Dietary (n-6) PUFA and Intestinal Tumorigenesis¹,²

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ABSTRACT Cancer is the second leading cause of death in the United States, and mortality due to colorectal cancer is only surpassed by lung cancer. Epidemiological studies demonstrate that dietary polyunsaturated fats can have a profound effect on colorectal cancer risk. Experimental data indicate that modulation of cellular (n-6) PUFA metabolism can affect the progression of the disease. This paper discusses the role (n-6) PUFA play in promoting intestinal tumorigenesis and how dietary PUFA from different families interact to modify the neoplastic process. Dietary PUFA that attenuate arachidonic acid metabolism [such as (n-3) PUFA] have antineoplastic properties, whereas those that augment arachidonic acid metabolism, such as linoleic, γ-linolenic, and arachidonic acids do not appear to enhance tumorigenesis when added to the Western diet but may diminish the beneficial effects of other dietary lipids. It is the relative contributions of the different dietary PUFA that may determine overall risk for and progression of the disease. J. Nutr. 134: 3421S–3426S, 2004.

KEY WORDS: • (n-6) PUFA • (n-3) PUFA • PGE₂ • arachidonic acid • linoleic acid • eicosapentaenoic acid • ApcMin⁻/⁻ mice

Colorectal cancer is the second leading cause of cancer death in the United States (1). The risk of colorectal cancer varies from country to country, and environmental factors such as dietary fat intake are related to the mortality statistics (2,3). Earlier studies focused on the relationships between risk and total fat and saturated fatty acids in the diet, but recently dietary highly unsaturated fatty acids (HUFA; in particular, those derived from fish oils)⁴ have received increasing attention (4). However, little attention has focused on HUFA from the (n-6) family. In particular, this paper explores the (n-6) HUFA arachidonic acid as both a dietary constituent and as a metabolic intermediate in intestinal tumorigenesis.

PUFA is a broad term for fatty acids with two or more double bonds, and HUFA are a subset of PUFA with four or more double bonds. There are two major families of dietary PUFA, the (n-6) and the (n-3) families. The (n-6) PUFA are derived from the parent compound linoleic acid [LA; 18:2(n-6)]. They are fatty acids containing at least two double bonds where the first double bond is 6 carbons from the methyl end of the molecule. After consumption, LA can be oxidized, stored in triacylglycerides, incorporated into membrane phospholipids, or elongated and desaturated to more unsaturated fatty acids (Fig. 1). During this latter process, LA is first converted to γ-linolenic acid [GLA; 18:3(n-6)] via the Δ-6 desaturase, the rate-limiting enzyme in this metabolic pathway, and then is rapidly elongated to dihomo-γ-linolenic acid [DGLA; 20:3(n-6)] with the addition of 2 carbons. DGLA is subsequently desaturated to arachidonic acid [AA; 20:4(n-6)] via the Δ-5 desaturase. AA is arguably the most important cell-signaling PUFA associated with membrane phospholipids. It has been reported that LA intakes of >2.5% of energy do not result in an appreciable increase of AA in human neutrophil phospholipids (5); thus, under typical intakes (~5–6% of energy), further conversion of LA to AA appears to be minimal.

LA is the most abundant PUFA in the Western diet, with median intakes of 12 and 17 g/d for women and men, respectively (6). Major dietary sources include vegetables; vegetable oils; and animal products, in particular, eggs and meats. The level of GLA in the diet is low and found primarily in specialty oils such as borage oil and evening primrose oil. AA is found exclusively in animal products, that is, meats and eggs, with daily intakes of ~170 mg/d; however, the accuracy of this number is questionable because of the unreliability of food

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⁴ Abbreviations used: AA, arachidonic acid; ALA, α-linolenic acid; COX, cyclooxygenase; DHA, docosahexaenoic acid; DGLA, dihomo-γ-linolenic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; HUFA, highly unsaturated fatty acid; LA, linoleic acid; NSAID, nonsteroidal anti-inflammatory drug; Min, ApcMin⁻/⁻ mouse model; PG, prostaglandin; SDA, stearidonic acid.

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composition databases for HUFAs (those PUFA that contain 4 or more double bonds) (7).

The (n-3) PUFA have at least three double bonds, where the first double bond is 3 carbons from the methyl end of the molecule; α-linolenic acid [ALA; 18:3(n-3)] is the parent compound for the (n-3) PUFA family. ALA is converted to stearidonic acid [SDA; 18:4(n-3)] via the Δ-6 desaturase and is subsequently elongated and desaturated to eicosapentaenoic acid [EPA; 20:5(n-3)]. When SDA is consumed, it is rapidly converted to EPA, because it enters the metabolic pathway after the rate-limiting step controlled by Δ-6 desaturase (8,9). Through a series of elongases, action by Δ-6 desaturase, and peroxisomal β-oxidation, EPA is converted to docosahexaenoic acid [DHA; 22:6(n-3)].

