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RISK, PREVENTION, AND SCREENING (DL HERSHMAN, SECTION EDITOR)

# Omega-3 Fatty Acids for Prevention of Breast Cancer: an Update and the State of the Science

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**Abstract** The quantity and makeup of dietary fat intake are known to affect human health. A variety of purported health benefits, including cancer prevention, have focused increased attention on use of omega-3 (ω-3) polyunsaturated fatty acid (PUFA) supplements. Preclinical evidence has been encouraging, and recent studies have increased our understanding of mechanisms by means of which ω-3 PUFAs may protect against breast cancer. However, epidemiological studies have yielded mixed results. Recent population studies have attempted to determine factors, for example total fat intake and the ratio of ω-3 to ω-6 PUFA intake, that may affect the action of ω-3 PUFAs. Several clinical trials, some currently ongoing, are investigating strategies to favorably alter endogenous fatty acid profiles in an attempt to develop clinically feasible prevention methods. Identification of well-defined subpopulations who are most likely to benefit from a targeted prevention approach will probably be crucial to this effort.

**Keywords** Breast cancer · Fish oil · Prevention · Fatty acids · Omega-3 polyunsaturated fatty acids · Omega-6 polyunsaturated fatty acids · Diet · Metabolism · Obesity · Risk factors · Inflammation · Nutrition

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## Introduction and Background

Omega-3 (ω-3) and omega-6 (ω-6) polyunsaturated fatty acids (PUFAs) are essential fatty acids that have been shown to have an important effect on several chronic illnesses [1]. Epidemiological data suggest that changes in total fat intake and a shift in ratio favoring ω-6 over ω-3 PUFAs in the Western diet have paralleled the rise of cardiovascular disease, obesity, diabetes, and other chronic diseases as leading contributors to morbidity and mortality [2]. Obesity is a well-known risk factor for development of several epithelial malignancies, and it is becoming increasingly apparent that inflammation may be an important mediator of this obesity–cancer link [3]. Partly for this reason, manipulating fatty acid composition has attracted increasing scientific attention as a potential cancer prevention and adjunctive treatment strategy.

Dietary intake, predominantly from cold-water fish, is the most reliable source of the long-chain ω-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [1, 4]. Data from several preclinical models suggest that these ω-3 PUFAs have an antitumor effect by means of a variety of steric and molecular interactions that inhibit cellular proliferation and promote cell death. DHA, for example, becomes inserted into the phospholipid cell membrane, thereby altering permeability, cell elasticity, and the function of many membrane-embedded receptors and proteins. Attenuation of inflammatory pathways and activation of the peroxisome proliferator-activated receptor-γ (PPAR-γ) have also recently been implicated [5••, 6].

Epidemiological data regarding associations between ω-3 PUFA intake and cancer development are inconsistent. Observational studies suggest a protective effect of ω-3 PUFAs against colon, prostate, and other cancers [7, 8]. However, the protective effect of ω-3 PUFAs against breast cancer (BC) is controversial. Because of mixed epidemiological observations, additional, larger population studies

are needed to determine whether there is an association between ω-3 PUFA intake and BC development consistent with an anti-cancer effect. Promising preclinical data have motivated several clinical prevention trials, which are currently ongoing. This review will provide an update on advances made in recent years regarding use of ω-3 PUFAs for prevention of BC.

## Population Studies

Despite numerous epidemiological reports, conflicting data have delayed consensus regarding any preventive effect of ω-3 PUFAs against BC development [9–12]. A large systemic review, reported in 2006, analyzed the findings of 38 studies that examined the effect of ω-3 PUFA intake on incidence of a variety of cancers in geographically heterogeneous populations [8]. Overall cancer risk was not affected by ω-3 PUFA intake. Some studies reported reduced risk of BC, whereas others reported increased risk. Therefore, the authors concluded that the epidemiological data do not support an association between ω-3 PUFA consumption and BC incidence. Since this report several additional population studies have been conducted, and will be reviewed below.

In a large prospective study including over 35,000 postmenopausal women enrolled in the VITamins and Lifestyle (VITAL) cohort, current use of fish oil supplements was associated with decreased risk of localized invasive ductal carcinomas, but not of lobular carcinomas or of regional or distant disease (hazard ratio, HR 0.68, 95 % confidence interval, CI 0.50–0.92; $P$=0.02) [13••]. The specific form of ω-3 PUFA taken (i.e. DHA and/or EPA) was not reported. Inconsistent reporting of PUFA composition, background total fat intake, and ω-3 to ω-6 ratio may partly explain the conflicting findings of epidemiological studies. To address this possibility, several studies have examined the specific makeup of consumed fatty acids.

