The effect of dietary n-3 and n-6 polyunsaturated fatty acids on the expression of cyclooxygenase 1 and 2 and levels of p21\textsuperscript{ras} in rat mammary glands

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Dietary n-6 polyunsaturated fatty acids (PUFAs) promote rat mammary cancer while n-3 PUFAs are inhibitory. The purpose of this study was to determine whether the fats exert their effects by altering the expression of genes that affect cancer development. Therefore, we have examined the effect of PUFAs on the expression of the cyclooxygenase (COX) 1 and 2 genes that are involved in prostaglandin biosynthesis. We also investigated the effect of dietary PUFAs on the expression of the p21\textsuperscript{ras} protein and Ha-ras mRNA. Rats were fed either low- (7% ; LF) or high- (21% ; HF) fat diets that were rich in either n-6 PUFAs (safflower oil, S) or n-3 PUFAs (menhaden oil, M) for 3 weeks. COX-1 mRNA levels were approximately the same in groups fed diets containing either level of menhaden oil, but were increased by ~30% in the LFS and HFS groups (P < 0.05). Transcripts of the inducible COX-2 gene were not detectable in the menhaden oil groups, but this gene was expressed in animals fed either level of safflower oil and in the HFS group was associated with increased levels of COX enzymatic activity and production of PGE\textsubscript{2}. Animals fed safflower oil had elevated levels of p21\textsuperscript{ras} protein compared to animals fed menhaden oil. Ha-ras mRNA was increased by ~35% in animals fed HFS compared to the group fed HFM (P < 0.05). These results demonstrate that dietary n-6 PUFAs upregulate COX-2 and, to some extent, COX-1 expression. There was a concomitant increase in COX enzyme activity and PG synthesis in the mammary glands of rats fed high levels of n-6 PUFAs. Together with associated changes in p21\textsuperscript{ras} expression, these results may explain, at least in part, the promoting effects of dietary n-6 PUFAs on mammary carcinogenesis.

**Introduction**

Dietary fats are known to modulate mammary gland carcinogenesis in experimental animals (1,2) and may have similar effects in human breast cancer development (3). Several studies in rodents have shown that vegetable oils rich in n-6 polyunsaturated fatty acids (PUFAs\textsuperscript{*}) promote mammary carcinogenesis whereas similar levels of marine oils rich in n-3 PUFAs, appear to inhibit (1,4). A number of mechanisms have been suggested to explain the association between dietary fatty acids and breast cancer (for review see ref. 5). Among these, particular attention has been paid to their effects on prostaglandin (PG) synthesis (6).

It is well known that malignant breast tumours and tumour cell lines from humans and rodents contain elevated levels of PGs compared to the normal tissues from which they arise (7–9). Inhibiting PG synthesis by non-steroidal anti-inflammatory drugs (NSAIDs) has been shown to hinder the development of chemically-induced as well as transplantable mammary cancer (10–13). Evidence linking the effects of dietary fat on mammary carcinogenesis to PG synthesis derives mainly from the observation that NSAIDs inhibit the promoting effects of diets rich in n-6 PUFAs (10,14). Furthermore, the n-6 PUFAs linoleic acid (LA) is a precursor of the PG 2 series that are known to be mitogenic for normal human and rodent mammary epithelial cells (6). On the other hand, the n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are precursors of the PG 3 series that lack such a mitogenic effect. Associations have also been made between dietary fatty acids, PG synthesis and ras gene expression (8).

The Ha-ras gene is commonly mutated in rat mammary adenocarcinomas and is over expressed in many human breast cancers (for review see ref. 15).

The key regulatory enzyme in PG biosynthesis is prostaglandin H-synthase/cyclooxygenase (COX) that catalyses the conversion of arachidonic acid (AA) to PGs (16). There are two COX isoforms, COX-1 and COX-2, both of which are usually inhibited by NSAIDs. In order to provide further information on the mechanism by which dietary fats modulate breast cancer development, the present study examines the effect of feeding rats different levels of n-3 and n-6 PUFAs on the expression of the COX-1 and COX-2 genes, as well as the Ha-ras gene in mammary glands.

