RESEARCH COMMUNICATION

Pharmacodynamics of Fish Oil: Protective Effects Against Prostate Cancer in TRAMP Mice Fed with a High Fat Western Diet

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Abstract

Numerous epidemiological studies suggest that frequent consumption of fish would decrease certain major inflammatory-related chronic diseases including cancer. Aims: To investigate the cancer chemoprotective effect of fish oil (FO) in Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) mice fed a FO diet (10% Menhaden fish oil; FO group) versus a 20% high fat diet (HF group; typical of a Western diet), both with a total content of 20% fat and equal calories. Methods: For each diet, two experimental arms were performed. The mice were put on diet at 8th or 12th week of age for periods of 14 and 10 weeks, the experiments being terminated when the mice reached 22 weeks of age. The animals were monitored weekly for health, and upon necropsy were examined for whole body metastasis, and prostate tissues were confirmed with histopathology. Results: At the end of the study, the FO group had significantly reduced prostate tumor weight ($p<0.05$) compared to the HF group. The incidence of palpable tumors and carcinomas was also lowered. Finally, there was no metastasis found in the FO group, whereas in the HF group, 16.7% of the mice were found to have metastases. Conclusions: This is the first study showing the beneficial effects of FO against prostate cancer having a HF diet, suggesting potential beneficial effects of FO in humans consuming HF in their diet.

Keywords: Fish oil - high fat western diet - TRAMP - prostate cancer - prevention - PUFA

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Introduction

Prostate cancer (PCa) is a major health concern for men in the United States (Jemal et al., 2009). Presently, metastatic PCa is not curable and the mean survival is 2-3 years. The progression of localized prostatic cancer to metastatic and invasive disease is characterized by a long latent period from the precursor lesions called prostatic intraepithelial neoplasia (PIN) to invasive carcinoma and metastasis typically associated with elderly men (Nelson, 2004). Therefore, to decrease the incidence of PCa or delay the neoplastic development or slow the progression of PCa, it would be logical to intervene with relatively non-toxic dietary or chemical compounds, which would offer tremendous clinical benefits to men.

A follow-up of 30 years shows that men who eat no fish have a 2- to 3-fold higher frequency of PCa than those who eat moderate or high amounts (Terry et al., 2001). The most recent meta-analysis has concluded that the benefit of fish consumption resulted in a 63% reduction of prostate cancer-specific mortality (Szymanski et al., 2010). It was further suggested that epidemiologic studies would probably benefit from the assessment of specific fatty acids in the diet, including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and the ratio of these to n-6 fatty acids, dietary constituents that are not typically studied individually (Terry et al., 2003).

It is postulated that fish are rich in omega-3 polyunsaturated fatty acids (PUFA), which may lower PCa risk and progression via reducing the inflammatory processes (Chan et al., 2005). The beneficial effects of DHA and EPA from fish oil products have led to the FDA approval of Lovaza@ (previously Omacor@, omega-3-acid ethyl esters) for lowering triglycerides (McKenney and Sica, 2007). We have previously reported that PUFA such as DHA and EPA are indeed anti-inflammatory (Saw et al. 2010) and that the nuclear factor (erythroid-derived 2)-like 2 (Nfe2L2 or Nrf2) signaling pathway appears to be involved (Wang et al., 2010). Nrf2 is a key regulator of the antioxidant response element (ARE)-mediated genes that would protect against oxidative stress- and inflammation-induced diseases including PCa (Hu et al., 2010). In this study, we conducted a long-term feeding experiment in Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) mice by providing them specially designed diet containing 10% Menhaden fish oil (FO) supplemented with 10% corn

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oil (FO group) versus a second group of mice fed with a typical Western-type of high fat (HF) diet containing 20% fat (HF group), both diets contained 20% fat content and equal calories.

