Fish oil slows prostate cancer xenograft growth relative to other dietary fats and is associated with decreased mitochondrial and insulin pathway gene expression

JC Lloyd1,2, EM Masko1,2, C Wu1,2, MM Keenan3, DM Pilla3, WJ Aronson4, J-TA Chi3 and SJ Freedland1,2,5

BACKGROUND: Previous mouse studies suggest that decreasing dietary fat content can slow prostate cancer (PCa) growth. To our knowledge, no study has yet compared the effect of multiple different fats on PCa progression. We sought to systematically compare the effect of fish oil, olive oil, corn oil and animal fat on PCa progression.

METHODS: A total of 96 male severe combined immunodeficient mice were injected with LAPC-4 human PCa cells. Two weeks following injection, mice were randomized to a Western diet based on fish oil, olive oil, corn oil or animal fat (35% kilocalories from fat). Animals were euthanized when tumor volumes reached 1000 mm³. Serum was collected at death and assayed for PSA, insulin, insulin-like growth factor-1 (IGF-1), IGF-1-binding protein-3 and prostaglandin E-2 (PGE-2) levels. Tumors were also assayed for PGE-2 and cyclooxygenase-2 levels, and global gene expression was analyzed using Affymetrix microarrays.

RESULTS: Mice weights and tumor volumes were equivalent across groups at randomization. Overall, fish oil consumption was associated with improved survival relative to other dietary groups (P = 0.014). On gene expression analyses, the fish oil group had decreased signal in pathways related to mitochondrial physiology and insulin synthesis/secretion.

CONCLUSIONS: In this xenograft model, we found that consuming a diet in which fish oil was the only fat source slowed tumor growth and improved survival compared with that in mice consuming diets composed of olive oil, corn oil or animal fat. Although prior studies showed that the amount of fat is important for PCa growth, this study suggests that the type of dietary fat consumed may also be important.

Prostate Cancer and Prostatic Disease (2013) 16, 285–291; doi:10.1038/pcan.2013.19; published online 23 July 2013

Keywords: prostatic neoplasms; diet; fat

INTRODUCTION

Prostate cancer (PCa) is the second leading cause of cancer-related death among men in Western society.1,2 Geographical differences in incidence rates suggest that environmental factors play a significant role in the progression and development of PCa. As diet varies significantly across nationalities and is an easily modified, low-cost intervention, many prior studies have queried the role of dietary fat in the progression and development of PCa.3–6 Differences in incidence rates suggest that environmental factors may also be important.

Previous mouse studies suggest that decreasing dietary fat content can slow prostate cancer (PCa) growth. To our knowledge, no study has yet compared the effect of multiple different fats on PCa progression. We sought to systematically examine this question and perhaps identify mechanisms by which more ‘prostate-healthy’ fats exert their effects. We hypothesized that high in both ω-3 fatty acids and olive oil-derived fats may protect against many forms of cancer, including prostate.12 On the contrary, other studies have suggested that increased consumption of ω-6 fatty acids13 and animal fats (saturated fat)14 can increase the risk of PCa.

Similarly, in vivo animal studies have suggested that increased dietary fat promotes PCa growth.15–17 However, the vast majority of these studies have focused on the amount of dietary fat and neglected to examine the type of fat consumed. Of major dietary fat sources, fish oil is the most heavily studied source. Previous mouse xenograft studies have suggested that diets high in fish oil (an ω-3 fatty acid), relative to diets with a greater proportion of corn oil (an ω-6 fatty acid), may slow tumor growth and prolong survival.16,19 Prior studies by our group have suggested that decreasing dietary saturated fat levels (animal fats, such as milk fat and lard) do not prolong survival in either intact or castrated mouse PCa xenograft models.20,23 However, to our knowledge, never before has a head-to-head trial of the effect of multiple types of dietary fat on tumor progression and PCa survival been undertaken. As such, we sought to systematically examine this question and perhaps identify mechanisms by which more ‘prostate-healthy’ fats exert their effects. We hypothesized that...
Fish oil and olive oil-based diets would slow tumor progression relative to the ‘less healthy’ corn oil- and animal fat-based diets.