**Materials and methods**

**Animals and diets**

Pathogen-free female Sprague-Dawley rats (38–43 days old) purchased from Charles River Laboratories (St Constant, Quebec, Canada) were housed at 24 ± 2°C and 50% humidity, with a 12-h light/dark cycle. They were acclimatized for 7 days with food and water provided ad libitum. The animals were then randomized into four groups (five per group) and were fed one of four experimental diets for 3 weeks. The composition of the experimental diets is shown in Table I. The diets, purchased from Dyets Co. (Bethlehem, PA), were based on the American Institute of Nutrition standard reference diet AIN-93G (17) with the addition of menhaden oil or safflower oil at the expense of carbohydrate (Table I). Soybean oil was added at 1 or 3% to the low and high fat diets, respectively, to provide essential fatty acids. All diets were stored in dark, air-tight containers at 4°C. Animals were killed by cervical dislocation, and mammary tissues were frozen in liquid N\textsubscript{2} and stored at −80°C.

**Northern analysis of COX-1 and Ha-ras mRNA**

Total RNA (20–25 µg) from the mammary glands of individual rats, isolated by the TRIzol reagent (Life Technologies; Grand Island, NY), was subjected to Northern blot analysis (18) using Zeta-Probe nylon membranes (Bio-Rad; Hercules, CA). The hybridization solution contained 50–100 ng of the probe labelled with [\textsuperscript{32}P]dCTP using random hexamer priming (Ambion Co., Austin, TX) to a specific activity of ~10\textsuperscript{8} c.p.m./µg. The probe for the Ha-ras gene was the 460 bp EcoRI fragment from plasmid BS9 described.

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**Abbreviations:** PUFAs, polyunsaturated fatty acids; PG, prostaglandin; NSAIDs, non-steroidal anti-inflammatory drugs; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; COX, cyclooxygenase; AA, arachidonic acid; LF, low fat menhaden; HFM, high fat menhaden; HFS, low fat safflower; LFS, low fat safflower.

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Carcinogenesis vol.19 no.5 pp.905–910, 1998
previously (19). The murine COX-1 probe was a 1.7 kb DNA fragment containing the coding sequences, but not the 5’ and 3’ untranslated flanking sequences (Oxford Biomedical Research, Oxford, MI). Following hybridization and autoradiography, membranes were rehybridized to a 32P-labelled probe (19) to verify equal RNA loading. Autoradiograms were integrated and normalized to the corresponding level of β-actin.

**RT-PCR analysis of COX-2 mRNA**

We used PCR primers that have already been described for COX-2 and GAPDH (20) (COX-2 5’ primer CTTATCGGCAGGAACTGG, 3’ primer ACTTTGCTTGATGGTGTGCTTCT; GAPDH 5’ primer CTCTATTTGAACTCACAT, 3’ primer GAGAGGCACCATCCAGTGAC). These primer pairs yield amplified products of 279 bp for COX-2 and 574 bp for GAPDH.

Total RNA (1 µg) was converted to single-stranded cDNA using 50 U of MMLV-reverse transcriptase with 2.5 µM random hexamers. cDNA samples were split into two equal aliquots and amplified using 0.2 µM primers. The cycling programme was an initial 5 min for melting (94°C) followed by 30 cycles of melting (94°C, 1 min), annealing (55°C, 1 min) and extension (72°C, 10 min). PCR products were separated in 2% agarose gels and visualized by ethidium bromide staining.

