Systematic Review

Effects of omega-3 and vitamin D supplementation in patients with breast cancer: a systematic review

Subtitle: Supplementation and breast cancer patients

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Abstract: Breast cancer (BC) is the leading cause of death among women with cancer worldwide. Nevertheless, key challenges must be addressed, as new therapies can be employed to support the main treatment and enable a better quality of life, survival and prognosis. In this paper, we described the role of omega-3 and vitamin D supplementation in patients during the main treatment. This is a systematic review of the literature from randomized clinical trials, following the PRISMA and PICOS guidelines for elaborating the guiding question and constructing the results. Our findings in this review reveal and support that omega-3 and vitamin D supplementation can help women with metastatic disease who are not receiving hormone therapy during BC treatment for HR+ (hormone receptor positive). They improve immunity and antioxidant capacity and decrease cardiometabolic effects. This intervention is safe and can be employed as an adjuvant to the other main treatments.

Keywords: Breast neoplasms, Omega-3, Vitamin D, Supplementation
Introduction

Breast cancer (BC) is the leading cause of death among women worldwide. According to estimates by the International Agency for Research on Cancer (IARC), almost 3.1 million women worldwide diagnosed with BC in 2023, with a higher incidence in parts of Europe, North America and some countries in South America (Brazil) and the African continent [1].

However, mortality behaves oppositely, as countries in South America and Africa present a worsening of this scenario compared to the other continents mentioned. Some factors related to this are: developing countries still have lower rates of early diagnosis, a low Human Development Index (HDI) and associated risk factors such as obesity, physical inactivity, smoking and alcohol consumption [1, 2].

Low-cost alternative therapies can be employed to benefit the clinical treatment of these patients [2]. Therefore, some research is being conducted to understand the benefits of supplementation of nutritional compounds during the treatment of breast neoplasms. The most studied nutrients in recent years include omega-3 and vitamin D [3-5].

Polysaturated fatty acids such as omega-6 (n-6), represented by: arachidonic acid and linoleic; and omega-3 (n-3), represented by: alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid, have several beneficial properties for healthy humans as they are linked to cell membrane structures and can act on hormone binding and cellular transport activities [5, 6].

In cancer treatment, it has been found that n-3 modulates the inflammatory and immune response and thus improve prognosis. Additionally, it can play an important role in cell differentiation and growth, and inhibit tumor growth in some molecular subtypes [7-9].

Vitamin D is available in two forms, vitamin D2 (ergocalciferol), which is found primarily in plant compounds such as algae and mushrooms, and vitamin D3 (cholecalciferol), which can be obtained, through the diet by consuming animal sources such as fish (tuna and salmon), meat, eggs, milk and dairy products, or through sun exposure [10].

The metabolically active form of vitamin D in the body is 1,25(OH)2 or calcitriol. For the effects of calcitriol's biological activity to occur, its vitamin D receptor (VDR) is required, which is expressed in various tissues, including breast tissue. Therefore, calcitriol can benefit patients by inhibiting vascular endothelial growth factor (VEGF) and thus interfering with the angiogenesis process [4,11,12].

Although there are several benefits of vitamin D and n-3 in BC treatment, these benefits remain uncertain in certain populations and molecular subtypes [13,14]. Thus, the aim of this study is to describe and analyze the benefits of supplementation in BC patients during treatment.

Methodology

This systematic review follows the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [15], was registered in PROSPERO (International Prospective Register of Systematic Reviews) CRD42023442158, and its guiding question, formed by the acronym PICOS, is What is the evidence of the benefits of omega 3 and vitamin D supplementation during the treatment of women with BC?

The first step of the review was to determine the databases and keywords to be used. Therefore, electronic searches were carried out using the descriptors listed in Table 1 in three databases: PubMed, Direct Science and Capes. The keywords defined by the Health Sciences Descriptors (DeCS) in Portuguese were used as a search strategy in English according to the National Library of Medicine (NLM) with the corresponding MeSH to expand the retrieval of the largest possible number of studies.