In a case–control study of a Korean population, 358 patients with BC and 360 healthy control subjects underwent dietary assessment by questionnaire and interview to determine fat intake amounts and types [14]. Both pre and postmenopausal women in the highest quartile of fatty-fish intake had a lower incidence of BC (odds ratio, OR 0.23, 95 % CI 0.13–0.42; $P$<0.001). EPA and DHA intake was estimated on the basis of the fish consumed. No association was found between EPA and/or DHA intake and BC risk for premenopausal women, but a protective effect was observed for postmenopausal women (EPA intake ≥0.101 g day$^{-1}$: OR 0.38, 95 % CI 0.15–0.96; $P$=0.035; DHA intake ≥0.213 g day$^{-1}$: OR 0.32, 95 % CI 0.13–0.82; $P$=0.010). Therefore specific patient characteristics (i.e. menopausal status), in addition to the makeup of dietary fat intake, may be important considerations.

In contrast with the Korean study, a case–cohort study of a Danish population did not find any association between total or specific ω-3 PUFA intake and BC risk [15]. Importantly, the Danish study quantified specific ω-3 PUFA levels in gluteal adipose tissue donated by women who prospectively enrolled in the Diet, Cancer, and Health study during the 1990s. Tissue levels of total marine ω-3 PUFAs, EPA, and DHA were compared between women who went on to develop BC ($n$=463) and a subcohort of healthy subjects ($n$=1,098). However, total levels of marine ω-3 PUFAs were low for all subjects, which may account for discrepancies between the ω-3 PUFA effect for this population versus that for populations that consume marine-rich diets. Interestingly, an earlier report on the same study population found a greater incidence of estrogen receptor (ER)-positive BC associated with higher fish consumption, as determined by use of a detailed food–frequency questionnaire [16]. This apparent discrepancy suggests that measurement of endogenous PUFA levels may be important in assessing the true effect of PUFAs.

Other population studies have investigated interactions between ω-3 and ω-6 PUFAs. In general, ω-6 PUFAs are believed to have pro-inflammatory effects, whereas ω-3 PUFAs are anti-inflammatory. Therefore, quantifying consumption of both fatty acids may be an essential step in isolating an ω-3 PUFA affect. In a French study population comprising >56,000 women followed for eight years, no association was found between total ω-3 (or other) PUFA intake and BC risk [17]. However, increased ω-3 PUFA intake was protective against BC for women who consumed the highest amounts of ω-6 PUFAs (HR 0.62, 95 % CI 0.44–0.86; $P$ *interaction*=0.042). Similarly, in the Shanghai Women's Study, which included over 72,000 women who were cancer-free at baseline and were followed prospectively, there was no association between ω-3 PUFA intake and BC risk [18••]. However, women who consumed the highest amounts of ω-6 PUFAs (>7.28 g day$^{-1}$) and the lowest amounts of ω-3 PUFAs (≤0.045 g day$^{-1}$) were at highest risk of developing BC (relative risk, RR 2.06, 95 % CI 1.27–3.34; $P$ *interaction*=0.008) compared with women who had the lowest ω-6 to ω-3 PUFA intake ratios. These findings were replicated in a case–control study including a cohort of Mexican women comprising 1,000 patients with BC and 1,074 healthy subjects [19••]. Increased ω-3 PUFA intake was associated with decreased risk of BC for obese women, defined as those with body mass index (BMI) ≥30 (OR 0.58, 95 % CI 0.39–0.87; $P$=0.008). These population studies show the importance of investigating the interactions between dietary fats and specific patient characteristics, including BMI and menopausal status, to better understand the complex relationships between ω-3 PUFAs and BC risk (Table 1). Additional studies to identify at-risk populations who may benefit from specific alterations to dietary fat