**Table I. Composition (%) of fat in experimental diets**

<table>
<thead>
<tr>
<th>Diet ingredient</th>
<th>AIN-93G</th>
<th>Menhaden oil (LFM)</th>
<th>High fat (HFM)</th>
<th>Safflower oil (LFS)</th>
<th>High fat (HFS)</th>
</tr>
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<tbody>
<tr>
<td>Cornstarch</td>
<td>40</td>
<td>40</td>
<td>28</td>
<td>40</td>
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</tr>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
<td>23</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>Dex-CS</td>
<td>13</td>
<td>13</td>
<td>9</td>
<td>13</td>
<td>9</td>
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<td>Sucrose</td>
<td>10</td>
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<td>7</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Alphacel</td>
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<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
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<td>Vitamin mix</td>
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<td>3.5</td>
<td>4</td>
</tr>
<tr>
<td>t-cystine</td>
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<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
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<tr>
<td>Choline bitartrate</td>
<td>0.25</td>
<td>0.25</td>
<td>0.29</td>
<td>0.25</td>
<td>0.29</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
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<tr>
<td>Safflower oil</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>Menhaden oil</td>
<td>0</td>
<td>6</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Energy (kcal/100 g)</td>
<td>360</td>
<td>360</td>
<td>416</td>
<td>360</td>
<td>416</td>
</tr>
<tr>
<td>n-3:n-6</td>
<td>0.15</td>
<td>2.6</td>
<td>2.6</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Western blotting of p210^hox protein**

Mammary glands were homogenized in ice-cold PBS with 0.2 mM PMSF and 0.1 mM leupeptin using a Polytron homogenizer at high speed for 10 s. Homogenates were centrifuged at 800 g for 10 min and supernatants were centrifuged at 40 000 g for 30 min. Plasma membranes were solubilized in homogenization buffer containing 10 mM sodium-deoxycholate and 1% Triton X-100 then centrifuged at 40 000 g for 30 min. Equivalent amounts of protein were applied to 12.5% SDS-polyacrylamide gels, separated by electrophoresis and electrotransferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA). Membranes were blocked with 5% non-fat dry milk and probed with a pan-reactive anti-ras monoclonal antibody (Oncogene Science, Cambridge, MI) in 1% non-fat dry milk. After incubation with a peroxidase-conjugated anti-mouse antibody (Sigma Chemicals, Oakville, ON), signals were detected by enhanced chemiluminescence (Amersham, Arlington Heights, IL).

**COX enzymatic activity**

Mammary glands from individual animals were homogenized in 100 mM Tris-HCl buffer (pH 7.4) and centrifuged at 9000 g at 4°C for 15 min. The supernatant was then centrifuged at 100 000 g for 1 h and the resulting microsomal fraction was resuspended in 50 mM potassium phosphate buffer (pH 7.4). COX activity was assayed essentially as described previously (21). Briefly, resuspended microsomes (150–200 µg protein) were incubated with 60 µM [1-14C]arachidonic acid (AA; 45–50 mCi/mM, ABC Inc., St Louis, MO) and cofactors (1 mM glutathione, 50 mM phosphate buffer, pH 7.4 and 1 mM epinephrine) at 37°C for 15 min. The reaction was terminated by acidification followed by a brief centrifugation. The supernatant containing the radioactive products and the unreacted [1-14C]AA, was extracted twice with 3 vol of chloroform. The extracts were combined, evaporated to dryness under N2, reconstituted in benzene/dioxane:acetic acid/formic acid (82:14:1:1). Metabolites of AA were visualized by autoradiography, excised and the radioactivity was determined by Cerenkov counting. COX activity was determined by relating the counts attributable to metabolites to the counts of all bands and expressed as pmol prostanoids/mg protein/min (21,22).

**Measurement of mammary gland PGE2**

Analysis of the mammary gland PGE2 was performed as described earlier (23). Briefly, tissues (200–300 mg) were minced, sonicated in 0.1 M Tris-HCl buffer (pH 7.4), and centrifuged at 800 g for 15 min to separate cell debris and the fat layer. The resulting supernatant was acidified to pH 3.5 with glacial acetic acid and extracted three times with 3 vol of ethyl acetate. Extracts were combined, evaporated to dryness under N2, resuspended in 200 µl of the enzyme immunoassay (EIA) buffer and stored at −80°C until analysed. EIA was carried out using a Correlate-EIA kit (Assay Designs Inc., Ann Arbor, MI). Colour intensity was measured at 405 nm using a Bio-Rad 550 microplate reader and the levels of PGE2 were expressed as ng/g wet tissue.

