Dietary n-3 Polyunsaturated Fatty Acids Enhance Hormone Ablation Therapy in Androgen-Dependent Prostate Cancer

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Hormone ablation therapy typically causes regression of prostate cancer and represents an important means of treating this disease, particularly after metastasis. However, hormone therapy inevitably loses its effectiveness as tumors become androgen-independent, and this conversion often leads to death because of subsequent poor responses to other forms of treatment. Because environmental factors, such as diet, have been strongly linked to prostate cancer, we examined the affects of dietary polyunsaturated fatty acids (PUFAs; at 1.5 wt%) on growth of androgen-dependent (CWR22) and androgen-independent (CWR22R) human prostatic cancer xenografts, the acute response of CWR22 tumors to ablation therapy, and their progression to androgen independence. Significant diet-induced changes in tumor n-3 or n-6 PUFA content had no affect on CWR22 or CWR22R tumors growing with or without androgen support, respectively. However, dietary changes that increased tumor eicosapentaenoic acid and linoleic acid content enhanced responses to ablation therapy, measured by cancer cell apoptosis and mitosis. In addition, relapse to androgen-independent growth (measured by renewed increases in tumor volume and serum prostate-specific antigen after ablation) positively correlated with tumor arachidonic acid content. There was no correlation between expression of 15-lipoxygenase isozymes or their products and tumor growth or responses to ablation. In conclusion, dietary n-3 PUFA may enhance the response of prostate cancer to ablation therapy and retard progression to androgen-independent growth by altering tumor PUFA content. (Am J Pathol 2008, 173:229–241; DOI: 10.2353/ajpath.2008.070989)

It is estimated that in 2007 prostate cancer in the United States will be the second most common cause of cancer-related mortality in men and that more than 218,000 new cases will be diagnosed, far more than any other form of cancer.1 Androgen ablation therapy remains the most effective means of treating metastatic tumors and is often used with localized lesions that have a more aggressive phenotype. Prostate cancer can be divided into four clinically important phases based on disease progression and response to therapy:

1) Androgen-dependent (AD) growth. Prostatic cancer initially requires the androgen hormone testosterone for development and growth.2 Metastasis commonly occurs during this phase of the disease, before detection and what might be otherwise definitive treatment by surgery or radiation.

2) Regression caused by androgen-withdrawal therapy. Androgen ablation therapy has been the mainstay of therapy for prostate cancer since the link between testosterone and prostate cancer was recognized decades ago and remains the most effective means of treating metastatic disease today.2,3 It is unlikely that many individuals die from prostate cancer without the benefit of androgen ablation therapy, at least as a palliative measure.

3) Progression to androgen-independence. Essentially all prostate cancers in individuals treated by ablation therapy will eventually develop the capacity to thrive in...
4) **Androgen-independent (AID) growth.** Because prostate cancer responds very poorly to current chemotherapeutic treatments, AID tumors represent the final phase of the disease. In 2007 prostate cancer may cause more than 27,000 deaths in the United States alone.1

Environmental factors contribute substantially to prostate carcinogenesis and a number of epidemiological studies have focused on diet in this regard.2,4 Asian and northern cultures with diets rich in marine oils and fish have less mortality from prostate cancer than western cultures where animal and land-based fats are consumed at higher levels. Additionally, migration of men from Asia to the United States is associated with an increase in prostate cancer to approximately that found in their adopted western culture, a strong evidence for an environmental influence on this disease. Although not all epidemiological studies have supported a relationship between dietary polyunsaturated fatty acids (PUFAs) and prostate carcinogenesis, laboratory studies indicate that higher levels of n-6 (animal and land-based) PUFAs generally drive prostate cancer growth whereas fish oils [n-3 long chain (LC) PUFA] are protective.5–9

Arachidonic acid (AA, 20:4 n-6) is arguably the most biologically important n-6 PUFA of cell membrane phospholipids. Fatty acids released from cell membranes by phospholipase directly effect cell signaling pathways and serve as a substrate for the cyclooxygenase (COX) and lipoxygenase (LOX) enzymatic pathways to produce bioactive eicosanoids such as prostaglandin (PG) E2. Eicosanoids act as ligands for nuclear and cytoplasmic membrane receptors to effect physiological and pathophysiological cell behavior through secondary messenger systems. Tissue levels of AA increase directly from consumption of animal meat and are maintained at basal levels by regulated conversion of linoleic acid (LA, 18:2 n-6), an essential n-6 fatty acid common in corn oil and itself a substrate for production of eicosanoids important in prostate cancer cell growth.10–13 Increasing dietary LA above that obtained in a typical Western diet (or mouse chow) has no significant affect on tissue AA levels.13 Eicosapentaenoic acid (EPA, 20:5 n-3) is a n-3 LC PUFA that can be increased in membrane phospholipids by consumption of fish oil or its n-3 LC PUFA precursor stearidonic acid (SDA, 18:4 n-3), which is common only in atypical oils (ie, echium, blackcurrent) and genetically designed canola oil.14 EPA replaces AA in membrane phospholipids and competes for metabolic pathways resulting in significantly lower levels of AA in all tissues and therefore reduced production of 2-series eicosanoids.15–19

A number of studies have linked PUFA metabolism to prostatic epithelial cell biology and tumor growth. For instance, AA stimulates proliferation and inhibits apoptosis of prostatic cancer cells through the 5-LOX product 5-hydroxyeicosatetraenoic acid (HETE), and promotes neovascularization and proliferation through the production of 12-HETE by 12-LOX.20,21 Metabolism of LA by 15-LOX-1 to form 13-hydroxyoctadecadienoic acid (HODE) increases with prostate cancer whereas 15-LOX-2 and its AA metabolic product, 15-HETE, are reported to be higher in normal prostatic tissue and may inhibit carcinogenesis.12,22,23 PUFA can also directly effect cell signaling pathways relevant to prostatic carcinogenesis.24,25 Although these and many other studies have clearly demonstrated that PUFAs and their bioactive metabolites can modulate prostate neoplasia, the majority of laboratory data supporting the antithetic relationship between n-6 and n-3 PUFAs in prostatic cancer is based on human prostatic cell lines that do not require androgen for sustained growth (advanced stage cancer) or rodent tumors with questionable relevance to the human disease. The effect of dietary manipulation on experimental prostate cancer is complex and outcomes are likely based on a variety of competing factors. Experimental design is critical in elucidating the relationship between dietary factors and disease end points in experimental models and, of particular importance, extrapolation of these relationships to humans. There have been no studies to date that directly examine the effects of dietary PUFAs on early phases of human prostatic cancer, in particular the dynamic response of AD tumors to hormone ablation therapy.

