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13. ABSTRACT (Maximum 200 Words)

There is a strong correlation between dietary fat and mortality attributed to prostatic cancer, but little is known about how this nutrient modifies tumor biology to enhance fatal malignancy. Arachidonic acid (AA) is an n-6 polyunsaturated fatty acid (PUFA), which directly mediates cell signaling and is the substrate for generation of bioactive eicosanoids; AA and eicosanoids regulate cell growth and cell death in a variety of experimental settings. Substitution of n-3 PUFA (i.e. fish oil) for AA significantly reduces tissue AA content and modifies eicosanoid profiles, thereby effecting changes in cell biology. As expected, changes in dietary PUFA content significantly altered the fatty acid profile in early and late stages of prostatic cancer; surprisingly, this was not associated with changes in tumor growth. In contrast, the same dietary enrichment of tissues for n-3 PUFA significantly enhanced the beneficial response to androgen-ablation therapy and delayed conversion (progression) to androgen-independent growth (relapse) in comparison to dietary AA. If these findings hold true in subsequent studies performed in our laboratories and others, modulation of prostatic cancer biology with innocuous dietary intervention could represent a very important adjunct to prostate cancer therapy.

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INTRODUCTION

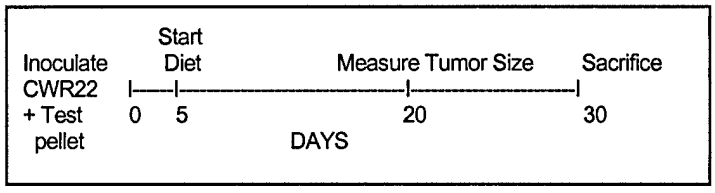
Epidemiologic studies indicate that dietary fat has a strong correlation with mortality rates caused by prostatic cancer, but little influence on establishment of the disease.[1-3] Cell proliferation and cell death (apoptosis) are two key biologic features of neoplasia which change during regression of androgen-dependent prostatic tumors induced by androgen ablation therapy, and on emergence of androgen-independent growth.[4-7] Arachidonic acid (AA) is an n-6 polyunsaturated fatty acid (PUFA) which directly mediates cell signaling mechanisms and is the substrate for generation of diverse eicosanoids through the AA cascade; AA and these eicosanoids have been shown to regulate cell proliferation and apoptosis in a variety of experimental settings. We have shown that substitution of n-3 PUFA (as in fish oils) for AA in membrane phospholipids significantly alters the AA cascade and therefore the biologic effects of this signaling pathway.[8-13] Clinical studies suggest that higher levels of fats (AA and other n-6 PUFA) in the diet promote prostatic carcinogenesis while fish oils have a protective effect, but these studies have not been definitive on this issue.[14] Previous work in prostatic carcinogenesis supports the antithetic relationship between n-6 and n-3 PUFA on growth of prostatic cancer cells, but the prostatic cell lines used represent an advanced stage of this cancer and no laboratory studies have examined the effects of n-6 PUFA on earlier phases. We are therefore interested in the effects of modulating tissue arachidonic acid levels on the early stages of prostatic cancer and whether altering tissue PUFA content can affect cancer progression. We have chosen an *in vivo* xenograft system which uniquely models prostatic cancer in humans. CWR22 is a primary human prostatic cell line which responds to androgen-withdrawal by regressing in the same manner as early stage androgen-dependent tumors in humans.[15] CWR22 cells progress to androgen-independent growth (CWR22R) when subjected to the same selective forces (androgen ablation) responsible for this change in men with the disease.[16] Demonstration of changes in human prostatic cancer cell growth mediated by manipulation of dietary PUFA would explain some of the environmental factors linked to this disease, provide a rationale for nutritional modulation of cancer growth, response to therapy, and progression in affected individuals, and supply a basis for more definitive mechanistic molecular and biochemical studies in the future.

BODY

Task (Experiment) 1 Months 1-10 Complete

The first set of experiments set forth by our proposal was to examine the effects of tissue enrichment for either n-6 or n-3 PUFA on growth of CWR22 xenografts in male mice with normal testosterone levels (i.e. early stage, androgen dependent cancer).

- CWR22 cells were propagated in athymic nude (*nu/nu*) mice supplemented with subcutaneous testosterone pellets and harvested as previously described.[15;17] The tumor masses were enzymatically dissociated and filtered to obtain a uniform cell suspension of known density (viable cell count) for injection in experimental mice. The first experimental protocol was performed as follows.
- Fifteen, 5 week old, male nude mice were randomly divided into 3 groups of 5 mice each and housed in microisolator cages with free access to standard mouse chow and water. After acclimatizing for a week, sustained release testosterone pellets were implanted and $5x10^5$ CWR22 cells were injected subcutaneously in matrigel. Experimental diets were started five days after implanting the tumor cells in



