

Effect of Omega-3 Fatty Acids on the Progression of Metastases after the Surgical Excision of Human Breast Cancer Cell Solid Tumors Growing in Nude Mice¹

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ABSTRACT

We showed previously that a diet rich in linoleic acid (LA), an omega-6 fatty acid, stimulates the growth and metastasis of human breast cancer cells in athymic nude mice. In contrast, diets supplemented with eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), omega-3 fatty acids, exert suppressive effects. We have now assessed EPA and DHA as adjuvant nutritional therapy in the nude mouse model and compared the responses when the intervention was commenced 1 week before (“neoadjuvant”) or immediately after (“postoperative adjuvant”) surgical excision of the primary tumor. Female nude mice received a high-fat, 8% LA diet beginning 7 days before 10⁶ MDA-MB-435 human breast cancer cells were injected into a thoracic mammary fat pad. As the tumor surface areas approached 0.7 cm², the mice were assigned to either continue on the LA-rich diet or to commence one containing 8, 4, or 2% EPA or DHA. Seven days later, the mammary fat pad tumors were excised; the mice still consuming the 8% LA diet were then allocated sequentially to either continue this diet or commence one of the six postexcision omega-3 fatty acid dietary interventions. Eight weeks later, the mice were necropsied and evaluated for local recurrence and lung metastases. Although there were no differences in the incidence of local recurrence between groups, EPA and DHA both inhibited the development of lung metastases. When the dietary interventions were commenced 7 days before surgery, the severity of lung metastasis was reduced by the two omega-3 fatty acids in a dose-dependent manner; at all three levels, the suppressive effects were statistically significant ($P < 0.05$). Postexcision EPA treatment produced small, statistically insignificant effects, but lung involvement was reduced significantly by feeding DHA at the 2 and 4% levels ($P < 0.05$). Overall, these results suggest that omega-3 fatty acids may have a place as adjuvant nutritional therapy in breast cancer and particularly as part of a neoadjuvant regimen.

INTRODUCTION

Several epidemiological studies have related a high fat consumption to a poor outcome in breast cancer patients, (1–4) and in two of these it was observed that relatively high intakes of omega-6 fatty acids were associated with both more advanced disease at the time of diagnosis, (4) and reduced survival (2). In support of these clinical observations, we showed that a high-fat diet rich in LA,³ an omega-6 fatty acid, stimulates the growth of the MDA-MB-435 human breast cancer cell line in the mfp of athymic nude mice and enhances its capacity to metastasize to the regional lymph nodes and lungs (5–7); EPA and DHA, the long-chain omega-3 fatty acids that are present at high concentrations in some fish oils, exert partial suppressive effects on tumor progression in this model (8).

We now report the results of a study in which the two omega-3 fatty acids were fed as adjuvant therapy prior to or immediately after surgical excision of the mfp tumors. Mice were fed a high-fat, LA-rich diet during the initial period after the MDA-MB-435 cell injections. The study was then divided into two segments; in one, these primary tumors were excised, and groups of mice were then fed one of three levels of EPA or DHA, whereas in the other, the “neoadjuvant therapy” group, the omega-3 fatty acid diets were commenced 1 week before surgery. The control group remained on the high-LA diet throughout the experiment.

MATERIALS AND METHODS

Animals. Female athymic nude mice (NCr-nu/nu) ages 3–4 weeks, were purchased from Harlan Sprague Dawley (Madison, WI). They were housed in a dedicated nude mouse facility that is accredited by the American Association for Accreditation of Laboratory Animal Care. All procedures were performed in a unidirectional laminar airflow hood.

