

The Conundrum of Omega-3 Fatty Acids in Cancer Prevention Studies: Which One? How Much? What Biomarkers?

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Abstract

Marine omega-3 fatty acids promote resolution of inflammation and have potential to reduce risk of obesity-related breast cancer. For prevention trials in obese women, inflammatory cytokines, aromatase, and measures of breast immune cell infiltration are logical, as are biomarkers of growth factor, adipokine, and estrogen signaling. Where best to look for marker change: in the circulation (easiest), in benign breast

tissue (most relevant), or in visceral adipose (inflammation often most marked)? A null biomarker modulation trial may reflect limitations in design, source and dose of fatty acids, or biomarkers and should not lead to premature abandonment of marine omega-3 fatty acids for cancer prevention. *Cancer Prev Res*; 11(4); 1–4. ©2018 AACR.

See related article by Gucalp et al., p. 203

Chronic inflammation is recognized as playing a role in the development of breast cancer, which in obese women is thought to be the result of inflammatory cell expansion and infiltration and excess cytokine production in adipose tissue. In addition to influence on proliferative, proangiogenic, and survival pathways, proinflammatory intermediates can influence estrogen-related signaling via increases in aromatase.

In obesity, excess free fatty acids are initially stored predominately in subcutaneous adipocytes and as these depots fill, in visceral adipose. Hypertrophied adipocytes become stressed, reduce secretion of adiponectin, and increase secretion of leptin. These hypoxic adipocytes also secrete MCP-1, resulting in immune cell infiltration. Increases in circulating bacterial lipopolysaccharides (LPS) as a result of an altered intestinal wall barrier as well as free fatty acids trigger immune cell phenotypic change in the obese adipose stromal vascular compartment. These include increases in CD8⁺ cytotoxic and CD4⁺ helper T cells, a decrease in T regulatory cells, and a shift from M2 to M1 activated macrophages. The so-called crown-like structures (CLS) are the result of necrotic adipocytes surrounded by phagocytizing macrophages. Increased secretion of proinflammatory cyto-

kines and adipokines from adipocytes and adipose stromal cells results in NF- κ B activation, nuclear translocation and transcription of MCP-1, IL6, TNF α , and IL1 β in a positive feedback loop (1). IL6, TNF α , and IL1 β also increase COX-2 and synthesis of proinflammatory eicosanoids, such as prostaglandin E2 (PGE2). PGE2, IL6, TNF α , and IL1 β upregulate aromatase and estrogen formation from androgens. IL6 can also induce tissue C reactive protein (CRP; Fig. 1; ref. 2). As obesity progresses, blood adiponectin falls and clinical insulin resistance develops with increasing fatty acid storage in liver and muscle (1). Systemic increases in IL6 result in liver production of CRP and systemic rises in this cytokine (2). Low blood adiponectin or adiponectin:leptin ratio, and higher systemic levels of CRP and estrogen, have generally been found to be associated with postmenopausal breast cancer (3).