The daily median intake for ALA is estimated to be 1.1 and 1.6 g/d for women and men, respectively (6), and the major dietary sources are vegetable oils, i.e., canola and soybean oils. The level of SDA in the diet is low, and SDA is found primarily in fish oil and some specialty oils such as echium (10) and black currant (11,12) oils. EPA and DHA are commonly referred to as the fish oil (n-3) PUFA. Typical intake for a combination of these fatty acids is estimated to be approximately 200 mg/d, but again, there is uncertainty in this number, and it does not take into account the amounts of docosapentaenoic acid [22:5(n-3)] in the diet (7). As observed with the conversion of LA to AA, ALA appears to be poorly converted to DHA when ALA intakes are increased above current levels (13,14).

The (n-3) and (n-6) fatty acids are not metabolically interconvertible but share the same enzymes when elongated and desaturated. As such, the consumption of one family of fatty acids can attenuate the metabolism of the other. Therefore, the competition between these two families of fatty acids helps to define their relationship with cancers, including colorectal cancer.

Murine model for intestinal tumorigenesis

To investigate intestinal tumorigenesis, we conducted a number of studies using the ApcMin/− (Min) mouse model.

This model has a germ-line mutation in the tumor suppressor gene Apc (15). The animals are heterozygous for the mutation, and spontaneous loss of heterozygosity results in the formation of tumors throughout the large and small intestines. Most human colorectal cancers occur spontaneously (i.e., sporadic cancers), and, in most, a mutation in APC is one of the earlier events linked to the process. A relatively small number of individuals are predisposed to the disease as a result of a germ-line mutation in one allele. These individuals, familial adenomatous polyposis patients, develop hundreds to thousands of tumors throughout the intestinal tract after loss of the wild-type allele. Min mice recapitulate this human condition and are considered a good model for studying the effects of diet on intestinal neoplasia.

All studies discussed in this paper were conducted with diets that contained LA at human-equivalent doses based on percentage of energy to better mimic human intake patterns. When individual fatty acids were introduced into the experimental diets, they were done so at the expense of oleic acid, because oleic acid was neutral for all end points of interest; therefore if any changes occurred, they could only be attributed to the substituted fatty acid. In addition, direct substitution maintained energy balance in all diets.

The link between AA metabolism and colorectal cancer

In 1991, Thun (16) reported that the risk for colorectal cancer was reduced by as much as 50% in individuals who took the nonsteroidal anti-inflammatory drug (NSAID) aspirin regularly. Subsequent studies demonstrated similar effects with a variety of NSAIDs. The common link among these studies was the fact that NSAIDs inhibited AA metabolism and prostaglandin (PG) formation.

After the release of AA from membrane phospholipids by a variety of phospholipases, it can be enzymatically oxidized to PGG2 and PGH2, the parent compounds of PGs, by cyclooxygenase (COX). Formation of PGH2 is the committed step in the biosynthetic pathway. There are two COX isoforms, COX-1 and COX-2. COX-1 is constitutively expressed at low...
levels in most tissues and is thought to have cellular housekeeping functions. COX-2 is not normally expressed in most tissues (with a few exceptions) and is considered to be the inducible form of the enzyme. Its expression is upregulated in leukocytes, fibroblasts, and epithelial cells in response to cytokines, growth factors, mitogens, and tumor promoters. COX-2 has been detected in virtually all cancers, and its expression is variable and dependent on the stage of the neoplasia (17). Induction of COX-2 appears to be important in maintaining tumor integrity, promoting angiogenesis, and contributing to the metastatic process (18–22).

AA is a preferential substrate for PG biosynthesis. Two PGs in particular have been linked to colorectal cancer, PGE2 and PGI2. Expression of COX-2 in nonneoplastic stroma (macrophages and fibroblasts) of benign adenomas and malignant epithelium of carcinomas is attributed with driving the production of excess PGs. When PGs are produced, they act locally on cell-surface receptors in a paracrine or autocrine manner to mediate their pathologic effects (19). PGE2 mediates tumorigenesis via 4 G-protein coupled receptors (EP 1–4) that modify intracellular calcium or cAMP (23). Cytoplasmic fluxes in either cAMP or calcium can have broad downstream effects on colorectal neoplasia; however, the precise molecular mechanisms have yet to be clarified. PGI2 appears to mediate its protumorigenic effects via the activation of peroxisomal proliferator-activated receptor-γ (24), which is upregulated in colorectal tumors, promoting tumorigenesis and inhibiting apoptosis (24–26). Therefore, the neoplastic process involves COX-2 metabolites and integrated signaling among the stroma, tumor epithelial cells, and the vascular endothelium (18,22).