MATERIALS AND METHODS

Cell culture

LAPC-4 human PCa cells were a generous gift from William J Aronson, University of California (UCLA) School of Medicine. This cell line was developed at UCLA by direct transfer of cancer cells from a patient with advanced prostate adenocarcinoma. LAPC-4 produces PSA, has a wild-type androgen receptor and shows features of hormone-dependent growth and metastasis.22 This cell line has been frequently used to model localized androgen-sensitive disease.23–25 We specifically chose this cell line for its hormone dependence, as we aim to model the effect of dietary intervention on early-stage disease, which is typically androgen sensitive. Cells were maintained in Iscove's modified medium with 10% fetal bovine serum and supplemented with the synthetic androgen R1881 at 1 nM. Cells were grown in 5% CO2 at 37°C and harvested by trypsinization at ~80% confluence in log-phase growth.

Animal studies

After approval from the Duke University Institutional Animal Care and Use Committee, 96 male severe combined immunodeficient (SCID) (CB.17 scid/scid) mice, aged 8 weeks, were purchased from Taconic Farms (Hudson, NY, USA). Animals were housed five to a cage and fed an ad libitum diet of standard mouse chow (20% protein, 9% fat and 71% carbohydrate kilocalories) for a 1-week acclimation period. Following acclimation, mice were injected in the flank with $1 \times 10^7$ LAPC-4 cells in 0.1 ml of Matrigel (Becton Dickinson, Franklin Lakes, NJ, USA) and returned to group housing with ad libitum feeding using standard mouse chow. Eleven days after injection, mice were transitioned to single housing. Given the importance of energy balance in modulating tumor growth, all mice were housed one per cage for the duration of the study to permit precise measurement of caloric intake.26

Given that changes in housing can be stressful, we waited 3 additional days before randomizing the mice to the four-diet arms at 2 weeks after injection. We elected to randomize the mice at 2 weeks following injection to ensure that we were studying the effect of diet on PCa progression rather than disease initiation. By 14 days after injection, all tumor xenografts were likely to have taken and become metabolically active, allowing us to model treatment of early stage disease. Mice were randomized to one of four diets (all 16% protein, 35% fat, 49% carbohydrate kilocalories), which differed only in their dietary fat source. Fat sources were fish oil, olive oil, corn oil and animal fat (lard/milk fat). The diets were prepared by TestDiet (Indianapolis, IN, USA) (Table 1). Primary fatty acid compositions were 30–40% ω-3 for fish oil, 65–80% oleic acid for olive oil, 54% ω-6 for corn oil and 56% saturated fat for the animal fat (Table 2). In a pilot study (data not shown), we determined that fish oil-fed mice consumed fewer calories on average than the other groups. As such, the fish oil group was fed ad libitum, and in the other groups were fed via a modified pair-feeding protocol to maintain isocaloric intake between the groups.26 Mice were weighed twice weekly to ensure equal body weights across groups.

When tumors became palpable, their dimensions were measured using digital caliper. Tumor volumes, measured twice weekly, were calculated using the following formula: width × height × length × 0.5236.27 At 3 weeks after randomization to the diets (5 weeks post-tumor injection), mice were bled via the facial vein, and blood glucose was measured using a handheld Accu-Chek Active glucometer (Roche Diagnostics, Indianapolis, IN, USA). Glucose measurements were taken immediately prior to feeding, which likely reflected a partially fasted state, as the majority of the animals consumed all of their prescribed diet prior to food allocation each day. However, we did not remove the diet from the animals’ cages at any given time before assessing glucose levels, so we cannot comment on how long the animals were fasted prior assessment.

Animals were euthanized using a lethal dose of pentobarbital when tumor volumes reached 1000 mm3 or when the health of the animal appeared compromised per institutional criteria (ruffled fur, hunched posture, lethargy, weight loss, etc.). Serum was obtained via cardiac puncture. All tumor tissue was surgically excised from the subcutaneous flank pockets in which it was growing. Serum and tumor samples were snap frozen at ~80°C for subsequent analysis.

Serum from eight median-surviving mice from each group (total 32 mice) was assayed for levels of murine insulin, insulin-like growth factor (IGF)-1, IGF-1-binding protein-3 (IGFBP-3) and prostaglandin E-2 (PGE-2) using mouse-specific enzyme-linked immunosorbent assays (Linco, Billerica, MA, USA; R&D Systems, Minneapolis, MN, USA; and Neogen, Lexington, KY, USA, respectively). Serum was also assessed for human PSA produced by the LAPC-4 xenograft using enzyme-linked immunosorbent assay (Abzyme, Needham, MA, USA).