Diets. The isocaloric experimental diets, which were based on the purified AIN-76A diet (9, 10), were prepared by BioServ, Inc. (Frenchtown, NJ). The EPA and DHA were supplied as ethyl esters by the Vitamins and Fine Chemicals Division of Hoffmann-La Roche, Ltd. (Basel, Switzerland). Each diet contained 20% (w/w) fat, with LA-rich safflower oil and saturated fatty acid-containing coconut oil proportioned to provide 8% LA, or substitutions of these oils with 8%, 4%, or 2% EPA or DHA ethyl esters (at 8% w/w this is equivalent to 7.4% omega-3 fatty acid). As supplied, the omega-3 fatty acids were supplemented with α -tocopherol, an antioxidant, at a concentration of 3 mg/g, to protect against oxidation. After formulation of the diets was completed, more additions were made to the 2

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³ The abbreviations used are: LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; mfp, mammary fat pad.

Table 1 Primary mfp tumor surface areas 7 days before excision and on the day of excision, and the excised tumor weights

Dietary group (no. of mice)	Tumor surface area (cm ²)		Tumor surface area (cm ²) increase over 7 days	Excised tumor weight (g)
	7 days preexcision	At excision		
Preexcision 8% LA ^a (148)	0.65 ± 0.02	1.13 ± 0.02	0.48 ± 0.02	0.91 ± 0.02
Preexcision 8% EPA (19)	0.78 ± 0.05	1.13 ± 0.09	0.32 ± 0.06 ^b	0.81 ± 0.09 ^c
4% EPA (17)	0.78 ± 0.04	1.09 ± 0.05	0.29 ± 0.06 ^b	0.71 ± 0.05 ^c
2% EPA (18)	0.75 ± 0.05	1.25 ± 0.07	0.49 ± 0.05	0.93 ± 0.07
8% DHA (18)	0.83 ± 0.03	1.07 ± 0.07	0.24 ± 0.06 ^d	0.83 ± 0.07
4% DHA (20)	0.78 ± 0.04	1.17 ± 0.07	0.37 ± 0.06 ^e	0.83 ± 0.06
2% DHA (18)	0.74 ± 0.03	1.18 ± 0.07	0.43 ± 0.05	0.91 ± 0.09

^a Comprises all mice fed the 8% LA until the tumors were excised.

^b Tumor surface area increase over 7 days prior to excision significantly less than that in mice fed 8% LA diet: $P < 0.01$.

^c Excised tumor weight significantly less than in the 8% LA dietary group: $P < 0.01$.

^d Tumor surface area increase over 7 days prior to excision significantly less than that in mice fed 8% LA diet: $P < 0.001$.

^e Tumor surface area increase over 7 days prior to excision significantly less than that in mice fed 8% LA diet: $P < 0.05$.

and 4% omega-3 fatty acid diets as required to obtain an identical (240 mg/kg) concentration of the vitamin; the same level was added to the 8% LA-containing control diet.

The pelleted sterile diets were placed in plastic bags that were flushed with N₂ gas and stored in the dark at -20°C to protect against lipid peroxidation (8). Each sealed bag was opened only once. Every other day, the feed trays were emptied and refilled with fresh diet. Autoclaved drinking water was provided ad libitum.

Cell Line. The culture requirements and the invasive and metastatic characteristics of the estrogen-independent MDA-MB-435 breast cancer cell line have been described elsewhere (6, 11).

Experimental Procedure. The mice were all fed the high-fat, 8% LA-containing diet for 7 days, after which they received injections of MDA-MB-435 cells as described previously in detail (6). Briefly, a right-sided thoracic mfp was exposed surgically while the mouse was under general anesthesia, 10⁶ cells were injected, and the incision was closed with a skin clip. Once palpable, the mfp tumors were measured once or twice a week (5), and, based on our previously published experience (12), as the calculated tumor surface areas approached 0.70 cm², the mice were assigned sequentially to either continue on the 8% LA diet for an additional 7 days or to commence a diet containing 8, 4, or 2% EPA or DHA ("preexcision neoadjuvant dietary group"). One week later, the tumors were all excised, and the mice in the group that had continued on the high-LA diet were immediately assigned sequentially to either continue on that diet (control group) or to begin treatment with one of the six omega-3 fatty acid-containing diets ("postexcision adjuvant dietary group").