Because the genetic mutational basis of prostatic cancer is still being defined, use of actual human prostatic cancer cells for experiments related to tumor growth and progression may still be more informative than animal models generated by chemical or genetic manipulation. There are very few human prostatic cancer cell lines that are truly AD.26 The CWR22 xenograft model has been well characterized in this regard and responds to androgen withdrawal in the same manner as AD tumors in men, with increased apoptosis, reduced cell proliferation, and tumor regression accompanied by decreasing levels of circulating prostate-specific antigen (PSA).27 These xenografts also progress (relapse) to AID growth (CWR22R) when subjected to the same selective forces (hormone ablation) that cause this change in men with the disease.28,29 CWR22 has the simplest reported karyotype of all isolates and the profile of gene expression in CWR22 and CWR22R tumors is representative of that seen in clinical samples, further validating this model system.29

We have used the CWR22 model system to show that dietary AA and EPA, fed at levels relevant to the upper range of human consumption, significantly affect the PUFA content of tumor membrane phospholipids, the production of bioactive eicosanoids, and the progression of prostate cancer to AID growth, which might translate into a clinically significant adjuvant affect in hormonal treatment of this disease.

**Materials and Methods**

**Prostate Cancer Xenografts**

CWR22 and CWR22R cells were generously provided by Dr. Thomas Pretlow (Case Western Reserve University, Cleveland, OH) and maintained as previously de-
scribed.27,30 The AID CWR22R (relapse) line was developed directly from AD CWR22 tumors that started growing again after castration of host mice. Xenograft tumors were propagated in 8-week-old castrated adult male athymic nude (nu/nu) mice (Harlan, Indianapolis, IN) housed in sterile microisolator cages in a positive pressure Hepa filter containment unit and fed a standard autoclavable rodent chow diet (Harlan Teklad 8656). Tumor cells in minced tissue fragments were dissociated with pronase, counted, and 3 × 10⁶ viable cells in 200 μl of Matrigel inoculated subcutaneously over the lumbar region. Animals receiving AD CWR22 cells were implanted with subcutaneous sustained release 12.5-mg testosterone pellets (Innovative Research of America, Sarasota, FL), whereas CWR22R xenografts were grown without this testosterone supplementation. Androgen ablation was accomplished by surgical removal of testosterone pellets under isoflurane anesthesia. All animal manipulations were approved by the University of Tennessee Institutional Animal Care and Use Committee and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (1996, NRC).

**Diets**

Experimental diets were premixed and stored at −70°C under an atmosphere of N₂ as previously described.19 To the AIN-76A base diet (Dyets Inc., Bethlehem, PA) was added 1.5 wt% of the following fatty acids, oleic acid (OA; 18:1 n-9, 99% pure), AA (20:4 n-6, 99% pure) (Nu-Chek Prep, Elysian, MN), or one of two LC n-3 PUFA diets, SDA (18:4 n-3, 85% pure) (Monsanto, St. Louis, MO) or EPA (20:5 n-3, 97% pure) (Nu-Chek Prep). The supplemented fatty acids were provided in the esterified form. The OA was provided as the triglyceride, triolein, whereas AA, EPA, and SDA were provided as ethyl esters. Our previous work has demonstrated that the addition of LC n-6 or n-3 PUFA at these levels in experimental diets of mice affects significant changes in the biochemistry of the AA cascade.15–17,19,31 The monounsaturated fatty acid OA is neutral with respect to influencing tissue AA levels and 2-series eicosanoid biosynthesis and therefore was included as a control. Because of an unexpected limited availability of SDA, we were required to switch to EPA after the first AD experiment. However, SDA is an immediate precursor for EPA and is considered a pro-EPA n-3 PUFA because of its rapid conversion to EPA after consumption. Studies in experimental models, including our own, and humans demonstrate similar effects between SDA and EPA.19,32 In all experiments, the study diets were provided to the mice only after detection of subcutaneous tumor growth (~0.5 cm diameter). Fresh diet was provided daily *ad libitum*. Food consumption and body weight were monitored on a daily and weekly basis, respectively.

**Experimental Design**

**Affects on Growth of AD and AID Xenografts**

To determine whether dietary PUFAs affect short-term responses to hormone ablation AD CWR22 tumors were established, as above, and the precastrated, testosterone-supplemented mice were started on experimental diets containing OA, AA, or EPA (*n* = 11, 14, and 13, respectively). Testosterone pellets were removed after mice had been on experimental diets for 7 days. After 5 more days on experimental diets the samples were harvested for analysis. This end point was selected based on our preliminary studies (not shown) and the work of others who previously demonstrated that CWR22 xenografts respond to androgen ablation within this time frame.

For the long-term hormone ablation experiment, all animals were placed on the same experimental regimen as described for the short-term hormone ablation experiment but were maintained for up to 6 months after removal of testosterone pellets (*n* = 8 for OA dietary group, and 10 for both EPA and AA groups). Throughout this period tumor volume and serum PSA levels were monitored every 2 to 3 weeks and relapse was determined to have occurred with renewed increases in measured tumor volumes or PSA levels, at which point the individual mice were euthanized and samples harvested for analysis. Xenograft tumor growth was monitored by caliper measurements (volume = (length × width × height) × 0.5236). PSA levels were measured by enzyme-linked immunosorbent assay according to the manufacturer’s instructions (Biomedia, Foster City, CA). As in humans with prostate cancer, regression of CWR22 xenografts occurs after removal of androgen stimulation and, after an extended period, some will resume growth (relapse) independent of testosterone, modeling progression to the final clinical stages of this cancer. This experiment was designed to determine whether dietary PUFA would have an effect on time required to transition from androgen-dependent to AID growth (relapse). At the end of each experiment samples from every tumor were immediately fixed in neutral buffered formalin, submerged in RNAlater (Ambion, Austin, TX), and/or snap-frozen in liquid nitrogen and stored at −80°C.