Table 1. Complete search strategy in electronic databases, 2023

<table>
<thead>
<tr>
<th>Terms</th>
<th>Descriptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 Cancer</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>#2 Intervention</td>
<td>Omega-3 fatty acids and/or vitamin D</td>
</tr>
<tr>
<td>#3 Studies</td>
<td>Randomized Clinical Trials</td>
</tr>
<tr>
<td>Combination</td>
<td>#1 AND #2 OR #3 AND</td>
</tr>
</tbody>
</table>

The second step of this review was to define the eligibility criteria for the studies. These criteria were defined according to the PICOS acronym, which takes into account population, intervention, comparison and study design (Table 2). The studies eligible for this review were: (a) randomized clinical trials; (b) conducted in adults (>18 years old); (c) female; (d) during contingency management (CM) treatment and post-treatment; (e) published in English, Spanish and Portuguese in the last 10 years; (f) studies investigated the benefits of omega-3 and/or vitamin D supplementation in the treatment of patients with BC, with abstract and full text available from April 7, 2023 to June 21, 2023; (g) studies that described the dosage of omega-3 and vitamin D administered to patients.

Information about the number of articles retrieved in the search is described by the reviewers in Figure 1, which is shown in the flowchart, describing the number of articles retrieved at each stage of the search, selection, inclusion and exclusion process.

The search, selection, inclusion and exclusion stages were carried out by two trained reviewers. During the search process, the articles were initially classified and analyzed by title, manually by two blind and independent reviewers, and articles that did not meet the inclusion criteria were excluded.

Next, the abstracts were read and incongruent or duplicate articles were also removed. After screening, the articles were read in full to complete the process of study selection. Discrepancies were resolved by a third reviewer.

After conducting a search for studies for the systematic review and the inclusion criteria, studies that addressed the...
effects of omega-3 and/or vitamin D supplementation on the health of patients with BC were included. Additionally, the reference lists of all relevant articles were examined to identify other eligible studies.

The investigation and discussion of the results, data regarding authors, year of publication, journal, number of databases and search period were extracted and assigned with the PRISMA Checklist with 27 items (yes or no). Information was then obtained on the objective, total sample size, age of participants, intervention groups, control group, study location, polymorphism studied, and characteristics of the intervention.

For the risk of bias analysis, the Cochrane tool (RoB 2) was used to evaluate all 7 articles included in this study. It was found that 1 (14%) study had a low risk of bias, 4 (57%) had some concerns about risk of bias and 3 (42%) had a high risk of bias (Figure 2).

Table 2. Criteria for inclusion and exclusion of studies according to PICOS, 2023

<table>
<thead>
<tr>
<th>Criteria for inclusion</th>
<th>Criteria for exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>Adult women undergoing treatment and post-treatment for BC, over the age of 18.</td>
</tr>
<tr>
<td>Intervention</td>
<td>Omega-3 and/or vitamin D supplementation in BC patients.</td>
</tr>
<tr>
<td>Comparison</td>
<td>Control group, Case group.</td>
</tr>
<tr>
<td>Results</td>
<td>Effects of omega-3 and/or vitamin D supplementation on the health of BC patients.</td>
</tr>
<tr>
<td>Studies</td>
<td>Randomized Clinical Trials</td>
</tr>
</tbody>
</table>

Results

In the databases with the keywords and filters applied, 393 studies were found, with 224 articles in Science Direct, 110 in Periodic of Capes and 59 in PubMed. After reviewing the titles, 274 were excluded because they did not meet the objective of the study. Four articles were excluded in the second stage, because they did not meet the inclusion criteria, with other methodological approaches, animal studies, and studies that investigated individuals with other comorbidities, under 18 years of age, and without the intervention of physical activity and/or physical exercise in patients with BC. In the final analysis, 36 studies were read in full, and 29 were excluded because they presented different outcomes from the main investigation, reduplicated, or used different methodologies. As a result, 7 articles were included in this systematic review.

All study participants were diagnosed with BC in the
ductal and/or lobular regions of the breast. The age of the patients in the studies varied widely, mainly because BC most commonly affects women over the age of 50 [2]. Thus, the largest age range was 50 to 60 years, with four studies showing an average age of 51 years, while two studies were conducted with women under 50 years, whose average age was 47 years. Only one study did not include information on the average age of participants.

