil as a single capsule<br>Control group: no supplements   | Blood pressure<br>Reduced SBP (p<0,05)<br>Dysfunction endothelial biomarkers<br>Decreased hs-CRP (p<0.01) in intervention group.  |
| Tousoulis et al. <sup>30</sup> (2014)                   | Greece  | 44.31/NR years    | 12 weeks | n= 29 | Oral treatment with n-3 PUFAs: dose of 2 g/day in capsules<br>Placebo: identical formulations (capsules)   | Dysfunction endothelial biomarkers<br>n-3 PUFAs improved significantly IL-6 levels (p<0.001) and increased PAI-1 levels (p<0.001).  |
| Augustine et al. <sup>31</sup> (2014)                   | USA     | 47.7/40–69 years  | 16 weeks | n= 61 | Patients were randomized in four groups<br>1) 2 g/d extended-release nicotinic acid (ERN)<br>2) 4 g/d prescription n-3 FAs ethyl ester (P-OM3)<br>3) Combination therapy<br>4) Placebo | Blood pressure<br>P-OM3 induced changes in EPA within low-density lipoproteins and very low-density lipoproteins were associated with beneficial effects on mean arterial pressure (p<0.05).              |
| Tardivo et al. <sup>32</sup> (2015)                     | Brazil  | 55.05/45-70 years | 6 months | n= 63 | Diet plus n-3 supplementation: received 900 mg/day omega-3, in capsule of marine PUFAs extract<br>Diet alone   | Blood pressure<br>Reduced SBP and DBP in intervention group (p<0.05)<br>Dysfunction endothelial biomarkers<br>Reduced IL-6 concentrations (p= 0.034).   |

...continuation table 2.

| Author, year                          | Country | Age, mean/range | Duration | N     | Intervention  | Main Results   |
|---------------------------------------|---------|-----------------|----------|-------|---|--|
| Yamada et al. <sup>34</sup><br>(2008) | Japan   | 51.6/ NR years  | 3 months | n= 60 | EPA group: diet plus highly purified EPA (1.8g/daily)<br>Control group: Diet alone  | Blood pressure<br>No effects for SBP<br>Dysfunction endothelial biomarkers<br>sICAM-1 and sVCAM-1 concentrations were significantly reduced after EPA administration (p<0.01). |
| Dewell et al. <sup>35</sup><br>(2011) | USA     | 50/≥18 years    | 8 weeks  | n= 96 | 1) Low dose of plant flaxseed oil supplements (2.2 g/d ALA)<br>2) High dose of flaxseed oil (6.6 g/d ALA)<br>3) Low dose fish oil supplements (1.2 g/d EPA and DHA)<br>4) High dose fish oil (3.6 g/d EPA and DHA)<br>5) 4 or 6 g soybean oil/d | Dysfunction endothelial biomarkers<br>No significant differences among groups for MCP-1, IL-6 and sICAM-1 values   |

NR: not reported; SBP: systolic blood pressure; IL-6: interleukin 6; hs-CRP: high-sensitivity C-reactive protein; TNF- $\alpha$ : tumor necrosis factor-alpha; EPA: eicosapentaenoic acid; DBP: diastolic blood pressure; PUFAs: polyunsaturated fatty acids; DHA: docosahexaenoic acid; PAI-1: plasminogen activator inhibitor-1; g/d: gram/day; FAs: fatty acids; sICAM-1: soluble intercellular adhesion molecule-1; sVCAM-1: soluble vascular cell adhesion molecule-1; ALA: alpha-linolenic acid; MCP-1: monocyte chemoattractant protein-1.

### Studies with mixed interventions

In the analysis, 8 studies were included (1950 subjects in total), with diet and supplements combination (Table 3). In 5 CCTs the same strategy was reported, characterized by four mixed diets with the same number of calories but with variations in the fat amount. Two high-fat diets, one rich in saturated fat and the other rich in monounsaturated fat and two low-fat, high-complex carbohydrate diets; supplemented with 1.2 g/day of n-3 PUFAs (intervention group); or 1g/day high oleic sunflower oil (control group)<sup>36,37,38,39,40</sup>. Su et al.<sup>41</sup> (2015) divided the intervention group based on calorie-restriction, calorie-restriction meal-replacement diet, calorie-restriction diet with fish oil supplementation (2.13 g/day of n-3 PUFAs), and calorie-restriction meal-replacement diet with supplementation. Shabrina et al.<sup>42</sup> (2020), in a study derived from previous work, divided the intervention in two groups (only caloric restriction and caloric restriction with fish oil supplementation). The study design of Simão et al.<sup>43</sup> (2012) included diets with soy-derived products, supplementation with fish oil and a combination of these two strategies.