**Results**

**Expression of COX-1 and COX-2**

Figure 1a (upper panel) shows a representative Northern blot of COX-1 mRNA isolated from the mammary glands of rats fed diets containing different levels of menhaden oil or safflower oil for 3 weeks. The 2.7 kb transcripts from five normal tissues (24–26), we used RT-PCR to detect this transcript in the mammary tissues. COX-2 mRNA was not changed in groups fed low fat menhaden oil (LFM) or high fat menhaden (HFM). Groups fed safflower oil at either level, however, had small, but significant increases in COX-1 expression (−35%; P < 0.05) when compared to either the low or high fat menhaden oil group.

Since we were unable to detect COX-2 mRNA by Northern analysis due to its absence or low abundance in normal tissues (24–26), we used RT-PCR to detect this transcript in the mammary tissues. COX-2 mRNA was
**Effect of PUFAs on mammary gland cyclooxygenases**

**Fig. 1.** Expression of COX-1, COX-2 and Ha-ras genes in the mammary glands of rats fed diets containing different levels of menhaden or safflower oil. (a) Northern blot analysis of total RNA for COX-1 (upper panel) and Ha-ras (middle panel). Cohybridization with a β-actin probe (lower panel) served to control for the amount of RNA loaded per lane. (b) RT-PCR analysis of COX-2 mRNA. Single stranded cDNA was reverse transcribed from total RNA from the mammary glands and used for PCR amplification with COX-2- (upper panel) and GAPDH- (lower panel) specific primers. Lanes are: 1, LFM; 2, HFM; 3, LFS; and 4, HFS.

**Fig. 2.** Levels of COX-1 mRNA measured by Northern blot analysis and quantified as arbitrary density units (ADU) by measuring the band intensity relative to β-actin. Data are means ± SD for five animals. Groups with different letters are significantly different from each other \( P < 0.05 \) using Student’s \( t \)-test.

**Table II.** The effect of different levels of menhaden or safflower oil on COX activity, and on the corresponding levels of PGE\(_2\) in rat mammary glands

<table>
<thead>
<tr>
<th>Group</th>
<th>COX activity(^b) (pmol prostanoids/mg/min)</th>
<th>( P^c )</th>
<th>PGE(_2)^b (ng/g wet tissue)</th>
<th>( P^c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFM</td>
<td>( 65 \pm 4.6 )</td>
<td></td>
<td>( 13.3 \pm 1.1 )</td>
<td></td>
</tr>
<tr>
<td>HFM</td>
<td>( 51 \pm 5.0 )</td>
<td>0.01 v. LFM</td>
<td>( 8.5 \pm 1.0 )</td>
<td>0.01 v. LFM</td>
</tr>
<tr>
<td>LFS</td>
<td>( 69 \pm 2.9 )</td>
<td></td>
<td>( 15.5 \pm 2.1 )</td>
<td></td>
</tr>
<tr>
<td>HFS</td>
<td>( 76 \pm 3.1 )</td>
<td>0.01 v. HFM</td>
<td>( 18.3 \pm 1.0 )</td>
<td>0.03 v. HFM</td>
</tr>
</tbody>
</table>

\( ^a \)For diet compositions see Table I.

\( ^b \)Values represent mean ± S.E.M. of five animals per group.

\( ^c \)Statistically significant differences were determined using Student’s \( t \)-test.

Undetectable in the mammary glands of the groups fed either level of menhaden oil, but the gene was clearly expressed in groups fed safflower oil (Figure 1b). Figure 1b is a representative blot of COX-2 transcripts from one animal per dietary group, but the same pattern of expression was obtained in four sets of samples.

**COX enzymatic activity and levels of PGE\(_2\)**

COX enzymatic activities were analysed by measuring the conversion rate of \([1-\text{C}]\text{AA}\) to prostanoids using a previously published radio-TLC technique (21,22). As shown in Table II, animals fed HFM had significantly lower COX activity in the mammary glands than those fed LFM. The group fed HFS had a significantly higher level of COX activity than that fed HFM.