A total of 449 women were included in the studies, with the small study involving 29 participants and the largest including 150 participants. In terms of stage of BC, two studies recruited patients in stages II to III, four studies were conducted with patients in stages I to III and one study was conducted with women in stage I only.

During the intervention, studies were conducted at different stages of treatment, one after surgery, one before surgery, one carried out during chemotherapy (the article does not specify which chemotherapy was used), one study conducted during adjuvant chemotherapy, two during neoadjuvant chemotherapy, and one after surgery, chemotherapy and radiotherapy.

The year with the most publications was 2019 with four studies. This was followed by 2018 and 2017 with one study each, and the most recent study was published in 2022.

The studies were conducted on several continents: three in North America (USA, Mexico), one in South America (Brazil), two in Asia (Iran, Indonesia), and only one in Africa (Egypt).

All studies had a control group. In the omega-3 studies, the control groups were given capsules containing corn, sunflower and soybean oil. In the studies with vitamin D, the control group was administered with edible paraffin in one article, while in another article the control group was administered with a dose of vitamin D to act as a placebo. In the last study, no intervention was made in the control group.

With respect to omega-3 interventions, all selected studies used fish oil as a source. The period that the intervention group remained receiving supplementation ranged from 4 weeks (only one study) to 24 weeks (one study), with two studies performing the intervention for 6 and 7 weeks.

Regarding the frequency of intake, it was at least once daily in all studies, twice daily in three studies and once daily in only one study. In all studies with omega-3 supplementation, EPA and DHA were included in the composition.

Regarding vitamin D interventions, all selected studies used vitamin D3 as a source. The period that the intervention group remained receiving supplementation ranged from 6 weeks (only one study) to 12 weeks (one study), with one study providing the intervention for 8 weeks.

In terms of frequency, two studies conducted the approach at least once a day, and only one study performed the intervention once a week.

For a better explanation and visualization of the results described above, four Tables were created, Table 3 and Table 4 refer to the characteristics of the studies with omega-3, and Table 5 and Table 6 refer to the studies with vitamin D supplementation and describe the specific characteristics of each selected randomized clinical trial.

There is an important point to be clarified regarding the heterogeneity of the studies included in this review. The first fact to be mentioned concerns the different time points at which the treatment with supplementation was initiated for BC. Furthermore, the different mechanisms that each medication implemented in chemotherapy may affect the results of vitamin D and omega-3 supplementation are still not well understood.

It is also worth highlighting that the majority of studies presented a moderate risk of bias, and only a single selected article had a low risk of bias, which could affect the results described here.

**Table 3. Specificities of studies testing omega-3 supplementation, eligible on sample, control/intervention group, and time of treatment, 2023**

<table>
<thead>
<tr>
<th>Author/Year/Country</th>
<th>Total sample size</th>
<th>The average age of groups</th>
<th>The average age of groups</th>
<th>Time of treatment of patients during the intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Da Silva Paixão et al., 2017 (BRASIL)</td>
<td>37 participants</td>
<td>Average age: 51 years</td>
<td>Intervention Group: Received fish oil gel capsules. Control Group: Received mineral oil concentrate.</td>
<td>After surgery</td>
</tr>
<tr>
<td>2 Da Silva Paixão et al., 2017 (BRASIL)</td>
<td>52 participants</td>
<td>Average age: 50.1 years</td>
<td>Intervention Group: Received fish oil gel capsules. Control Group: Received capsules containing sunflower oil.</td>
<td>During neoadjuvant chemotherapy</td>
</tr>
<tr>
<td>3 Darwito et al., 2019 (INDONESIA)</td>
<td>48 participants</td>
<td>Average age: 47.5 years</td>
<td>Intervention Group: Received fish oil gel capsules. Control Group: Received an unspecified placebo.</td>
<td>During neoadjuvant chemotherapy</td>
</tr>
<tr>
<td>4 Peppone et al., 2019 (EUA)</td>
<td>81 participants</td>
<td>Average age: 59.7 years</td>
<td>Intervention Group 1: Received a high dose of omega-6 (soybean oil). Intervention Group 2: Received omega-3 (fish oil). Control group: Low dose of omega-3.</td>
<td>After surgery, radiotherapy and chemotherapy</td>
</tr>
</tbody>
</table>
### Table 4. Details of the selected articles investigated omega-3 supplementation, on the aspects of interventions, physical and psychological health, time of intervention, frequency, amount and source of omega-3, and main outcomes