Materials and Methods

Animals

Female hemizygous C57BL/TGN TRAMP mice, line PB Tag 8247NG, and male C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The animals were bred on same genetic background, genotyped and maintained in the Laboratory Animal Service facility at Rutgers University as we have described previously (Barve et al 2008; 2009; 2010; Keum et al 2009; Khor et al 2009; Yu et al 2010), in accordance with the guidelines established by the University's Animal Research Committee consistent with the NIH Guidelines for the Care and Use of Laboratory Animals.

Diet and study design

The diet consumed by the mice before entering the study was PicoLab® Rodent Diet 20, 5053 from WF Fisher & Son Inc. (Somerville, NJ). The experimental diets were prepared by Research Diets Inc. (New Brunswick, NJ). The doses of HF and FO were selected based on previous published studies that 5.9 to 17.6 % FO diet was shown to be effective in preventing the incidence of colon adenocarcinoma when compared to 23.5% corn oil treated rats (Reddy and Sugie 1988). Later it was also reported that high fat diet containing 20% mixed lipids promoted colon tumorigenesis as compared to the normal AIN-76A diet and 17% fish oil diet supplemented with 3% corn oil diet (Rao et al 2001). For our current study, the animals were fed with AIN-76A-based HF diet consisted of 5.4% corn oil and 14.6% mixed lipids, abbreviated as HF (the diet has a total of 20% w/w fat content), or FO diet containing 10% Menhaden FO and 10% corn oil (each diet had a total of 20% w/w fat content), abbreviated as

Table 1. Compositions of the Diets

Macronutrient (%)	AIN-76A	HF	FO
Protein	20	24	24
Carbohydrate	66	46	46
Fat	5	20	20
kcal/g (approximate)	3.9	4.6	4.6
Diet ingredients (g)	-	-	-
Casein	200	235	235
DL-Methionine	3	3.5	3.5
Corn Starch	150	257	257
Sucrose	500	-	-
Maltodextrin 10	-	100	100
Dextrose	-	90.2	90.2
Cellulose	50	59	59
Corn oil	50	54	100
Mixed lipid[a]	-	146	-
Menhaden Oil	-	-	100
Mineral mix	35	41.1	41.1
Vitamin mix	10	11.8	11.8
Choline bitartrate	2	2.4	2.4

[a]Mixed lipid contains 32g beef fat, 20g lard, 24g milk fat, 60g hydrogenated coconut oil and 10g peanut oil.

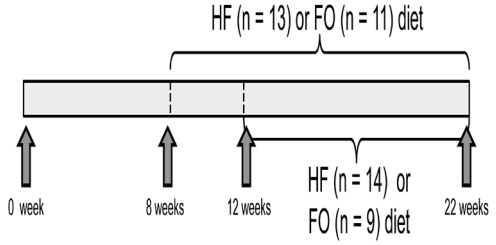

Figure 1. Overall Experimental Design. Eight or twelve week-old of TRAMP males were put on experimental HF diet or FO diet. The experimental diets were given for 14 weeks and 10 weeks respectively, till the age of 22 weeks.

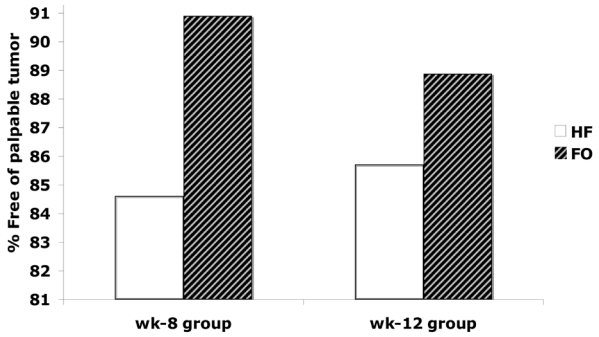

Figure 2. Percent Free of Palpable Tumor. The percentage of TRAMP mice free of a palpable tumor at the end of the experimental is presented. Mice started in 8 weeks and 12 weeks old fed with FO diet has a higher percent free of palpable tumor than those fed with HF diet.