**Phospholipid Fatty Acid Analysis**

Xenograft tissue phospholipid fatty acid analyses was performed as described.17 After extraction, isolation, and methylation, fatty acids were quantified using a Hewlett Packard 5890 series II gas chromatograph with a DB23 capillary column (0.25 mm × 30 m) (J&W Chromatography, Folsom, CA). Hydrogen was used as
the carrier gas and fatty acids were identified by comparing peaks with known standards (Nu Chek Prep), and quantified using an internal standard, 1,2-di-heptadecanoyl-sn-glycerol-3-phosphocholine (50 µg; Avanti Polar Lipids, Alabaster, AL), added to each sample before processing.

Eicosanoid Analysis

For eicosanoid analysis tumor samples were homogenized in ice-cold (4°C) 0.1 mol/L Tris-HCl buffer (pH 7.4) and an aliquot used to determine protein concentration with a modified Lowry assay. On-line liquid chromatography of extracted samples was performed with Agilent (Santa Clara, CA) 1100 Series capillary high performance liquid chromatography. Separations were achieved using a Phenomenex (Torrance, CA) Luna C18(2) column (5 µm, 150 × 2 mm), which was held at 40°C. The flow rate was 350 µL/minute. Mobile phase A was 0.1% acetic acid in water. Mobile phase B was 0.1% acetic acid in 85:15 acetonitrile:ethanol. Gradient elution was used and the mobile phase percent B was varied as follows: 15% B at 0 minutes, ramp from 0.5 to 2 minutes to 30% B, ramp from 2 to 5 minutes to 55% B, ramp from 5 to 20 minutes to 70% B, ramp from 20 to 100% B, hold from 21 to 26 minutes at 100% B, ramp from 26 to 27 minutes down to 15% B, and hold from 27 to 33 minutes. Samples were spiked with an internal standard solution to achieve a final concentration of 4 pg/µL PGF₂α-d₄, 30 pg/µL 13-HODE-d₅, and 30 pg/µL 15-HETE-d₈. Injection volume was 15 µL. Samples were analyzed in triplicate.

Electrospray ionization tandem mass spectrometry (ESI/MS/MS) was used for detection. Analyses were performed on an MDS (Mississauga, Canada) Sciex API 3000 equipped with a TurboSpray source. Turbo desolvation gas was heated to 350°C at a flow rate of 6 L/minute. All analyses were monitored as negative ions with the instrument equipped with a TurboIonSpray source. Turbo desolvation gas was heated to 350°C at a flow rate of 6 L/minute. All analyses were monitored as negative ions with the instrument equipped with a TurboIonSpray source. The optimum cycle number was determined for each gene to obtain amplification products from test samples within a linear range. Reaction products were separated by agarose gel electrophoresis with ethidium bromide and visualized under UV light. Densitometry measurements were performed with J-Image software (National Institutes of Health, Bethesda, MD) using captured images of the gels and normalization to the GAPDH internal standard.

RNA Extraction and Semiquantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Analysis

Total RNA was extracted from tumor samples stored in RNEater using TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer’s instructions, and resuspended in diethyl pyrocarbonate-treated water. Residual genomic DNA was removed by incubating RNA with DNase1 (Invitrogen), following the manufacturer’s instructions. The quality and quantity of RNA were determined by UV light spectrometry.

RNA was reverse-transcribed in a reaction containing Oligo(dT) Primer RETROscript (Ambion), dNTP mix, RNaseOUT ribonuclease inhibitor (Invitrogen), and AMV reverse transcriptase (Fisher Scientific, Pittsburgh, PA). cDNA was amplified in a 20-µL PCR reaction containing 0.5 µmol/L primers and 10 µL REDTaq ReadyMix with MgCl₂ (Sigma, St. Louis, MO). The cycles consisted of 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 1 minute. The optimum cycle number was determined for each gene to obtain amplification products from test samples within a linear range. Reaction products were separated by agarose gel electrophoresis with ethidium bromide and visualized under UV light. Densitometry measurements were performed with J-Image software (National Institutes of Health, Bethesda, MD) using captured images of the gels and normalization to the GAPDH internal standard.

Western Blot Analysis

Protein samples were obtained from tissues homogenized in RIPA buffer (50 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 1 mmol/L phenylmethyl sulfonlfy fluoride, 1 mmol/L ethylenediaminetetraacetic acid, 5 µg/ml aprotonin, 5 µg/ml leupeptin, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate). Protein was quantified with BCA protein assay kit (Pierce, Rockford, IL) and equal amounts were loaded into sodium dodecyl sulfate-polyacrylamide gel electrophoresis for electrophoresis and transferred onto a nitrocellulose membrane. The membranes were blocked with Tris-buffered saline.
and 0.05% Tween 20 (TBST) containing nonfat dry milk, washed with TBST, and incubated at 4°C overnight with primary antibody (15-LOX-2, LX 25; Oxford Biomedical Research, Oxford, MI) in nonfat milk/TBST. After washing, membranes were incubated with secondary antibody and protein bands detected with enhanced chemiluminescence Western blotting detection reagents (Amer sham, Piscataway, NJ) exposed to Blue X-Ray film (Ph enix Research Products, Hayward, CA). Membranes were then incubated in stripping buffer (62.5 mmol/L Tris-HCl, 100 mmol/L β-ME, 2% sodium dodecyl sulfate, pH 6.7) at 50°C for and reprobed with anti-actin antibody (sc-1615; Santa Cruz Biotechnology, Santa Cruz, CA) to confirm equal loading. We tested several commercially available antibodies for 15-LOX-1 but none detected this protein on our samples or positive control lysates.

**Tumor Cell Proliferation and Apoptosis**

Formalin-fixed samples were routinely processed for histology and relative levels of cell proliferation and apoptosis were determined by counting the number of mitotic figures and apoptotic bodies among 2000 to 10,000 tumor cells in hematoxylin and eosin-stained sections using established morphological features, as previously described. Apoptotic cells in xenograft tumors stained for cleaved caspase-3 by immunohistochemistry but this technique did not improve sensitivity or specificity (not shown).

**Statistics**

Statistical analysis was performed with SAS software (JMP; Cary, NC) using analysis of variance with Dunnett’s method to compare results between groups and Spearman’s ρ for correlation analysis. The Kaplan-Meier survival platform was used to analyze affects of diet progression of tumors to AID growth. Data are expressed as mean ± SEM. In all cases, a P value less than 0.05 was interpreted as significant.