<table>
<thead>
<tr>
<th>Author/Year/Country</th>
<th>Intervention Group</th>
<th>Total duration (number of weeks)</th>
<th>Frequency</th>
<th>Quantity (mg/g)</th>
<th>Source of omega-3</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Da Silva Paixão et al., 2017 (BRASIL)</td>
<td>18 patients received n-3 PUFA</td>
<td>4 weeks</td>
<td>2 × daily (1 capsule)</td>
<td>1 capsule contained 470mg EPA and 390mg DHA</td>
<td>Fish oil</td>
<td>No difference was found between the two groups on Interleukin6 (IL-6), TNF-α, and IL-1 β. However, GI patients did not show oscillation in hs-CRP and CD4+ mononuclear lymphocyte levels. In the CG, an increase in hs-CRP was observed compared to the baseline (p = 0.024). Indicating a higher inflammatory profile in response to the tumor.</td>
</tr>
<tr>
<td>De la Rosa Oliva et al., 2019 (MÉXICO)</td>
<td>26 patients received n-3 PUFA</td>
<td>24 weeks</td>
<td>2 × daily (2 capsules)</td>
<td>1 capsule contained 2.4g EPA/DHA with a 1:2 ratio.</td>
<td>Fish oil</td>
<td>The IG showed a significant decrease in xerostomia episodes compared to the CG. (p = 0.032).</td>
</tr>
<tr>
<td>Darwito et al., 2019 (INDONÉSIA)</td>
<td>24 patients received n-3 PUFA</td>
<td>7 weeks</td>
<td>1 × a day (1 capsule)</td>
<td>1 capsule contained 2.4g EPA/DHA with a 1:2 ratio.</td>
<td>Fish oil</td>
<td>GI obtained lower expression of ki-67 and VEGF compared to CG (p = 0.032 and p = 0.041) indicating lower cell proliferation, angiogenesis, and lymph angiogenesis.</td>
</tr>
<tr>
<td>Darwito et al., 2019 (INDONÉSIA)</td>
<td>30 patients received n-3 PUFA</td>
<td>6 weeks</td>
<td>2 × daily (3 capsules)</td>
<td>1 capsule contained 325mg of EPA and 225 of DHA</td>
<td>Fish oil</td>
<td>GI receiving omega-6 had significant improvement regarding FRC and BIF total score compared to GI receiving omega-3 (p &lt; 0.01 and p = 0.04) respectively. In addition, the omega-3 GI showed a statistically significant decrease regarding IL-6, PTGES2, and IFNγ, compared to omega-6 GI.</td>
</tr>
</tbody>
</table>

**Subtitles:** hs-CRP, High Sensitivity C-Reactive Protein; IG, Intervention Group; CG: Control Group.

### Table 5. Specificities of studies testing vitamin D supplementation, eligible on sample, control/intervention group, and time of treatment, 2023

<table>
<thead>
<tr>
<th>Author/Year/Country</th>
<th>Total sample size</th>
<th>The average age of groups</th>
<th>Intervention/Control Group</th>
<th>Time of treatment of patients during the intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mohseni et al., 2019 (IRÁ)</td>
<td>52 participants</td>
<td>Average age 47 years</td>
<td>Intervention Group: Received vitamin D capsules. A placebo Group: Received edible paraffin.</td>
<td>During chemotherapy</td>
</tr>
<tr>
<td>El-Bassiouny et al., 2022 (EGITO)</td>
<td>150 participants</td>
<td>Average age 52 years</td>
<td>Intervention Group: Received vitamin D capsules. A placebo Group: No intervention was performed.</td>
<td>During adjuvant chemotherapy</td>
</tr>
<tr>
<td>Going et al., 2018 (EUA)</td>
<td>29 participants</td>
<td>Group 1: Received high doses of vitamin D. Group 2: received low-doses of vitamin D (behaving as placebo).</td>
<td>Before surgery</td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Details of the selected articles investigated vitamin D supplementation, on aspects of interventions, physical and psychological health, timing of intervention, frequency, amount and source of vitamin D, and main outcomes