order to permit uniform initial "seeding" across all groups. Powdered diets were premixed to contain 1.5% (by weight) AA, stearidonic acid (SDA; an n-3 PUFA), or oleic acid (OA; control fat) and stored at -70° C under nitrogen gas. We have previously shown that SDA and AA fed at these levels significantly affect tissue AA concentrations along with the lipid metabolites (i.e. prostaglandins) of this n-6 PUFA.[13] We had proposed to use eicosapentaenoic acid (EPA) as our source of dietary n-3 PUFA but switched to SDA after finding that SDA has an identical effect on tissue AA content and biologic activity.[13] OA was used to control for dietary fat content because it is neutral with respect to tissue AA and eicosanoid levels. We decided not to use linoleic acid (which is metabolized to AA *in vivo*) as a n-6 control for the first experiment because it was deemed unnecessary in addition to OA. Fresh diet was fed daily, and food intake, animal health and xenograft tumor growth monitored daily. Ears were notched for identification and mice were weighed weekly. Tumor growth was identified 3 weeks after implanting CWR22 cells and dimensions measured using a caliper. Volume was determined by calculating (H x L x W) x 0.5236. The experiment was ended 10 days later because some of the tumors had enlarged beyond the 1 cm diameter stipulated to represent the maximum tolerated by the experimental protocol and IACUC regulations. At sacrifice, tumor dimensions were again measured, serum was obtained for PSA levels (which correlates with tumor volume), tumors were excised, weighted, and samples either fixed in 10% neutral buffered formalin for histology, or snap frozen in liquid N_2 for fatty acid and eicosanoid analysis.

- Hematoxylin and eosin (H&E) stained sections of CWR22 tumors were evaluated for mitosis and apoptosis according to morphologic criteria (i.e. apoptotic bodies and mitotic figures per 1000 tumor cells) and the degree of lymphocytic infiltrate was graded according to a scoring scheme: 1 = a scant infiltrate, 2 = mild, 3 = moderate, and 4 = severe. Data were evaluated by ANOVA and nonparametric Rank Sums analysis for significance and summarized in Table 1.

TABLE 1:

	Mean Change Tumor Volume ** (day 20-30)	Final Tumor Weight (gm)	Mean Mitotic Index	Mean Apoptosis Index	Mean Ratio Apoptosis/ Mitosis (x 100)	Mean Lymphocyte Score
OA (Control)	72	148	35	8	27	1.4
AA (n-6)	305	390	34.6	8.4	26	2.4 *
SDA (n-3)	323	630	37.6	11.6	32	3.3 *

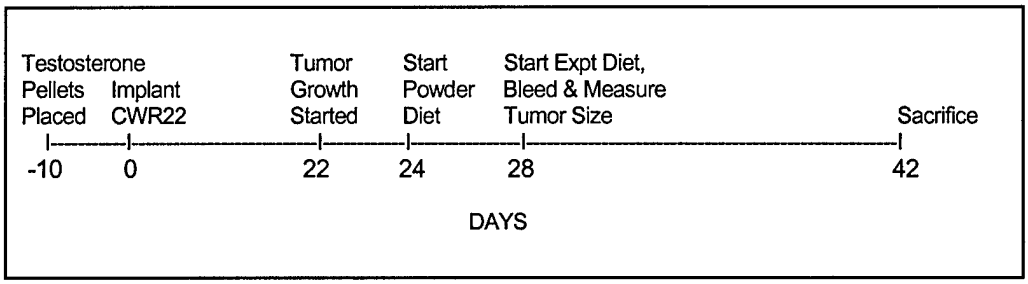
** in mm^3

* Significantly different from OA (Control) group ($P < 0.05$)

Although the apoptosis/mitosis ratio was slightly greater in the SDA group, there was no significant effect of dietary PUFA on measured growth of xenografts. Tumors appeared and grew at different rates, regardless of dietary group and despite the fact that every effort was made to innoculate the same number of tumor cells in every animal. Dr. T. Pretlow (the originator of the CWR22 cell lines at Case Western Reserve University) was consulted about this variability because of his extensive experience with this xenograft model. It has been his experience (personal communication) that CWR22 cells grow as well, if not better, in nude mice as in any other strain of immunodeficient mouse, but he is of the opinion that immune surveillance may nonetheless affect uniformity of growth from animal to animal. Dr. Pretlow noted that lymphocytic infiltrates can be found

associated with some xenografts, so we decided to determine the relative lymphocyte density in tissue sections as a surrogate for this putative immune response. As shown in Table 1, the score (relative lymphoid infiltrate) actually increased significantly in tumors from animals fed either n-6 or n-3 PUFA in comparison to the OA diet control. The infiltrate also increased significantly in larger tumors (data not shown) suggesting that this lymphoid response did not impair growth.

It was clear from our first experiment that substitution of the dietary n-6 PUFA AA with the n-3 PUFA SDA, which we've previously shown to significantly effect tissue AA content,[13] did not have a profound affect on growth of CWR22 xenografts. However, because we were concerned that the relatively large variation in growth of xenografts in individual mice might mask more subtle effects, we redesigned the experiment as follows to eliminate any possible controllable variable which might have a impact on tumor growth between mice.



Thirty-six male nude mice were implanted in 2 subcutaneous sites with 10^6 CWR22 cells suspended in matrigel, after placement of testosterone pellets, and diet was started after tumors were well established.

The new experiment was designed to ensure that:

1. circulating testosterone levels were the same in all mice when cells were implanted
    i. testosterone levels increase to experimental levels within 5-7 hours of pellet implantation (Innovative Research of America, technical communication) so it seemed unlikely that this brief transition period would significantly affect uniformity of tumor growth between animals, but this was a modifiable aspect of the experimental protocol so pellets were placed before injection of CWR22 cells
2. the number of cells implanted was adequate for robust and uniform tumor growth
    i. while 5×10^5 cells is more than adequate to initiate tumor growth, T Pretlow suggested that implanting greater numbers might at least partially circumvent effects of host mouse immunity
3. all mice ate the experimental diets as fed
    i. starting powder diet (without PUFA) before adding the PUFA components might help with acclimatization (i.e. change from standard pellet chow diet)
4. there would be adequate number of mice with tumors to detect relatively small effects of the dietary manipulation on tumor growth
    i. by seeding 36 mice with CWR22 cells in two locations we hoped to obtain 9-10 similarly growing xenograft tumors per dietary group
5. only mice with robust tumors were included in the experimental groups
    i. if immunity (or some other innate factor) was responsible for individual variation in tumor growth between mice in the first experiment, then using only mice with actively growing tumors might help eliminate this effect(s).