We next measured the concentration of mammary gland PGE\(_2\) by enzyme immunoassay (Table II). There was generally a good correlation between changes in COX activity and the concentration of PGE\(_2\) in the corresponding groups \( (r = 0.64, \ P < 0.05) \).

**Levels of Ha-ras mRNA and p21\(^{ras}\) protein**

Figure 1a (middle panel) shows a representative Northern blot of Ha-ras mRNA from mammary glands of rats fed the four diets. The 1.4 kb transcripts from five animals per group from each group were quantified in ADU by measuring band intensities relative to the intensity of the corresponding β-actin bands. As shown in Figure 3b, Ha-ras mRNA only showed a significant increase in animals fed HFS (~35%, \( P < 0.05 \)). Levels of immunodetectable p21\(^{ras}\) were higher in the animals fed both levels of safflower oil compared to those fed menhaden oil as shown in the Western blot in Figure 3a.

**Discussion**

In an effort to elucidate the mechanisms by which dietary fat modulates carcinogenesis in rat mammary glands, we have examined the effect of diets rich in n-3 and n-6 PUFAs on the expression of the COX-1 and COX-2 genes and the Ha-ras proto-oncogene. The experimental diets were based on the AIN-93G standard reference diet (17). The 7% soybean oil in the AIN-93G diet was replaced by 1% soybean oil (to ensure adequate amounts of LA) plus either 6% menhaden oil or safflower oil in the low fat diets, or 3% soybean oil plus either 18% menhaden oil or safflower oil in the high fat diets (Table I). In the high fat diets, the oils were added at the expense of carbohydrates such that the caloric density of the high fat diet was 416 kcal/100 g compared to 360 kcal/100 g for the low fat diet. Menhaden oil and safflower oil are rich...
The blot is representative of three observations. (a) Expression of p21ras protein measured by Western blot analysis using a pan-reactive anti-ras monoclonal antibody. Lanes are: 1, LFM; 2, HFM; 3, LFS; and 4, HFS. The blot is representative of three observations. (b) Levels of Ha-ras mRNA measured by Northern blot analysis and quantified as arbitrary density units (ADU) by measuring band intensities relative to β-actin. Data are means ± SD for five animals. Groups with different letters are significantly different from each other (P < 0.05) using Student’s t-test.

Fig. 3. Expression of the Ha-ras gene in mammary glands of rats fed diets containing different levels of menhaden or safflower oil. (a) Expression of p21ras protein measured by Western blot analysis using a pan-reactive anti-ras monoclonal antibody. Lanes are: 1, LFM; 2, HFM; 3, LFS; and 4, HFS. The blot is representative of three observations. (b) Levels of Ha-ras mRNA measured by Northern blot analysis and quantified as arbitrary density units (ADU) by measuring band intensities relative to β-actin. Data are means ± SD for five animals. Groups with different letters are significantly different from each other (P < 0.05) using Student’s t-test.

suggestions that elevated levels of PGE2 are produced by increases in COX-2 expression activity or PGE2 levels. The discrepancy between COX-2 mRNA levels and COX enzymatic activity in the high fat and low fat groups is likely to be due to differences in the stability of the mRNA. It is known that COX-2 mRNA is highly unstable (35), but its stability can be increased by factors known to increase PG synthesis such as interleukin (IL)-1β (36), IL-1β (37) and transforming growth factor-β (28). Furthermore, several studies have shown elevated accumulation, increased stability and accelerated post-transcriptional processing of various genes expressed in the mammary glands of animals fed high fat compared to low fat diets (38–41). We observed significant reductions in both COX activity and PGE2 levels in animals fed HFM compared to LFM. This is likely to be because EPA and DHA are not oxygenated as rapidly as AA and act as competitive inhibitors of COX endoperoxide activity (42). Our results agree with those of Karmali et al. (8) who reported that dietary n-3 PUFAs were associated with lower levels of PGE2 in normal and cancerous mammary glands from DMBA-treated rats compared to dietary n-6 PUFAs.