**Results**

Terminal body weights (in g) of animals implanted with CWR22 or CWR22R cells were: 29.4 ± 0.5 for mice fed OA, 29.4 ± 0.6 for AA, and 29.7 ± 0.6 for those fed either SDA or EPA. Food consumption patterns were monitored for each cage (containing four to five animals per cage). Although there appeared to be no differences in food intakes among cages, it was impossible to monitor individual food intake, and thus this individual variation could not be controlled.

**Affects of Dietary PUFA on Phospholipid Fatty Acid Composition in AD and AID Tumors**

Tumor tissue samples were harvested for analysis after the mice had been on experimental diets for 14 days. In previous studies, we determined that fatty acid levels in diverse tissues reach equilibrium ~5 to 6 days after changing dietary fatty acid composition (J.W., unpublished). Addition of AA to the diet dramatically enriched AA-containing phospholipids by 66% and 67% in CWR22 (Table 1) and CWR22R (Table 2) prostate tumors, respectively, without affecting the n-3 LC PUFA content. Concomitantly, LA (18:2 n-6) levels in the tumor phospholipids significantly declined by 25 to 26% in both tumor types; displacement of LA by AA in tissue phospholipids is a hallmark of dietary AA supplementation. Thus, a critical impact of dietary AA was an increase in tissue AA content at the expense of LA, modifying the AA to LA ratio in phospholipids significantly (J.W., unpublished).

<table>
<thead>
<tr>
<th>PUFA</th>
<th>OA diet, n = 6</th>
<th>AA diet, n = 10</th>
<th>SDA diet, n = 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>17.53 ± 0.07</td>
<td>17.49 ± 0.24</td>
<td>17.89 ± 0.29</td>
</tr>
<tr>
<td>18:0</td>
<td>18.36 ± 0.14</td>
<td>20.38 ± 0.18</td>
<td>19.69 ± 0.17</td>
</tr>
<tr>
<td>18:1 n-9</td>
<td>28.51 ± 0.42</td>
<td>24.15 ± 0.40</td>
<td>24.22 ± 0.41</td>
</tr>
<tr>
<td>18:1 n-7</td>
<td>5.47 ± 0.13</td>
<td>4.51 ± 0.13</td>
<td>4.42 ± 0.13</td>
</tr>
<tr>
<td>18:2 n-6</td>
<td>9.15 ± 0.21</td>
<td>6.75 ± 0.28</td>
<td>11.63 ± 0.33</td>
</tr>
<tr>
<td>18:3 n-3</td>
<td>0.01 ± 0.03</td>
<td>ND</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>18:4 n-3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>20:3 n-6</td>
<td>2.35 ± 0.13</td>
<td>1.35 ± 0.06</td>
<td>3.03 ± 0.12</td>
</tr>
<tr>
<td>20:4 n-6</td>
<td>9.38 ± 0.30</td>
<td>15.63 ± 0.25</td>
<td>4.90 ± 0.23</td>
</tr>
<tr>
<td>20:5 n-3</td>
<td>0.02 ± 0.02</td>
<td>0.04 ± 0.02</td>
<td>2.55 ± 0.12</td>
</tr>
<tr>
<td>22:4 n-6</td>
<td>1.33 ± 0.07</td>
<td>2.91 ± 0.10</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>22:5 n-6</td>
<td>0.70 ± 0.05</td>
<td>1.16 ± 0.05</td>
<td>0.05 ± 0.00</td>
</tr>
<tr>
<td>22:6 n-3</td>
<td>ND</td>
<td>ND</td>
<td>3.97 ± 0.07</td>
</tr>
<tr>
<td>Total n-3 PUFA</td>
<td>1.50 ± 0.04</td>
<td>1.21 ± 0.056</td>
<td>3.04 ± 0.07</td>
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<tr>
<td>Total n-6 PUFA</td>
<td>22.91 ± 0.44</td>
<td>27.80 ± 0.44</td>
<td>19.86 ± 0.46</td>
</tr>
<tr>
<td>(n-3)/(n-6) ratio</td>
<td>0.07 ± 0.00</td>
<td>0.04 ± 0.00</td>
<td>0.49 ± 0.02</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>26.00 ± 0.41</td>
<td>30.05 ± 0.46</td>
<td>30.31 ± 0.44</td>
</tr>
</tbody>
</table>

ND, not detectable.
tent resulting in an AA to LA ratio of 0.4, a 5.8-fold difference compared to the AA-fed group. The n-3 LC PUFAs are potent antagonists of tissue AA content by displacing AA from tissue phospholipids and inhibiting the conversion of LA to AA, with accompanying increases in LA content. In each type of tumor (CWR22 and CWR22R), EPA and total n-3 PUFA content in phospholipids increased equivalently to 2.5% and 10% of total fatty acids, respectively. Dietary EPA and SDA both tended to increase tissue docosahexaenoic acid (DHA, 22:6 n-3), but to a lesser extent than tumor EPA content. SDA was not detected in any tumors, including those from mice fed SDA, consistent with its very rapid conversion to EPA. These results are similar to those previously reported for dietary EPA and SDA supplementation.19

Affects of Dietary PUFA on Growth of AD and AID Tumors

CWR22 and CWR22R tumors (from androgen-intact and depleted mice, respectively) in all dietary groups were obviously growing and this was reflected by a ratio of apoptosis/mitosis less than one (Figures 1 and 2). There were, however, no significant differences in tumor growth parameters between experimental groups with the single exception of a significant reduction in proliferation in the CWR22R tumors from EPA-fed mice, compared to the OA control group ($P = 0.048$, apoptosis was not significantly affected). Otherwise, apoptosis and mitosis of tumor cells was essentially the same in mice whether they were fed n-3 or n-6 PUFA, and there were no significant differences in the percent change in tumor volume throughout the experimental periods (not shown). CWR22R xenografts grew much faster than CWR22 tumors after inoculation of the same number of cells, as previously reported.35 and this was reflected in a lower ratio of apoptosis/mitosis across all dietary groups at harvest (Figures 1 and 2). The lack of a dietary PUFA effect on CWR22 cells was recapitulated, in part, during preliminary experiments when PUFA diets were introduced 1 week after inoculation of CWR22 cells, before tumor growth was visually apparent, with no differences in growth of tumor masses, cell proliferation, or apoptosis after 30 days on these experimental diets (results not shown). We conclude from these experiments that dietary enrichment of CWR22 human prostatic cancer xenografts with n-3 or n-6 PUFA has little or no significant affect on neoplastic cell growth characteristics regardless of whether they are early AD or late-stage AID tumors.