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Intervention Group</th>
<th>Total duration (number of weeks)</th>
<th>Frequency</th>
<th>Quantity (mg/g)</th>
<th>Source of omega-3</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mohseni et al., 2019 (IRÅ)</td>
<td>26 patients received Vitamin D</td>
<td>8 weeks</td>
<td>Once per week</td>
<td>50.000 UI</td>
<td>Vitamin D3</td>
<td>No difference was found between the two groups on inflammatory markers. However, GI patients had a significant result regarding TAC compared to GP (p=0.017).</td>
</tr>
<tr>
<td>El-Bassiouny et al., 2022 (EGITO)</td>
<td>75 patients received Vitamin D</td>
<td>12 weeks</td>
<td>Once a day</td>
<td>5.000 UI</td>
<td>-</td>
<td>GI patients had a significant difference compared to CG regarding serum LDH, IL-6, and cTnT levels (p &lt; 0.001).</td>
</tr>
<tr>
<td>Going et al., 2018 (EUA)</td>
<td>15 patients received Vitamin D</td>
<td>6 weeks</td>
<td>Once a day</td>
<td>10.000 UI</td>
<td>Vitamin D3</td>
<td>Patients who received high doses of vitamin D showed decreased serum levels of 27HC. However, there was no significant difference compared to the low-dose group.</td>
</tr>
</tbody>
</table>

Subtitles: hs-CRP, High Sensitivity C-Reactive Protein; IG, Intervention Group; CG: Control Group; TAC, Total Antioxidant Capacity; LDH, Lactate Dehydrogenase; cTnT, Cardiac Troponin T; IL-6, Interleukin 6; 27HC, 27-Hydroxycholesterol.

Discussion

To our knowledge, this is the first detailed review conducted on RTC that evaluated the efficacy of omega-3 and vitamin D in patients with BC. Our findings in this review reveal and support that omega-3 and vitamin D supplementation can help women who are metastasis-free and not receiving hormone therapy during treatment for HR+ (Hormone Receptor Positive) BC. Thus, this intervention is safe and can be used adjunctively to other main treatments.

The main benefits of omega-3 supplementation include: attenuation of the inflammatory profile in response to the tumor due to the decrease in IL-6, IFNγ and PTGES2, decreased cell proliferation, angiogenesis, and lymphangiogenesis, through low expression of ki-67 and VEGF (Vascular Endothelial Growth Factor), and decreased xerostomia. In addition, immunity is improved by maintaining normal serum levels of CD4+ T lymphocytes (cytotoxic T lymphocytes) and hs-CRP (high-sensitivity C-reactive protein) [16-19].

Vitamin D can assist in the suppression of RH+ tumors, as well as mitigate cardiometabolic effects by reducing IL-6, LHD, and cTnT, improving total antioxidant capacity (TAC), and preventing tumor growth through 27HC (27-Hydroxycholesterol) and CYP27A1 [20-22].

Effects of omega-3 supplementation

Omega-3 polyunsaturated fatty acids (PUFAs) have several benefits in different types of cancer in adults and children [23-26]. The beneficial mechanisms include modulation of the inflammatory response, mitigation of cell proliferation (metastasis and angiogenesis), and a role in gene expression that drives changes in cell metabolism. In addition, omega-3 PUFAs suppress the production of COX2, through the main omega-3 fatty acids (alpha-linolenic, EPA and DHA) [27, 28].

Tumors with positive hormone receptors, especially BC omega-3, can modulate estrogen metabolism, leading to a lower growth stimulus of hormone-dependent neoplastic cells, and decreasing arachidonic acid, which in turn leads to a decrease in derivatives of thromboxane, leukotriene and prostaglandin E2, which may reduce tumor cell survival [29, 30].