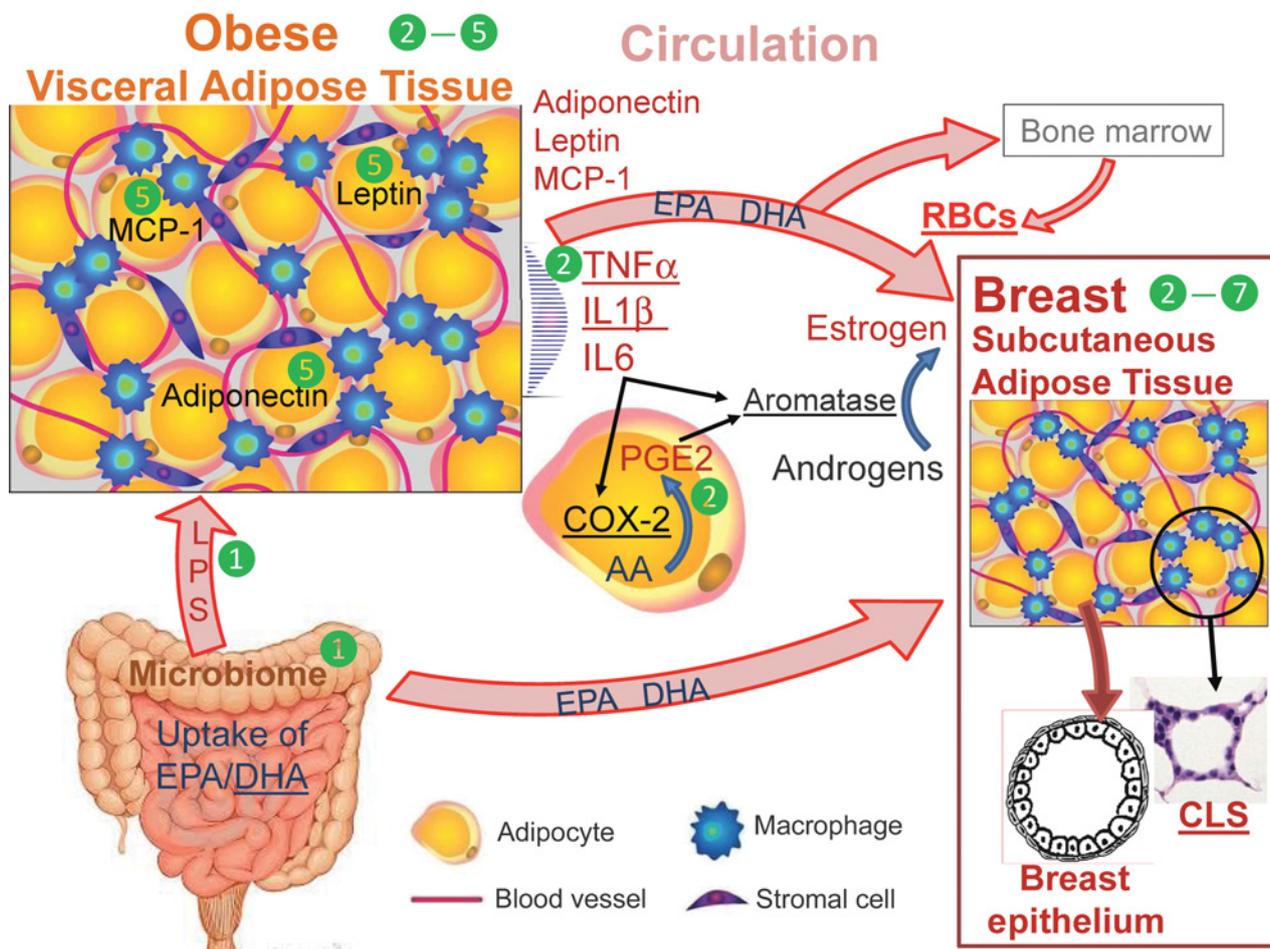
There are multiple preclinical and epidemiologic studies suggesting that the marine omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) help resolve inflammation and reduce the risk for breast cancer (reviewed in refs. 4–6), particularly in the obese individual. Potential mechanisms schematically represented in Fig. 1 (coded by number) include the following: (i) improved gut microbiota diversity and restoration of epithelial barrier function reducing proinflammatory LPS; (ii) reduced adipose COX-2 and proinflammatory cytokine gene expression via inhibition of NF κ B; (iii) reduction in substrate for proinflammatory eicosanoids via replacement of arachidonic acid (AA) in phospholipid membranes with EPA and DHA; (iv) reduction in tissue estrogen via effects on aromatase expression and activity; (v) increased adiponectin

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**Figure 1.**

Pathways of obesity-related inflammation that may be relevant to breast cancer and may be modified by supplementation with marine omega-3 fatty acids such as DHA. Of a variety of potential biomarkers that may be assessed in blood or in specific tissues, those addressed by Gucaip et al. (7) are indicated by an underline.

transcription and translation as a result of increased peroxisome proliferator-activated receptor (PPAR) activity; (vi) reduction in EGFR and other growth factor-induced signaling as a result of DHA and EPA incorporation into lipid rafts; and (vii) resolvins and protectins generated from EPA and DHA (1, 5, 6).

In this issue, Gucaip and colleagues (7) reported no evidence of modulation of expression of several benign breast tissue proinflammatory genes (TNF α , IL1 β , and COX-2), aromatase, or CLS following 3 months of an intermediate-level dose of DHA of 2 g/day. The cohort consisted of 57 biomarker-evaluable overweight and obese pre- and postmenopausal high-risk women or breast cancer survivors, some of whom were on aromatase inhibitors or NSAIDs. What might be responsible for these null findings? Whenever clinical findings do not match basic science expectations, the answer is often found in (i) formulation; (ii) dose; (iii) cohort differences; (iv) biomarker selection and measurement; and (v) sample size and heterogeneity.