**Table 1** Selected recent population studies of ω-3 PUFAs and breast cancer risk

| Author | Subjects | Number | Instrument | Variable | Magnitude of effect | 95 % Confidence interval | *P* value |
|---|---|---|---|---|---|---|---|
| Brasky [13••] | Cohort | 35,016 | Questionnaire | Current fish oil supplement intake | 0.68[b] | 0.50–0.92 | 0.02[a] |
| Kim [14] | Case | 358 | Questionnaire | High fatty fish intake (≥15.39 g $day^{-1}$) vs. low intake (<3.42 g $day^{-1}$) | 0.23[c] | 0.13–0.42 | <0.001 |
| | Control | 360 | | High EPA intake (≥0.101 g $day^{-1}$) by postmenopausal women | 0.38[c] | 0.15–0.96 | 0.035 |
| | | | | High DHA intake (≥0.213 g $day^{-1}$) by postmenopausal women | 0.32[c] | 0.13–0.82 | 0.01 |
| Witt [15] | Case | 463 | Adipose tissue biopsy | Adipose tissue levels (per 1 % increase): | | | |
| | Control | 1,098 | | Total ω-3 PUFAs | 1.00[b] | 0.95–1.05 | n.s. |
| | | | | DHA | 1.00[b] | 0.91–1.10 | n.s. |
| | | | | EPA | 0.94[b] | 0.73–1.21 | n.s. |
| Thiebaut [17] | Cohort | 56,007 | Questionnaire | Highest vs. lowest quintile total ω-3 PUFA intake | 0.99[b] | 0.84–1.15 | n.s. |
| | | | | Highest vs. lowest quintile long-chain ω-3 PUFA intake by women with highest ω-6 PUFA intake | 0.62[b] | 0.44–0.86 | 0.04 |
| Murff [18••] | Cohort | 72,571 | Questionnaire | Highest (0.20 g $day^{-1}$) vs. lowest (0.02 g $day^{-1}$) marine ω-3 PUFA intake | 0.74[d] | 0.52–1.06 | n.s. |
| | | | | Lowest (≤0.045 g $day^{-1}$) vs. highest (>0.10) ω-3 PUFA intake by women with highest ω-6 PUFA intake (>7.28 g $day^{-1}$) | 2.06[d] | 1.27–3.34 | 0.008 |
| Chajes [19••] | Case | 1,000 | Interview and questionnaire | Highest vs. lowest tertile total ω-6 PUFA intake by premenopausal women | 1.92[c] | 1.13–3.26 | 0.02 |
| | Control | 1,074 | | Highest vs. lowest tertile ω-3/ω-6 PUFA intake by obese premenopausal women | 0.58[c] | 0.39–0.87 | 0.008 |

[a] Effect on risk of invasive ductal carcinoma, not including other histology

[b] Hazard ratio

[c] Odds ratio

[d] Relative risk

n.s., not significant

intake via supplementation or lifestyle changes, and studies to determine the biological mechanisms that mediate the relationship between ω-3 PUFAs and BC, will be essential for guiding clinical prevention trials.

## Pre-clinical Studies

Unlike the epidemiological evidence, preclinical data from a variety of experimental models are promising regarding possible use of ω-3 PUFAs to prevent BC. In general, ω-3 PUFAs are believed to disrupt carcinogenesis by means of a variety of mechanisms leading to cell death and/or inhibition of cellular proliferation. Possible mechanisms include alteration of the phospholipid cell membrane and associated receptors, interruption of signal transduction pathways, anti-inflammatory effects, altered estrogen and insulin metabolism, and production of free radicals including reactive oxygen species (ROS) [7]. Several studies that increase our understanding of mechanisms of action of ω-3 PUFAs and BC are reviewed below.

### Actions of ω-3 PUFAs In Vitro

It has been well established that ω-3 PUFAs can suppress development of cancers by inhibiting cellular proliferation and inducing cell death [7]. In-vitro studies investigating the effects of ω-3 PUFAs on murine and human BC cells provide important insights into the mechanisms underlying this inhibition of tumorigenesis. Alteration of the lipid membrane by ω-3 PUFAs, and related disruption of pro-inflammatory eicosanoid synthesis by cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) enzymes, has recently been revealed in mouse and human BC cell lines [20–23]. Treatment of ER-positive and ER-negative human BC cell lines with both EPA and DHA was found to induce apoptosis and reduce cell viability [24•]. This was paralleled by

decreased or absent expression of Bcl2 and by increased procaspase-8 expression in EPA and DHA-treated cells compared with control cells. Bcl2 is an important apoptosis-regulator protein, and its overexpression has been implicated in several cancers. Cleavage of pro-caspase 8 amplifies the caspase cascade, thereby promoting apoptosis. Corsetto and colleagues also revealed inhibition of epidermal growth factor receptor (EGFR) activation by DHA and EPA, and a slight reduction in EGFR expression as a result of DHA exposure. EGFR is overexpressed in several malignancies, including those of the breast and lung, and promotes cell growth and migration via several downstream-signaling proteins. Treatment of ER-negative human BC cells with DHA has been shown to induce apoptosis via increased caspase-3 activity [25•]. DHA also reduced the migratory ability of these cells, suggesting that DHA may also prevent tumor cell invasion.