Simão et al.<sup>43</sup> (2012) showed a significant decrease in SBP in the intervention group with fish oil and soy-derived products (kinako), and a significant decrease in DBP in the control group, soy-derived products group and fish oil group after interventions. About the three CCTs with additional supplements of n-3 PUFAs, two of them reported a reduction in hypertension prevalence<sup>41,42</sup> and the other study showed a reduction in both SBP and DBP in subjects in the lowest category of homeostatic model assessment of insulin resistance (HOMA-IR)<sup>40</sup>.

In relation to endothelial markers, hs-CRP and IL-6 concentrations were significantly reduced in Su et al.<sup>41</sup> (2015) and Shabrina et al.<sup>42</sup> (2020) studies, with fish oil supplementation, while the intervention with 1.2 g/day of n-3 PUFAs reported in Yubero-Serrano et al.<sup>40</sup> (2015), only evidenced changes in IL-6 concentrations with lower HOMA-IR. Adiponectin and NO levels were significantly increased after the mixed diet with soy products and fish oil<sup>43</sup>. Lastly, Perez-Martinez et al.<sup>36</sup> (2010) reported a stronger effect on sICAM-1 reduction concentrations with a MUFAs (monounsaturated fatty acids) rich diet, compared to n-3 PUFAs diet.



**Table 3.** Studies assessing effects of n-3 PUFA utilizing mixed interventions.

| Author, year                               | Country | Age (years)       | Duration | N      | Intervention  | Main results   |
|--|---------|-------------------|----------|--------|---|--|
| Perez-Martinez et al. <sup>36</sup> (2010) | Spain   | 56.17/35-70       | 12 weeks | n= 74  | Participants were randomly assigned to one of four isoenergetic diets:<br>1) High-fat: SFAs-rich diet (HSFA)<br>2) High-fat: MUFAs-rich diet (HMUFA)<br>3) Low fat, high-complex carbohydrate diets supplemented with 1.24 g/day n-3 PUFAs (LFHCC n-3)<br>4) Low-fat, high-complex carbohydrate diets with 1 g/day high oleic sunflower oil-Placebo (LFHCC) | Dysfunction endothelial biomarkers<br>sICAM-1 levels were lower during the HMUFA than during the HSFA and LFHCC n-3 diets (p= 0.022)   |
| Su et al. <sup>41</sup> (2015)             | Taiwan  | NR                | 12 weeks | n= 136 | Participants were assigned to one of four dietary interventions:<br>1) Calorie restriction diet (CR)<br>2) Calorie restriction meal replacement diet (CRMRF)<br>3) Calorie restriction diet with fish oil supplementation (CRF)<br>4) Calorie restriction meal replacement diet with fish oil supplementation (CRMRF).                                      | Dysfunction endothelial biomarkers<br>IL-6 and CRP significantly decreased in CRF and CRMRF groups (p<0.05)  |
| Simão et al. <sup>4</sup> (2012)           | Brazil  | 47.9/NR years     | 3 months | n= 65  | Patients were randomly assigned to four groups:<br>1) Kinako group: received 29 g/d of kinako (toasted ground soya bean)<br>2) Fish oil group: received 3 g/d of fish oil capsules<br>3) Fish oil and kinako group: received 3 g/d of fish oil and 29 g/d of kinako<br>4) Control group: maintain usual diet  | Blood pressure<br>Decreased SBP in the group that received kinako and fish oil concomitantly (p<0,05)<br>DBP reduced in fish oil group (p<0.01), kinako group (p<0.01) and control group (p<0.05)<br>Dysfunction endothelial biomarkers<br>Increase in adiponectin (p<0.01) and NO values (p<0.05) in the kinako and fish oil groups |
| Paniagua et al. <sup>37</sup> (2011)       | Spain   | 55.27/35-70 years | 12 weeks | n= 337 | Subjects were randomly assigned to one of four isoenergetic diets:<br>1) High-fat: SFAs-rich diet (HSFA)<br>2) High-fat: MUFAs-rich diet (HMUFA)<br>3) Low fat, high-complex carbohydrate diets supplemented with 1.24 g/day n-3 PUFAs (LFHCC n-3)<br>4) Low-fat, high-complex carbohydrate diets with 1 g/day high oleic sunflower oil-Placebo (LFHCC)     | Blood pressure<br>Elevated blood pressure was reduced with LFHCC (p= 0.02) and LFHCC n-3 diets (p= 0.03)   |

...continuation table 3.