Western blot analysis was conducted on the xenograft tumor tissue of six median-surviving mice from each group (total 24 mice). Tissue lysate was first prepared using the QProteome Mammalian Protein Preparation kit (Qiagen, Valencia, CA, USA). A total of 1 ml cell lysis buffer was added to 50–60 μg of prostate xenograft tumor tissue and processed using a mechanical tissue homogenizer. Homogenates were centrifuged at 10,000 r.p.m. for 20 min to clarify the lysates, and total protein concentration was determined using the BCA Protein Assay Reagent (Thermo Fisher Scientific, Rockford, IL, USA). All lysates were stored at ~80°C until further analysis.

To conduct the western blot analysis, denatured samples of tissue homogenates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and subsequent immunoblotting using the Fast Western Blot kit (Pierce–Thermo Scientific, Rockford, IL, USA) to determine expression of target proteins. The primary antibody used for immunoblotting was anti-cyclooxygenase-2 (COX-2) (160112) from Cayman Chemical (Ann Arbor, MI, USA). Given the need to run 24 samples and given that the gels hold 12 samples, we ran samples of three mice per group from four groups on each gel, requiring a total of two gels to be analyzed. These experiments were repeated twice. Densitometric analysis was performed using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

RNA isolation and microarray analysis

Total RNA from the excised xenograft tumors of each of the six median-surviving mice per group was purified with TRIzol (Invitrogen, Grand Island, NY, USA) and verified to be intact by Bioanalyzer. Extracted RNA was then hybridized to Affymetrix Human genome 133A 2.0 arrays (Affymetrix, Santa Clara, CA, USA) using a standard protocol described previously.28 All data have been deposited into .cel files (GEO: GSE40654).

Statistical analysis

The primary end point was survival, defined as time from randomization to death, which was examined using the log-rank test. Graphically, survival was represented using Kaplan–Meier curves. Comparisons of secondary outcomes including body weight, tumor volume, serum levels of PSA, IGFI/GFII and insulin were performed using Student’s t-test. All data are represented using Kaplan–Meier curves. Comparisons of secondary outcomes including body weight, tumor volume, serum levels of PSA, IGFI/GFII and insulin were performed using Student’s t-test. All data are represented using Kaplan–Meier curves.
axis hormones, glucose and PGE-2 were made, and tumor COX-2 levels were determined using the Kruskal–Wallis test.

For microarray analyses, all .cel files were normalized by the robust multi-average method (Expression Console, Affymetrix). Given the distinct biological response of the fish oil, we compared differences in biological pathways between the six median-surviving fish oil-fed mice and the six median-surviving mice from each of the other dietary groups combined (18 samples total). Data were analyzed with gene set enrichment analysis using the Broad public server to identify biological pathways affected by dietary fat source.29

All statistical analyses were performed using STATA 10.0 (Stata, College Station, TX, USA), with \( P \leq 0.05 \) considered statistically significant.

## RESULTS

### Body weight

At randomization, mouse weights were equivalent across arms \(( P = 0.99)\). A pair-feeding protocol allowed for isocaloric feeding between groups. Despite careful titration of caloric intake, mouse body weights differed significantly across groups at a small number of time points (Figure 1). Of note, during the first 20 days, fish oil-fed mice were slightly lighter than the other groups (typically \(< 1 \text{ g} \) difference in body weight), with no appreciable differences during the next 20 days. By day 40, fish oil-fed mice