When n-3 PUFAs are used in patients receiving hormone therapy (aromatase inhibitors - AI), an improvement in pain and arthralgia can be observed, but the results are still contradictory [31-33].

In addition, no significant results were found in reducing the inflammatory profile of BC patients receiving AI. This result may be justified due to the duration of supplementation, as well as the side effects of AIs, which might inhibit the anti-inflammatory effect of omega-3 [33, 34].

At the same time, it is necessary to shed light on the dietary profile of these patients and how this food intake may interfere with treatment. In a recent study, it was possible to verify that BC patients are women who have a poor intake of essential nutrients and good fats, such as omega-6 and 3. This is reflected in their nutritional status and characterizes these women as overweight and obese, with a more inflammatory profile and a worse prognosis [35].

In addition, the Mediterranean diet (MD), which is rich in omega-3, is associated with a protective factor against...
The importance of vitamin D in the treatment of BC patients

Regarding vitamin D, it is worth noting that most patients with BC have low serum levels of 25-(OH) D (25-hydroxyvitamin D) and an obesogenic profile [39-41]. However, supplementation is an effective method to restore serum levels to baseline. It is important to consider whether vitamin D has a protective effect on patients before they develop CM and what the benefits of vitamin D has in the treatment of patients with CM, but the results are still uncertain [42].

Several studies have revealed that vitamin D can improve overall disease-free survival, and clinical prognosis, as well as mitigate inflammation and regulate antiproliferative pathways and cell differentiation [43-45]. The most widely accepted mechanism for the benefits of calcitriol in BC is that calcitriol exerts its role best when it binds to its receptor VDR. This VDR receptor is widely distributed in breast tissue and can modulate and control cellular mechanisms, such as cell proliferation, invasion, metastasis, angiogenesis and apoptosis [46,47].

A study conducted by Swedish researchers on women with an average age of 54 years found that a higher presence of this receptor in breast tissue indicated a better prognosis and a lower risk of death. In contrast, women with low levels of RVR expression had a high rate of cell proliferation as measured by ki-67 (p < 0.001), a larger tumor size (p = 0.002) and a high Nottingham grade (p < 0.001) [45].

Some limitations in this review need to be better addressed, including in cohort studies and randomized trials, among others. Particularly in relation to the interventions of omega-3 and vitamin D, the optimal dose and the time of intervention, as there seems to be no minimum period for supplementation established in the literature. Furthermore, in some studies only a single serum dosage was administered, which may obscure the true value of 25-(OH) D and omega-3. Moreover, the studies examined include interventions in different ethnic cities with different molecular subtypes of BC, as the different subtypes generate different pathologic and molecular responses, which consequently interferes with the prognosis and the natural course of the disease. It is also worth nothing that some metabolic factors may affect the bioavailability of these compounds, as well as nutritional status, dietary intake and sedentary lifestyle, among others.

From future perspectives, randomized clinical trials separated by a certain molecular subtype should be developed to investigate the efficacy of omega-3 and vitamin D supplementation during the treatment of women with BC. It is also important that new interventions are separated according to different stages of treatment, as chemotheraphy drugs can mask the real positive effects of supplementation, or even decrease their molecular activities.

Conclusion

Omega-3 can assist in the adjuvant treatment of BC, as this nutrient has shown several positive effects on the health of these patients by improving immunity and inflammatory markers.

In respect of calcitriol, it can also be used during chemotherapy in RE+ patients. It can improve the cardiovascular metabolic profile and prognosis, as well as inhibit the progression of CM through 27HC.

Finally, longer longitudinal studies and randomized clinical trials in women with only one molecular subtype are needed to determine the optimal dose and timing of vitamin D and omega-3 intervention. This will further clarify the results found in this review and add to the literature. It is further emphasized that the more low-cost therapies investigated, the better the clinical treatment options for patients with breast neoplasms.

Authors' contribution

Lailton Oliveira da Silva and Victor da Silva collected the research data, in addition to filling in the results for tabulating this data, as well as wrote the article. Anderson Weiny Barbalho Silva and José Juvenal Linhares supervised the work, provided support services in data collection, as well as gave guidance on writing the research.

Conflict of interests

Nothing to declare.
The research did not receive funding.

References