Tissue PUFA content stabilizes within 5-7 days after changing dietary intake (J. Whelan, unpublished observations) and it was therefore our belief that 2 weeks on experimental diet would be adequate for cellular changes to manifest as a result this shift in PUFA content. The expected changes in xenograft PUFA content with the feeding of experimental diets were, in fact, observed. As shown in Table 2 there was significant modulation of tissue AA (20:4 n-6) and n-3 PUFA content resulting a substantial increase in the ratio of n-3/n-6 PUFA in CWR22 tumors from SDA fed mice.

TABLE 2: CRW22 PROSTATE TUMOR POLYUNSATURATED FATTY ACID CONTENT

	AA DIET, n = 10	OA DIET, n = 6	SDA DIET, n = 9
16:0	17.49 ± 0.242	17.53 ± 0.068	17.89 ± 0.288
16:1	1.02 ± 0.159	1.12 ± 0.272	1.56 ± 0.199
18:0	20.38 ± 0.184	18.36 ± 0.137	19.69 ± 0.175
18:1 n-9	24.15 ± 0.405	28.51 ± 0.425	24.22 ± 0.412
18:1 n-7	4.51 ± 0.132	5.47 ± 0.135	4.42 ± 0.127
18:2 n-6	6.75 ± 0.282	9.15 ± 0.212	11.63 ± 0.333
20:3 n-6	1.35 ± 0.065	2.35 ± 0.134	3.03 ± 0.117
20:4 n-6	15.63 ± 0.251	9.38 ± 0.297	4.90 ± 0.230
20:5 n-3	0.04 ± 0.022	0.02 ± 0.016	2.55 ± 0.116
22:4 n-6	2.91 ± 0.097	1.33 ± 0.067	0.25 ± 0.019
22:5 n-6	1.16 ± 0.055	0.70 ± 0.051	0.05 ± 0.004
22:5 n-3	ND	ND	3.97 ± 0.074
22:6 n-3	1.21 ± 0.057	1.50 ± 0.043	3.04 ± 0.073
Total (n-3) FA	1.25 ± 0.061	1.54 ± 0.034	9.66 ± 0.241
Total (n-6) FA	27.90 ± 0.436	23.18 ± 0.440	19.89 ± 0.460
(n-3)/(n-6) ratio	0.04 ± 0.002	0.07 ± 0.0005	0.49 ± 0.018

Data are mole % and are expressed as mean ± SEM. ND indicates not detectable.

Table 3 shows mean levels of several eicosanoids in xenograft tissue homogenates. Levels of PGE_2 were essentially zero (relative to previous analyses of colorectal tumors known to express Cox-2), suggesting that significant upregulation of arachidonic acid metabolism by cyclooxygenase in CWR22 tumors was absent and western blot analysis of tumor lysates failed to detect Cox-2 protein. Significant levels of both 15-HETE and 13-HODE were detected. While there was a trend towards changes in 15-HETE, as expected to occur with dietary PUFA manipulation, these were not significant. We are currently seeking to confirm this data by gas chromatography-mass spectrometry (GC-MS, pending). 15-HETE is derived primarily from AA via 15-

lipoxygenase (LO)-2 activity, while 13-HODE arises from linoleic acid (LA; 18:2 n-6) metabolism by 15-LO-1. These data would suggest that 15-LO-2 activity in the CWR22 xenograft is higher than that of 15-LO-1, with greater sensitivity to dietary modulation of resultant lipid products. Western analysis of tumor samples from the 3 dietary groups did not reveal any differences in expression of 15-LO-2; we could not detect 15-LO-1 with available antibodies (effective 15-LO-1 antibodies are apparently rare and we are still seeking access to such material).

TABLE 3: CWR22 Xenograft Tissue Eicosanoid Levels (Mean, pg/mg protein)

Dietary Group	PGE_2	15-HETE	13-HODE
OA (Control)	0	590	8
AA (n-6 PUFA)	20	752	6
SDA (n-3 PUFA)	4	475	5

Tumor growth response to changes in tissue PUFA levels are presented in Table 4. Although it may appear that dietary n-3 PUFA (SDA) provides a slight growth benefit, this is not close to being a significant effect ($p > 0.5$). If dietary PUFA actually were to promote CWR22 xenograft growth in comparison to the oleic acid control, it would not likely be due to a difference in oxidative stress. PUFAs, such as SDA and AA, may enhance oxidative stress and free radical generation over that caused by feeding OA but this is most often attributed with inhibition of tumorigenesis, rather than promotion of growth. There was no significant difference in percent tumor growth (not shown) or in apoptosis or mitosis (Table 4). Tumors in all 3 dietary groups were clearly growing, however, as evidenced by a ratio of mitosis/apoptosis ratio > 1.