| Carbon atoms | Formal name | Corn oil (%) | Fish oil (%) | Olive oil (%) | Animal fat (%) |
|--------------|-------------|--------------|--------------|---------------|----------------|
| C4:0         | Butyric     | <0.06        | —            | —             | 1.04           |
| C6:0         | Caproic     | <0.06        | —            | —             | 1.21           |
| C7:0         | Heptanoic   | —            | —            | —             | <0.1           |
| C8:0         | Caprylic    | <0.06        | —            | —             | 0.9            |
| C9:0         | Nonanoic    | <0.06        | —            | —             | <0.1           |
| C10:0        | Capric      | <0.06        | —            | —             | 2.25           |
| C11:0        | Undecanoic  | —            | —            | —             | 0.23           |
| C12:0        | Lauric      | <0.06        | —            | —             | 2.72           |
| C13:0        | Tridecanoic | —            | —            | —             | 0.15           |
| C14:0        | Myristic    | <0.06        | 6.85         | 0.01          | 9.47           |
| C14:1n5      | Myristoleic | <0.06        | —            | —             | 0.660          |
| C15:0        | Pentadecanoic | <0.06    | 0.46         | —             | 0.95           |
| C15:1n5      | 10-Pentadecenoic | <0.06 | —            | —             | <0.1           |
| C16:0        | Palmitic    | 10.3         | 14.8         | 11.9          | 28.2           |
| C16:1n7      | Palmitoleic | 0.12         | 9.74         | —             | 1.51           |
| C17:0        | Margaric    | 0.08         | 0.38         | 0.1           | 0.64           |
| C17:1n7      | Margaroleic | <0.06        | —            | 0.1           | <0.1           |
| C16:2        | Hexadecadienoic | —      | 1.62         | —             | <0.1           |
| C18:0        | Stearic     | 1.98         | 2.55         | 3.4           | 13.8           |
| C18:1n9T     | Elaidic     | 0.08         | —            | —             | 2.96           |
| C16:3n4      | Hexadecatrienic | —        | 1.51         | —             | <0.1           |
| C18:1n9C     | Oleic       | —            | 9.58         | 77.2          | 22.8           |
| C18:1n7C     | Vaccenic    | —            | —            | —             | 0.94           |
| C18:1       | Other cis isomers | —        | —            | —             | 1.4            |
| C19:0       | Nonadecanoic | —            | —            | —             | 0.24           |
| C18:2      | Other trans isomers | 0.40  | —            | —             | 0.94           |
| C16:4n1     | Hexadecatetraenoic | —        | 1.53         | —             | <0.10          |
| C18:2n6     | Linoleic    | 53.7         | 1.93         | 3.9           | 3.16           |
| C20:0       | Arachidic   | 0.40         | 0.17         | 0.3           | 0.16           |
| C18:3       | Trans isomers | <0.06      | —            | —             | <0.1           |
| C13:3n6     | g-Linolenic | —            | —            | —             | <0.1           |
| C18:3     | Octadecatrienic | —        | —            | —             | <0.1           |
| C20:1n11    | Eicosenoic  | —            | 1.48         | 0.2           | 0.15           |
| C18:3n3     | Linolenic   | —            | 1.48         | 0.7           | 0.34           |
| C21:0      | Heneicosanoic | —         | —            | —             | <0.1           |
| C18:4n3     | Octadecatetraenoic | —    | 3.09         | —             | <0.1           |
| C20:2n6     | Eicosadienoic | <0.06   | 0.18         | —             | <0.1           |
| C22:0       | Behenic     | 0.12         | 0.10         | 0.1           | <0.1           |
| C20:3n6     | Homo-gamma-linolenic | —   | —            | —             | 0.12           |
| C22:1n11    | Docosanoic  | —            | —            | —             | <0.1           |
| C22:1n9     | Erucic      | <0.06        | 0.33         | —             | <0.1           |
| C20:3n3     | Eicosatrienoic | <0.06  | 0.37         | —             | <0.1           |
| C20:4n6     | Arachidonic | <0.06        | 2.09         | —             | 0.12           |
| C23:0       | Tricosanoic | —            | —            | —             | <0.1           |
| C20:4n3     | Eicosatetraenoic | —     | —            | —             | <0.1           |
| C22:2n6     | Docosadienoic | —        | —            | —             | <0.1           |
| C24:0       | Lignoceric  | 0.17         | 0.60         | Trace         | <0.1           |
| C25:2n3     | Eicosapentaenoic | <0.06 | 14.16        | —             | <0.1           |
| C21:5       | Nervonic    | —            | —            | —             | <0.1           |
| C22:5n6     | Heneicosapentaenoic | —       | 0.76         | —             | <0.1           |
| C22:5n3     | Docosapentaenoic | <0.06  | 2.82         | —             | <0.1           |
| C22:6n3     | Docosahexaenoic | <0.06    | 12.2         | —             | <0.1           |
| C22:4       | Adrenic     | —            | 0.24         | —             | <0.1           |
| C24:1       | Selacholeic | —            | 0.22         | —             | <0.1           |
| Others      | —            | —            | —            | —             | 2.7            |
were the heaviest and remained so for the majority of subsequent time points until only a few mice were still alive, near day 95. Overall, all mice consumed their prescribed diets without observed toxicity in any group.

**Tumor growth and survival**

Median tumor volumes at randomization (day 14) were equivalent across all four groups ($P = 0.99$; Figure 2). As the study progressed, disparities in tumor growth became apparent. From approximately day 28 (14 days after randomization), fish oil-fed mice demonstrated significantly smaller tumors than mice in each of the other dietary groups. This trend continued throughout the duration of the study. Although fish oil-fed mice had smaller tumors at every time point, these differences reached statistical significance only at certain, but not all, time points. Of the 96 mice included in the study, 11 (12%) were required to be killed prior to their tumor volumes reaching 1000 mm$^3$ due to apparent compromise in overall health. Of these mice, two were from the corn oil group, three from fish oil, three from olive oil and three from the animal fat group.