Affects of Dietary PUFA on Short-Term Response of AD CWR22 Tumors to Androgen Ablation

In these experiments, we duplicated the initial steps of the CWR22 experiments outlined above. After the full establishment of tumors, we placed the mice on experimental diets containing AA, OA, or EPA for 7 days (ensuring stable changes in fatty acid compositions), and then surgically removed the testosterone pellets (hormone ablation). As noted above, we have previously established that steady state levels of tissue phospho-

Table 2. AID CRW22R Prostate Tumor Polyunsaturated Fatty Acid Content (mol %)

<table>
<thead>
<tr>
<th>PUFA</th>
<th>OA diet, $n = 9$</th>
<th>AA diet, $n = 13$</th>
<th>EPA diet, $n = 6$</th>
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<td>16:0</td>
<td>11.70 ± 0.21</td>
<td>11.46 ± 0.39</td>
<td>11.50 ± 0.31</td>
</tr>
<tr>
<td>18:0</td>
<td>23.84 ± 0.24</td>
<td>24.44 ± 0.88</td>
<td>23.50 ± 0.43</td>
</tr>
<tr>
<td>18:1 n-9</td>
<td>26.52 ± 0.33</td>
<td>23.34 ± 0.83</td>
<td>22.22 ± 1.18</td>
</tr>
<tr>
<td>18:1 n-7</td>
<td>4.40 ± 0.11</td>
<td>3.91 ± 0.08</td>
<td>3.54 ± 0.16</td>
</tr>
<tr>
<td>19:2 n-6</td>
<td>13.77 ± 0.29</td>
<td>10.36 ± 0.38</td>
<td>16.29 ± 0.40</td>
</tr>
<tr>
<td>18:3 n-3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>20:3 n-6</td>
<td>0.70 ± 0.01</td>
<td>0.43 ± 0.02</td>
<td>0.63 ± 0.04</td>
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<tr>
<td>20:4 n-6</td>
<td>10.49 ± 0.21</td>
<td>17.52 ± 0.28</td>
<td>7.88 ± 0.61</td>
</tr>
<tr>
<td>20:5 n-3</td>
<td>ND</td>
<td>ND</td>
<td>2.46 ± 0.08</td>
</tr>
<tr>
<td>22:4 n-6</td>
<td>0.64 ± 0.04</td>
<td>1.45 ± 0.23</td>
<td>0.38 ± 0.06</td>
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<td>22:5 n-6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>22:6 n-3</td>
<td>3.23 ± 0.07</td>
<td>3.53 ± 0.68</td>
<td>4.14 ± 0.34</td>
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<td>Total n-3 PUFA</td>
<td>3.23 ± 0.07</td>
<td>3.53 ± 0.68</td>
<td>9.90 ± 0.59</td>
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<td>Total n-6 PUFA</td>
<td>25.60 ± 0.32</td>
<td>29.76 ± 0.66</td>
<td>25.18 ± 1.03</td>
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<td>(n-3)/(n-6) ratio</td>
<td>0.13 ± 0.00</td>
<td>0.12 ± 0.02</td>
<td>0.39 ± 0.02</td>
</tr>
</tbody>
</table>

ND, not detectable.

A Figure 1. Mean ratio of apoptosis/mitosis (bar graph) and apoptosis and mitosis levels in AD CWR22 xenograft tumors after 14 days on experimental diet with androgen stimulation.
lipid fatty acid composition are achieved within 5 to 6 days of initiating changes in dietary PUFA (J.W., unpublished data). Mice were maintained on the experimental diets for 5 days after hormone ablation before obtaining samples for analysis.

The removal of androgen stimulation resulted in regression of this hormone-dependent tumor with apoptosis/mitosis ratios >1.0 in all dietary groups (Figure 3), contrasting with sustained growth and ratios <1 in the earlier experiments. However, in these experiments apoptotic cell death was significantly greater and cell proliferation was significantly lower in tumors harvested from mice fed EPA in comparison to mice fed OA or AA. As a result the ratio of apoptosis/mitosis in tumors from this dietary group dramatically increased (Figure 3). There was a highly significant positive correlation between the ratio of apoptosis/mitosis and tumor content of LA, EPA, total n-3 PUFA, and the n-3/n-6 PUFA ratio, and an inverse correlation with tissue AA and total n-6 PUFA content (Table 3).

**Affects of Dietary PUFA on Progression of AD CWR22 Tumors to AID Growth (Relapse) after Androgen Ablation**

As in humans with prostate cancer, CWR22 xenografts often start growing again (relapse) after a postablation regression nadir, modeling progression to the final clinical stages of this cancer. To determine whether the affects of dietary PUFA on responses to androgen ablation might translate into a prolonged period between ablation and relapse to AID growth, we repeated the earlier short-term ablation experiment but maintained the mice on their respective diets for up to 6 months after removal of the testosterone pellets. Throughout this period tumor volume and serum PSA levels were monitored and relapse was determined to have occurred with renewed increases in measured tumor volumes or PSA levels.

PSA levels were positively and highly correlated with tumor volume (Figure 4 and Table 4). Tumor volume and PSA levels decreased after ablation therapy in all but one AA-fed mouse, in which we failed to detect any such nadir (the growth nadir likely occurring before the first postablation measurements were taken). As indicated by

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**Table 3.** Multivariate Correlation Analysis between CWR22 Xenograft Tumor PUFA Content and the Ratio of Apoptosis/Mitosis 5 Days after Androgen Ablation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation with apoptosis/mitosis</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>0.843</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AA</td>
<td>-0.933</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EPA</td>
<td>0.920</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total n-6 PUFA</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total n-3 PUFA</td>
<td>0.912</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>n-3/n-6 PUFA ratio</td>
<td>0.923</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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**Figure 2.** Mean ratio of apoptosis/mitosis (bar graph) and apoptosis and mitosis levels in AID CWR22R xenograft tumors after 14 days on experimental diet without androgen stimulation. Although there was less mitosis in tumors from the EPA group compared to OA fed mice (*P < 0.05) there was no significant difference in tumor growth as indicated by the ratio of apoptosis/mitosis.

**Figure 3.** Mean apoptosis/mitosis ratios in AD CWR22 xenograft tumors 5 days after androgen ablation (bar graph) and mean for apoptosis and mitosis levels (*Significantly different from OA and AA dietary groups).