Should EPA have been given along with DHA? Although the two appear to have similar biologic functions, there are differences in uptake that may be dependent on doses, ratio of the two, concomitant intake of AA; and cell type. In preclinical studies, EPA appears to have higher proportional absorption into membranes of ER⁺ MCF-7 cells than DHA, whereas DHA may have greater proportional absorption in triple-negative cell lines and may be superior in reducing EGFR signaling due to better lipid raft absorption and spatial receptor disruption (8). Given the promising preclinical data in ER⁻ cell lines and early reports that DHA may be superior to EPA in reducing inflammation, some early-phase breast cancer prevention studies, such as that reported in this issue, have used DHA alone, whereas others following more traditional epidemiologic leads have used combinations of EPA and DHA (9–11). A recently reported clinical trial in a relatively homogeneous cohort with abdominal obesity and baseline CRP evidence of inflammation found little difference in whole blood inflammatory gene expression between 2.7 g/day of EPA

compared with DHA. Both were better than controls for changing mRNA of at least two genes involved in NF- κ B and PPAR signaling (12). Although gene expression was measured in whole blood, a number of investigators have noted the correlation between omega-3–induced changes in RBC and other blood cellular component phospholipids and those in adipose (including the breast) given sufficient exposure time (2, 5, 6).

Animal model supplementation studies often first induce an inflammatory environment with a high fat diet and then administer combinations of DHA and EPA at 4% to 10% of feed by weight, usually in the range of 10% of calories for EPA and/or DHA (5, 8). Human supplementation studies with EPA and DHA, in contrast, often do not preselect for baseline inflammation and then dose in the range of 1% to 2% of calories (5). Those trials showing consistent modulation of tissue blood inflammatory biomarkers are generally in individuals with active inflammation and employ combined EPA + DHA doses of 3.4 to 4.0 g/day or EPA or DHA alone \geq 2.7 g/day (5, 12). There is little evidence of inflammatory biomarker modulation at doses $<$ 2 g/day (1, 5, 6). Not all obese individuals as defined by BMI have evidence of metabolic dysfunction and inflammation. Selecting the cohort for high visceral adipose or high CRP, both of which are associated with inflammation and obesity-related women's cancers, might be one way of ensuring the cohort has sufficient baseline inflammation such that resolution is readily measurable (13).

Biomarker selection from a number of possibilities is perhaps the thorniest issue and investigators in the trial reported by Gucalp and colleagues chose markers from 3 of the 7 potential mechanistic areas. Reduction in the frequency of breast tissue CLSs would provide morphologic evidence of decreased inflammation but as evidenced in their study (7), baseline CLS prevalence is likely too low to be helpful in the small needle biopsy samples obtained in prevention trials. Baseline Ki-67 as a measure of proliferation is often too low to see improvement in postmenopausal women. Change in gene expression in benign breast tissue, although attractive, is fraught with problems given the variation in proportion of epithelial cells, adipocytes, and stroma in sequential biopsies and likely posttranscriptional nature of many omega-3 effects. Differences in selection of primer-probes and housekeeping genes can make it difficult to compare different studies. However, if mRNA assessments are to be used, MCP-1, IL6, TNF α , and IL1 β are logical as they are upregulated by NF- κ B activity

and reduced by EPA and DHA (1). The recently reported finding that EPA or DHA in doses of 2.7 g/day had favorable effects on PPAR α , and CD14 whole-blood mRNA, which would be expected to inhibit NF- κ B activity, deserves follow-up and exploration in breast tissue (12). Adiponectin and adiponectin receptor mRNA are also logical candidates as they are reduced in obesity and upregulated by PPAR α , which is increased by both EPA and DHA. Furthermore, adiponectin and leptin have opposing roles in inflammation and breast cancer development such that assessing the ratio of the two makes biologic sense and helps compensate for proportional differences in cell types in sequential breast biopsies (3, 5). Measurement of phosphorylated proteins likely to be affected by disruption of spatial organization of growth factor receptors in lipid rafts is another approach but differences in antibodies and platforms can also make it difficult to compare results across studies. Blood biomarker assessments are generally the most reproducible. Lower levels of CRP and other blood inflammatory markers associated with breast cancer risk have been inversely correlated with EPA and DHA intake or blood levels in cross-sectional studies (14), but are often not observed in interventional studies lasting $<$ 6 months unless there is evidence of baseline inflammation and higher doses of DHA and/or EPA are used (12).

The null results reported for benign breast tissue inflammatory gene expression for short duration intermediate dose DHA by Gulcap and colleagues (7) should not lead us to abandon the study of marine omega-3 fatty acids for prevention of breast cancer. Epidemiologic leads should encourage us to continue to investigate this class of compounds particularly as public acceptance for this type of natural product/supplement seems high. Certainly, formulation and dose require further study.

Importantly, given our incomplete understanding of the predominant mechanisms by which marine omega-3 fatty acids might work to prevent cancer, perhaps we have just not been fishing with the right bait.

Disclosure of Potential Conflicts of Interest

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