At surgery, every effort was made to ensure that identifiable residual mfp tumor was not left *in situ*; however, in 20 cases (6%), this was clearly not possible because of infiltration around the subclavian artery or deep invasion into the pectoral muscles, and these mice have been excluded from the present report. Also, after several fatalities due to postoperative hemorrhage, no additional attempt was made to excise the regional lymph nodes. The excised tumors were weighed, trimmed to remove necrotic tissue, and immediately frozen and stored in liquid nitrogen for later phospholipid fatty acid analyses.

After the tumors had been excised, the mice were weighed once a week for an additional 8 weeks; they were then killed by cervical dislocation. Tumor-free mice, or those with mfp tumors that had failed to achieve the required surface area within 8 weeks of the cell injections, were excluded from the study. When these mice, plus those known to have had residual tumor tissue after surgery, and deaths in the immediate postoperative period were excluded, there were 110 mice in the preexcision dietary segment of the study, 128 in the postexcision dietary segment, and 20 in the 8% LA-fed control group.

At necropsy, body weights and the weights of any mfp tumor recurrences were recorded. The extent of macroscopic lung metastasis was assessed as described previously (5). The lungs were then fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 μm, stained with H&E, and examined by light microscopy.

Tumor Fatty Acids. Tumor lipid extractions were performed using the method of Folch *et al.* (13). The fatty acids were then transesterified and the fatty acid methyl esters were separated by gas chromatography (8). The individual fatty acids are presented as a percentage of the total fatty acids assayed.

Statistical Analyses. In the tables, the data are presented as the mean values ± SE. The incidence differences were evaluated by the χ^2 test, and the lung macroscopic metastasis total volumes were assessed by the nonparametric Mann-Whitney *U* test. Other statistical comparisons utilized Student's unpaired *t* test. Values for $P < 0.05$ were considered significant.

RESULTS

Primary Tumor Size. The mfp tumor surface areas 7 days before excision and on the day of excision, the increases in surface areas over that 7-day period, and the excised tumor weights are summarized in Table 1. The combined mean tumor surface area for the 110 mice that were treated with omega-3 fatty acid for 7 days prior to the excisions was 0.78 ± 0.02 cm² at the time of the dietary change. Despite the sequential assignment of tumor-bearing mice to the omega-3 fatty acid or LA-fed control groups, when entry into the study was completed, it was found that the corresponding mean surface area for the 148 mice fed a high-LA diet until the tumors were excised, which in-

Table 2 Body weight and locoregional tumors in athymic nude mice with prior tumor excision and fed either 8% LA diet throughout, or omega-3 fatty acid either continuously starting 7 days before surgery (preexcision), or commencing immediately after surgery (postexcision)

Dietary group	Body weight (g)		Locoregional tumors			
	Preexcision	Postexcision	Incidence (%)		Weight (g)	
			Preexcision	Postexcision	Preexcision	Postexcision
8% LA	26.3 ± 0.05		14/20 (70%)		0.52 ± 0.12	
8% EPA	28.9 ± 0.7 ^a	29.8 ± 0.7 ^a	10/19 (53%)	14/19 (74%)	0.07 ± 0.04 ^b	0.19 ± 0.04
4% EPA	29.0 ± 0.4 ^c	29.1 ± 0.8 ^a	8/17 (47%)	15/22 (68%)	0.11 ± 0.05 ^b	0.19 ± 0.04
2% EPA	27.7 ± 0.5 ^b	28.7 ± 0.6 ^a	10/18 (56%)	16/22 (73%)	0.32 ± 0.13	0.26 ± 0.10
8% DHA	29.0 ± 0.3 ^c	28.3 ± 0.6 ^b	13/18 (72%)	10/19 (53%)	0.27 ± 0.06	0.08 ± 0.03 ^b
4% DHA	28.4 ± 0.6 ^a	28.6 ± 0.7 ^a	16/20 (80%)	15/23 (65%) ^d	0.17 ± 0.05	0.23 ± 0.08
2% DHA	28.3 ± 1.0	28.7 ± 0.3 ^a	13/18 (72%)	16/22 (73%)	0.25 ± 0.08	0.19 ± 0.04

^a Significantly different from the 8% LA dietary group: $P < 0.01$.