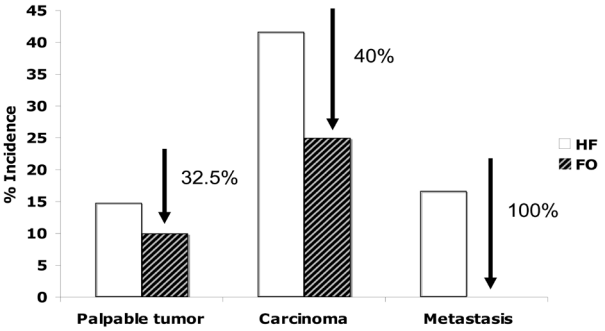

Figure 3. The Overall Percent of Palpable Tumor, Carcinoma and Metastasis in TRAMP Mice. Compared to mice fed with HF diet, mice fed with FO diet has lower incidence of palpable tumor and carcinoma, as well as without metastasis. The number of animals studied for palpable tumor (HF, n = 27 and FO, n = 20), and pathologically assessed carcinoma and metastasis (HF, n = 12 and FO, n = 8).

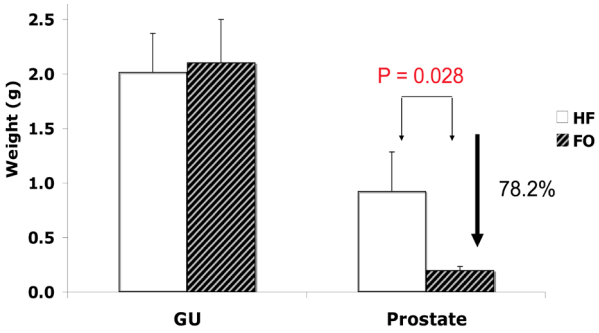

Figure 4. Tumor Weight of Genitourinary Apparatus (GU) and Prostate in TRAMP Mice Fed with FO or HF Diets. Prostate tumor weight was significantly lower in the FO group than the HF group (p = 0.028).

FO (Table 1). Both HF and FO diets were equal calories and the calories coming from fat were the same, i.e. 20%.

Male TRAMP mice received HF or FO diet starting at 8 weeks old (wk 8 group, n = 13 for HF; n = 11 for FO) and from 12 weeks old (wk 12 group, n = 14 for HF; n = 9 for FO). Small tumors or PIN lesions presumably started to form beginning at 8 weeks old, a time corresponding to sexual maturity as reported by Greenberg et al. (Greenberg et al 1995) (Figure 1). The mice were weighed weekly and their general health was monitored on a regular basis. All mice were sacrificed at the age of 22 weeks for HO and FO and the genitourinary apparatus (GU) consisting of seminal vesicles, prostate, and bladder were isolated for further analyses as described below.

Histopathology

The dorso-lateral prostate was excised and fixed in 10% formalin overnight and then transferred to 70% ethanol for additional 24 h. Tissue processing, slicing and evaluation were performed as previously in our laboratory (Barve et al., 2008; 2009; 2010; Khor et al., 2009) according to the established scale for TRAMP mice. The number of animals studied for palpable tumor (HF, n = 27 and FO, n = 20) and pathohistologically assessed carcinoma and metastasis (HF, n = 12 and FO, n = 8).

Statistical analysis

Values were reported as mean ± standard error of the mean (SEM). The student's *t*-test was used to determine the statistical differences of GU and prostate tumor weights. *P* value of smaller than 0.05 is considered statistically significance.

Results

TRAMP mice fed with FO diet had higher percentage free of palpable tumor and lower incidence of histopathologically confirmed carcinoma

The composition of dietary lipids has an effect on the formation of palpable tumors, carcinoma as well as metastasis (Figures 2 and 3). FO group had higher percentage of mice free of palpable tumor in both the wk-8 and wk-12 groups as compared to the HF diet (Figure 2). Figure 3 shows the effects of FO and HF on the incidence of palpable tumor, carcinoma and metastasis when both the wk-8 and wk-12 groups were combined. Higher incidence of palpable tumor in the HF group with a total of 11 out of 12 mice that were confirmed histolopathogically with prostate tumors and lesions (Figure 3). Additionally, HF fed TRAMP mice also had higher incidence of carcinoma (examined by a pathologist in a blinder fashion) as compared to the FO fed group. Furthermore, in the HF group, two mice had metastatic tumors and one mouse had a necrotic palpable tumor. In contrast, none of the mice in the FO group had necrosis or metastasis (Figure 3).