| Author, year                                  | Country                    | Age (years)          | Duration | N      | Intervention   | Main results  |
|---|----------------------------|----------------------|----------|--------|--|---|
| Tierney et al. <sup>38</sup><br>(2011)        | Ireland                    | 54.9/35-70<br>years  | 12 weeks | n= 417 | Subjects were randomly assigned to four isoenergetic diets:<br>1) High-fat: SFAs-rich diet (HSFA)<br>2) High-fat: MUFAs-rich diet (HMUFA)<br>3) Low fat, high-complex carbohydrate diets supplemented with 1.24 g/day n-3 PUFAs (LFHCC n-3)<br>4) Low-fat, high-complex carbohydrate diets with 1 g/day high oleic sunflower oil-Placebo (LFHCC)         | Dysfunction endothelial biomarkers<br>Reducing dietary SFA had no effect on several inflammation biomarkers.  |
| Gulseth et al. <sup>39</sup><br>(2010)        | Norway                     | 54.65/35-70<br>years | 12 weeks | n= 428 | Subjects were randomly assigned to one of four isoenergetic diets:<br>1) High-fat: SFAs-rich diet (HSFA)<br>2) High-fat: MUFAs-rich diet (HMUFA)<br>3) Low fat, high-complex carbohydrate diets supplemented with 1.24 g/day n-3 PUFAs (LFHCC n-3)<br>4) Low-fat, high-complex carbohydrate diets with 1 g/day high oleic sunflower oil- Placebo (LFHCC) | Blood pressure<br>No differences on SBP and DBP values (p= 0.52 and 0.24 respectively) between groups   |
| Yubero-Serrano et al. <sup>40</sup><br>(2015) | 8<br>European<br>countries | 53.3/35-70<br>years  | 12 weeks | n= 472 | Subjects were randomly assigned to one of four isoenergetic diets:<br>1) High-fat: SFAs-rich diet (HSFA)<br>2) High-fat: MUFAs-rich diet (HMUFA)<br>3) Low fat, high-complex carbohydrate diets supplemented with 1.24 g/day n-3 PUFAs (LFHCC n-3)<br>4) Low-fat, high-complex carbohydrate diets with 1 g/day high oleic sunflower oil- Placebo (LFHCC) | Blood pressure<br>Decreased DBP and SBP after LFHCC n-3 diet, only in subjects with the lowest HOMA-IR (p<0.05)<br>Dysfunction endothelial biomarkers<br>In subjects with lower HOMA-IR (tertiles 1 and 2), IL-6 concentrations were reduced after consumption of HMUFA and LFHCC n-3 diets (p<0.05). |
| Shabrina et al. <sup>42</sup><br>(2020)       | Taiwan                     | 46.9/≥ 30<br>years   | 12 weeks | n= 21  | Caloric restriction (CR): calorie-controlled lunch and dinner with a reduction of 500–800 kcal/d<br>Caloric restriction with fish oil supplementation (CRF): CR diet and fish oil capsules that provided 2.13 g/day n-3 PUFAs  | Blood pressure:<br>Decreased DBP and SBP in CRF group (p<0.05)<br>Dysfunction endothelial biomarkers<br>Decreased IL-6 and CRP in CRF group (p<0.05)  |

SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; sICAM-1: soluble intercellular adhesion molecule-1; NR: no report; IL-6: interleukin 6; CRP: C-reactive protein; SBP: systolic blood pressure; DBP: diastolic blood pressure; NO: nitric oxide; HOMA-IR: homeostasis model assessment of insulin resistance; MS: metabolic syndrome.

## DISCUSSION

This study, a systematic review about the potential protective role of n-3 PUFAs on arterial blood pressure and endothelial dysfunction in MS, provides convincing evidence of the beneficial influence of n-3 PUFAs on endothelial damage evaluated via the analysis of several well-known serum inflammatory markers.

We identified 29 relevant and suitable studies published from 2004 to 2020. These studies provided evidence from 3981 persons from several countries, contributing to elucidating new dietary therapies to improve cardiovascular health.

The studies involving dietary interventions to increase n-3 PUFAs through food sources, such as the Mediterranean-style diet and vegetable oils rich in ALA, reported a significant reduction in both SBP and DBP<sup>14,16,19-21,22,23,24,25</sup> as well as lower levels of CRP concentrations<sup>14,20,16,22</sup>. In the same sense, the PREDIMED Study (Prevención Dieta Mediterránea) reported an inverse association between the adherence to Mediterranean dietary pattern and blood pressure<sup>44</sup>. The Greek ATTICA study found an inverse correlation between adherence to the Mediterranean diet and CRP levels<sup>45</sup>. The beneficial effect of the Mediterranean-type diet on endothelial function may be attributed to the synergic action of various components of the diet, such as olive oil, fish, red wine, vegetables, and fruits<sup>46,47,48</sup>. Specifically, fresh fish provides EPA and DHA, long-chain n-3 PUFAs and a high intake of marine n-3 PUFAs has been associated to improved insulin sensitivity in humans<sup>49,50</sup>. On the other hand, olive oil is a main source of fat in the Mediterranean diet, a source of n-9 oleic MUFAs. The combination of MUFAs, tocopherols, polyphenols, a low saturated fat intake and well-balanced n-6/n-3 FAs ratio, improve the immune system and reduce inflammatory responses<sup>51</sup>.