**Figure 4.** Kaplan-Meier curves indicating days to AID relapse growth, determined by increases in tumor volume after the postablation ablation nadir. y axis = percentage of animals with AD tumors in remission after ablation (ie, no evidence of renewed AID growth). Drop lines indicate where 50% of the tumors in this study had relapsed to AID. AID growth occurred sooner when mice were fed AA (*P < 0.05). Inset indicates correlation of PSA levels with measured tumor size (R² = 0.6035).
Kaplan-Meier survival curves (Figure 4), time to renewed growth of CWR22 tumors after ablation therapy (AID relapse) occurred significantly sooner in mice fed AA than in those fed either OA or EPA, with no significant difference between the latter two groups after 6 months. As expected, relapse to AID growth determined by increasing PSA levels gave similar results (not shown). Therefore, consumption of AA is associated with a significant reduction in the time it takes for AD tumors to start growing again independent of testosterone, and would result in a more rapid progression to the final stage(s) of this cancer. The reduction in time to relapse had the highest correlation with increased tissue levels of AA (Table 4); tissue levels of LA, EPA, total n-3 PUFA, and the n-3/n-6 PUFA ratio were positively correlated with the number of days to tumor relapse.

**Effect of Dietary PUFA on Eicosanoid Production in Xenograft Tumors**

As shown above, relatively modest changes in dietary fatty acids can dramatically affect PUFA content in human prostatic cancer xenografts, with similar relative changes in both AD CWR22 and AID CWR22R tumors. Although tissue PUFAs can themselves mediate cell signaling pathways, which might contribute to the affects of diet on the response of tumors to ablation therapy, we were interested in examining the extent to which PUFA were metabolized to eicosanoids in AD CWR22 tumors, grown with testosterone, and in AID CWR22R tumors, grown without testosterone. To do this, we used high performance liquid chromatography-tandem mass spectrometry to quantify tissue eicosanoid levels and semi-quantitative RT-PCR to determine relative levels of mRNA expression, focusing on COX and 15-LOX enzymatic pathways, which have been previously implicated in prostatic carcinogenesis.11,22,23

Levels of PGE$_2$ in CWR22 xenograft tissue homogenates were near the limits of quantitation, as were other PGs and thromboxanes (not shown), indicating minimal COX metabolic activity in these tumors. The most abundant COX metabolite was PGD$_2$, with significantly higher levels in tumors from AA-fed mice than in the SDA-fed group (AA diet = 1.7 ± 0.4 pg/µg; OA diet = 0.7 ± 0.3 pg/µg; SDA diet = 0.08 ± 0.03 pg/µg). These results are consistent with substrate (AA) availability in the tumors (Table 1). AID CWR22R tumors contained similarly low levels of prostaglandins, and PGD$_2$ was again the most abundant COX metabolite (not shown).

15-HETE and 13-HODE were both more abundant than the COX metabolites in xenograft tumor tissues (Figure 5). Once again, tissue levels of 15-HETE reflected tissue levels of AA, in which the AA-fed animals had the highest levels of this eicosanoid and the SDA-treated animals had the lowest. Between the OA- and AA-fed groups, the levels of 13-HODE (15-LOX derivative of LA) reflected the tissue levels of LA. In contrast, the levels of 15-HETE in the SDA-fed group was lower than the other two groups despite having the highest levels of LA in the tumor tissue. The 15-HETE to 13-HODE ratio reflected relative tumor AA and LA content (Figure 5) and was higher in the AA group than the other two dietary groups. Essentially the same results were obtained from CWR22 tumors harvested 5 days after ablation (Figure 5). The relative levels of 15-HETE and 13-HODE and their ratio (Figure 5) in CWR22R tumors from the OA group contrasted with the AA group and reflected the relative abundance of AA and...
LA substrates in tumor phospholipids (Table 2). The 15-HETE to 13-HODE ratio was highest in the AA group and lowest in the n-3 PUFA group in both CWR22 and CWR22R tumors.

Effect of Dietary PUFA on Enzyme mRNA Expression in Xenograft Tumors

Cyclooxygenase

COX-2 mRNA expression was detectable in some of the CWR22 tumors and AID CWR22R tumors (Figure 6). However, CWR22 and CWR22R tumor lysates were uniformly devoid of COX-2 protein as determined by Western blot analysis and immunohistochemistry (not shown). Based on prostaglandin profiles and protein expression, it appears that there is minimal COX enzymatic activity in the CWR22 and CWR22R prostate cancer models.

Lipoxygenase

In AD CWR22 tumors, we could easily detect 15-LOX-2 gene expression by RT-PCR and Western blot analysis, whereas we observed relatively low levels of 15-LOX-1 expression (Figure 6). N-6 and n-3 PUFA supplementation to the diet did not appear to affect mRNA expression of 15-LOX-1, 15-LOX-2, or their relative abundance in the AD CWR22 tumors 5 days after ablation (not shown). In comparison to AD CWR22 tumors, AID CWR22R tumors contained more 15-LOX-1 mRNA and less 15-LOX-2 (Figure 6). The difference in relative levels of 15-LOX-2 isozyme expression in CWR22 and CWR22R tumors was confirmed by Western blot (Figure 6) and is consistent with the relative abundance of metabolites. These results suggest a shift in dominance of one isozyme to the other in the conversion to androgen-independent growth, from higher 15-LOX-2 activity in the AD CWR22 xenograft to higher 15-LOX-1 in AID CWR22R tumors.

To further examine the apparent change in 15-LOX isozyme expression between AD and AID tumors, we measured relative levels of mRNA in the tumors that relapsed during the course of our long-term ablation studies. Results showed a mixed pattern of isozyme expression with a majority of relapsed tumors exhibiting reduced relative expression of 15-LOX-2 mRNA (as seen in AID CWR22R xenografts) whereas some retained higher levels characteristic of AD CWR22 xenografts (Figure 7). There was no apparent relationship between increased expression of one enzyme or the other and diet, suggesting this switch is unrelated to dietary influences on progression to androgen independence and that metabolism through this pathway may not explain the beneficial PUFA affects after ablation therapy. In fact, there was no correlation between tissue levels of 15-HETE or 13-HODE and the time required for CWR22 tumors to begin growing independent of androgen (Table 4). Therefore, the 15-LOX metabolic pathway does not appear to play a role in the modulation of responses to androgen ablation therapy associated with dietary PUFA.