^b Significantly different from the 8% LA dietary group: $P < 0.05$.

^c Significantly different from the 8% LA dietary group: $P < 0.001$.

^d Data missing for one mouse.

cluded the dietary LA control group, was significantly less, $0.65 \pm 0.02 \text{ cm}^2$ ($P < 0.001$). However, despite the larger size of the tumors in the groups that were subsequently fed an omega-3 fatty acid, the increases in combined mean surface areas after 7 days of feeding 8 or 4% EPA or DHA were significantly less than those of the 8% LA-fed mice (Table 1), indicating that some growth retardation had occurred during the presurgery omega-3 fatty acid treatment. Furthermore, the weights of the excised mfp tumors for the 8% EPA and 4% EPA groups were significantly less than those from mice fed the 8% LA diet ($P = 0.007$ and $P = 0.003$, respectively).

Body Weight Gains. The body weights at necropsy for the 13 dietary groups are summarized in Table 2 and show no evidence of food avoidance or toxic side effects in mice fed the omega-3 fatty acid diets; indeed, in all but one instance (2% DHA-fed preexcision group), the mean body weights were significantly higher than that of mice fed the 8% LA-containing diet.

However, in the group of mice commencing the 8% DHA diet after the excision of the primary tumor, there was a temporary weight loss that occurred during the 8th week of the study and averaged 4.4g compared with the previous week. This was associated with inadvertent atmospheric heat-related deterioration of the omega-3 fatty acid during shipment, was limited to this particular diet and treatment group, and was reversed completely by the end of the experimental period for each mouse (2–4 weeks later; Table 2).

The explanation for the relatively low body weights of mice in the 8% LA dietary group is unclear. One possibility that we considered was that this was due to the effects of severe metastatic disease in these animals, but this was not supported by an examination of body weights at necropsy in relation to macroscopic lung metastasis. We found that, for 8% LA-fed mice with a mean total metastatic volume of $4 \pm 2 \text{ mm}^3$, the corresponding body weight value was $25.8 \pm 0.7 \text{ g}$, and for those with a mean volume of $108 \pm 62 \text{ mm}^3$, it was $26.6 \pm 0.5 \text{ g}$.

Locoregional Tumors. In most treatment groups, the situation arose in which, both at necropsy and during later histological examination, it was not possible to distinguish between an unexcised axillary lymph node that had been completely replaced by tumor and a regrowth of residual cancer at

the excision site. Consequently, both have been categorized together as "locoregional tumors".

The omega-3 fatty acids had no significant effects on the incidence of recurrent mfp tumors and/or axillary lymph node involvement, and although there were obvious trends for the weights of these locoregional tumors to be less than those from the 8% LA-fed mice, these differences were statistically significant only for the 8% and 4% EPA preexcision dietary groups and for the 8% DHA postexcision dietary group (Table 2; $P < 0.05$).

Lung Metastases. These data are all summarized in Tables 3 and 4. At necropsy, 75% of the mice fed the high-fat diet containing 8% LA were found to have macroscopic metastases, with an average of five lung surface deposits per mouse, and a mean metastatic volume of $60.4 \pm 32.7 \text{ mm}^3$. When the omega-3 fatty acid dietary modifications were initiated 7 days before the mfp tumor excisions, there were significant inhibitions in the development of lung metastasis (Table 3). At levels of 8 or 4%, both EPA and DHA were effective in suppressing macroscopic metastasis formation and in reducing the severity of the pulmonary metastatic involvement when it did occur; there were no significant differences in efficacy between either the two omega-3 fatty acids or these two levels of intake. At a level of 2%, EPA still clearly produced a partial inhibition of the metastatic process. Although 2% DHA appeared to be ineffective, this was due to the influence of a single mouse with 22 nodules and a total metastatic volume of 735.8 mm^3 . When this outlier is excluded, the mean number of metastatic nodules per mouse is 3.1 ± 1.0 in the 2% DHA preexcision group; the mean total metastatic volume ($21.8 \pm 12.9 \text{ mm}^3$) is then not significantly different from that of the 2% EPA postexcision group, but it is less than that of the 8% LA control group ($P < 0.05$).