DHA has also been shown to increase intracellular ROS in ER-positive BC cells [26•]. Kang and colleagues revealed DHA-induced ROS accumulation, leading to caspase-8 activation and resulting apoptotic cell death. This cytotoxic effect was negated by pharmacological inhibition of specific caspases and knockdown of caspase-8 by siRNA transfection. Therefore, EPA and DHA's anti-tumor effects seem to act via multiple mechanisms, including disruption of the cell membrane and embedded receptors, alteration of signaling pathways involved in cellular proliferation and migration, dysregulation of apoptosis, and production of cytotoxic oxidizing molecules.

### Animal Models—In Vivo Effects of ω-3 PUFAs

Increased dietary intake of ω-3 PUFAs has been shown to have a substantial anti-tumor effect in animal models. However this effect has been poorly reflected in prevention of human carcinogenesis, as shown by the epidemiological data reviewed earlier. To provide more information in support of human clinical trials, more recent studies investigated the mechanisms by which ω-3 PUFAs inhibit tumorigenesis in animals. In the study by Kang et al. discussed above, ER-positive breast cancer mouse xenografts were fed a fish-oil-supplemented diet to determine whether in-vivo effects of ω-3 PUFAs reflected in-vitro findings [26•]. At six weeks of feeding, plasma levels of DHA and EPA were substantially higher for mice fed a 5 % fish-oil diet. DHA and EPA levels increased several hundredfold for both normal and tumorous mammary tissue. Substantially smaller tumors were observed for the mice receiving the fish-oil-supplemented diet compared with those receiving the control diet. As supported by in-vitro experiments, intracellular ROS levels increased in tumor tissue from the fish-oil-fed mice compared with control-diet-fed animals. Increased apoptotic and reduced proliferative indices were observed in tumor tissue of fish-oil-fed mice. Notably, little to no effect of ω-3 PUFAs was observed in ER-negative BC cells or xenografts. This suggests the anticancer effect of ω-3 PUFAs may be limited to specific tumor types; decreased ERα and ERβ expression was observed in female rats fed a fish-oil-rich diet [27]. Additionally, decreased mitosis and increased apoptosis were observed in the mammary gland and colon with increased fish oil feeding, indicating a possible preventative, anti-neoplastic effect of ω-3 PUFAs.

An effect of ω-3 PUFAs on development and progression of hormone-insensitive tumors was investigated in a series of studies by Manni and colleagues [28•, 29, 30]. The investigators hypothesized that the combination of tamoxifen and ω-3 PUFAs, which also act as PPAR agonists, would prevent development of ER-negative tumors by suppression of synergistic interactions between ER and PPARγ pathways. In a rat model of carcinogen-induced mammary tumors, the combination of tamoxifen and an ω-3-PUFA-rich diet strongly inhibited mammary tumor development [28•]. Importantly, the antitumor effect of combined fish oil and tamoxifen was more pronounced than that seen with tamoxifen or ω-3 PUFAs alone. Administration of a fish-oil-rich diet with sub-optimum doses of tamoxifen was also effective in curtailing the development of mammary tumors, whereas suboptimum-dose tamoxifen alone had no protective effect. However, in a later study using a different animal model of ER-negative breast tumors, fish-oil feeding did not provide an additional protective effect when combined with tamoxifen [29]. To better isolate a preventive effect, the investigators next examined the effect of tamoxifen and fish oil on pre-malignant, hyperplastic lesions in their carcinogen-induced rat mammary tumor model [30]. Although fish-oil feeding substantially reduced proliferative marker Ki-67 in hyperplastic lesions, development of these preneoplastic lesions was not inhibited. Notably, in two of these three studies, tissue accumulation of ROS was observed in the fish oil-supplemented animals, indicating increased oxidative stress as a potential anticancer mechanism. In the most recent study, tissue and plasma levels of arachidonic acid were suppressed by intake of ω-3 PUFAs, implicating alteration of COX and LOX pathways in the antitumor effect.