Overall, there were trends toward an association between diet and survival, although this did not reach statistical significance ($P = 0.093$, log-rank test; Figure 3). When examined in two-way analyses, mice in the fish oil group survived longer than mice in any other group ($P = 0.014$ for fish vs corn, $P = 0.017$ for fish vs olive, $P = 0.090$ for fish vs animal fat). After combining the three non-fish oil groups, all of which had similar survival times, fish oil was associated with a significant improvement in survival vs non-fish oil diets ($P = 0.014$, log-rank test; Figure 4).

**Gene expression profiles and gene set enrichment analysis**

To understand the mechanisms underlying the slower growth of xenograft tumors in mice consuming the fish oil diet, we performed global gene expression analysis using Affymetrix U133 A2 arrays to analyze six xenograft tumors excised from each of the four treatment groups for a total of 24 samples. The microarray data were normalized by the robust multi-average method, and the differences in the pathway composition between the 6 fish oil and 18 non-fish oil diet samples were compared using gene set enrichment analysis. The gene set enrichment analysis applied a Kolmogorov–Smirnov statistic to determine whether specific biological processes (represented as 4850 gene sets in the Molecular Signature Database (MSigDB) http://www.broadinstitute.org/gsea/msigdb/collections.jsp#C2) were enriched or depleted in tumors of mice taking fish oil. This analysis revealed 41 gene sets that were depleted in fish oil samples with a false discovery rate of <25%. No gene set was enriched in the fish oil samples. Among the gene sets
most significantly depleted in the fish oil group, two biological processes were particularly prominent: (1) regulation of insulin synthesis and secretion (e.g., gene sets related to Reactome insulin synthesis, glucose regulation of insulin secretion and diabetes pathways) (Figure 6b) and (2) mitochondrial activity (e.g., gene sets related to the mitochondrial gene module and mitochondrial oxidative phosphorylation) (Figure 6c).

DISCUSSION

Epidemiologic studies suggest that increased dietary fat consumption has a negative effect on PCa outcomes; however, it is unclear to what extent the type of dietary fat consumed influences disease initiation and progression. Indeed, both population-based and xenograft studies have suggested that fish oil consumption decreases PCa risk. Other studies have shown that,
Fish oil slows prostate cancer growth

JC Lloyd et al

when using a corn oil-based diet, decreasing total dietary fat increases survival in a xenograft model. In contrast, another study demonstrated no improvement in PCA outcome when the amount of dietary fat was decreased using a saturated fat-based diet. This raised the hypothesis that, in addition to the amount of dietary fat being important, the type of fat mattered. Thus, we sought to systematically examine the effects of various types of fat on PCA progression. We found that male mice xenografted with LAPC-4 tumors fed a diet in which the dietary fat source was fish oil outlived mice fed diets composed of corn oil, olive oil or animal fat; survival was similar across the non-fish oil groups. Gene expression analyses revealed decreased signal related to mitochondrial and insulin synthesis/secretion pathways in the fish oil-fed mice relative to other groups, suggesting that these pathways may be important for mediating the anti-PCA activity of our fish oil-based diet.

Numerous but not all population-based studies have demonstrated a correlation between high dietary fat intake and PCA initiation/progression. However, these studies comment only on the amount, not the type, of dietary fat consumed; most of these studies assessed people eating a primarily ‘Western’ diet high in fats from vegetable oils, meat and dairy sources, all of which are thought to promote PCA development and progression. Thus, it is noteworthy that other studies have shown both a diet high in cold-water fish oil and the Mediterranean diet (rich in plant foods, fish and olive oil) to decrease PCA risk. Moreover, a prospective phase II randomized trial of men undergoing radical prostatectomy showed that a low-fat diet with fish oil supplementation reduced tumor Ki67 expression compared with Western diet controls, although promising future studies are required to apply this intervention in a longer-term setting.