TABLE 4: CWR22 Xenograft Growth and Cell Biology - Effects of Dietary n-3 and n-6 PUFA

	Mean Change Right Tumor Volume ** (day 28-42)	Mean Change Left Tumor Volume ** (day 28-42)	Mean Change Total Tumor Volume *** (day 28-42)	Mean *Apoptosis* Index* * (± SEM)	Mean *Mitosis* Index* * (± SEM)
OA (Control)	391	858	624	7.5 ± 0.8	15.8 ± 1.1
AA (n-6 PUFA)	709	602	655	8.2 ± 0.7	18.0 ± 2.2
SDA (n-3 PUFA)	599	926	762	7.1 ± 1.1	18.9 ± 1.6

** in mm^3: volume = (L x H x W) x 0.5236; change = volume day 42 - volume day 28
** mean of all tumor volume changes (i.e. right and left)
* *Mean Apoptosis and Mitosis Indexes were determined as in Experiment 1
* Significantly different from OA (Control) group ($P < 0.05$)

Task (Experiment) 2 Months 3-12 Complete

Forty-five Male nude mice were implanted with androgen-independent CWR22R cells and subjected to the same experimental protocol as in the redesigned version of Experiment 1 (above) except that mice were castrated prior to tumor implantation (versus testosterone supplementation) and EPA was used as the dietary n-3 PUFA in place of SDA (SDA production was unexpectedly discontinued by the supplier). As noted, we have previously shown that SDA and EPA have the same effects on tissue n-3 PUFA content and are essentially interchangeable in the diet for this purpose.[13]

- As for Experiment 1, there was no difference in the final body weights of xenograft-bearing mice fed the 3 experimental diets (not shown)
- Table 5 shows that CWR22R tumor tissue PUFA content was modified by dietary n-3 or n-6 PUFA manipulation as in Experiment 1 with significant differences in n-3 and n-6 fatty acids, including arachidonate.

TABLE 5: CRW22R PROSTATE TUMOR POLYUNSATURATED FATTY ACID CONTENT

	TO Group, n = 9	AA Group n = 13	EPA Group, n = 6
16:0	11.70 ± 0.207	11.46 ± 0.388	11.50 ± 0.306
16:1	0.48 ± 0.019	ND	0.51 ± 0.030
18:0	23.84 ± 0.242	24.44 ± 0.882	23.50 ± 0.433
18:1 n-9	26.52 ± 0.327	23.34 ± 0.834	22.22 ± 1.178
18:1 n-7	4.40 ± 0.106	3.91 ± 0.083	3.54 ± 0.165
18:2 n-6	13.77 ± 0.290	10.36 ± 0.385	16.29 ± 0.396
20:3 n-6	0.70 ± 0.008	0.43 ± 0.016	0.63 ± 0.043
20:4 n-6	10.49 ± 0.213	17.52 ± 0.285	7.88 ± 0.608
20:5 n-3	ND	ND	2.46 ± 0.079
22:4 n-6	0.64 ± 0.043	1.45 ± 0.235	0.38 ± 0.064
22:5 n-6	ND	ND	ND
22:5 n-3	ND	ND	3.30 ± 0.229
22:6 n-3	3.23 ± 0.067	3.53 ± 0.680	4.14 ± 0.340
Total (n-3) FA	3.23 ± 0.067	3.35 ± 0.680	9.90 ± 0.594
Total (n-6) FA	25.59 ± 0.323	29.76 ± 0.662	25.19 ± 1.026
(n-3)/(n-6) ratio	0.13 ± 0.003	0.12 ± 0.021	0.39 ± 0.025

Data are mole % and are expressed as mean ± SEM. ND = not detectable.

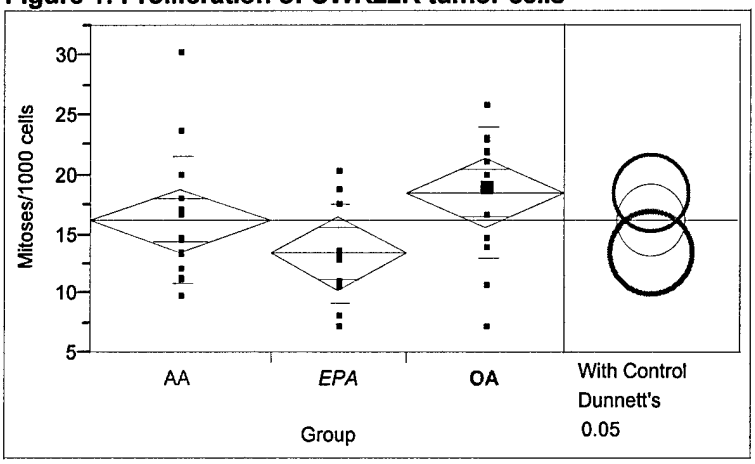
- As for CWR22 tumors, there was no appreciable PGE_2 production in CWR22R tissues and 15-HETE levels appeared to be more sensitive to dietary manipulation than 13-HODE (Table 6), but there was no significant difference between dietary groups.

TABLE 6: CWR22R Xenograft Tissue Eicosanoid Levels (Mean, pg/mg protein)

Dietary Group	PGE_2	15-HETE	13-HODE
OA (Control)	26	319	8
AA (n-6 PUFA)	16	355	6
EPA (n-3 PUFA)	14	143	6

- Although n-3 PUFA enriched tumors had a statistically significant reduction in proliferation compared to the OA control ($P = 0.048$), mitosis in EPA tumors was not different from that in AA fed mice (Figure 1). There was no significant difference in final tumor volume or apoptosis in tumors growing in mice supplemented with the 3 different dietary fatty acids (Table 7).