Population studies on the effect of ω-3 PUFA intake may often be confounded by changes in the ω-3 to ω-6 PUFA intake ratio. The effects of varying this ratio have been investigated by use of animal models. Exposure to high levels of ω-6 PUFAs in utero, via increased maternal dietary intake, led to a greater incidence of chemically-induced mammary tumors in female rats [31]. Adding fish oil to the maternal diet substantially protected against the ω-6 PUFA effect. In another rat model of chemically-induced

mammary tumors, a high dietary ω-3 to ω-6 PUFA ratio was found to reduce cellular proliferation by 60 % and to increase apoptosis in mammary carcinomas compared with a low ω-3 to ω-6 PUFA ratio [5••]. Multiple complementary mechanisms may explain this finding for the high ω-3 to ω-6 PUFA ratio group, including:

- attenuation of inflammatory activation, evidenced by reduced phosphorylation of nuclear factor kappa-B (NFκB);
- reduced lipid synthesis;
- metabolic alterations, including reduced adiponectin levels and reduced insulin-like growth factor-1 (IGF-1) signaling; and
- alterations of signal transduction pathways, including activation of AMP-activated protein kinase (AMPK) and suppression of mammalian target of rapamycin (mTOR).

A high ω-3 PUFA dose was used to achieve a high ω-3 to ω-6 PUFA intake ratio, which would probably not be achievable by fish intake alone. This study suggests that dietary modification and ω-3 PUFA supplementation in combination may be a useful clinical strategy for prevention of BC. The heterogeneous mechanisms underlying the anticancer activity of ω-3 PUFAs indicate the need to investigate a differential effect on the basis of BC molecular subtype.

Methods other than dietary supplementation have been shown to increase ω-3 PUFA levels in animal models. A calorie-controlled diet plus treadmill exercise led to weight loss, reduced body fat, and altered epidermal phospholipid composition in mice [32]. Specifically, elevated DHA levels were found in skin tissues of mice that were fed a calorie-consistent diet and which underwent treadmill exercise compared with sedentary and libitum-fed mice, and with exercise-alone mice. Most preclinical data have revealed anti-tumorigenic effects resulting from increasing ω-3 PUFA levels via dietary intake and via other methods. The animal studies described above have suggested multiple mechanisms that may contribute to a protective affect of ω-3 PUFAs against neoplastic transformation.

## Clinical Trials

Encouraging animal data supporting an involvement of ω-3 PUFAs in prevention of BC have led to a number of human trials. The relatively innocuous side-effect profile of ω-3 PUFA supplements has made this approach particularly attractive. Dietary DHA and EPA supplementation cause a dose-dependent increase of these ω-3 PUFA levels in the serum and breast adipose tissue [33], reflecting animal models. Increasing BMI, however, reduced the dose-responsiveness of DHA and EPA levels in serum, and of DHA levels in breast adipose tissue. High doses of DHA and EPA (up to 7.56 g combined) were well tolerated, with high compliance (92.9±9.2 %). Reflecting pre-clinical findings, methods other than dietary supplementation have been shown to increase endogenous ω-3 PUFA levels. In a pilot study including 40 women receiving chemotherapy for localized BC, diet and exercise counseling was associated with a rise in blood ω-3 PUFA levels [34•]. All women in this study received written information and bimonthly newsletters on diet and exercise, and a pedometer. Women randomized to the intervention group also received telephone counseling with a registered dietician encouraging increased fruit and vegetable intake, reduced fat intake, and increased physical activity. Specific counseling regarding ω-3 or ω-6 PUFA intake was not provided. Over the 12-month study period, ω-3 PUFA levels increased in the blood, although there was no statistically significant difference between the control and intervention groups. Therefore, in addition to supplementation, improved diet and exercise can alter the ω-3 to ω-6 PUFA ratio in favor of ω-3 PUFAs. A multifaceted approach, including lifestyle and behavioral changes and ω-3 PUFA supplements, may be a more effective prevention strategy than supplementation alone.

Several studies have been conducted with the objective of identifying blood and urine-based biomarkers that may be reflective of cancer risk and that can be modified by altering the volume and composition of endogenous lipids via a variety of interventions. The relationship between blood ω-3 PUFA levels, determined via erythrocyte fatty acid level, and development of benign fibrocystic conditions of the breast was investigated in a case-control study in Shanghai [35]. Proliferative fibrocystic conditions of the breast are associated with increased BC risk. Women with the highest EPA levels had a substantially reduced risk of BC, as compared with proliferative fibrocystic conditions, in relation to women with the lowest EPA concentrations (OR 0.51, 95 % CI 0.27–0.94; $P$=0.003). These findings support theories of the preventive effect of EPA against development of BC, and of EPA's protective effect against progression of preneoplastic lesions to cancer. This study also identified erythrocyte fatty acid level as a potential biomarker for fatty acid intake and composition, which may be a useful tool for diet and supplement intervention.