When the omega-3 fatty acid dietary interventions were initiated immediately after the mfp tumor excisions (Table 4), the effect of EPA on the subsequent development of macroscopic lung metastases was less pronounced; even at the 8% level of intake, the difference compared with the 8% LA-fed mice was not statistically significant ($P = 0.072$). In contrast, DHA was at least as effective as when treatment began 7 days before the tumor excisions. However, the results obtained with 8% DHA are suspect because of the complication of high

Table 3 Macroscopic and microscopic lung metastases in athymic nude mice with prior primary tumor excision and maintained on 8% LA throughout the experiment or fed omega-3 fatty acid commencing 7 days before surgery (preexcision)

Diet	Macrometastases			Micrometastases only
	Incidence	No./mouse	Metastatic volume (mm ³)	Incidence
8% LA	15/20 (75%)	5.0 ± 1.0	60.4 ± 32.7	1/20 (5%)
8% EPA	7/19 (37%) ^a	1.1 ± 0.4 ^b	4.4 ± 2.5 ^b	5/19 (26%)
4% EPA	8/17 (47%)	1.5 ± 0.7 ^b	9.4 ± 4.6 ^a	3/17 (18%)
2% EPA	7/18 (39%) ^c	2.8 ± 1.2 ^c	25.5 ± 16.4 ^c	5/18 (28%)
8% DHA	6/18 (33%) ^b	1.3 ± 0.5 ^b	10.3 ± 7.0 ^b	1/17 (6%) ^d
4% DHA	11/20 (55%)	2.0 ± 0.7 ^a	9.1 ± 3.5 ^a	4/20 (20%)
2% DHA	12/18 (67%)	4.2 ± 1.4	61.4 ± 41.5 ^c	2/18 (11%)

^a Significantly lower than the 8% LA group: $P < 0.02$.^b Significantly lower than the 8% LA group: $P < 0.01$.^c Significantly lower than the 8% LA group: $P < 0.05$.^d Histology was not available for one mouse.^e When one mouse with a total metastatic volume of 735.8 mm³ is excluded, the mean ± SE for the group is 21.8 ± 12.9 ($P < 0.05$, compared with the 8% LA group).**Table 4** Macroscopic and microscopic lung metastases in athymic nude mice with prior tumor excision and maintained on 8% LA throughout the experiment or fed omega-3 fatty acid commencing immediately after surgery (postexcision)

Diet	Macrometastases			Micrometastases only
	Incidence	No./mouse	Metastatic volume (mm ³)	Incidence
8% LA	15/20 (75%)	5.0 ± 1.0	60.4 ± 32.7	1/2 (5%)
8% EPA	10/19 (53%)	2.0 ± 0.7 ^a	24.3 ± 11.5	3/19 (16%)
4% EPA	9/22 (41%)	2.7 ± 0.9 ^a	51.4 ± 25.5	4/22 (18%)
2% EPA	15/22 (68%)	4.5 ± 1.0	47.8 ± 23.9	2/22 (9%)
8% DHA	6/19 (32%) ^b	1.1 ± 0.5 ^b	3.6 ± 1.4 ^c	5/19 (26%)
4% DHA	12/24 (50%)	2.4 ± 0.9 ^a	27.4 ± 13.5 ^a	4/24 (17%)
2% DHA	11/22 (50%)	2.3 ± 0.7 ^a	17.6 ± 6.6 ^a	4/22 (18%)

^a Significantly lower than the 8% LA group: $P < 0.05$.^b Significantly lower than the 8% LA group: $P < 0.01$.^c Significantly lower than the 8% LA group: $P < 0.001$.

peroxidation product formation in the diet (data not shown), which occurred late in the experiment and was most likely the cause of the temporary weight loss during the 8th week of the study in this dietary group.