Figure 1: Proliferation of CWR22R tumor cells



Dunnett's pairwise comparison with OA set as control and significance at $P < 0.05$

TABLE 7: CWR22R Xenograft Growth, Cell Proliferation & Death - Effects of Dietary n-3 and n-6 PUFA

	Mean Total Tumor Volume **	Mean *Apoptosis* Index** (± SEM)	Mean *Mitosis* Index** (± SEM)
OA (Control)	1135	5.0 ± 0.6	18.5 ± 1.5
AA (n-6 PUFA)	1312	4.1 ± 0.5	16.2 ± 1.4
EPA (n-3 PUFA)	763	4.4 ± 0.6	13.4 ± 1.3 *

** in mm^3: volume = (L x H x W) x 0.5236
* Significantly different from OA (Control) group ($P < 0.05$)
** Apoptosis and Mitosis Indexes were determined as in Experiment 1

We **conclude from Experiments 1 and 2** that enrichment of xenograft human prostatic tumors for n-3 or n-6 PUFA has no significant affect on neoplastic cell growth characteristics, regardless of whether they are early androgen-dependent or late androgen-independent cancers. The lack of a significant affect on eicosanoid metabolites with dietary manipulation is surprising and will be confirmed by mass spectrometry (pending). If true, this unexplained failure to significantly alter levels of 15-HETE and 13-HODE may explain the lack of a dietary affect on tumor growth.[18;19]

Task (Experiment) 3 Months 12-24 Incomplete

EXPERIMENT 3A: Androgen-dependent CWR22 cells were inoculated into 2 locations over the lumbar area of 45 castrated male nude mice which had been implanted with sustained release testosterone pellets. When tumor growth was clearly evident the mice were acclimatized to powdered diet (as in Expt 1 and 2), randomly divided into 3 groups and fed experimental diets containing 1.5% OA, AA, or EPA. Seven days after starting experimental diets testosterone pellets were removed under general anesthesia, tumors were measured and serum was sampled for later PSA analysis. Five days after testosterone ablation the mice were euthanized, tumors were measured and harvested for further analysis and serum was taken for PSA.

Experimental Design:

Castration	Implant Testosterone Pellets	Implant CWR22 Cells	Tumor Growth - Start Exptl Diet	Remove Testosterone Pellets	Sacrifice
-21	-3	0	40	47	52

DAYS

- As shown in Figure 2 and Table 8 there was no significant affect of dietary PUFA on changes in tumor size over a 5 day post-ablation period.

Figure 2: Changes in xenograft tumor volume post-ablation in left and right flanks

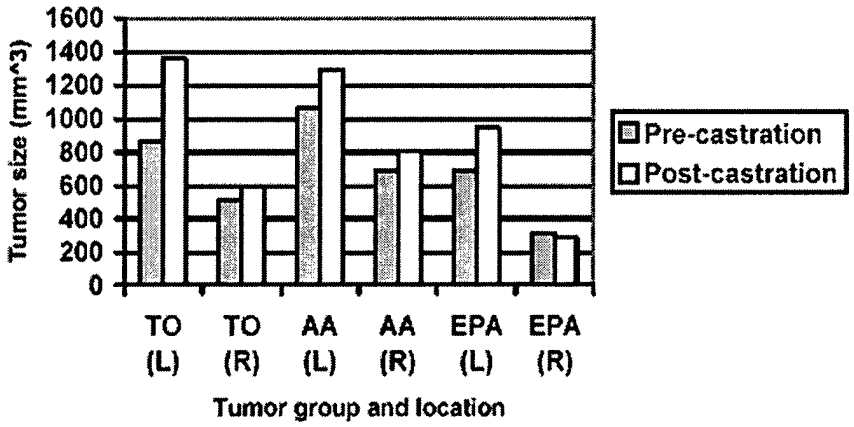


TABLE 8: Effects of Dietary n-3 and n-6 PUFA on CWR22 Xenograft Growth Five Days After Ablation

Right + Left Tumor Averaged	OA Control	AA Diet	EPA Diet
PRE-Ablation Tumor Volume	320	457	263
POST-Ablation Tumor Volume	442	550	321
VOLUME CHANGE	122	93	58
PERCENT VOLUME CHANGE	38 %	20 %	22 %