HF diet increases tumor weight whereas FO diet has anti-tumorigenesis effect with lower tumor weight

When the prostate weight was compared between the HF and the FO group, the FO group had significantly decreased the tumor burden in terms of prostate tumor weight by 78.2% as compared to that in the HF diet group ($p = 0.028$; Figure 4).

Discussion

Previously, it was reported that the Western-type of high-fat (HF) diet accelerated prostate tumor progression in TRAMP mice and increased tumor angiogenesis (Llaverias et al., 2010). We have also previously reported the efficacies of dietary phytochemicals including curcumin, phenylethylisothiocyanate (Barve et al., 2008), tocopherols (Barve et al., 2009), broccoli sprouts (Keum et al., 2009), dibenzoylmethane (Khor et al., 2009), tocotrienols (Barve et al., 2010) and indole-3-carbinol (Wu et al., 2011) in TRAMP mice. However, to date, no study has been reported on the cancer protective effect of fish oil (FO) in TRAMP mice. Menhaden FO is a rich source of the PUFA DHA and EPA. We have reported that very low doses of DHA and EPA that are achievable in human diet, could suppress inflammatory pathways with enhanced anti-oxidative stress gene heme-oxygenase 1 (HO-1) expression in RAW cells (Saw et al., 2010) and that the Nrf2 anti-oxidative stress signaling pathway plays a role in the anti-inflammatory activities of DHA and EPA using Nrf2 knockout macrophages (Wang et al., 2010). In our current study, we show that for the first time FO retarded the growth of prostate tumor in TRAMP mice when fed in conjunction with a HF diet. No apparent toxicity was observed for the mice receiving with the HF or FO diets.

We showed that by 22 weeks of age, four out of 27 TRAMP mice (14.8%) fed with HF diet developed palpable tumors (Figure 3). On the other hand, only two out of 20 mice (10%) fed with FO developed palpable tumors, representing a 32.5% of reduction of palpable tumor when compared to HF diet. There was also significant reduction (78.2%) in prostate weight in the FO group (Figure 4), correlates with the histopathological findings with increased carcinoma and metastasis in the HF group (Figure 3). Furthermore, most of the prostates found in the FO group appear to look normal, whereas in the HF group, there were several cases of severe carcinoma in that the seminal vesicles and bladder were not distinguishable from the prostate tumors (data not shown). Therefore, our findings show that FO has inhibitory effects on tumor formation and metastasis with significantly lowered prostate tumor weights, and no metastasis in TRAMP mice fed with 20% high fat diet consisted of 10% FO.

Recently, Nrf2 has emerged as a novel target for cancer prevention (Hu et al., 2010) and we have recently shown that Nrf2 is epigenetically silenced in prostate tumors in TRAMP mice (Yu et al., 2010). As prostate tumor progresses in TRAMP mice, there appears to be a progressive loss of expression of Nrf2 and its downstream target genes such as UDP-glucuronosyltransferase (UGT), glutathione S-transferase (GST) and HO-1 , which appears to correlate with human prostate cancer (Yu et al., 2010). Therefore, it is tempting to speculate that the anti-oxidative stress and anti-inflammatory actions of DHA and EPA in fish oil could possibly explain for the cancer chemopreventive effects observed in our current

study. However, further study would be needed to address these mechanisms.

In conclusion, using fish oil as a cancer chemopreventive agent in TRAMP mouse model, we show for the first time that even in a high fat Western diet, fish oil is able to attenuate prostate cancer formation and metastasis as compared to the non-fish oil high fat Western diet.

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