The level of AA in tissues and its conversion to PGs is influenced by the type and the level of dietary fat; however, establishing a definitive teleological link between diet, the AA cascade, and colorectal cancer has been equivocal in vivo. This is important because dietary PUFA, such as those from fish oils, can decrease this pathway similarly to NSAIDs (i.e., inhibition of PG biosynthesis), but unlike NSAIDs, some dietary PUFA (i.e., AA) can also augment (n-6) metabolism and PG formation. Therefore, based on epidemiological studies in humans and our previous work with NSAIDs in Min mice (27–29), we explored the relation of AA with intestinal tumorigenesis as a dietary constituent and metabolic intermediate in the disease process.

\[\text{AA} \rightarrow \text{COX-2} \rightarrow \text{PGs} \rightarrow \text{Tumors} \]

**FIGURE 2** The AA cascade, from LA to PGs. Overview of experiments evaluating the effect on tumorigenesis by modifying this pathway. For example, from a dietary perspective this pathway can be influenced by LA, ALA, and AA, with and without concomitant use of inhibitors. SC26196 inhibits Δ-6 desaturase and concomitant addition of dietary AA bypasses the inhibition. Similarly, NSAIDs inhibit PGE2 biosynthesis, and concomitant addition of PGE2 analogues bypass the inhibitions.

\[\text{Dietary LA} \rightarrow \text{SC26196} \rightarrow \text{NSAIDs} \rightarrow \text{Antibody against PGE2} \]

(n-6) PUFA and intestinal tumorigenesis

A series of studies were designed whereby the AA cascade was inhibited and enhanced at multiple points to establish a direct association with this metabolic pathway and intestinal neoplasia (Fig. 2). The biosynthetic pathway of AA is controlled by Δ-6 desaturase. Selective inhibition of Δ-6 desaturase with SC26196 reduced tumor number by ∼40%, but these results were reversed when the inhibition was bypassed with the concomitant addition of dietary AA (30). These results suggest that the biosynthetic pathway for AA from its precursors is important in supporting tumorigenesis.

Similarly, inhibiting the conversion of AA to PG with a variety of NSAIDs reduced tumor number by as much as 95% (27,29), and bypassing the inhibitions with stable analogues of PGE2 attenuated these responses (23). Furthermore, there was a ∼35% reduction in tumor number in Min mice (with preexisting tumors) after 4 d of treatment with a monoclonal antibody against PGE2 (23). Collectively, these data demonstrate that inhibiting any step in AA metabolism, from LA through PGs, has deleterious effects on intestinal tumorigen...
Dietary PUFA, AA metabolism, and intestinal neoplasia

The studies with selective inhibitors of (n-6) PUFA metabolism, coupled with bypass experiments, have important dietary implications. For example, our previous studies demonstrated that attenuating (n-6) PUFA metabolism reduced tumorigenesis, but augmenting (n-6) PUFA metabolism with GLA and AA failed to modify tumor number above control levels, even though AA metabolism was enhanced. However, as for the inhibitor studies, (n-6) PUFA metabolism can be attenuated with dietary (n-3) HUFAs. When Min mice were fed diets containing (n-3) HUFAs (i.e., EPA), tumor number and tissue levels of AA and PGs were reduced by as much as 50% (8); when AA was concomitantly added to an EPA-containing diet, tumor number did not change from control levels, despite the presence of EPA. These results are likely due to the fact that the addition of AA to the EPA-containing diet normalized tissue AA content and PG levels (Fig. 4) (31). These data demonstrate that dietary (n-3) HUFAs have beneficial effects as long as AA is not present in the diet, a condition that only exists in the diet of strict vegans (individuals who consume no meat, fish, or eggs).

Are all dietary (n-6) PUFA the same?

AA and its metabolism are important in the tumorigenic process in humans, but it is still unclear whether dietary LA, at current intakes, is an important factor because of the following data. The U.S. diet already contains LA at ~15 g/d (or ~5% of energy). LA is found in virtually all staple foods of the diet that contain fats: meat, dairy, eggs, fish, vegetable, dietary oils, etc. As such, it is an important essential nutrient in all typically healthy dietary groups—vegans, lacto-ovo vegetarians, and omnivores. Thus, it would be difficult to avoid or to

TABLE 2
Fatty acid composition of plasma phospholipids in individuals consuming low fat diets containing various levels of linoleic acid, arachidonic acid and (n-3) HUFA