In a mouse xenograft model, high consumption of ω-3 fatty acids (the primary fatty component of fish oil) has been shown to impair tumor cell proliferation, increase apoptosis and reduce tumor mass. These findings align with the results of the current study, in which fish oil-fed mice demonstrated prolonged survival relative to mice consuming diets in which the fat source was olive oil, corn oil or animal fat. The beneficial effects of fish oil are thought to stem from the ability of ω-3 fatty acids to suppress the production of arachidonic acid, which, in turn, suppresses the production of arachidonic acid-derived eicosanoids, in particular, prostaglandins and thromboxanes. Prostaglandins produced from arachidonic acid (specifically, PGE-2) tend to be pro-inflammatory and pro-proliferative in contrast to prostaglandins derived from ω-3 fatty acids, which tend to be less favorable for the growth and development of cancer cells. Moreover, incorporation of ω-3 fatty acids has been shown to suppress the production of COX-2, which further diminishes the production of pro-inflammatory prostaglandins.

In this study, however, we found no significant difference in serum PGE-2 and tumor COX-2 levels across the study groups. This may be due to the relatively small sample size or perhaps argues for an alternative mechanism underlying the positive effect seen with the fish oil diet. Alternatively, using samples at the time of death when all mice have large tumors, we may have missed the window of time wherein there were differences in PGE-2 and/or COX-2. However, in support of the idea that fish oil may alter PCA biology independent of COX-2 and PGE-2, we note a recent preprostatectomy randomized trial that showed that fish oil supplementation lowered tumor Ki67 levels without changes in COX-2 or PGE-2 levels. Therefore, as we noted that prior dietary studies by our group appeared to work via altering IGF/insulin hormone levels, we evaluated the insulin/IGF axis. However, again, no significant differences were noted.

Given the lack of corroborative data to support our two leading hypotheses for why fish oil-fed mice survived the longest, we turned to gene expression analysis. Using this approach, we found significant downregulation of pathways related to insulin synthesis and secretion as well as mitochondrial activity in tumors from mice fed fish oil. Although serum insulin levels were not different between the fish oil arm and other dietary groups, such a comparison may not fully reflect insulin activity within the tumor microenvironment. Thus, although we cannot easily explain how a fish oil diet may have altered the tumor microenvironment to specifically inhibit insulin signaling, the gene expression data are nonetheless hypothesis generating, suggesting the existence of such a difference in insulin signaling between the groups. Indeed, this may have contributed to prolonged survival of the fish oil-fed mice, given that insulin is a potent direct mitogen for PCs. Moreover, a recent study found that insulin increases de novo steroidogenesis, including androgen production, in PCs. Notably, cholesterol — the backbone of steroid synthesis — must be imported into the mitochondria for conversion to pregnenolone before steroidogenesis/androgen synthesis can occur. Thus, the results revealed in our gene expression analyses, namely, decreased insulin signaling and decreased mitochondrial activity, appear to fit with these external findings. Ultimately, further studies are needed to better elucidate the exact mechanisms through which a fish oil-based diet slows PCA xenograft growth.

This study must be viewed in light of its limitations. First, as with any single animal model, further studies in other models are needed before any generalizable conclusions can be drawn. More broadly, the use of an animal model is itself a limitation, given that to what degree animal studies model human PCs is unclear and ultimately these results need to be validated in human studies. Second, our study utilized a simplified diet in which all dietary fat was provided by a single source, which is unlikely to be reproduced in humans given the varied and complex nature of human dietary consumption. Moreover, evolving understandings of ethnic variations in bioavailability of dietary fatty acid metabolites must be considered; as such, it is possible that these interventions would require targeting to specific racial/ethnic groups for maximal efficacy. Third, we observed higher blood glucose in fish oil-fed mice at the time of death. This likely represents a slight overfeeding of fish oil-fed mice relative to other groups, an assessment supported by the observation that mice in the fish oil group were slightly but significantly heavier at time of death than mice in other groups. However, given that increased caloric intake and body mass are generally positively associated with larger and more aggressive PCA tumors, prolonged survival of the fish oil-fed mice in our study actually argues even more strongly for the beneficial effects of this diet.

CONCLUSION

We found that in a mouse PCA xenograft model, mice fed a diet in which fish oil provided the sole source of fat outlived mice fed diets composed of corn oil, olive oil or animal fat; survival was similar across the non-fish oil groups. On gene expression analysis, the fish oil-fed group demonstrated downregulation of pathways related to mitochondrial activity and insulin synthesis/secretion, suggesting the hypothesis that these pathways may be important in producing the benefits of the fish oil diet. The results of our study suggest that increased dietary intake of fish oil may slow PCA progression. Further research is needed to characterize the utility of this potentially low-cost, low-risk intervention in human populations as well as to better understand the potential mechanisms through which this intervention slows PCA progression.
CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGEMENTS
We would like to acknowledge the contributions of Jodi Antonelli, Jean-Alfred Thomas II and Tamarra T. E. Phillips. This study was supported by the Department of Veterans Affairs; Division of Urology, Department of Surgery, Duke University: Prostate Cancer Foundation; NIH Training Grant 1 TL1 RB024126; and NIH S01 CA131235-03.