Other eicosanoid metabolic pathways linked to prostatic carcinogenesis involve 5- and 12-LOX. Activity of these pathways was examined only by RT-PCR for relative enzyme mRNA levels in this study. For 5-LOX, we either failed to amplify a product or detected a faint band in a few samples (Figure 6). One relapse tumor from our long-term ablation study had a stronger signal, but overall there appeared to be little 5-LOX mRNA in any of the xenograft tumors, regardless of androgen status (not shown). In contrast, 12-LOX mRNA could be easily detected in most tumor samples but, again, there were no obvious differences in relative expression levels between AD and AID tumors (Figure 6). 5- and 12-LOX metabolite profiles were not examined because the literature suggested a more important link with the 15-LOX pathway, so it remains to be determined whether production of these eicosanoids might correlate with growth responses to dietary PUFAs. Given the fact that dietary AA has been shown to dramatically increase eicosanoid levels systemically and EPA has the opposite effect, it would be logical...
Discussion

Prostate cancer is initially dependent on androgens for growth and in most cases regression occurs in primary and metastatic sites with hormone ablation therapy. However, relapsed growth invariably occurs throughout time as tumors adapt to an environment lacking normal androgen stimulation; this conversion marks the terminal phase in most patients that die from this disease. Experimental models to date have typically explored the impact of dietary intervention during this terminal AID phase using immortalized cell lines (ie, DU-145 and PC-3), ignoring the earlier AD phase and responses to hormone manipulation. Hormone ablation typically results in an extended remission period (years) in which nutritional intervention is (arguably) most likely to be successful, yet there has been essentially no testing of dietary factors on this potentially sensitive phase of the disease. An extension of this remission phase would obviously reduce morbidity and mortality attributable to prostate cancer.

CWR22 xenograft cells share greater similarity with primary prostatic tumors than immortalized lines, such as PC-3 and LNCaP cells, and also mimic the natural progression of this disease by initially requiring testosterone, regressing and entering a remission phase after hormone ablation, and eventually commencing renewed growth (relapse) in the absence of normal androgen stimulation. We therefore used this model system to examine the impact of dietary PUFA, tumor tissue fatty acid metabolism, and the relationship of these factors to prostate cancer growth and progression.

Our dietary design allowed us to address a number of questions. We carefully chose to modify dietary AA in preference to its n-6 PUFA precursor, LA. Although LA is elongated and desaturated to form AA, this is a regulated process and the Western diet contains more LA than can be used to raise tissue AA content. The average US diet contains LA at ~6% of energy and for dietary LA to impact tissue AA levels it would have to be consumed at ≥2% of energy, which would require drastic changes in dietary composition. Therefore, from a practical standpoint, modifying LA in the human diet has little effect on either LA or AA content in tissue phospholipids, a response we can mimic in rodents with our designed experiments. Diets for our xenograft studies contained human equivalent doses of LA (~6% energy) and this was kept constant across all groups. AA is common in the typical Western diet and when included in diets containing a typical (saturating) level of LA virtually all tissue phospholipids become enriched with AA at the expense of LA, which is an unequivocal characteristic of dietary AA. The advantages of this experimental design are not only approximation of a more typical Western diet, but dramatic modification of the tissue AA to LA ratios and formation of downstream metabolites such as 15-HETE and 13-HODE. We also lowered tissue LA (without lowering tissue LA) by supplementing the diet with n-3 LC PUFA (EPA and SDA).

Growth of AD CWR22 or AID CWR22R tumors in mice was insensitive to dietary AA or n-3 LC PUFA, despite the fact that PUFA content and metabolism in these tumors was significantly modified. This was an unexpected finding based on previous in vivo and in vitro experimental studies, which suggest that n-3 PUFA inhibits and AA promotes growth. This inconsistency may be related to the distinct models used and/or differences in experimental design. For instance, Kobayashi and colleagues recently reported that growth of hormone-dependent prostatic LAPC-4 xenograft tumors was inhibited by replacing 50% of dietary fat (corn oil) with fish oil. In contrast to CWR22 tumors, the LAPC-4 cells were shown to express COX-2 and produce PGE2, which is a known cell-survival pathway in cancer inhibited by n-3 PUFA, and we examined the affects of dietary PUFA on established tumors rather than their establishment and subsequent growth. We did, however, show that dietary n-3 LC PUFA may significantly enhance the sensitivity of early AD prostatic cancer to therapeutic androgen ablation therapy and retard the inevitable and often fatal conversion to AID growth. Such dietary affects on the early dynamic responses to androgen ablation have not been examined in animal models or humans and we would suggest that re-evaluation of past epidemiological data and/or prospective analysis may reflect a dietary benefit of LC n-3 PUFA. Our data would indicate that the net effect of dietary LC n-3 PUFA in patients undergoing hormone ablation therapy would be a more rapid response and a significant prolongation of therapeutic benefits after ablation therapy, which could potentially translate into a longer lifespan for treated individuals.

Very few studies have investigated the effects of dietary PUFA on responses of cancer to hormonal manipulation. Importantly, our results provide significant additional data to this area of the literature. Dietary AA and EPA had opposing effects with regards to time to relapse and this observation could account, in part, for the beneficial effects of n-3 LC PUFA observed on latter stages versus earlier stages of prostate cancer. In our experiments 50% of the animals on the AA diet relapsed ~70 days after hormone ablation, compared to 85 days for the OA-fed group and 120 days for animals fed EPA (at which point 90% of the AA-fed animals had relapsed to AID growth). It remains to be determined whether tumor relapse in mice on OA and EPA diets might diverge significantly if followed longer than 6 months. Significantly, the AA animals progressively relapsed in a linear, time-dependent manner whereas it took 70 days for the first animals to relapse in either of the other two dietary groups. These results support studies suggesting dietary n-6 LC PUFA promotes and n-3 LC PUFA inhibits the progression of prostate cancer. Like CWR22 cells, AD LAPC-4 prostate xenograft tumors can also progress to AID growth after ablation. Ngo and colleagues reported that time to relapse of LAPC-4 tumors in animals fed isocaloric diets containing either 5% or 17.5% corn oil (in which LA was the only PUFA provided in the diet) was 25% longer in animals receiving lower LA diets (n-
creased from 16 to 20 weeks). In our experiments, higher levels of LA in tumor phospholipids significantly correlated with an improved response to ablation therapy and a prolonged interval to AID relapse growth, suggesting tissue content of this n-6 PUFA has anti-neoplastic affects in the context of hormone manipulation. In Ngo’s study, a threefold increase in dietary LA may have significantly driven up tumor AA content; whereas such a change in tissue AA levels would not occur in humans with a dietary increase in LA, mice may not limit conversion of LA to AA as strictly as humans when provided increasing amounts of LA in the diet. The reduction in time to relapse seen in Ngo’s study could be consistent with our results if the high LA diet significantly increased tumor AA content.