<table>
<thead>
<tr>
<th>Diet</th>
<th>Dietary LA g/d</th>
<th>Dietary AA g/d</th>
<th>Dietary (n-3) g/d</th>
<th>Day 0: start of diet</th>
<th>Day 14: end of experiment</th>
<th>% of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetarian (n = 10)</td>
<td>LA: 3.1 g</td>
<td>AA: 0.9 g</td>
<td>0 g (n-3): 0 g</td>
<td>Day 0</td>
<td>25.7 ± 0.7</td>
<td>9.2 ± 0.6</td>
</tr>
<tr>
<td>Southern fish (n = 10)</td>
<td>LA: 2.6 g</td>
<td>AA: 0.14 g</td>
<td>0.11 g (n-3): 1.33 g</td>
<td>Day 0</td>
<td>25.7 ± 1.0</td>
<td>10.8 ± 0.8</td>
</tr>
<tr>
<td>Tropical fish (n = 9)</td>
<td>LA: 2.4 g</td>
<td>AA: 0.56 g</td>
<td>0.90 g (n-3): 0.62 g</td>
<td>Day 0</td>
<td>25.7 ± 1.2</td>
<td>9.8 ± 0.7</td>
</tr>
<tr>
<td>Kangaroo (n = 8)</td>
<td>LA: 3.6 g</td>
<td>AA: 0.49 g</td>
<td>0.21 g (n-3): 2.33 g</td>
<td>Day 0</td>
<td>26.4 ± 0.7</td>
<td>10.8 ± 0.8</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. Statistical comparisons relative to the value above it.
2 P < 0.05.
3 P < 0.01.
4 P < 0.001 [derived from Sinclair et al. (32), with permission].
dramatically reduce LA intake. Even so, the conversion of LA to AA in humans may not modify tissue AA levels at dietary levels above 2.5% of energy (5); therefore moderate changes in current levels of LA consumption would not be expected to modify AA levels or its metabolism (5).

Dietary AA and (n-3) HUFAs, however, have profound effects on tissue AA and its metabolism (32,33), where adding (n-3) HUFAs to the diet would have a greater effect on AA metabolism than would reducing LA intakes. All omnivorous diets contain AA and (n-3) HUFAs, because they are found, to various degrees, in all meats (including fish) and in eggs (Table 1). Although the average daily intakes of AA and (n-3) HUFAs are approximately equal, an inequality is still present, because, unlike LA, increasing the levels of dietary AA has profound effects on tissue AA content, and (n-3) HUFAs are not very effective in preventing incorporation. For example, when individuals are provided diets containing both AA and (n-3) HUFAs, tissues are enriched with AA, despite the presence of (n-3) HUFAs (Table 2), and these changes are accompanied by an augmentation of AA metabolism (33). Furthermore, the ratio of AA to (n-3) HUFAs required for a neutral effect is unknown. Nevertheless, in isolation, increasing the consumption of (n-3) HUFAs would more likely result in a greater reduction of AA and its metabolism than would trying to reduce LA intake (34). Establishing dietary levels of (n-3) HUFAs that have predictable outcomes may have to be carefully balanced against the levels of AA in the diet.

Summary and conclusions

In summary, dietary AA potently enriches phospholipid AA content in virtually every tissue (35), and these levels are positively correlated with tumor number ($P < 0.025$). Tissue AA levels are also positively correlated with PG levels, and PG levels are positively correlated with tumor number (Table 3) (8,31). If AA is important in the tumorigenic process in humans, it is still unclear whether modifying dietary LA levels has an effect on AA-driven end points. In our model, adding GLA and AA to a diet already adequately supplied with LA had no additional effects on tumorigenesis (4), suggesting that a threshold exists for the tumorigenic effect of AA. This can be seen when intestinal phospholipid AA content is correlated with the average tumor number in Min mice fed diets containing different PUFA (Fig. 5).

More research is required to delineate the differences among the dietary (n-6) PUFA (i.e., AA vs. LA) with regard to their tumorigenic potential and their ability to antagonize beneficial PUFA, such as those from fish oils.

**Table 3**

<table>
<thead>
<tr>
<th>Correlation between tissue prostaglandin formation and AA levels and tumor number</th>
<th>Correlation coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue AA vs. prostaglandins</td>
<td>PGE$_2$</td>
<td>0.71</td>
</tr>
<tr>
<td>6-keto-PGF$_{1\alpha}$</td>
<td>0.73</td>
<td>0.0004</td>
</tr>
<tr>
<td>Prostaglandins vs. tumor number</td>
<td>PGE$_2$</td>
<td>0.27</td>
</tr>
<tr>
<td>6-keto-PGF$_{1\alpha}$</td>
<td>0.41</td>
<td>0.01</td>
</tr>
</tbody>
</table>

1Data derived from (8) and (31).

**FIGURE 5** Relationship between intestinal phospholipid AA levels and tumor number in Apc$^{Min^+1}$ mice.

**LITERATURE CITED**

20. Tsuji, M., Kawanoe, S., Tsuji, S., Sawaoka, H., Hori, M. & Dubois, R. N.