Tables 3 and 4 also show that treatment with the omega-3 fatty acids did not eliminate preexisting pulmonary micrometastases, but rather inhibited their subsequent progression. Although these were often limited to single or only a few foci, micrometastases in the absence of grossly visible lesions occurred with greater frequency in the omega-3 fatty acid compared with the 8% LA-fed group and did not show a relationship to the level of omega-3 fatty acids in the diet.

Tumor Phospholipid Fatty Acids. The fatty acid composition of the cell membrane phospholipids was determined for the mfp tumors from 12 mice fed 8% LA until the time of excision and the same number from mice fed 8 or 4% EPA or 8 or 4% DHA for 7 days prior to the tumor excisions (the preexcision or neoadjuvant groups). Table 5 shows that 7 days of feeding the omega-3 fatty acids were sufficient to produce significant increases in the EPA and DHA content of the phospholipids and corresponding decreases in arachidonic acid, an omega-6 fatty acid. LA was reduced significantly in the tumors from mice fed 8% EPA or 8% DHA ($P < 0.001$ and $P < 0.01$, respectively) as compared to tumors from mice on the 8% LA diet. These lower levels of LA, a metabolic precursor of arachi-

donic acid, did not result in significantly greater reductions in arachidonate in the groups fed 8% EPA or DHA compared with the groups fed 4% of the omega-3 fatty acids. Feeding one omega-3 fatty acid also resulted in the introduction of significant levels of the other long-chain omega-3 fatty acid into the phospholipids, notably of DHA derived from EPA (Table 5).

DISCUSSION

In an earlier experiment, in which the primary tumors were left *in situ* (8), we found that feeding a diet containing 8% EPA or DHA to nude mice from a week prior to the injection of MDA-MB-435 cells until necropsy caused a retardation of mfp tumor growth compared with that in mice fed an 8% LA-containing diet; at a level of 4%, the fatty acids produced only a small but statistically significant inhibition of growth late in the observation period. However, even in the presence of primary tumors with an expanding cell mass throughout the experiment, the dietary omega-3 fatty acids exerted inhibitory effects on the development of lung metastases, with 4% EPA showing greater efficacy than 4% DHA.

The present study was designed to evaluate the ability of dietary omega-3 fatty acid supplementation to suppress both the growth of unrecognized residual tumor after surgical excision of the primary tumor and the otherwise sustained development of

Table 5 The principal fatty acids in tumor cell phospholipid fractions as a percentage (mean \pm SE) of the total assayed fatty acid content^a

Fatty acid	Dietary group				
	8% LA ^b	8% DHA ^c	4% DHA ^c	8% EPA ^c	4% EPA ^c
Palmitic (16:0)	24.4 \pm 0.6	23.7 \pm 0.5	22.9 \pm 0.3 ^d	24.8 \pm 0.5	24.0 \pm 0.3
Stearic (18:0)	21.0 \pm 0.5	21.2 \pm 0.5	20.3 \pm 0.2	21.8 \pm 0.3	21.6 \pm 0.2
Oleic (18:1)	18.8 \pm 0.3	18.3 \pm 0.2	18.3 \pm 0.3	18.4 \pm 0.4	18.1 \pm 0.1
Linoleic (18:2)	18.3 \pm 0.5	16.4 \pm 0.4 ^e	18.8 \pm 0.2	15.7 \pm 0.3 ^f	18.8 \pm 0.1
Arachidonic (20:4)	10.5 \pm 0.3	6.8 \pm 0.3 ^f	7.2 \pm 0.3 ^f	6.9 \pm 0.3 ^f	7.6 \pm 0.2 ^f
Eicosapentaenoic (20:5)	0 ^g	0.9 \pm 0.1 ^f	0.6 \pm 0.1 ^f	4.9 \pm 0.3 ^f	2.8 \pm 0.2 ^f
Docosahexaenoic (22:6)	1.3 \pm 0.1	7.5 \pm 0.4 ^f	7.1 \pm 0.3 ^f	1.9 \pm 0.1 ^f	2.1 \pm 0.1 ^f

^a Twelve tumors were analyzed from each dietary group.