Eicosanoids can have potent biological affects that include modulation of cellular replication and sensitivity to apoptosis. Their production is dependent on expression of specific enzymes and the relative abundance of PUFA substrate, which we clearly modified with our experimental diets. The relative eicosanoid levels in xenograft tumors harvested from different dietary groups generally followed changes in tissue substrate with, for instance, higher PGD2 and 15-HETE levels in tumors from mice fed AA compared to those fed OA or n-3 LC PUFA. We chose to focus on the 15-LOX isozymes and COX-2 because of their reported importance in prostatic tumorigenesis. COX-2 protein expression was undetectable in either CWR22 or CWR22R tumors and addition of AA to the diets did not increase COX-2 expression, in contrast to what was observed when exogenous AA was added to cultured PC-3 prostate tumor cells. Given these findings and the fact that prostaglandin levels were much lower than in other tumors where PGE2 is known to play a role, it seems highly unlikely that dietary manipulation of the COX pathway contributed to our biological outcomes. Other eicosanoid metabolic pathways that have also been linked to prostatic carcinogenesis include 5- and 12-LOX. Although we found no significant relationship between mRNA levels and growth or responsiveness of xenografts to androgens, we cannot rule out their enzymatic activity and metabolic products in modulating these processes.

Patterns of 15-LOX expression in our initial studies suggested that progression of AD CWR22 prostate tumors to AID CWR22R-relapsed tumors was associated with a shift in the predominant expression pattern of the 15-LOX isoforms, from 15-LOX-2 in AD tumors to 15-LOX-1 in the more aggressively growing AID tumors (Figures 1, 2, 5, and 6). However, examination of tumors from our long-term hormone ablation studies clearly indicated that some tumors retained dominant expression of 15-LOX-2 over 15-LOX-1, suggesting this shift is not required for progression to AID growth. Uniquely, our dietary design allowed us to explore the substrate-based impact of altering 15-LOX product profiles in prostate cancer tissue and whether these effects were dependent on 15-LOX expression. Both 15-HETE and 13-HODE were formed in all tumors but when AA was provided in the diet tissue levels of AA dramatically increased at the expense of LA, increasing 15-HETE formation and reducing 13-HODE. These distinct diet-induced changes in 15-LOX substrates and metabolites in AD and AID tumors were not associated with any measurable differences in growth of tumors with and without androgen support, respectively. In contrast, a significant increase of LA (the substrate for 13-HODE) in AD tumors enhanced the anti-tumorigenic affects of androgen ablation, whereas an increase of AA (the substrate for 15-HETE) did not. Finally, we found that the type of dietary LC PUFA had no measurable affects on tumor levels of mRNA for 15-LOX, or any of the other enzymes we examined. In summary, we could find no discernable contribution of the metabolism of AA or LA through the 15-LOX pathway to growth of AD or AID prostate cancer xenografts, or responses of AD xenografts to hormone ablation with changes in dietary PUFA content.

The role of 15-LOX activity and its downstream metabolites in mediating the progression of prostate cancer is still unclear. Part of this is attributable to blatant promiscuity of the two isoforms (15-LOX-1 and 15-LOX-2) regarding substrate utilization. Both isoforms effectively oxidize AA and LA to generate 15-HETE and 13-HODE, respectively, and 15-LOX-1 is able to oxygenate both free and esterified fatty acids. The major difference in their activities is characterized by whether 15-HETE is the exclusive product when AA is the only substrate. Under this admittedly spurious condition, 15-LOX-2 exclusively generates 15(S)-HETE, whereas 15-LOX-1 forms 15(S)-HETE and 12(S)-HETE in a ratio of ~9:1. Because 15-LOX-2 expression is generally believed to be higher in normal or less advanced prostatic cancer tissue, this leads some to conclude that benign tissue exclusively produces 15-HETE and malignant prostatic tissue favors 13-HODE formation. This conclusion is tenuous given the kinetics surrounding substrate utilization. In addition, others report 15-LOX-2 mRNA expression to be the predominant isoform in both normal and malignant prostatic tissue. It is also possible that 15-LOX-2 is acting as a tumor suppressor protein lost early in prostate cancer development. Equivalent biological activities have been observed using catalytically active 15-LOX-2 and catalytically inactive splice variants of 15-LOX-2. Furthermore, overexpression of 15-LOX-2 and its stable, catalytically inactive splice variant suppressed PC-3 prostate tumor growth in vivo and cell proliferation in vitro, suggesting that 15-LOX-2 may have anti-neoplastic activities independent of its ability to oxygenate PUFA.

Mechanisms other than those related to direct affects or metabolism of tumor PUFA content might also be considered responsible for our results, in particular, the influence of dietary changes on tissue oxidative stress. We do not believe dietary changes in tissue oxidative stress are responsible for our data because the PUFA content and unsaturation indexes were similar for AA and n-3 LC PUFA diets, and significantly greater in both of these than in the OA diets, yet biological effects distinguished EPA-fed mice from both AA and OA in the short-term ablation experiments and AA from EPA and OA in the long-term studies. Additional indirect mechanisms may be affected by dietary PUFA manipulation. For in-
stance, tissue PUFA levels might affect responses to androgen ablation outside the neoplastic epithelium with altered patterns of cytokine and growth factor expression either systemically or in the tumor microenvironment that in turn affect tumor growth.

In summary, our results suggest that dietary AA reduces the time required for conversion of AD to AID prostatic tumor growth after androgen ablation therapy, and that dietary n-3 LC PUFA increases this remission period and the acute beneficial responses to hormone deprivation. This relatively innocuous supplemental therapy (ie, dietary n-3 LC PUFA) may have the added benefit of reducing the risk of other chronic diseases that have been reported to increase with androgen ablation, ie, type II diabetes and cardiovascular disease.32,53

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