In this review, the Nordic diet (characterized by intake of apples, pears and berries, root and cruciferous vegetables such as cabbages, whole grain and rye bread as cereals, high intake of fish, low-fat dairy products, potatoes and vegetable fats)<sup>52</sup> showed a trend to reduce SBP and DBP in the intervention group<sup>16</sup>, with a protective effect on endothelial dysfunction biomarkers<sup>17</sup>. The low sodium content of the Nordic diet probably contributes to improve blood pressure levels, with similar effect as the Dietary Approaches to Stop Hypertension diet (DASH)<sup>53,54,55</sup>. Several studies showed that a diet based on the Nordic food pattern may impact CVD risk through improving serum lipid profile and blood pressure levels and also, it has a beneficial effect to reduce low-grade inflammation<sup>18,56</sup>.

This review shows an effective intervention on blood pressure and endothelial markers with the addition of ALA-rich vegetable oils. Flaxseed oil, as a functional food, represents one of the major sources of n-3 PUFAs. Besides, rapeseed oil, is also employed in dietary interventions and it is rich in plant polyphenols, phytosterols, tocopherols, and other lipid bioactive compounds; all of these are nutrients with endothelial protective effects through the inhibition

of reactive oxygen species (ROS) production<sup>57</sup>. Both oils have been implicated as positive mediators against CVD and insulin resistance, being an ideal source of fat<sup>58,59,60</sup>.

The beneficial effects of fish on endothelial health in MS were also demonstrated in various studies included in this review. The study of Vázquez et al.<sup>24</sup> (2014) showed significant lowering effects on DBP with the daily intervention of white fish, compared with the control group<sup>24</sup>. Similarly, De Mello et al. (2011) found reduced levels of SELE and hs-CRP circulating levels with the addition of fish three times/week<sup>23</sup>. These results are consistent with the Nurse's Health Study, in which higher consumption of fish and n-3 PUFAs was related to lower risk of CVD and deaths derived from CVDs<sup>61</sup>. In the same sense, in the Japan Public Health Center's study, the CVD risk was approximately 40% lower among people eating fish eight times per week compared to participants eating fish only once per week<sup>6</sup>. On the other hand, a recent meta-analysis found that fish consumption was not significantly associated with reduced risk of elevated blood pressure<sup>62</sup>. Regardless of these contradictory results, fish contains a variety of nutrients that may contribute to cardiovascular health, such as protein, n-3 PUFAs, vitamin D, iodine, selenium, and taurine, all of these with beneficial effects for MS and CVD risk factors<sup>63</sup>.

Regarding CCTs with n-3 PUFAs supplementation, a significant decrease in SBP and/or DBP in the experimental group was reported in several studies<sup>27,29,32</sup>. The same effect was also observed in mixed interventions. In relation to endothelial dysfunction markers, the results were heterogeneous but, in general, a reduction of circulation levels of CRP, sICAM-1, PAI-1 and IL-6 was observed after fish oil and purified EPA supplementation<sup>29,30,32,33</sup>. Several articles have demonstrated the beneficial effects of n-3 PUFAs for the cardiovascular system, which include ameliorating uncontrolled inflammatory reactions, reduced oxidative stress and mitigating coagulopathy. The evidence suggests that n-3 PUFAs and its metabolites could modulate inflammatory signaling pathways and lower the production of inflammatory biomarkers<sup>64</sup>.

This review has some limitations. First, comparison between all studies are hindered by heterogeneity. Second, the possibility of change in biomarker values within an individual relative to the physiological intraindividual fluctuation. The design included studies that enrolled participants with MS (i.e., increased likelihood of elevated inflammation). However, this review employed a rigorous systematic approach to evaluate the effectiveness of n-3 FAs on endothelial health in a pathology such as MS with increasing incidence globally, offering a new treatment approach by nutritional supplementation.

## CONCLUSION

In conclusion, dietary interventions and supplementation with n-3 PUFAs constitute a relevant strategy to improve metabolic risk factors related to SM, such as blood pressure and inflammatory markers. Supplementation with n-3

PUFAs constitute an important tool to be integrated with other strategies to improve cardiovascular health, such as adherence to dietary recommendations, weight control, smoking cessation and regular physical activity. Furthermore, new dietary studies to pinpoint the causal relation with endothelial health are warranted.

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