Identification of other blood-based biomarkers may be useful for future intervention trials. One such biomarker may be hepatic stearoyl-CoA desaturase-1 (SCD-1), an important regulator of endogenous saturated fatty acid composition. Epidemiological studies have identified associations between reduced saturated fatty acid intake, characterized by lower SCD-1 levels, and reduced BC risk [36]. Diets rich in PUFAs suppress SCD-1 expression; SCD-1 levels may therefore be indicative of anticancer activity of PUFAs, although this has yet to be investigated.

Several ongoing clinical trials investigating the use of ω-3 PUFAs for BC prevention have attempted to disrupt

metabolic and inflammatory pathways implicated in carcinogenesis. The ubiquitous intercellular adhesion molecule-1 (ICAM-1) has been investigated as a potential biomarker for BC risk and ω-3 PUFA response [37]. ICAM-1 is activated by several pro-inflammatory cytokines and has been associated with BC risk in epidemiological studies [38, 39]. In a nested case–control study including 408 subjects with cancer diagnosed between 1994 and 2007 and 760 matched control subjects, elevated plasma ICAM-1 level was associated with increased BC risk for subjects with low ω-3 PUFA intake (OR 4.7, 95 % CI 1.6–13.4; $P$=0.004) [37]. Prospective randomized trials are needed to validate ICAM-1 as a biomarker for BC risk and its use in assessing risk-reductive effects of ω-3 PUFAs.

Addition of ω-3 PUFAs to endocrine chemoprevention strategies is currently being investigated. Signori et al. reported preliminary data from an ongoing trial in which postmenopausal women at increased risk of BC, because of increased breast density, were randomized to receive either no intervention, raloxifene 60 mg, raloxifene 30 mg, the ω-3 PUFA compound Lovaza 4 g, or Lovaza 4 g plus raloxifene 30 mg, for two years [40••]. The primary endpoint is reduction in breast density. After completing one year of the study ($n$= 46), women in all groups tolerated the interventions well, with high compliance. Raloxifene led to a dose-dependent reduction in serum IGF-1 level, which was not seen with Lovaza; nor was an augmented IGF-1 effect observed on addition of Lovaza to raloxifene. Levels of circulating inflammatory markers, including C-reactive protein (CRP) and interleukin-6, were not affected for any of the treatment groups. The final results of this study are awaited.

Identifying an at-risk group most likely to benefit from a specific intervention will likely yield a more effective prevention strategy than seeking a single approach for a diverse population. To this end, our group has been interested in identifying mechanisms linking obesity, a known risk factor for development of postmenopausal BC, and carcinogenesis [3, 41].

Obesity is associated with systemic, subclinical inflammation and with elevated levels of circulating pro-inflammatory cytokines [3, 42]. Several of these cytokines are known to be elevated in BC patients, and have been associated with BC development and progression [43, 44]. We recently identified histologically-evident inflammation of breast white adipose tissue, manifested as dead and dying adipocytes surrounded by a crown of infiltrating macrophages, now known as crown-like structures of the breast (CLS-B) [45]. The presence of these lesions in mouse models of obesity and in humans was associated with elevated levels of pro-inflammatory cytokines, NFκB activation, and increased aromatase activity [45, 46]. Increased COX-2 and prostaglandin $E_2$ ($PGE_2$) in the inflamed breast tissue of obese patients were found to contribute to increased aromatase activity [47]. Disrupting this newly-identified obesity→inflammation→aromatase axis represents a possible prevention strategy, and the anti-inflammatory properties of DHA make it an attractive agent to study in this high-risk group. Monitoring blood levels of pro-inflammatory cytokines associated with CLS-B, for example tumor necrosis factor-α, may be useful for assessing the benefit of investigational agents, including DHA, for patients identified to have breast inflammation. Targeted strategies such as these are needed to identify more effective ways of preventing BC.

## Conclusion

The effect of ω-3 PUFAs in reduction of BC risk remains unclear. Population studies have identified multiple factors, including total fat intake and dietary fat composition, which can modulate the endogenous effects of ω-3 PUFAs. Animal models and in-vitro data have provided insights into the complex pathways of breast carcinogenesis and the points at which ω-3 PUFAs may be effective in interrupting this process. These preclinical findings are being applied in new clinical prevention trials, which use refined strategies that take into account the increasingly recognized complexities of fatty acid biology. Disrupting the links between distinct risk factors and development of malignancies in specifically defined populations could bring the success of recent, so-called targeted treatments to the field of cancer prevention.

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