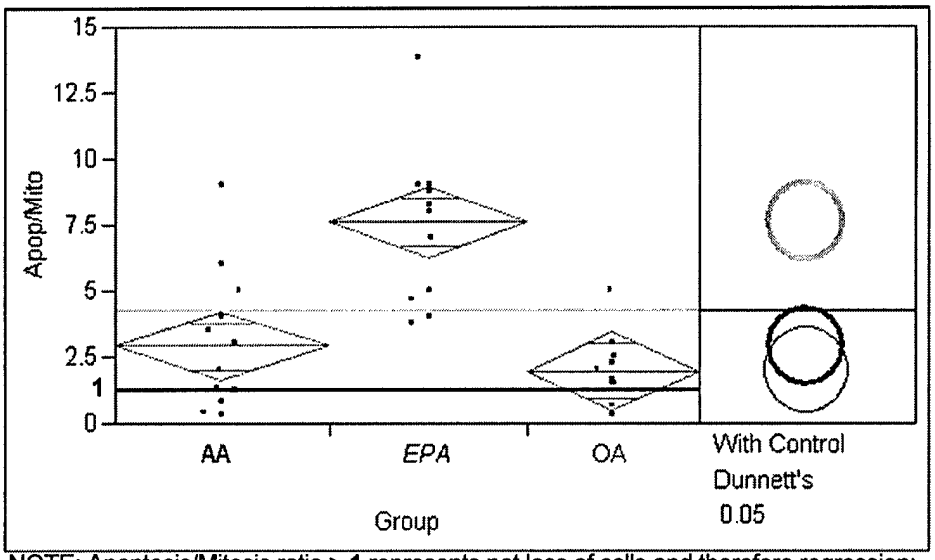
- As in Experiment 1, measured eicosanoid levels in CWR22 tumors 5 days after androgen ablation were not significantly different between the different dietary groups (not shown).
- There was no significant difference in PSA levels between the three dietary groups in comparing pre- and post-ablation serum samples. This is attributed to the large range of values between individual mice (not shown), in concert with the variable tumor size.
- ***Cell death was significantly greater*** and ***cell proliferation was significantly lower*** in androgen-dependent tumors harvested from mice fed the EPA diet (Table 9). Note in Figure 3 that the ratio of apoptosis/mitosis provides an indication of tumor growth (ratio < 1) or regression (ratio > 1).
- Non-neoplastic mouse prostatic tissues were also harvested in this study and, while not statistically significant, there was similar enhancement of apoptosis in this androgen-dependent tissue from EPA fed animals in comparison to those fed OA or AA (not shown).

TABLE 9: Effects of Dietary n-3 and n-6 PUFA on PSA Levels, Apoptosis, and Mitosis Five Days After Androgen Ablation

Dietary Group	Mean Change in PSA **	Mean Apoptosis Index**	Mean Mitosis Index**
OA (Control)	- 19	2.4	1.5
AA (n-6)	- 37	2.2	1.4
EPA (n-3)	3	4.0 *	0.5 *

** difference between pre- and post-ablation serum levels (U/ml)
* Significantly different from OA (Control) group ($P < 0.05$)
** Apoptosis and Mitosis Indexes were determined as in Experiment 1

Figure 3: Ratio of Apoptosis/Mitosis in CWR22 Prostate Tumors 5 Days Post-Ablation



NOTE: Apoptosis/Mitosis ratio > **1** represents net loss of cells and therefore regression; the larger the ratio the greater the cell loss. All CWR22 tumors from testosterone-supplemented mice (pre-ablation, Expt 1) had a ratio < 1 (not shown), indicating net growth in the presence of androgens.

EXPERIMENT 3B: Part two of Task 3 is an identical experimental design for evaluation of a second androgen-dependent cell line (PC82) for affects of dietary PUFA on ablation-mediated responses. When our frozen aliquots of PC82 cells failed to grow after implantation in testosterone supplemented male nude mice last year we asked our original source (laboratory of Dr. J. Isaacs at Johns Hopkins University) for another sample. Although they kindly agreed to provide more PC82 material, the Isaacs laboratory itself was having problems growing xenografts from their frozen stores, despite their years of experience using this line. We waited months but finally decided to look for an alternative cell line. In reviewing the literature again and talking to several researchers that work in this area it was concluded that: 1) PC82 cells would in fact be the best line to use for our studies, and 2) LACP9 cells (developed by Dr. C. Sawyers at UCLA Medical Center) would probably be the 3rd choice (after PC82 and CWR22 xenografts). An MTA was obtained and Dr. Sawyers kindly sent frozen LAPC9 cells, which successfully grew several months after implantation in male nude mice supplemented with testosterone, as for CWR22 cells. LAPC9 tumor tissue was transplanted into experimental mice and Experiment 3B was conducted as for 3A. Due to the delay incurred in turning to another xenograft model the animal work for this experiment has only just been completed and laboratory data is, unfortunately, not yet available.

Not surprisingly, there was no significant difference between dietary groups comparing pre- and post-ablation tumor volumes (Table 10). Histologic evaluation of cell proliferation and cell death should be completed in the next weeks and we plan to evaluate serum samples for PSA and tumor material for eicosanoids by GC-mass spectrometry as soon as possible.

TABLE 10: Effects of Dietary n-3 and n-6 PUFA on LAPC9 Xenograft Growth Five Days After Ablation

	OA Control	AA Diet	EPA Diet
PRE-Ablation Tumor Volume	142	317	473
POST-Ablation Tumor Volume	153	349	471
VOLUME CHANGE	11	31	-2
PERCENT VOLUME CHANGE	7.7 %	9.8 %	- 0.4 %

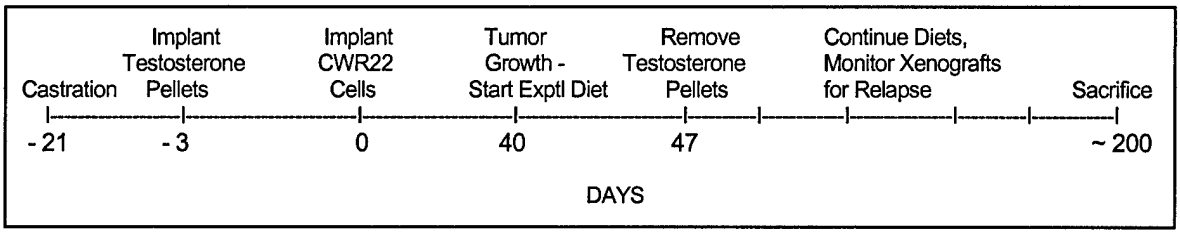
We **conclude** from Experiment 3A that enrichment of androgen-dependent prostatic cancer tissues with ***n-3 PUFA enhances the immediate beneficial effects of androgen-ablation therapy.***