REFERENCES
1 Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics. 2009. CA Cancer J Clin 2009; 59: 229–249.
2 Hsing AW, Tsao L, Devesa SS. International trends and patterns of prostate cancer incidence and mortality. Int J Cancer 2000; 85: 60–67.
3 Kolonel LN, Nomura AM, Cooney RV. Dietary fat and prostate cancer: current status. J Natl Cancer Inst 1999; 91: 414–428.
4 Villeneuve PJ, Johnson KC, Kreiger N, Mao Y. Risk factors for prostate cancer: results from the Canadian National Enhanced Cancer Surveillance System. The Canadian Cancer Registries Epidemiology Research Group. Cancer Causes Control 1999; 10: 355–367.
5 Kolonel LN. Fat, meat, and prostate cancer. Epidemiol Rev 2001; 23: 72–81.
6 Chan JM, Gann PH, Giovannucci EL. Role of diet in prostate cancer development and progression. J Clin Oncol 2005; 23: 8152–8160.
7 Clinton SK, Giovannucci E. Diet, nutrition, and prostate cancer. Ann Rev Nutr 1998; 18: 413–440.
8 Crowe FL, Key TJ, Appleby PN, Travis RC, Overvad K, Jakobsen MU et al. Dietary fat intake and risk of prostate cancer in the European Prospective Investigation into Cancer and Nutrition. Am J Clin Nutr 2008; 87: 1405–1413.
9 Norrish AE, Skeaff CM, Arribas GL, Sharpe SJ, Jackson RT. Prostate cancer risk and consumption of fish oils: a dietary biomarker-based case-control study. Br J Cancer 1999; 81: 1238–1242.
10 Terry P, Lichtenstein P, Feychting M, Ahlbom A, Wall A. Fatty fish consumption and risk of prostate cancer. Lancet 2001; 357: 1764–1766.
11 Augustsson K, Michaux DS, Rimm EB, Leitzmann MF, Stampfer MJ, Willett WC et al. A prospective study of intake of fish and marine fatty acids and prostate cancer. Cancer Epidemiol Biomarkers Prev 2003; 12: 64–67.
12 de Lorgeril M, Salen P, Martin JL, Monjaud I, Boucher P, Mamelle N. Mediterranean dietary pattern in a randomized trial: prolonged survival and possible reduced cancer rate. Arch Intern Med 1998; 158: 1181–1187.
13 Godley PA, Campbell MK, Gallagher P, Martinson FE, Mohler JL, Sandler RS. Biomarkers of essential fatty acid consumption and risk of prostatic carcinoma. Cancer Epidemiol Biomarkers Prev 1996; 5: 889–895.
14 Mills PK, Beeson WL, Phillips RL, Fraser GE. Cohort study of diet, lifestyle, and prostate cancer in Adventist men. Cancer 1989; 64: 598–604.
15 Wang Y, Corr JG, Thaler HT, Tao Y, Fair WR, Heston WD. Decreased growth of metastatic human prostate cancer to androgen independence in immunodeficient SCID mice. Nat Med 1997; 3: 402–408.
23 Gravina GL, Maramonop F, Guisti I, Carosa E, De Sante S, Ricevuto E et al. Differential effects of PDX101 (belinostat) on androgen-dependent and androgen-independet prostate cancer models. Int J Oncol 2012; 40: 711–720.
24 Emonds KM, Swinnen JV, Lerut E, Koole M, Mortelmans L, Mottaghy FM. Evaluation of androgen-induced effects on the uptake of [11C]choline and [11C]acetate in an androgen-sensitive and androgen-independent prostate cancer xenograft model. JNMMPM 2010; 31: 31.
25 Thomas R, Sharifi N. SOD mimetics: a novel class of androgen receptor inhibitors that suppresses castration-resistant growth of prostate cancer. Mol Cancer Ther 2012; 11: 87–97.
26 Mukherjee P, Sotnikov AV, Mangian HJ, Zhou JR, Visek WJ, Clinton SK. Energy intake and prostate tumor growth, angiogenesis, and vascular endothelial growth factor expression. J Natl Cancer Inst 1999; 91: 512–523.
27 Thomas 2nd JA, Antonelli JA, Lloyd JC, Masko EM, Poulton SH, Phillips TE et al. Effect of intermittent fasting on prostate cancer tumor growth in a mouse model. Prostate Cancer Prostatic Dis 2010; 13: 350–355.