^b The 8% LA diet was fed for 4–6 weeks.

^c The 8% LA diet was fed for 4–6 weeks, and the omega-3 fatty acids for 1 week.

^d Significantly different from the 8% LA group: $P < 0.05$.

^e Significantly different from the 8% LA group: $P < 0.01$.

^f Significantly different from the 8% LA group: $P < 0.001$.

^g None detected.

preexisting local and systemic metastases. Although we were unable to routinely excise the axillary lymph nodes, our results do show that there was a reduction in both locoregional progression and the emergence of macroscopic lung metastases when EPA and DHA were fed as an adjunct to surgery; the effect on systemic metastasis was evident even when the omega-3 fatty acids were fed at the 2% level. McCormick *et al.* (14) observed a somewhat analogous effect of combination therapy with bilateral ovariectomy and retinyl acetate in rats exposed to the mammary carcinogen dimethylbenz[*a*]anthracene. They found that treatment initiated after excision of the first tumor to develop prevented the emergence of new tumors at other mammary sites.

When the omega-3 fatty acids were fed for 7 days before surgery, a reduction in the growth of the primary tumors was observed compared with those in the 8% LA-fed control group over the same time interval. Also, although no consistent additional effect of prefeeding the omega-3 fatty acids on locoregional tumor progression was observed, we did find that when EPA or DHA was fed for 1 week prior to surgery, there was a greater suppression of lung metastatic involvement than that obtained when the dietary intervention was commenced after the excision of the mfp tumors. Thus, the therapeutic advantage obtained by commencing the omega-3 fatty acid dietary intervention prior to surgery (primary or neoadjuvant dietary therapy) was most likely due to an effect on one or more steps in the metastatic cascade that result in hematogenous spread. In this context, Choy and McCulloch (15) have demonstrated that breast cancer cells may be detected in effluent venous blood during surgery, and that this is associated with vascular invasion in the primary tumor. The size of tumor cell clumps that enter the circulation influence the successful completion of the metastatic process, and in an experimental model, a direct relationship was demonstrated between the size distribution of cancer cell aggregates in the venous drainage of a transplanted tumor and the number of macroscopic lung metastases (16).

In our earlier experiment with the nude mouse model (8), we showed that EPA and DHA inhibit tumor production of arachidonic acid-derived eicosanoids, including prostaglandin E₂ and 12-hydroxyeicosatetraenoic acid. These compounds are

involved in several steps of the metastatic cascade, including intravascular tumor cell-tumor cell and tumor cell-platelet interactions and aggregation (17). The present study showed that 7 days of feeding the omega-3 fatty acids was sufficient to produce an increase in tumor cell phospholipid EPA and/or DHA levels and concomitant reductions in LA-derived arachidonic acid content, and so reduced levels of these key eicosanoids may have been responsible for the therapeutic advantage seen with the preexcision dietary interventions. Unfortunately, tumor tissues were not available to perform these assays.

In addition to their influence on intravascular metastatic events, the omega-6 and omega-3 fatty acids have distinct and opposing effects on the invasive step of the metastatic cascade. For example, we have demonstrated stimulation of MDA-MB-435 cell invasion by LA in an *in vitro* assay and related this to induction of the M_r 92,000 type IV collagenase by 12-hydroxyeicosatetraenoic acid (18). Conversely, EPA and DHA inhibit invasion by the breast cancer cell line (19) and suppress type IV collagenase activity (20).

It has also been reported that omega-3 fatty acids inhibit the production of platelet-derived growth factor by vascular endothelial cells *in vitro* (21) and basic fibroblast growth factor production (22), and so EPA and DHA may also have suppressed the secretion of growth factors that, although involved in postsurgical wound healing, might also stimulate tumor cell proliferation and adhesion and/or the neovascularization and host stromal fibroblast proliferation that are essential for sustained growth at the metastatic site (23–25).

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