Because of this exciting data (obtained with CWR22 xenografts) and our conviction that PC82 cells would be particularly informative for these experiments, the originator of the PC82 line was contacted in the Netherlands early this spring and he kindly agreed to provide this model for our work. The international MTA has been time consuming, but has now been agreed to by all parties and we hope to be able to repeat Experiment 3B with this material. With the data from 3 distinct androgen-dependent prostatic cancers we believe this should provide a clear indication of whether or not tissue levels of n-3 PUFA may, in fact, modulate responses to ablation therapy in prostate cancer. Therefore, while we regret the unavoidable delays in this Task, we are enthusiastic about the potential for reaching exciting conclusions in the end.

Task (Experiment) 4 Months 17-36 Completed

CWR22 cells were inoculated into 45 testosterone-supplemented male nude. The protocol for this study duplicated Task 3 (see below) except that instead of undergoing testosterone ablation, animals were maintained on experimental diets and tumors were monitored until relapse (renewed growth, PSA secretion as indication of conversion to androgen-independence) or until 6 months after testosterone ablation, at which point the experiment was terminated. Figure 4 shows the changes in mean tumor volume of CWR22 tumors following androgen ablation. There was a distinct nadir in tumor volume (Figure 4) and serum PSA levels (not shown) for all but one mouse fed AA, where the tumor did not appear to stop growing. "Relapse" of androgen-dependent tumors to androgen-independent growth was defined as the first time point after ablation when tumor volume (TV) or serum PSA levels started to increase (Figure 5A and B, respectively). In many individual mice PSA levels started to rise before increases were noted in TV. Individual pairwise comparisons by t-test suggested that "relapse" was significantly sooner in AA fed mice than in EPA fed mice ($P = 0.04$).

Experimental Design:



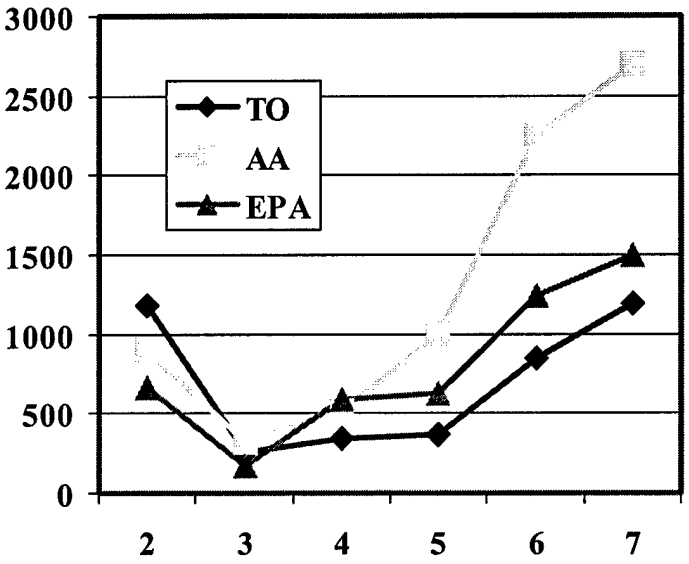


Figure 4: Changes in mean CWR22 tumor volumes after androgen ablation in animals fed oleic acid (TO), arachidonic acid (AA) or eicosapentaenoic acid (EPA).

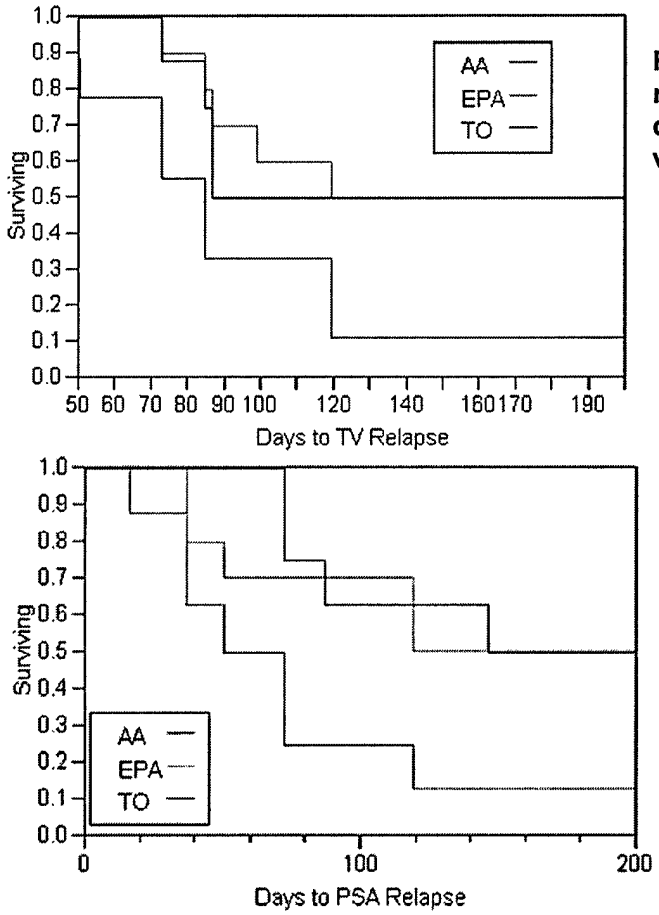


Figure 5A & B: Animals surviving with non-relapsing (androgen-dependent) tumors as defined by lack of renewed increases in tumor volume (TV) or PSA.