PG biosynthesis, particularly series 2, can stimulate cell proliferation and suppress the immune response and may thereby promote cancer development (43). Overproduction of PGs has been reported to occur in human breast cancers as well as in rat mammary adenocarcinomas (7–9), and has been suggested to play a role in mammary carcinogenesis (32). Recently, Tsubi and DuBois (44) examined the possible role of COX-2 in carcinogenesis using stably transfected rat intestinal epithelial cells overexpressing this gene. Interestingly, these cells had elevated Bcl2 protein expression, reduced transforming growth factor-β2 receptor levels and were resistant to apoptosis. Furthermore, the cells had elevated E-cadherin levels and increased adhesion to extracellular matrix proteins. Both phenotypic changes were reversed by sulindac sulphone, a COX inhibitor. As suggested by the authors, the phenotypic changes induced in these epithelial cells by COX-2 over expression could enhance their tumourigenic potential. In a similar manner, COX-2 induction by n-6 PUFAs that we observed in the mammary gland, might lead to enhanced persistence of preneoplastic cells.

In contrast to the effects of n-6 PUFAs, n-3 PUFAs have been shown to inhibit the growth of human breast cancer cells (45,46). These effects were attributed to the production of series 3 PGs, rather than series 2 and also to the ability of metabolites of the n-3 PUFAs to inhibit the metabolism of n-6 PUFAs at various stages in the AA cascade. The findings of the present study suggest that the inhibitory effect of n-3 PUFAs on mammary carcinogenesis might be due to their modulating effects on COX enzymatic activity.

Our results suggest, furthermore, that the tumour promoting activity of n-6 PUFAs may be related to their ability to increase levels of p21ras. The increases we observed in p21ras in the LFS and HFS groups compared to those fed LFM or HFM are in general agreement with those of Karmali et al. (8) who reported higher levels of p21ras in mammary tumours from DMBA-treated Sprague–Dawley rats fed corn oil compared to those fed fish oil. In their study, p21ras levels were not different ras harbouring preneoplastic hyperplastic alveolar nodules after treatment with n-6 PUFAs and decreased p21ras levels in these cultures treated with n-3 PUFAs.
The Ha-ras gene is commonly mutated in rat mammary adenocarcinomas and is over expressed in many human breast cancers (15,19,48). In the present study, however, increases in p21ras in the LFS and HFS groups were only accompanied by a significant increase in Ha-ras mRNA in the HFS group. This may be due to the involvement of the Ki- or N-ras genes or to the complexities of the post-translational modification of p21ras (49,50).

The correlation we have observed between dietary fatty acids and p21ras and PG levels in the mammary gland suggests a mechanistic association. Indeed, phospholipase A2 (PLA2) has been shown to be stimulated by activated ras (50). PLA2-mediated phospholipid hydrolysis can release arachidonic acid that can subsequently be converted into PGs. This suggests that ras is a primary control point for PG synthesis. In support of this suggestion, mammary epithelial cells transfected with the Ha-ras gene exhibit enhanced transcription of COX-2 and increased production of PGE2 (51). Moreover, factors that alter ras farnesylation can modulate the expression of the COX-2 gene (52).

In summary, we have shown that dietary n-6 PUFAs up-regulate COX-2 and, to some extent, COX-1 expression. There was a concomitant increase in COX enzyme activity and PG synthesis in the mammary glands of rats fed high levels of n-6 PUFAs. Together with associated changes in p21ras expression, these results may explain, at least in part, the promoting effects of dietary n-6 PUFAs on mammary carcinogenesis.

Acknowledgements

This investigation was supported by a grant from the Canadian Breast Cancer Research Initiative. MCA is the recipient of a Natural Sciences and Engineering Research Council of Canada (NSERC) Industrial research Chair and acknowledges support from the member companies of the Program in Food Safety (University of Toronto). LLS is the recipient of a National Institute of Nutrition (Canada) Postdoctoral Fellowship. AE-S is the recipient of an NSERC Graduate Student Scholarship.

References


Received on July 9, 1997; revised on November 7, 1997; accepted on December 12, 1997