28 Chen JL, Merl D, Peterson CW, Wu J, Liu PY, Yin H et al. Lactic acidosis triggers starvation response with paradoxical induction of TXNIP through Monoaok. PLoS Genet 2010; 6: 1–18.
29 Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA 2005; 102: 15545–15550.
30 Kolonel LN. Nutrition and prostate cancer. Cancer Causes Control 1996; 7: 83–94.
31 Lee MM, Wang RT, Hsing AW, Gu FL, Wang T, Spitz MR. Case–control study of diet and prostate cancer in China. Cancer Causes Control 1998; 9: 545–552.
32 Whittomere AS, Kolonel LN, Wu AH, John EM, Gallagher RP, Howe GR et al. Prostate cancer in relation to diet, physical activity, and body size in blacks, whites, and Asians in the United States and Canada. J Natl Cancer Inst 1995; 87: 652–661.
33 Park SY, Murphy SP, Wilkens LR, Henderson BE, Kolonel LN. Fat and meat intake and prostate cancer risk: the multiracial cohort study. Int J Cancer 2007; 121: 1339–1345.
34 Ramon JM, Bou R, Rromea S, Alzica ME, Jacas M, Ribles J et al. Dietary fat intake and prostate cancer risk: a case–control study in Spain. Cancer Causes Control 2000; 11: 679–685.
35 Trichopoulou A, Lagiou P, Kuper H, Trichopoulou D. Cancer and Mediterranean dietary traditions. Cancer Epidemiol Biomarkers Prev 2000; 9: 869–873.
36 Aronson WJ, Kobayashi N, Barnard RJ, Henning S, Huang M, Jardack PM et al. Phase II prospective randomized trial of a low-fat diet with fish oil supplementation in men undergoing radical prostatectomy. Cancer Prev Res (Phila) 2011; 4: 2062–2071.
37 Li Z, Aronson WJ, Arteaga JR, Hong K, Thames G, Henning SM et al. Feasibility of a low-fat/high-fiber diet intervention with soy supplementation in prostate cancer patients after prostatectomy. Eur J Clin Nutr 2008; 62: 526–536.
38 Connolly JM, Coleman M, Rose DP. Effects of dietary fatty acids on DU145 human prostate cancer cell growth in athymic nude mice. Nutr Cancer 1997; 29: 114–119.
39 Hardman WE. (n-3) fatty acids and cancer therapy. J Nutr 2004; 134(Suppl 12): 3427S–3430S.
40 Needleman P, Raz A, Minkes MS, Ferrendelli JA, Sprecher H. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. Proc Natl Acad Sci USA 1979; 76: 944–948.
41 McKeelhan WL, Adams PS, Rossier MP. Direct mitogenic effects of insulin, epidermal growth factor, glucocorticoid, cholera toxin, unknown pituitary factors and possibly prolactin, but not androgen, on normal rat prostate epithelial cells in serum-free, primary cell culture. Cancer Res 1984; 44: 1998–2010.
42 Hsing AW, Chua Jr S, Gao Y, Gentschev E, Chang L, Deng J et al. Prostate cancer risk and serum levels of insulin and leptin: a population-based study. J Natl Cancer Inst 2001; 93: 783–789.
43 Lubik AA, Gunter JH, Hendy SC, Locke JA, Adomat HH, Thompson V et al. Insulin increases de novo steroidogenesis in prostate cancer cells. Cancer Res 2011; 71: 5754–5764.
44 Armstrong P, Kelley DS, Newman JW, Staggers Sr FE, Hartiala J, Allayee H et al. Arachidonate 5-lipoxgenase gene variants affect response to fish oil supplementation by healthy African Americans. J Nutr 2012; 142: 1417–1428.
45 Caile EF, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003; 348: 1625–1638.
46 Whitley BM, Moreira DM, Thomas JA, Aronson WJ, TERRIS MK, PREST JR ET al. Preoperative weight change and risk of adverse outcome following radical prostatectomy: results from the Shared Equal Access Regional Cancer Hospital database. Prostate Cancer Prostatic Dis 2011; 14: 361–366.