It is **concluded that dietary supplementation with AA**, which significantly enriches cancer tissues in this PUFA in addition to modulating levels of other fatty acids, **significantly reduces the time to relapsed tumor growth** following androgen-ablation therapy of dependent tumors.

CONTINUING RESEARCH EFFORTS - PLANS

- Given the surprising lack of correlation between tumor PUFA content and eicosanoid levels, gas chromatography - mass spectrometry will be used to confirm eicosanoid data presented in the report.
- Given the exciting results from Task 3, mRNA from CWR22 tumor lysates are being analyzed by Affymetrix microarray technology to screen for expression of genes which differentially respond to androgen ablation in prostate cancer tissues enriched for n-3 or n-6 PUFA.
- PC82 are being obtained from the originator of this xenograft in the Netherlands (MTA in place as of this report date), in an attempt to re-establish this xenograft in our laboratory and test for responses to ablation in the context of different dietary PUFA (i.e. Experiment 3B).
- When results are obtained from LAPC9 xenograft analyses all data will be published, most likely in 2 manuscripts to include Experiments 1, 2 and 4 in the first and Expt 3 in a second. The PI and co-PI will likely seek further funding to continue this work, which may have to include any more extensive, costly animal and/or Affymetrix gene array experiments. There are a number of intriguing questions that arise from our data and the clinical biologic implications certainly warrant further work.

- A summary of results obtained from LAPC9 tumor analyses (Task 3B), microarray gene analysis of CWR22 tumors, GC-MS analyses for 15-LO metabolites and copies of published articles derived from these funded studies will be forwarded to the U.S. Army Medical Research and Materiel Command.

STATEMENT OF WORK - SUMMARY:

While we were disappointed in the unexpected failure to detect a significant affect of dietary PUFA on prostatic tumor growth in Tasks 1 and 2, the data from Tasks 3 and 4 are very exciting and consistent with our original hypothesis. Growth of androgen-dependent and -independent prostatic tumors appears to be insensitive to dietary manipulation of PUFA content that enriches these tissues for n-3 series lipids. However, the same dietary changes may significantly enhance the sensitivity of "early" androgen-dependent prostatic cancer to therapeutic androgen ablation therapy and retard the inevitable conversion to androgen-independent growth. The net effect of this could be significant prolongation of therapeutic effects and therefore the potential return to a normal lifespan in affected individuals. Although extremely encouraging, we recognize that the results will have to be verified with subsequent studies in our laboratory as well as that of others. We also recognize that many questions remain to be answered before any clinical recommendations can be made, but the potential importance of these studies should drive this work forward.

KEY RESEARCH ACCOMPLISHMENTS

- Altering dietary intake of n-3 and n-6 PUFA significantly affects tissue levels of n-3 and n-6 fatty acids in androgen-dependent and androgen-independent prostatic cancer without significantly altering levels of 15-lipoxygenase metabolites.
- Cyclooxygenase activity in CWR22 xenografts is unlikely to be of biologic significance since metabolites and COX-2 enzyme levels are very low or non-existent.
- Modification of tumor n-3 and n-6 PUFA content through dietary manipulation does not affect the growth of either androgen-dependent or androgen-independent prostatic cancer xenografts in nude (*nu/nu*) mice.
- Dietary n-3 PUFA significantly enhances the beneficial affects afforded by androgen ablation therapy in androgen-dependent CWR22 prostatic cancer xenografts.
- Dietary n-6 PUFA significantly reduces the time to cancer relapse following androgen ablation, and therefore the post-therapeutic lifespan of the host.

REPORTABLE OUTCOMES

- BrdU labeled prostates and epididymal fat from male mice archived from various dietary groups for future analysis of cell proliferation and fatty acid synthase activity/expression.
- Funding of this grant proposal contributed to promotion of PI to Associate Professor at the University of Tennessee and a job offer from Cornell University.
- An invention disclosure (pertaining to the last 2 bulleted items under Key Accomplishments) has been submitted to the University of Tennessee Office of Research, with their encouragement, and is currently under review as a potential patent application by the University.

- We expect to publish 2 manuscripts from this work and will have a database of differentially expressed genes following androgen ablation treatment of androgen-dependent prostatic tumors enriched for either n-3 or n-6 polyunsaturated fatty acids.

CONCLUSIONS

Dietary intake of n-3 and n-6 polyunsaturated fatty acids does not have a significant affect on growth of early or late stage prostatic cancer. However, manipulation of tumor PUFA content by these dietary components significantly enhances the response of "early" stage, androgen-dependent prostatic cancer to androgen ablation therapy by increasing short term beneficial biologic responses and prolonging the post-therapeutic time to relapse and androgen-independent growth. If proven true by subsequent studies in our laboratory and others, the net effect of optimizing dietary PUFA content in patients undergoing androgen-ablation therapy would be a prolongation of the inevitable conversion to androgen-independent and, ultimately, fatal cancer growth. As an adjuvant to hormone therapy, this form of dietary intervention would be quite innocuous and could potentially provide a normal lifespan for the cancer patient.

List of Salaried Personnel

Michael McEntee, PI Jay Whelan, co-PI
Technicians: Benjamin Johnson, Alysyn Gardner, Carol Ziegler

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